Exhibit B

Duke University
Research Description Document
"Duke DARPA Pandemic Prevention Platform (P3)"
Revision 3 (January 2020)

The Contractor shall furnish the necessary personnel, materials, facilities, and other services as may be required to manage and complete the contracted effort in accordance with this Research Description Document (RDD) and the schedule set forth herein. The work and services to be performed hereunder shall be subject to the requirements and standards contained in the above titled Contractor's proposal, copies of which are in the possession of both parties. In the event of an inconsistency between the provisions of this Cooperative Agreement and the Contractor's proposal, the inconsistency shall be resolved by giving precedence in the following order: (1) the attachments to the cooperative agreement, and (2) the Contractor's proposal. The contracted effort is unclassified.

(b)(6) Support for the project may not continue without the active direction of the PI. The PI shall keep the DARPA Program Manager, the Agreements Officer Representative (AOR) and the Agreements Officer informed on contract progress through submission of the reports and other deliverables identified in Exhibit A, DARPA Agency Specific Terms and Conditions (July 2016), Paragraph 5. Reports and Reports Distribution, as described below.

Quarterly R&D Status Report - The contractor shall provide quarterly progress reports as applicable, to the addressees identified in Cooperative Agreement Exhibit A, paragraph 5. b) Report Distribution. The purpose of these reports, 5-10 pages in length, is to present a summary of work completed by RDD tasking and milestones met; the advancement in the state-of-the-art on the research and development involved; discuss any problems encountered; update the program schedule, present the program financial status; and discuss remaining work, using the template below:

(i) A description of progress during the current quarterly reporting period. Please do not include extensive discussions of previous results.
(ii) A table with the following headings: Task (including sub-tasks); milestone/ deliverable; planned start date; planned completion date; percent completion; status (before-, on-, or behind schedule).
(iii) A summary of all problems or areas of concern.
(iv) Planned activities and milestones for the next reporting period.
(v) A description of any major equipment purchased or constructed during the reporting period.
(vi) Notification of any changes in key personnel associated with the contract during the reporting period.
(vii) A summary of substantive information derived from noteworthy trips, meetings, or conferences held in connection with the contract during the reporting period.
(viii) Related accomplishments (publications, patents, media coverage) since the last quarterly report.

Final Technical Report - The Final Technical Report and Final Financial Status Report shall address the topics set forth below and be delivered in accordance with Cooperative Agreement Exhibit A, paragraph 5. b) Report Distribution.

(i) Task Objectives

(b)(6)

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Exhibit B – Research Description Document has been updated in Month 28 (January 2020) to reflect feedback from DARPA Program Manager/SETA.

(b)(5)

(ii) Technical Problems
(iii) General Methodology (i.e., literature review, laboratory experiments, surveys, etc.)
(iv) Technical Results
(v) Important Findings and Conclusions
(vi) Significant HardwareDevelopment
(vii) Special Comments
(viii) Implications for FurtherResearch
(vix) Standard Form 298, August 1998

The Contractor shall also submit a MonthlyFinancialStatus to provide financial program progress and inform the DARPA Program Manager of any potential cost issues. The financial status shall be prepared using the Attachment (1) Revision 1 template and submitted to

(b)(6)@darpa.mil.

Reports and papers shall be marked using Distribution Statement A: "Approved for Public Release." Papers and articles published as a result of DARPA sponsored research shall include a statement reflecting the sponsorship. In addition, a bibliography of the titles and authors of all such papers are to be included in the Final Technical Report. The cover or title page of each of the above reports or publications prepared, will have the following citation:

Sponsored by:
Defense Advanced Research Projects Agency
Biological Technologies Office (BTO) Program: PandemicPreventionPlatform (P3) Issued by DARPA/CMO under Contract No. HR0011-17-2-0069

Travel is authorized for attendance at the Kickoff Meeting and PI Meetings.

Animal use is anticipated in this effort. The contractor shall obtain all necessary Institutional Animal Care and Utilization Committee (IACUC) approval and demonstrate this approval to the Government prior to beginning experimentation with animals. If animal use is no longer anticipated, or changes significantly from the approved IACUC then the PI must submit a letter stating the discontinuation of animal use for this effort and/or receive appropriate authorization for IACUC changes of previously specified protocols. Unless prior approval by DARPA is given, IACUC documentation must be provided prior to contract award. Absolutely no funds can be used for animal research and testing (including, but not limited to, animal purchase, housing and care) until DoD veterinary approval is granted.

1.0 SCOPE

For each Task Area below, we describe the scientific team, the task area approach, and proposed innovations. Innovation and platform development is expected to occur within an initial 24-month developmental phase with much of the development activities focused on the first 12 months and waning thereafter. A single capability demonstration is planned 24-30 months (with Duke-selected pathogen influenza).

Exhibit B – Research Description Document has been updated in Month 28 (Jan 2020) to reflect deletions and additions based on platform optimization and feedback from DARPA Program Manager/SETA.

Justification of Pathogens (Influenza and Chikungunya virus)

The World Health Organization (WHO) estimates that influenza transmission results in up to 500,000 deaths annually. Furthermore, influenza virus has been estimated to be responsible for an annual economic burden in excess of $87 billion. The current standard of care for influenza prevention is seasonal immunization with a multivalent cocktail of inactivated influenza viruses predicted to be antigenically representative of circulating strains (H1N1, H3N2 and B). The majority of influenza virus vaccine preparations available for human vaccination in the United States are generated via propagation in embryonated eggs using a procedure that has remained virtually unchanged since the mid-1900's. The generation/administration of vaccines in this way **1)** is laborious resulting in a minimum five to six-month lag between virus isolation and vaccine availability **2)** may select for egg-adaptation mutations resulting in a loss of immunogenicity with regard to circulating strains, and **3)** requires sufficient time post- immunization for the development of virus-specific antibodies (~2 weeks). Thus, there is a critical need for fast-acting antiviral countermeasures, such as therapeutic antibodies, that can be rapidly isolated, evolved, manufactured, and safely delivered to at risk individuals. As such, naturally occurring infection with seasonal or a weaponized, highly pathogenic, influenza strain remains a significant global challenge to both civilians and the US military.

Chikungunya virus (CHIKV) is an *Aedes spp.* mosquito-vectored RNA virus and the etiological agent of chikungunya fever and the associated polyarthralgia disease. In recent decades, CHIKV has been responsible for millions of human infections in Africa, the Indian Ocean Islands, India, South America, the Caribbean, and Southeast Asia. Genetic adaptation of CHIKV has facilitated efficient transmission of the virus by *Aedes albopictus* mosquitoes, which have a wide geographic distribution. Epidemic activity of CHIKV has resulted in the emergence of human cases into the United States and Europe with the potential for subsequent localized transmission. As such, CHIKV remains a significant global public health concern and threat to US troops because of the potential for rapid global expansion due to human/mosquito transmission cycles, coupled with the debilitating nature of infection, potential for long-term sequela, and lack of approved vaccines/antivirals. As with influenza virus, there is a critical need for fast-acting antiviral countermeasures against chikungunya virus that can be rapidly isolated, evolved, manufactured, and delivered to at-risk individuals.

Below, we outline the assumptions of expected samples to initiate the viral propagation (TA1), complete antibody evolution (TA2) and produce a countermeasure (TA3) as part of our proposed platform; we have developed a variety of mitigation strategies to ensure we can use a broad array of starting materials. Within the Project Work Description and Requirements section below, we provide clear tasks/subtasks and deliverables for each Task Area, with timelines for each.

Task Area 1 (TA1): Approach/Innovation for On-demand Platform to Grow Virus

We propose a comprehensive P3 virus propagation platform capable of rapid and broad screening of potential culture systems and subsequent down selection for the generation of large-scale virus stocks to support downstream activities in TA2/3. Platform development and optimization will occur at Duke during the Developmental Phase (0-24 months). We will participate in a capability demonstration with influenza (Base).

