From: Peter Daszak
Sent: Thu, 15 Jun 2017 00:10:56 +0000
To: Park, Eun-Chung (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]
Cc: Kevin Olival, PhD; Anthony Ramos; Coleman, Amanda (NIH/NIAID) [C]
Subject: RE: Potential media interest from a Nature paper in press based on our work funded by two NIAID R01s

Thanks all – great comments which we'll include when we re-post tomorrow.

We're just beginning reaching out to journalists and will use a version with these comments incorporated (including the full grant numbers).

Also – when I get the final corrected proofs back from *Nature*, we'll send them on to you.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. (b)(6)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Park, Eun-Chung (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, June 14, 2017 5:19 PM
To: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Cc: Kevin Olival, PhD; Anthony Ramos; Coleman, Amanda (NIH/NIAID) [C]
Subject: RE: Potential media interest from a Nature paper in press based on our work funded by two NIAID R01s

Peter,
I am attaching the document containing comments from our communication office.

Sincerely,

Eunchung

Eun-Chung Park, PhD
Program Officer,
NIAID, NIH
PH:(b)(6)
(b)(6)

From: Peter Daszak (b)(6)
Sent: Wednesday, June 14, 2017 12:30 PM
To: Park, Eun-Chung (NIH/NIAID) [E] (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Kevin Olival, PhD (b)(6) Anthony Ramos (b)(6) Coleman, Amanda (NIH/NIAID) [C] (b)(6)
Subject: RE: Potential media interest from a Nature paper in press based on our work funded by two NIAID R01s
Importance: High

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Sent: Wednesday, June 14, 2017 10:36 AM
To: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Cc: Kevin Olival, PhD; Anthony Ramos; Coleman, Amanda (NIH/NIAID) [C]
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Subject: RE: Potential media interest from a Nature paper in press based on our work funded by two NIAID R01s
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Contact: Anthony M. Ramos
1.212.380.4469
ramos@ecohealthalliance.org

FOR IMMEDIATE RELEASE – DRAFT FOR APPROVAL

ECOHEALTH ALLIANCE MAPS GLOBAL DISTRIBUTION OF 'MISSING' VIRUSES ACROSS WILDLIFE SPECIES

Scientists Identify Highest Risk Mammal Species and Locations for Emerging Viruses

NEW YORK – June X, 2017 – EcoHealth Alliance, a global nonprofit organization working at the intersection of environmental, animal and public health, announced a paper published online in the journal, *Nature*, highlighting the first comprehensive analysis of all viruses known to infect mammals. The study shows that bats carry a significantly higher proportion of viruses able to infect people than any other group of mammals; and it identifies the species and geographic regions on the planet with the highest estimated proportion of yet-to-be discovered, or 'missing', viruses likely to infect people. This work provides a new way to predict where and how we should work to identify and pre-empt the next potential viral pandemic before it emerges.

The study team built a comprehensive database of all known viruses infecting over 700 mammal species (including people). They used mathematical models to identify the host species characteristics associated with having a larger number of viruses capable of infecting people (zoonotic viruses). They show that zoonotic potential is predicted by a host species evolutionary relatedness to humans, the degree of human-wildlife contact, and other factors including the taxonomic order it belongs to. They used this analysis to demonstrate for the first time that, after correcting for uneven research effort and other variables, bats harbor the highest proportion of zoonotic viruses of any mammal group. "In 2005, our team showed that SARS originates in bats. Ever since that finding, scientists have wondered whether bats are 'special' reservoirs for viruses. We now show definitively that bats carry a higher estimated proportion of yet-to-be-identified viruses of potential risk to people than any other mammal group," says EcoHealth Alliance's President and senior author on the study, Dr. Peter Daszak. The paper points out that viral discovery research on wild bats could help prevent pandemics. Bats are important to ecosystem health, through pollinating tropical fruits, removing crop pests like moths and disease vectors like mosquitoes, and providing other critical ecosystem services globally. "While the data show bats carry potentially important viruses, it's important to remember that the only way

Commented [NIAID1]: Hard to understand this concept of missing viruses.

Commented [ED2]: This seems like a massive database—might be impressive to include the approximate number of viruses studied!

Commented [NIAID3]: Unclear what this is.