Virus Propagation – (Task Areas 1.1, 1.2)

A major technical hurdle that must be overcome is the design and implementation of a virus propagation system

with sufficient breadth of susceptibility such that rescue and high titer growth of a diverse range of human viruses (DNA, RNA, enveloped, non-enveloped, etc.) is reasonably assured. Classically, the selection of culture systems for virus rescue/amplification has been predicated on tropism assumptions and knowledge of the virus group of interest. Arboviruses, for example, are inoculated into suckling mice, as well as mammalian and arthropod in vitro culture. Clinical/diagnostic virology laboratories often employ commercially available eukaryotic cells for the isolation and identification of virus in clinical samples. The cells used for viral diagnostics, including primary monkey kidney, human foreskin fibroblast, human embryo fibroblast, rabbit kidney, as well as various cancer/immortalized lines (MDCK, A549, HEp-2, etc.) are chosen because of their broad susceptibility to numerous well-characterized human viruses. Positive cultures are then identified visually (lethality, cytopathic effect (CPE), etc.) or immunocytochemically (ICC; cross-reactive antibodies). These techniques, though effective, are slow and laborious. Furthermore, although cell types used for diagnostics may be susceptible to infection with numerous viruses, they may or may not be competent for the production of sufficient quantities of virus progeny to support antibody discovery, efficacy, and evolution studies. **We therefore propose to develop in TA1 a "Thaw-and-Infect" eukaryotic cell culture array comprised of cell types/lines competent for the isolation and high titer growth of a variety of known and unknown viral isolates**.

Thaw-and-Infect culture array: We will identify appropriate cell types capable of supporting replication and virus stock propagation for all virus families known to induce significant disease in humans. The identified cell types/lines will be curated from appropriate commercial vendors/national repositories (ATCC, BEI Resources, etc.) and amplified (RBL Virology Unit) to generate in-house cryopreserved seed-lots in accordance with vendor recommendations. As possible, we will develop/adapt cell lines to a minimum number of standard growth conditions (medium composition, temperature, atmospheric conditions, etc.) to optimize downstream efficiency of culture array setup and viral growth competency screens. SOPs will be developed for seed stock generation, recovery, and propagation as part of the TA1 virus propagation platform.

We anticipate the final virus culture platform array format to consist of a single or multiple multi-well culture plates. For all cell lines, we will identify a seeding density that results in an infection-appropriate confluence in this format and use this information to generate working frozen stocks of appropriate density that can achieve this confluence within 18-24 h of recovery from cryostasis. The "shelf- life" of working lots of all lines, in terms of percent viability, will be evaluated approximately every 3 months and after cryopreservation. Once we have generated seed and working lots of all relevant cell lines, we will evaluate the ability of the culture array to rescue selected viruses. The focus of effort in the Base agreement will be influenza and CHIKV.

In the event of a public/military health crisis, we recognize that the "virus" could be supplied as: **1)** infectious seed stock of known or unknown sequence/identity, **2)** clinical/surveillance sample from an infected individual, or **3)** viral sequence information without infectious material. However, we envision scenarios 1 and 2 being far more probable (i.e., sponsor-supplied infectious material for subsequent propagation). Therefore, we will evaluate the ability of the Thaw-and-Infect cell array to rescue selected viruses in the following contexts: direct inoculation of infectious seed stock (infectious culture supernatant) and virus "spiked" into an appropriate clinical/surveillance milieu, such as Zika virus in mosquito homogenate or human rotavirus in a fecal suspension.

Because we recognize the potential that during a pandemic outbreak only electronic viral sequence information may be available, we will work with Synthetic Genomics Vaccine, Inc. (b)(6) Task 1.2) to optimize their protocols for the synthesis of error-free viral infectious clone genome for direct transfection Synthesis demonstrations will help to identify areas that can be improved to reduce the time required to synthesize varied virus genomes. Once synthesized, the genomes will be sequence-verified and delivered to Duke for virus rescue. Additionally, we will quantify transfection efficiency of all cell lines in the panel using up to five commercially available transfection reagents and DNA/RNA expressing a standard fluorescent protein.

Using our Thaw-and-Infect cell array platform, we will rapidly identify permissive cell lines and use visual estimation of replication metrics (kinetics of spread and median fluorescence intensity; MFI) to select permissive lines for propagation of large-scale cultures. Briefly, 18-24 h prior to inoculation the cell array will be rescued from cryostasis, re-fed with appropriate growth medium and maintained at standard growth conditions (1 day).

We anticipate the sponsor supplying a minimum of 1.0 mL virus source material, which will be divided evenly for identification and propagation. The virus source material will be uniformly suspended in an appropriate volume of infection medium (~5.0 mL representing a maximum 10-fold dilution), sterile filtered if necessary, and used to directly inoculate our culture array. The inoculum will remain in contact with the cell monolayers for 1-2 h and may involve centrifugation- enhanced inoculation as this has been shown to enhance rescue of some viruses. The inoculum will then be removed, and the cells refreshed with appropriate virus growth medium. Viral replication in the cell culture panel will be imaged every 8-12 h, as described below, using virus sequence-specific Fluorescently-tagged Oligonucleotide Probe (FOPs) hybridization to viral nucleic acid and live cell imaging or monitored more efficiently with a Virus Particle Counter. Virus seed stocks will be harvested at ~72 h from up to n=5 cell lines based on the results of the kinetics/MFI analysis as supernatants/cell lysates (3 days). In addition, culture supernatants will be sent for sequence verification.

Virus detection: Classic methodologies for the visualization of virus infected cells (CPE or ICC) are slow requiring days to weeks for sufficient replication/antigen expression and the availability of appropriate immunological reagents. We propose to significantly expedite this process by instead directly detecting replicating viral nucleic acids via the hybridization of viral sequence-specific (commercially purchased) FOPs in permeabilized live cultures coupled with fluorescent live single cell imaging using a high-throughput multimodal plate reader or by using a Virocyte Virus Particle Counter. During the development phase, and in parallel with our cell culture array curating activities, we will develop/optimize conditions for these detection methodologies using our selected viruses (influenza, chikungunya) and appropriately matched cell culture systems, which we will subsequently include during our cell culture panel qualification tests.

Approximately every 8-12 h following inoculation, the virus growth media will be removed and stored in a matched multi-well plate, followed by reversible permeabilization of the cell monolayers using streptolysin O or comparable reagent. To achieve signal specificity and mitigate background, following permeabilization, we will incubate cultures with tagged oligo-nucleotide pairs capable of fluorescence resonance energy transfer similar as to previously described. We anticipate evaluating n=3-5 probe pairs per virus to determine if there is a preferable target(s) based on virus-specific replication parameters/kinetics. Following completion of this live cell imaging, the monolayers will be washed, virus growth medium returned, and culture incubation continued. Although it may be necessary to incubate propagation cultures for multiple days to obtain peak titers, we anticipate the ability to detect viral nucleic acid using FOPs by 24-48 h post-infection. Once this methodology is fully optimized, we anticipate the ability to rapidly quantify titer (tissue culture infectious dose 50%; TCID50 or focus forming assay; FFA) of in-house generated stocks using readily available commercial reagents (**2 days**).

During the optimization work (0-12 months) new rapid virus particle counting technology became available that could eliminate the process described above with FOPs and accelerate TA1 virus growth assessment. This technology will be incorporated into the TA1 platform workflow and is anticipated to reduce the overall timeline by 20-25%.

Culture scale-up: We will aim to generate virus working stocks of 500 mL per lot at a titer of $\geq 10^6$ FFU/mL to support Plaque Reduction Neutralization Test 90% (PRNT90) assays, fluor-labeling of virus for cell sorts, **w**hole virus ELISA assays and animal model challenge studies**.** We will compare 72 h titers of the top 5 first round cultures down select three candidate cell lines for the generation of large scale working stocks. To decrease the temporal lag between seed lot generation/titration and working lot amplification, we will rescue larger cultures of all five preliminary cell lines from cryostasis while titration plates are incubating/developing. However, only the three "best" lines (based on kinetics of spread, MFI, and titer) will be inoculated to generate the large working lots. Eukaryotic cell culture expansion is the major time-limiting step in large-scale virus propagation. It is standard practice (RBL Virology Unit) to cryopreserve eukaryotic cells at a density between $1x10^6$ and $2x10^6$ cells/mL. Starting with this cell density, we estimate it would take 7-8 days of *in vitro* culture expansion to reach a sufficient cell count ($\geq 1x10^8$) to seed a multi-layer flask (~1,720 cm^2) capable of supporting the growth of a ~500 mL culture. Therefore, to reduce this lag time, we propose to identify conditions for the generation of high density frozen cell stocks (~10^8 cells/mL) similar to as previously described.

For all lines included in the Thaw-and-Infect array, cells will be frozen at various densities (up to 10^8 cells/mL) and the effects of freezing density on viability upon recovery following 7-14 days of cryostasis will be evaluated. If necessary, we will optimize the composition of the freezing medium to maximize the viability of high density cell stocks. Working lots (25 x 1.0 mL aliquots) will be generated for all cell lines at a density that can support virus propagation (up to 500 mL in volume) 24-48 h after sample acquisition, or at the maximal density that does not have a negative impact on post-thaw viability. We will also evaluate the "shelf-life" of working lots of all lines in terms of percent viability every 3 months after cryopreservation.

Prior to inoculation, high density cultures will be rescued from cryostasis, re-fed with appropriate growth medium and maintained at standard growth conditions (1-2 days). The respective virus seed stocks will be uniformly suspended in an appropriate volume of infection medium, sterile filtered if necessary, and used to directly inoculate large cultures. Following an appropriate infection time (1-2 h), the inoculum will then be removed and the cells refreshed with appropriate virus growth medium, and the cultures maintained under standard conditions. Virus yield in large cultures will be monitored every 8-12 hours via rapidly developed quantitative real-timePCR or virus particle counting. Cultures will be harvested upon reaching a copy number of $\geq 10^{10}$ copies/mL (~10^6 infectious units/mL, assuming a 10,000:1 genome to infectious unit ratio) or when less than a 10-fold increase is observed in three subsequent measurements (3-5 days). Working lots of virus will be harvested as cell culture supernatant/lysate and quantified via TCID50 or FFA. Additionally, the genetic diversity/drift of working virus lots relative to source material (or earliest passage for which sufficient material is available to support this analysis) will be evaluated by sequencing.

As mentioned above we will also explore the use of a ViroCyt Virus Particle Counter to monitor virus growth across all aspects of the TA1 platform. This innovative new piece of equipment would be used to count virus particles in culture in order to rapidly evaluate virus growth during virus stock propagation. We will conduct several demo runs to evaluate the ability of this machine to meet our needs. This includes monitoring growth of several different viruses at multiple MOIs, growth media and time points. If this instrument proves to be more efficient at monitoring cell growth, as predicted, we will use this strategy in place of quantitative RT-PCR and FOPs to accelerate viral growth evaluation, saving several hours of time per platform run.

The TA1 platform strategy will result in generation of a working lot of virus sufficient to begin antibody identification and evolution (TA2) and can be used for further amplification/scale-up as necessary. **This will be achieved as follows (10-15 days):**

- Sequence identification of sponsor provided source material and synthesis of FOPs
- Primary down-selection of n=5 virus culture systems and generation of n=5 putative virus seed stocks (passage +1 stocks)
- Infectious unit quantification of virus seed lots and secondary down-selection of n=3 culture systems for working lot amplification
- Virus working lot propagation (passage +2 stocks), 500 mL culture volume and titer $\geq 10^6$ infectious units/mL

If both virus sequence and virus source material are available at the start then an educated jump into a permissive cell line can reduce this timeline to 6-10 days.

TA1 Analytics: To show progeny viruses are bio-identical to original isolate (or earliest passage for which sufficient material is available to support this analysis), Duke will utilize proteomic analyses to characterize virus isolates and progeny viruses used for subsequent countermeasure development. We have established methods in the DHVI/CGMP Analytics group to evaluate virus protein profiles from samples including SDS-PAGE, western blot and RP-UPLC to resolve and detect individual virus proteins that can be compared between stocks. Furthermore, when paired with mass spectrometry, these methods can verify protein identity, sequence (in containment ABI sequencer), and detect glycosylation and other protein modifications. The Duke proteomics facility (https://genome.duke.edu/cores-and-services/proteomics-and- metabolomics), led by (b)(6) (b)(6) is equipped to support this analysis, as required. Similarly, virus stocks can be evaluated using LC-MS methods as a reliable and reproducible way to identify and quantify their lipid composition . Data analysis will

be supported by the DHVI/DARPA P3 statistics team as needed.

Innovation: Our approaches for virus amplification and quantification are built upon proven classic methodologies. However, we believe the combination of rapid quasi-real-time imaging of replicating viral nucleic acid combined with a culture array comprised of representative cell lines capable of supporting replication of all known human virus families will greatly expedite virus replication/quantification. Specifically, this virus growth platform will **1)** rapidly identify and down-select putative culture systems, **2)** reduce incubation times for virus quantification assays, **3)** reduce the lag time between seed and working stock propagation, **4)** enable quick/accurate multiplicity of infection calculation for optimal growth in subsequent virus preps, and **5)** facilitate rapid turnaround neutralization assays for the support of TA2/TA3 activities.

Task Area 2 (TA2): Approach/Innovation for System to Isolate/Evolve Antibodies

The Duke DARPA P3 TA2 platform assumes two possible sample input scenarios based on real- world pandemic experiences (convalescent PBMC/plasma from an infected human or viral isolate/clinical specimen). Our current and innovative platform approaches to antibody isolation, evolution, screening and candidate selection, accommodating both pandemic sample types, are detailed below. During months 1-12 we made significant progress on our overlapping approaches and with DARPA guidance have down-selected and streamlined for focused development of a final TA2 platform:

Convalescent PBMC sample (Tasks 2.1-2.2)

If a peripheral blood sample from an infected (acute/convalescent) individual is available, we propose a streamlined approach. The (b)(6) laboratories have developed a suite of technologies that currently enable the identification and affinity optimization/evolution of neutralizing antibodies from memory B cells and plasma cells. (b)(6) has converted this approach to high-throughput 384 well plate platform and also built out the entire pipeline within BSL3/Select Agent containment. Together these technology platforms/approaches synergize to provide an unprecedented ability to rapidly isolate human antibodies and provide innovation opportunities to shorten the time of antibody isolation and countermeasure delivery.

The (b)(6) lab (UT Austin) will no longer be developing plasma Ab isolation components of the platform. Rather they will be reduced to a warm-ready consulting/content expert role in support of our isolation and evolution approaches. The (b)(6) lab has world-class approaches and tools for isolating plasma antibodies and for analyzing B cell lineages form NGS. These capabilities may benefit Duke P3 in the future as things evolve.

Isolation Approach: Pathogen-specific Memory B or Plasma Cells ((b)(6) *Duke):* During the 24- month developmental phase, fluorophore-labeled whole virions (provided by TA1 team), or other recombinant protein/VLP hooks will be used to sort influenza and chikungunya, specific memory B cells for antibody isolation. In addition, plasma cells will be sorted as single cells. The VH and VL genes of both single pathogen-specific memory B cells or single plasma cells will be amplified by PCR, and the full Abs will be produced by transient transfections using overlapping PCR. Bulk plasma cell sorting will also allow us to eliminate the need for use of fluorophore-labeled virions for sorting.