Commented [NIAID4]: Not sure about using "definitive" given this is all based on predictive theory.

these viruses can emerge in people is if we make contact with bats, alter their environment, hunt them or otherwise disturb their ecology" -- BCI or us.

The study uses the analyses to produce detailed maps showing where on the planet we are most likely to find as-yet-undiscovered viruses that could emerge in people, or 'missing zoonoses.'. These maps differ among mammal groups. For example, hotspots of 'missing zoonoses' for bats are in South and Central America and parts of Asia, for primates in tropical Central America, Africa, and southeast Asia. "The holy grail in pandemic prevention is to understand where the next zoonotic virus is likely to emerge and from what species. Our study provides the first ever predictive map of where these undiscovered zoonoses can be found across the world. This information will be critical to guide future surveillance to identify and stop the next pandemic before it has chance to emerge," says Dr. Kevin Olival, lead author on the study. Finally, the paper provides a new way to estimate how likely a newly-discovered virus from wildlife could be to infect people. It shows that measuring the evolutionary breadth of its host species can predict its potential to infect people. This approach is already being used as part of a multi-country project to identify new viruses in wildlife and help prevent their emergence – the USAID PREDICT program (http://www.ecohealthalliance.org/program/predict).

Commented [ED5]: Does it count as a "new way" if USAID is already using it? Would it be better to recast this as confirming that USAID's techniques work?

This work was funded by grants from the National Institute of Allergy and Infectious Diseases (NIH-NIAID, https://www.niaid.nih.gov/) and from the USAID Emerging Pandemic Threats program (www.usaid.gov//what-we-do/global-health/pandemic-influenza-and-other-emerging-threats).

Commented [NIAID6]: Please include specific grant numbers.

About EcoHealth Alliance

Building on over 45 years of groundbreaking science, EcoHealth Alliance is a global, nonprofit organization dedicated to pandemic prevention and ecosystem health. Approximately 60 percent of emerging infectious diseases like Ebola, HIV, Zika, SARS, and MERS originated in animals before spilling over to human populations. Using environmental and health data covering the past 60 years, EcoHealth Alliance scientists created the first-ever, global disease hotspots map that identified at-risk regions to determine where field programs can help predict and prevent the next pandemic crisis. That work is the foundation of EcoHealth Alliance's rigorous, science-based approach working in more than 30 countries worldwide.

For more information, please visit www.ecohealthalliance.org

From: Coleman, Amanda (NIH/NIAID) [C]
Sent: Wed, 14 Jun 2017 12:46:10 -0400
To: 'Peter Daszak'; Park, Eun-Chung (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]
Cc: Kevin Olival, PhD; Anthony Ramos
Subject: RE: Potential media interest from a Nature paper in press based on our work funded by two NIAID R01s

Thank you, Peter!

Amanda Coleman [C]
Phone: (b)(6)

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To: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
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That is great. I will share your good news with our front office.

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Host and viral traits predict zoonotic spillover from mammals

Kevin J. Olival1, Parviez R. Hosseini1, Carlos Zambrana-Torrelio1, Noam Ross1, Tiffany L. Bogich1 & Peter Daszak1

The majority of human emerging infectious diseases are zoonotic, with viruses that originate in wild mammals of particular concern (for example, HIV, Ebola and SARS)1–3. Understanding patterns of viral diversity in wildlife and determinants of successful crossspecies transmission, or spillover, are therefore key goals for pandemic surveillance programs4. However, few analytical tools exist to identify which host species are likely to harbour the next human virus, or which viruses can cross species boundaries5–7. Here we conduct a comprehensive analysis of mammalian host–virus relationships and show that both the total number of viruses that infect a given species and the proportion likely to be zoonotic are predictable. After controlling for research effort, the proportion of

zoonotic viruses per species is predicted by phylogenetic relatedness to humans, host taxonomy and human population within a species range—which may reflect human–wildlife contact. We demonstrate that bats harbour a significantly higher proportion of zoonotic viruses than all other mammalian orders. We also identify the taxa and geographic regions with the largest estimated number of 'missing viruses' and 'missing zoonoses' and therefore of highest value for future surveillance. We then show that phylogenetic host breadth and other viral traits are significant predictors of zoonotic potential, providing a novel framework to assess if a newly discovered mammalian virus could infect people.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. (b)(6)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