Innovation: Currently, memory B or plasma cell sorting and pathogen-specific Ab isolation, specificity identification and Ab production takes ~17 days. We propose to innovate to decrease this time to at most 12 days. Within the first six months we plan to streamline our existing RT-PCR process by combining PC primers used in the nested PCR process. This will reduce the number of PCR plates in our first and second round of PCR. Furthermore, the streamlined process will reduce the number of plates that need to be sequenced (in containment ABI sequencer). Reductions in PCR and sequencing will speed up isolation of candidate countermeasure antibodies.

Evolution Approach:. Improving antibody neutralization potency H/L Chain Swapping (b)(6) (b)(6) *Duke):* Concurrent with fluorescent whole virus-specific single B cell sorting experiments, unpaired antibody variable heavy (VH) chain gene and variable light (VL) chain gene next generation sequencing (NGS) will be performed. Once neutralizing antibodies have been identified from the whole virus-specific single B

cell sorts, Cloanalyst (PMID 24795717) will be used to probe the NGS dataset for additional clonally related VH and VL chain gene sequences. The [redacted] laboratory (Duke) will use Antigen Receptor Mutation Analyzer for Detection of Low-Likelihood Occurrences (ARMADILLO) (Pubmed 29861171) to computationally determine clade independent somatic hypermutations that have been strongly selected for by the immune system. Mutation events will be ranked by frequency and up to twenty-two of the most frequent amino acid mutations will be selected. Combinatorial libraries containing all mutation pairings possible (up to 1 x10^6 VH or VL chain gene sequences) will be in-house made and rapidly synthesized on the Synthetic Genomics BioXP3200. VH + VL chain gene pairs will be transiently transfected into mammalian cells. The cells with the highest binding to fluorescent whole virus (TA1) will be sorted for RT-PCR of VH + VL chain genes. These antibodies will be tested in neutralization assays (TA1).

Innovation: Instead of using random mutagenesis this approach relies upon next generation sequencing and computational methods to identify mutations selected by the host immune system. Using the Synthetic Genomics BioXP3200 enables large combinatorial libraries to be rapidly synthesized. Combined with mammalian cell display and fluorescent whole-virus single-cell sorting, synergistic mutations that are likely to increase antibody binding affinity - hence increase neutralization potency – can be identified. The improved neutralizing antibody VH/VL chain gene sequences will be transferred to the TA3 team for pre-production analysis.

Task Area 3 (TA3): Approach/Innovation for Countermeasure Delivery

Messenger RNA (mRNA) is a promising new therapeutic delivery platform. While its application to therapeutic targets including infectious diseases is still in its infancy, work by our team and others has shown that it is potentially transformative. We have established an RNA platform that has the combined benefits of potent delivery, safety and straightforward, rapid production suitable to deliver medical countermeasures.

We have shown that our nucleoside-modified mRNA is a safe and rapid platform for delivery of therapeutic antibodies. Systemic administration of 1.4 mg/kg of nucleoside-modified mRNA encapsulated in lipid nanoparticles encoding the anti-HIV-1 antibody VRC01 resulted in plasma antibody titers of ~170 µg/mL 24 h post-injection in humanized mice. Protective antibody titers were maintained for >1 week following a single administration and were maintained at ~40 µg/mL with repeat administration for over five weeks. Importantly, treatment with half this dose was sufficient to provide full protection from intravenous HIV-1 challenge, demonstrating it is a viable delivery platform for passive immunotherapy. In total, these results show that the current modified-mRNA platform approach meets pre-determined success criteria (criteria 1, 2, 3 and 4). We expect that appropriate Ab evolution to increase potency (TA2) will significantly reduce dosing requirements. Under the Duke DARPA P3 program, we will improve and apply the mRNA platform for delivery of antibodies as effective medical countermeasures for emerging infectious diseases.

RNA Platform for Countermeasure Delivery: We are advancing our RNA platform for a number of clinical applications including therapeutics and vaccines. Scalable GMP processes have been established for mRNA and lipid nanoparticle production in partnership with BioNTech GmbH and Acuitas Therapeutics, respectively. Clinical products using these technology platforms have been safely evaluated in humans (ClinicalTrials.gov identifier: NCT03014089). To facilitate seamless integration into the overall Duke DARPA P3 program, we will transfer these processes and associated release methods to the DHVI CGMP facility. This facility can produce RNA countermeasures at the necessary scale (>1,000 doses) and has the required manufacturing, analytical testing, quality, regulatory and clinical expertise to support the program.

The RNA component of the product is produced *in vitro* from a DNA template (using sequences provided by TA2) through a series of enzymatic reactions utilizing bacterially-expressed enzymes that are commercially available. To initiate the production process, template plasmid DNA (produced in *E. coli* or synthesized on a BioXP) is linearized to allow synthesis of runoff transcripts with the desired 3' end. Alternatively, we may use Rolling Circle Amplification (SGI).

Next, mRNA is synthesized with ribonucleotide triphosphates (rNTPs) by T7 DNA-dependent RNA polymerase. Modified nucleoside triphosphates are included in the reaction mixture for incorporation into the RNA product. The template DNA is then degraded by incubation with DNase. Finally, the mRNA is

enzymatically capped by vaccinia capping enzyme, and the cap1 structure is formed by addition of the enzyme 2'-O-methyltransferase. Incorporation of modified nucleosides, such as pseudouridine and 1-methylpseudouridine, and HPLC purification to remove contaminating double-stranded RNA, increases protein translation while rendering the mRNA immunologically silent. CGMP runs up to three-gram scale have been routinely produced and can be scaled-up further, as needed.

Lipid nanoparticles (LNPs) containing an ionizable lipid have proven to be excellent RNA carriers *in vivo* and are currently in clinical trials for siRNA delivery. Purified RNA will be formulated into lipid nanoparticles using a self-assembly process in which an aqueous solution of RNA at pH 4.0 is rapidly mixed with a solution of lipids dissolved in ethanol. This process is conducted using a two pump system to combine the RNA solution and lipid solution in a controlled reaction using a T-junction connector. The ethanol is then removed from the resulting LNP preparation, and the product is exchanged into final formulation buffer by tangential flow diafiltration. Finally, the LNPs are filtered through a 0.2 µm sterilizing grade filter. This process is readily scalable and has routinely been utilized to produce LNP at the one gram scale.

The production processes for RNA and LNPs are rapid and straightforward and can be conducted in less than a week, which makes the platform uniquely positioned for rapid response. The rate- limiting steps of the process are the production of sufficient plasmid-DNA template encoding the biologic of interest and safety testing of the product. We will evaluate opportunities to make improvements in both of these areas as the program is developed (see platform improvements below).

Innovation: In order to develop an RNA-based medical countermeasure with optimal potency, half-life and ease of use in the field; we will evaluate a number of improvements to the platform during the 24- month developmental phase of the project and implement those that support this objective in the capability demonstrations planned throughout the Base. Potential improvements to be evaluated include the following: mRNA modifications such as 5' cap, optimized 5'- and 3'-UTRs and coding sequence, and different lengths of poly(A)-tail, SMART RNA replicons (Synthetically Modified Alpha Replicon Technology), optimized lipid nanoparticle formulations, improved delivery to utilize less invasive routes of administration, and platform integration improvements. Detailed plans for each improvement area are described below.

Optimize LNP delivery formulation: Our first generation formulation showed that high levels of circulating Ab could be produced with intravenous delivery of RNA/LNPs. Since a simpler route of administration and more potent formulation is desired, we will evaluate alternative delivery routes including both systemic and inhalation to identify an optimal method that will deliver a simpler and more potent product. Formulations that increase circulating Ab titer (i.e., ≥ 20% of peak titer), and/or increase duration of expression (i.e., titer > 10 ug/mL for 45 days post-administration) though a simpler delivery route, will be advanced for integration into pre-clinical and clinical testing. Our collaborators, Acuitas Therapeutics and Arcturus Therapeutics, continue to optimize LNP formulation to increase both liver translation and for use with alternative routes of delivery. We will select the best formulations that have also passed their toxicity tests by the criteria above.

So far, we have delivered modified mRNA-LNPs by the intradermal (i.d.), intramuscular (i.m.), intravenous (i.v.), intraperitoneal (i.p.) subcutaneous (s.c.), and inhaled (i.n.) routes with very high levels of protein translation. Based on these data, we intend to move forward with s.c. administration as the primary route of delivery, since it yields high protein expression, is simple, relatively non-invasive with a straightforward regulatory pathway for clinical use. We will examine delivery of modified mRNA-LNP therapeutics using wild type or FcRn-/- hFcRn mouse model and will select the delivery route based on favorable expression profile, ease of administration, and clear path for clinical implementation (see Animal Testing).

Optimize the RNA (b)(6) *Duke;* (b)(6) *UPenn;* (b)(6) *SGVI):* The ability of an RNA to be translated at the highest level (peak and duration) determines the dose of that RNA required for a therapeutic effect. Increasing inherent translation of RNA therapeutics reduces the dose required to reach protective Ab concentrations. The optimization of protein translation from RNA, therefore, becomes an important component. The mRNA encoding each new therapeutic Ab developed needs to be optimized, including the cap structure, 5' and 3' untranslated regions (UTRs), coding sequence conformation, nucleoside modifications, and poly(A) tail length, whose optimums can differ for every coding sequence and the target cell type. Such

improvements can increase translation by over 50-fold compared to the wild-type mRNA. As these optimizations are not possible on a 60-day time scale, we propose to perform a formal optimization for the cell types targeted by the delivery route to be used determined above using three existing DHVI human monoclonal antibodies. Our plan is to identify the five best optimizations using these three model mAbs to establish an approach to sequence optimization. Using this platform we can quickly screen/select optimal sequences to deliver countermeasures identified in TA2 for CGMP production.

First, the optimal cap structure (enzymatic, NRcap, "CleanCap (Trilink)", isopropylidene dinucleotide cap analogs, and phosphorothioate caps) and poly(A) tail length will be analyzed for the three model mAb coding sequences using primary cells that translate delivered mRNA for the site of injection followed by *in vivo* delivery. Next, various optimal UTRs and coding sequence optimizations will be analyzed. We have developed a set of plasmids containing a 5' sequence that ensures complete enzymatic capping and 3 different 5' and 2 different 3' UTRs that allows optimization of UTRs for any coding sequence using 6 plasmids. This yields a set of plasmids containing the best UTRs combined with the optimal poly(A) tail length. Two different codon optimizations are cloned into each plasmid and each plasmid is linearized and 3 different mRNAs are made containing Ψ, m1Ψ, or Ψ + m5-cytosine nucleoside modifications. The resulting mRNAs will be used to transfect primary cells that represent the tissues exposed though the selected delivery route (i.e., hepatocytes for IV delivery). A platform including the five best mRNA coding sequences will be defined through testing using model human mAbs. This will allow us to rapidly deploy the best modified mRNA constructs for expression of new human mAbs for delivery as countermeasures once the platform is fully developed.

In addition to improvements in modified mRNA, we will also evaluate whether RNA replicons can increase peak Ab titer and extend Ab expression in vivo. SGVI has developed a self-amplifying RNA vector based on an alphavirus derived from the attenuated TC-83 strain of Venezuelan equine encephalitis virus that can overcome innate immune response shutdown (vector termed SMART: Synthetically Modified Alpha Replicon Technology). Whole body IVIS imaging of mice injected with either SMART or TC-83 replicon RNA expressing luciferase protein revealed that the SMART RNA expressed significantly more luciferase on days 1, 3 and 7 post-injection and remained higher than the TC-83 replicon until day 14. In addition, luciferase was detected at time points out to 28 days post SMART RNA injection demonstrating significant duration of expression.

To select the RNA improvements to be incorporated into the overall Duke DARPA P3 platform for capability demonstration, we will evaluate RNA construct designs. Head to head evaluations of the two most potent RNA designs encoding model mAbs will be first evaluated *in vitro* the relevant human primary cell substrate followed by *in vivo* studies in wild-type or FcRn-/- hFcRn mice to evaluate pharmacokinetics and in ferrets as a Flu disease challenge model (see Animal Testing, **Table 1**). The selected platform improvements will then be prepared for incorporation into development runs and scaled up for use in capability demonstrations and clinical trial material production.

Platform Integration Improvements (b)(6) *Duke:* (b)(6) *SGVI):* We will also evaluate platform improvements aimed at seamlessly integrating RNA production into the overall response and shortening timelines for delivery. The two areas with the most significant opportunity for improvement are reducing the time from Ab sequence to template DNA and the time for product release. We will work to address these bottlenecks using proven new technologies from SGVI (*in vitro* gene assembly, BioXP) and others (rapid sterility testing). Details of the approaches are below. Heavy and light chain Ab genes can be engineered into RNA very rapidly. The process consists of designing overlapping oligos, synthesis of error corrected genes, assembly of the genes into the RNA vector and synthetically amplifying the vector DNA. The amplified DNA can then enter the RNA production process as a critical reagent. Time from Ab gene sequence to template DNA is less than one week; construction of multiple RNA vectors can occur in parallel, so the requirement for more than one construct does not increase time to generate. We will compare performance of synthetically assembled DNA to the traditional plasmid DNA approach in year one to verify resulting RNAs are of identical quality and potency using the proposed analytical release panel and *in vivo* testing. If selected, the synthetic process would be scaled up to support capability demonstrations and GMP clinical trial lot production.

Animal Testing: Animal studies in support of this program could include a number of animal models including

wild-type, FcRn-/- hFcRn mice, IFN-α/βR-/- mice, ferrets, rabbits and non-human primates (NHPs), as well as other appropriate animal models based on the DARPA-provided challenge virus (**Table 1**).

Table 1: Proposed Animal Models for Duke DAPRA P3		
Platform Development Phase		
Model	**Purpose**	**Rationale**
WT or FcRn-/- hFcRn Mice	• RNA comparisons • LNP formulation • Dosing optimization • Route optimization	• Used for pK studies with human IgG • N >= 10 per group; 35 day study duration • Reduced induction of anti-human IgG responses
Ferret	• Human Influenza challenge studies	• Clinical symptom/disease model of seasonal influenza virus infection, • N=10 per group (control, countermeasure + challenge) • 1° Endpoints: weight loss, clinical score
Capability Demonstrations / Integration Phase		
WT or FcRn-/- hFcRn Mice	• Dosing study for pre- clinical data	• Used for pK studies with human IgG • N=10 per group, 12 groups for dose finding • Reduced induction of anti-human ○ IgG responses
Ferret	• Human Influenza challenge studies (Capability Demonstration #1)	• Clinical symptom/disease model of seasonal influenza virus infection • N=10 per group (control, countermeasure + challenge) • 1° Endpoints: weight loss, clinical score,
Non-Human Primate	• Determine countermeasure peak concentration • Determine countermeasure variability (<10%)	• Large animal studies suited to replicating human physiology • N=5 per group (control, countermeasure + challenge); 60 day study • Endpoint: TBD

Studies during platform development phase are planned to assess *in vivo* pharmacokinetics (pK) and potency. The kinetics of the countermeasure *in vivo* will be determined by ELISA using population pK analysis of multiple animals. We will also conduct challenge studies during development as needed.