Contact: Anthony M. Ramos
1.212.380.4469
ramos@ecohealthalliance.org

FOR IMMEDIATE RELEASE – DRAFT FOR APPROVAL

ECOHEALTH ALLIANCE MAPS GLOBAL DISTRIBUTION OF 'MISSING' VIRUSES ACROSS WILDLIFE SPECIES

Scientists Identify Highest Risk Mammal Species and Locations for Emerging Viruses

NEW YORK – June X, 2017 – EcoHealth Alliance, a global nonprofit organization working at the intersection of environmental, animal and public health, announced a paper published online in the journal, *Nature*, highlighting the first comprehensive analysis of all viruses known to infect mammals. The study shows that bats carry a significantly higher proportion of viruses able to infect people than any other group of mammals; and it identifies the species and geographic regions on the planet with the highest proportion of yet-to-be discovered, or 'missing', viruses likely to infect people. This work provides a new way to predict where and how we should work to identify and pre-empt the next potential viral pandemic before it emerges.

The study team built a comprehensive database of all known viruses infecting over 700 mammal species (including people). They used mathematical models to identify the host species characteristics associated with having a larger number of viruses capable of infecting people (zoonotic viruses). They show that zoonotic potential is predicted by a host species evolutionary relatedness to humans, the degree of human-wildlife contact, and other factors including the taxonomic order it belongs to. They used this analysis to demonstrate for the first time that, after correcting for uneven research effort and other variables, bats harbor the highest proportion of zoonotic viruses of any mammal group. "In 2005, our team showed that SARS originates in bats. Ever since that finding, scientists have wondered whether bats are 'special' reservoirs for viruses. We now show definitively that bats carry a higher proportion of yet-to-be-identified viruses of potential risk to people than any other mammal group", says EcoHealth Alliance's President and senior author on the study, Dr. Peter Daszak. The paper points out that viral discovery research on wild bats could help prevent pandemics. Bats are important to ecosystem health, through pollinating tropical fruits, removing crop pests like moths and disease vectors like mosquitoes, and providing other critical ecosystem services globally. "While the data show bats carry potentially important viruses, it's important to remember that the only way these viruses can emerge in

people is if we make contact with bats, alter their environment, hunt them or otherwise disturb their ecology" -- BCI or us.

The study uses the analyses to produce detailed maps showing where on the planet we are most likely to find as-yet-undiscovered viruses that could emerge in people, or 'missing zoonoses'. These maps differ among mammal groups. For example, hotspots of 'missing zoonoses' for bats are in South and Central America and parts of Asia, for primates in tropical Central America, Africa, and southeast Asia. "The holy grail in pandemic prevention is to understand where the next zoonotic virus is likely to emerge and from what species. Our study provides the first ever predictive map of where these undiscovered zoonoses can be found across the world. This information will be critical to guide future surveillance to identify and stop the next pandemic before it has chance to emerge", says Dr. Kevin Olival, lead author on the study. Finally, the paper provides a new way to estimate how likely a newly-discovered virus from wildlife could be to infect people. It shows that measuring the evolutionary breadth of its host species can predict its potential to infect people. This approach is already being used as part of a multi-country project to identify new viruses in wildlife and help prevent their emergence – the USAID PREDICT program (http://www.ecohealthalliance.org/program/predict).

This work was funded by grants from the National Institute of Allergy and Infectious Diseases (NIH-NIAID, https://www.niaid.nih.gov/) and from the USAID Emerging Pandemic Threats program (www.usaid.gov//what-we-do/global-health/pandemic-influenza-and-other-emerging-threats).

About EcoHealth Alliance

Building on over 45 years of groundbreaking science, EcoHealth Alliance is a global, nonprofit organization dedicated to pandemic prevention and ecosystem health. Approximately 60 percent of emerging infectious diseases like Ebola, HIV, Zika, SARS, and MERS originated in animals before spilling over to human populations. Using environmental and health data covering the past 60 years, EcoHealth Alliance scientists created the first-ever, global disease hotspots map that identified at-risk regions to determine where field programs can help predict and prevent the next pandemic crisis. That work is the foundation of EcoHealth Alliance's rigorous, science-based approach working in more than 30 countries worldwide.