Studies during the capability demonstrations will assess countermeasure concentration using multiplex Luminex bead-based assays to determine peak human antibody levels and half-life. We will also determine the inter-NHP antibody concentration variance with the expectation that it will not exceed 10%. Appropriate animal models will be selected for the capability demonstration challenge studies (see **Table 1**) and model-specific (animal model and virus) quantifiable metrics will be defined prior to challenges studies (i.e., death, temperature, weight loss, etc.).

Task Area 4: Approach/Innovation for Platform Integration

Across the 30-month program we will develop a fully-integrated end-to-end platform that can start with unknown samples from a viral outbreak and be prepared to produce an efficacious and safe CGMP medical countermeasure scalable to 20,000 doses within 60 days (see Figure 2, below). To achieve successful platform integration, all task areas will work together harmoniously to ensure work is proceeding in an efficient manner. Having activities centered at DHVI with contributions from subcontractors will be key to this efficiency.

TA1 will initiate with viral propagation at the Duke RBL. We are confident our TA1 team will have the

ability in the end-to-end platform to rapidly identify unknown viral pathogens. Furthermore, the "Thaw and Infect" approach coupled with methods to rapidly quantify virus developed by (b)(6) will enable viral propagation. TA1 and TA2 may be initiated concurrently. For TA2, we have proposed multiple, innovated approaches through the Base that can be used regardless of source samples. Furthermore, by centering isolation/evolution strategies at Duke, we can have concurrent activities to yield the most potent antibodies without compromising time. While the antibodies are evolved/isolated, the TA2 analytics teams will be preparing for binding and neutralization testing.

While TA1 and TA2 proceed, TA3 will initiate preparation for CGMP production. Once the top five Ab sequences are selected, the TA3 teams at UPenn/SGVI will verify optimal vector sequence and design in parallel with final potency evaluation. Once potency and vector optimization is complete the Duke team will initiate RNA production of the best RNA mAb countermeasure candidate. LNPs will then be produced to encapsulate the RNA and the RNA:LNP product will undergo fill/finish and release testing will be done. Concurrent to these activities the regulatory/clinical and/or animal study teams will be preparing for subsequent human or animal studies.

Our approach is unique in that we are combining the best of academia and industry to move pandemic preparedness forward with established platform methodologies and novel innovative approaches to accelerate medical countermeasure development and delivery. Process development and capability demonstration work progressing through the three Task Areas above will be coordinated and synergized by a well-established administrative team of forward leaning project managers, financial/contracts managers, regulatory experts, clinical investigators, etc.

Background: Biologic countermeasures, such as monoclonal antibodies (mAbs), are a rapid/effective means of controlling and containing outbreaks of emerging pathogens where no licensed therapeutic or vaccine is available. However, standard paradigms for their production and delivery limits their use as a first- line prophylactic or therapeutic, since the process for their identification, optimization and production can take months to years. The Duke DARPA Pandemic Prevention Platform (P3) team seeks to apply its experience, innovations, cutting-edge research portfolio, and in-house CGMP manufacturing capabilities to greatly expedite mAb countermeasures for future pandemics. The fully integrated platform will be a major advancement in rapid pandemic countermeasure development and will address the significant global challenge pandemic outbreaks have on both civilian and military populations.

The Duke University DARPA P3 team will be centrally based at the Duke Human Vaccine Institute (DHVI), a 220+ person organization for bench-to-bedside research, including a 38,000 gross sq.ft. BSL-2/3 NIAID-built regional biocontainment facility, in-house CGMP manufacturing facility, and clinical trials capabilities, including the capacity for first-in-man phase I clinical trials at the Duke-NIAID Vaccine and Treatment Evaluation Unit (VTEU). The DHVI has, for the past decade, carried out innovative basic and translational research through highly effective national and international collaborations in the fight against emerging infectious diseases. For the DARPA P3 initiative, Duke investigators have partnered with outstanding collaborators with RNA vaccine and therapeutic platforms and manufacturing expertise ((b)(6) UPenn, and (b)(6) Synthetic Genomics Vaccines Inc.). This team is unique because: a) it has been working together for 12+ years; b) members are highly respected leaders in their fields; and c) the team can work quickly and expeditiously in an atmosphere of trust and selfless collaboration.

This multi-disciplinary team has the expertise, novel technology and ability to collaborate to attain the DARPA P3 goals. Duke's approach to meeting these goals is to focus on developing platform approaches drawing upon experience in growing a variety of different viruses, performing single-cell Ab isolation and evolution, and developing rapid and efficient RNA CGMP capabilities eliciting active and passive antibody protection.

Goals and Impact: The Duke DARPA P3 program will combine world-class expertise in virology, immunology and CGMP manufacturing to create a fully integrated platform capable of responding to a viral pandemic within 60 days. Within each Task Area, we identify the current state of the art of the platform proposed and how to improve performance and efficiency of the process. In doing so, we have struck a balance between innovation and taking reasonable risks to achieve workable solutions given the short timeframe. Our approach is unique in

that we are combining the best of academia innovation and industry production to move pandemic preparedness forward. Once the platform is developed, we will seek to publish advances to disseminate this important knowledge, as well as commercialize the platform to ensure a global impact to address the significant challenge pandemic outbreaks have on both civilian and military populations.

Our overall strategy (Base) to develop a fully integrated platform that will start with a viral isolate and peripheral blood from an outbreak subject and produce an efficacious and safe current good manufacturing practice (CGMP) medical countermeasure scalable to 20,000 doses within 60 days. Shown are our task area-specific objectives and deliverables. Duke DARPA P3 innovations include novel methods for identifying and culturing known/unknown viruses; creating innovative reagents to isolate protective antibodies; novel computational mAb engineering/evolution platforms; the use of nucleoside-modified mRNA and viral replicons to safely express therapeutic antibodies; and rapid RNA manufacturing and lipid nanoparticle formulation for active and passive antibody delivery. Assembly of existing platform technologies with these low and moderate to high risk innovative approaches will enable the integrated Duke DARPA P3 consortium to ensure product delivery that rises above the current state of the art in the development of pandemic medical countermeasures.

2.0 PROJECT WORK DESCRIPTION AND REQUIREMENTS

Task Area 1: On-Demand Platform to Grow Virus

The goal of the work in Task Area 1 will to be to develop methods to support viral propagation, so that virus can be used for downstream Tasks (i.e., whole virus sorts, ELISA, neutralization assays). Within Task Area 1, there are two subtasks (Task 1.1 and Task 1.2). The **updated (mod. Oct 2018)** task area approach, deliverables, and timeline are provided in the below.

1.1. Virus Propagation System Approach (Duke, (b)(6)

- "Thaw-and-Infect" eukaryotic cell culture array comprised of cell types/lines competent for the isolation and high titer growth of a variety of known and unknown viral isolates.
  - Identify optimal seeding/freezing density for infection-appropriate confluence between 24-36 hours following recovery from cryostasis.
  - ~25 aliquots per cell line.
  - Final format will consist of a single or multiple multi-well culture plates.
- Viral propagation monitored using Fluorescently-tagged oligonucleotide probe (FOPs) hybridization to viral nucleic acid, quantitative real-time PCR or Virus Particle Counter.
- Analysis of virus stocks to ensure quality
- High density frozen cell stocks of the "Thaw and Infect" culture array capable of supporting virus culture scale up.
  - Cell lines chosen based on influenza and CHIKV susceptibility in literature
  - Identify optimal conditions to generate high density frozen cell stocks (up to 10^8 cells/mL) that can support virus propagation (up to 500 mL culture volume) 24-48 hours following recovery from cryostasis.
  - ~25 aliquots per cell line.
  - Final format will be multi-layer flask.
- The "shelf-life" of working lots of all cell lines, in terms of percent viable recovery, will be evaluated for the first 12 months, then annually thereafter.
- Evaluate the ability of the Thaw-and-Infect culture array to rescue influenza virus (ss segmented -RNA) and CHIKV (181/25 vaccine strain; ss +RNA).
- Quality control - Progeny viruses will be sequenced (in containment ABI sequencer) to monitor drift. As directed by DARPA we will utilize proteomic and other biochemical analyses to characterize virus isolates (lipid, protein, and carbohydrate) using established methods in the DHVI/CGMP Analytics group, including SDS-PAGE, western blot and RP- Furthermore, when paired with mass spectrometry, these methods can verify protein identity, sequence, and detect glycosylation and other protein modifications.

1.1 Deliverables and Timelines

(b)(6)

Commented Minor edits made to align with current activity

Subtask	Deliverable	Timeline (months)
1.1.1	• Initial development of "Thaw and Infect" eukaryotic cell culture array reduced panel capable of supporting influenza virus and CHIKV propagation.	0-6
1.1.2	• Continued development of "Thaw and Infect" eukaryotic cell culture array full panel.	6-18
1.1.3	• FOPs specific for influenza virus detection/titration or alternative Virus Particle Counter approach • FOPs specific for CHIKV detection/titration or alternative Virus Particle Counter approach • Optimized methods/data report	6-12
1.1.4	• Quality-controlled seed stocks of influenza virus to support DARPA P3 tasks • Quality-controlled seed stocks of CHIKV to support DARPA P3 tasks	9-15
1.1.5	• High density frozen cell stocks of 15 cell lines in "Thaw and Infect" culture array capable of supporting Flu and CHIKV propagation within 24-48 hours after thaw.	9-37
1.1.6	• Quality-controlled working stocks of influenza virus to support DARPA P3 tasks • Quality-controlled working stocks of CHIKV to support DARPA P3	12-18

(b)(6)
Commented (b)(5)
(b)(5)

1.2. Viral Infectious Clone Genomes Approach (SGVI, (b)(6)

- SGVI will demonstrate their ability to rapidly synthesize error-free viral infectious flu genomes should a situation arise where sponsor can only provide viral sequence and not infectious source material.
- Overlapping oligonucleotides composing the Flu Hemagglutinin and Neuraminidase genes will be pooled, ligated and amplified by PCR. Amplified PCR products will then be error corrected using enzymatic methods.
- Next promoter ant terminator regions will be added by assembling into linearized plasmid encoding these elements and the final gene expression construct will be amplified.
- Once synthesized, the HA and NA genes will be sequence-verified and delivered to Duke along with plasmids encoding the other 6 Flu genes for virus rescue.
- Quality Control - Progeny viruses will be sequenced (in containment ABI sequencer) to monitor drift and ensure that the progeny are bio-identical to traditionally produced flu. . As directed by DARPA we will utilize proteomic and other biochemical analyses to characterize virus isolates using established methods in the DHVI/CGMP Analytics group, including SDS-PAGE, western blot and RP-UPLC to resolve and detect individual virus proteins that can be compared between stocks.

1.2 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
1.2.1	• One Influenza virus infectious clone genome	0-12

(b)(6)
Commented NEW TASK Months 31-48, (b)(5)
(b)(5)

1.3 Expansion of HD Cell Line Stocks

- Generate HD stocks of the remaining 15 cell lines would be made (≥ 25 aliquots) to ensure full array is ready

and transferable
- Each aliquot would be sufficient to seed 2 x 5-layer 875 cm^2 cell culture flasks
- Cells would be ready for infection within 48 hours
- For each cell line, density testing would be performed to identify optimal freeze media and density based on viable recovery after thaw

1.3 Deliverables and Timelines – OPTIONAL TASK

Subtask	Deliverable	Timeline (months)
1.3.1	• Expand High density frozen cell stocks to 30 cell lines for the "Thaw and Infect" culture array capable of supporting Flu and CHIKV propagation within 24-48 hours after thaw.	31-48

1.4 Virus Specific Impacts on Viral Growth platform

- Run additional virus types through the platform to de-risk virus-dependent differences and make it virus-independent
- We would utilize ~3 different viruses from different families
- This would strengthen the platform, add to knowledge base by assessing virus growth kinetics and cell susceptibility by qPCR along an infection time course
- This would also help to de-risk the platform by allowing us to identify potential differences with viruses other that influenza and CHIKV
- We have noted higher assay variability with flu than with CHIKV (FISH neut assay)
- Some viruses require cell disruption for harvest, which would affect the virus growth monitoring by qPCR

1.4 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
1.4.1	• Virus specific impacts on Viral Growth platform	31-48

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Task Area 2: System to Isolate and Evolve Antibodies

The goal of the work in Task Area 2 will to be to isolate neutralizing antibody to Flu and CHKV from acute/vaccinated/convalescent PBMC, improve the antibody ~100-fold in function by *in vitro* antibody evolution and transfer antibody sequence to Task Area 3 for production and delivery optimization/testing. Within Task Area 2, there are two subtasks (**Tasks 2.1 and 2.2**). The task area approaches, deliverables and timelines are provided below.

2.1. Optimize Antibody Isolation from Peripheral Blood Cells and Plasma

- ***Down-selected Isolation Approach.*** *Pathogen-specific Memory B or Plasma Cells (Duke, [(b)(6)]* Fluorophore-labeled whole virions (provided by TA1 team) or recombinant labeled protein will be used to sort influenza and chikungunya specific memory B cells for antibody H/L chain isolation. In addition, plasma cells may be sorted as single cells. The VH and VL genes of both single pathogen-specific memory B cells or single plasma cells will be amplified by PCR, and the full Abs will be produced by transient transfections using innovative and rapid overlapping PCR.
  - mAb candidates will be produced in small batches and progress through high-throughput binding assays (e.g. whole virus ELISA) and be down-selected for pathogen neutralization in collaboration with TA1 to establish baseline potency prior to handing off to evolution team.

2.1 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)

2.1.1	• Pathogen-specific antibody sequences from PBMCs from Flu-infected subjects using single memory B cell sorts or bulk plasma cells	0-6
2.1.2	• Pathogen-specific antibody sequences from PBMCs from CHKV-infected subjects using single memory B cell sorts or bulk plasma cells	6-12
2.1.3	• Method for sorting antigen-specific B cells using whole viruses (TA1)	6-12
2.1.4	• Pathogen-specific antibody sequences from PBMCs from Flu and CHKV-infected/vaccinated subjects using single B cell sorts using whole viruses	6-12

2.2. Optimize In Vitro Antibody Evolution

- ***Down-selected and Modified Evolution Approach.*** H/L Chain Swapping *(Duke, (b)(6) (b)(6)*:
  - We have down-selected an Ab evolution scheme focused on H/L chain swapping and augmentation of the H/L chain pool form NGS.
  - Concurrent with fluorescent whole virus-specific single B cell sorting experiments, unpaired antibody variable heavy (VH) chain gene and variable light (VL) chain gene next generation sequencing (NGS) will be performed.
  - Once neutralizing antibodies have been identified from the whole virus-specific single B cell sorts, Cloanalyst (PMID 24795717) will be used to probe the NGS dataset for additional clonally related VH and VL chain gene sequences.
  - The (b)(6) laboratory (Duke) will use Antigen Receptor Mutation Analyzer for Detection of Low-Likelihood Occurrences (ARMADILLO) (Pubmed 29861171) to computationally determine clade independent somatic hypermutations that have been strongly selected for by the immune system.
  - Mutation events will be ranked by frequency and up to twenty-two of the most frequent amino acid mutations will be selected. Combinatorial libraries containing all mutation pairings possible (up to 1 x10^6 VH or VL chain gene sequences) will be in-house made and rapidly synthesized on the Synthetic Genomics BioXP3200.
  - VH + VL chain gene pairs will be transiently transfected into mammalian cells. The cells with the highest binding to fluorescent whole virus (TA1) will be sorted for RT-PCR of VH + VL chain genes. These antibodies will be tested in neutralization assays (TA1).

2.2 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
2.2.1	• Whole virus binding ELISA and neutralization assays for Flu and CHKV	0-18
2.2.2	• Evolved antibody sequences from Flu-infected subjects	12-18
2.2.3	• Evolved antibody sequences from CHKV-infected subjects	18-24

2.3 Duke DARPA P3 platform Binding Assays and DNA Plasmids for Antibody *In Vitro* Transcription

- Perform ELISAs/Binding assays on samples from all pre-clinical IND-enabling animal studies in TA3
- Provide low-endotoxin Duke DARPA P3 antibody (H/L) plasmids for In Vitro Transcription to generate RNA for pre-clinical IND-enabling animal studies in TA3

2.3 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
2.3.2	• Duke DARPA P3 platform ELISAs/Binding Assays	31-48

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2.3.3	• DNA plasmid templates for Antibody *In Vitro* Transcription	31-48

Task Area 3: Deliver Medical Countermeasures

To develop an RNA-based medical countermeasure with optimal potency, half-life and ease of use in the field; we will evaluate a number of improvements to the platform during the 24-month performance period and implement those that support this objective in a pre-clinical capability demonstration using ferrets and NHPs. Potential improvements to be evaluated include mRNA modifications, SMART RNA replicons (Synthetically Modified Alpha Replicon Technology), and optimized lipid nanoparticle formulations. Within Task Area 3, there are five subtasks (**Subtasks 3.1 - 3.5)**. The task area approaches, deliverables and timelines are provided below.

3.1. Optimize RNA Platform Approach (Duke, UPenn, SGVI)

- We will develop modified RNA using model antibodies to deliver optimal expression.
- Parameters to be evaluated include the following: mRNA modifications such as 5' cap, optimized 5'- and 3'-UTRs and coding sequence, and different lengths of poly(A)-tail. We will develop SMART RNA replicons (Synthetically Modified Alpha Replicon Technology), evaluating 5' cap, UTR improvements as well as replicon improvements. Included in this effort we will compare template DNA derived from standard plasmid minipreps or with synthetic DNA produced by SGVI's gene assembly method (BioXP).
- Using DHVI model influenza monoclonal antibody sequences UPenn will optimize mRNA modifications; SGVI will develop RNA replicon platform; Duke will perform head-to- head-comparison studies in wild type and/or FeRn-/-hFcRn line 32 Tg mice (T32).

3.1 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.1.1	• Optimize RNA for in vivo production of model antibodies	0-9
3.1.2	• Evaluation of modified mRNA and RNA replicon platforms in cell based in vitro models	0-9
3.1.3	• Head-to-head comparison of modified mRNA and RNA replicon platforms in wt or FcRn-/- hFcRn line 32 Tg mice (T32)	9-12

3.2. Optimize LNP formulation Approach

- Our first generation formulation showed that high levels of circulating Ab could be produced with intravenous delivery of mRNA/LNPs. We have evaluated delivery of mRNA-LNPs by the intradermal (i.d.), intramuscular (i.m.), intravenous (i.v.), intraperitoneal (i.p.) subcutaneous (s.c.), and inhaled (i.n.) routes with very high levels of protein translation.
- Based on these data, we intend to move forward with IM, SC and IV administration.
- Formulations that increase circulating Ab titer (i.e., ≥ 20% of peak titer), and/or increase duration of expression (i.e., titer > 10 ug/mL for 45 days post-administration) in mice experiments will be advanced for integration into the final capability demonstrations in Ferrets and NHPs.

3.2 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.2.1	• Evaluate potential RNA formulations with current LNP providers (eg Acuitas, Arcturus for packaging /stabilizing RNAs	0-9
3.2.2	• Test formulations in small animal models (Mice) including wt and FcRn-/- hFcRn Mice	9-24

3.3. Platform Transfer to DHVI-GMP

- At the end of the effort described in Tasks 3.1 and 3.2, we will deliver an optimized process for production of RNA encoded antibodies and an effective system for delivery. For this task, the RNA and LNP production platforms will be implemented at DHVI allowing future options for development and clinical trial material production to be conducted in DHVI's GMP facility.

3.3 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.3.1	• Platform transition to DHVI (Duke) - Method / Transfer Package	6-15
3.3.2	• Produce countermeasure material for flu ferret and NHP in vivo studies	12-34*

* The time to produce the countermeasure is not expected to take this duration. However, the timeline must align with 2.2.3 and 2.2.4, which is extending the time period.

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3.4. Approach to Challenge/pK Animal Studies in Ferret

- After development and down selection of the best RNA and LNP candidates in small animal experiments using model antibodies, we will develop and deliver a Flu countermeasure to Ferrets as a relevant disease model.
- Studies are planned to assess in vivo pharmacokinetics (pK) and potency.
- The kinetics of the countermeasure in vivo will be determined by ELISA using population pK analysis of multiple animals. We will also conduct virus challenge study.
- Studies during this animal capability demonstration will assess countermeasure concentration using multiplex Luminex bead-based assays to determine peak human antibody levels and half-life.

3.4 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.4.1	• In vivo pK RNA/LNP data for Flu counter-measure • Reproducibility data on RNA/LNP for Flu counter-measure	18-24
3.4.2	• In vivo protection capability of Flu counter-measure in ferret challenge model	18-24

3.5. Approach to pK studies conducted in NHP with developed countermeasure

- After testing the selected Flu countermeasure in Ferrets as a relevant disease model, studies will be done to assess in vivo pharmacokinetics (pK) in NHPs as a suitable animal model for human countermeasure expression.
- The kinetics of the countermeasure in vivo will be determined by ELISA using population pK analysis of multiple animals.
- Studies during this capability demonstration will assess countermeasure concentration using multiplex Luminex bead-based assays to determine peak human antibody levels and half- life.
- We will also determine the inter-NHP antibody concentration variance with the expectation that it will not exceed 10%.

3.5 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)

3.5.1	• In vivo pK RNA/LNP data for Flu counter-measure • Reproducibility data on RNA/LNP for Flu counter-measure	18-39

3.6 Development and Testing of IM Delivery of mRNA-LNP countermeasure

- LNP vendor (Acuitas) will formulate model anti-Flu Ab RNA in a panel of 5 new LNP formulations to screen for improved Ab expression in mice via IM route (versus IV route).

3.6 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.6.1	• In vivo pK RNA/LNP data for IM delivered Flu counter-measure • Reproducibility data on RNA/LNP for IM delivered Flu counter- measure	31-48

Task Area 4: Platform Integration

4.1. Capability Demonstration #1 Approach

- Capability demonstration performed with influenza virus at Duke.
- Virus received by Task Area 1 team at Duke.
- Initiate propagation of virus in thaw and infect arrays to develop virus for isolating antigen-specific B cells and for testing antibody neutralization/binding.
- Virus will be provided to Task Area 2 team.
- PBMCs received by Task Area 2 team at Duke.
- Duke will work on antibody isolation from B cells.
- Antibody sequences will be provided to UPenn and/or SGVI for RNA modification.
- Duke will make final RNA sequence selected and enclose the RNA in an LNP (produced at Duke).
- Resulting countermeasure will be tested in vivo in the Ferret Flu challenge model.

4.1 Deliverables and Timeline

Subtask	Deliverable	Timeline (months)
4.1.0	• Data Summary and Report, and Countermeasure	24-30

(b)(5)

4.2 Deliverables and Timeline

Subtask	Deliverable	Timeline (months)
4.1.0	• Required reports, invoices and supporting documentation	31-48

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Overall Timeline