For more information, please visit www.ecohealthalliance.org

From: Normil, Carine (NIH/NIAID) [C]
Sent: Thu, 1 Jun 2017 12:50:54 -0400
To: Aleksei Chmura
Cc: Stemmy, Erik (NIH/NIAID) [E]; Dr. Peter Daszak; Smith, Philip (NIH/NIAID) [E]; Alison Andre
Subject: RE: Publication compliance for Grant Number: 5R01AI110964 - 04 PI Name: DASZAK, PETER

Thank you, Aleksei! This information is very much appreciated.

Best,
Carine

Carine Normil

Grants Management Specialist (Contractor)

Grants Management Program, DEA, NIAID, NIH, HHS
5601 fishers Lane, Rm 4G46, Bethesda , Maryland 20892

Phone: (b)(6)

Fax: (301)-493-0597
Email: (b)(6)

NIH National Institute of Allergy and Infectious Diseases

From: Aleksei Chmura (b)(6)
Sent: Wednesday, May 31, 2017 10:59 AM
To: Normil, Carine (NIH/NIAID) [C] (b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Dr. Peter Daszak (b)(6) Smith, Philip (NIH/NIAID) [E] (b)(6) Alison Andre (b)(6)
Subject: Re: Publication compliance for Grant Number: 5R01AI110964 - 04 PI Name: DASZAK, PETER
Importance: High

Dear Carine,

Please find the attached documentation of this publication being in compliance with NIH Public Access Policy.

Many thanks most,

Sincerely,

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
(mobile)
(b)(6) (Skype)

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Visit our blog: www.ecohealthalliance.org/blog

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On 23 May 2017, at 13:12, Normil, Carine (NIH/NIAID) [C] (b)(6) wrote:

Good afternoon:

Your progress report for the above referenced award has a non-compliant publication. Please take the necessary steps to bring "*Ge XY, Li JL, Yang XL, Chmura AA, Zhu G, Epstein JH, Mazet JK, Hu B, Zhang W, Peng C, Zhang YJ, Luo CM, Tan B, Wang N, Zhu Y, Crameri G, Zhang SY, Wang LF, Daszak P, Shi ZL. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 November 28;503(7477):535-8. PubMed PMID: 24172901*" into compliance with the NIH Public Access Policy.

To comply with the policy, please reply to this email and provide a PDF generated report from My NCBI that includes evidence of compliance (PMCID number) for this publication. If you believe the above referenced publication does not fall under the Public Access Policy, please provide a brief explanation. A response is appreciated by **June 15, 2017**.

If you have questions about the Policy, feel free to contact me via email at (b)(6) or send a note toPublicAccess@nih.gov.

Best regards,
Carine

Carine Normil

Grants Management Specialist (Contractor)

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Phone: (b)(6)

Fax: (301)-493-0597
Email: (b)(6)

From: Peter Daszak
Sent: Wed, 24 May 2017 21:21:21 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Hongying Li; Aleksei Chmura; Alison Andre
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Great – thanks Erik. We'll follow up with the forms, and will make sure we're there early on the day.

Look forward to seeing you in June.

Cheers,

Peter

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get it back to me by June 19th or 20th that would be great. Also, please let them know they should bring their passports with them. Everyone else will need a photo ID as well.

Let me know if you need directions to our building. I would suggest planning to arrive between 8:15 and 8:30, as there can be a line at security if there are other public meetings occurring that day. There is no visitor parking at our facilities, but there is a public parking garage on our block that I can get validation stickers for if you'll be driving. We are also a short walk from the Twinbrook Metro stop, if you plan to travel by train.

Erik

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Thank you!
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Sent: Wed, 24 May 2017 19:16:23 +0000
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Attachments: 5601 Foreign Visitor Form.xlsx

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NIAID FOREIGN VISITOR AUTHORIZATION

MEETING START DATE	Thursday, June 29, 2017
MEETING START TIME	8:30 AM
MEETING ENDING DATE	Thursday, June 29, 2017
MEETING ENDING TIME	12:00pm
NAME OF MEETING	DMID Forum
BUILDING(S) & ROOM NUMBER(S) TO BE VISITED	5601 Fishers Lane 8f100
WILL CRITICAL INFRASTRUCTURE AND/OR LABORATORIES BE VISITED?	No
HOSTING OFFICIAL (Federal Employee) **Name** **IC/Organization** **Title** **Telephone Number**	Dr Erik Stemmy RDB/DMID/NIAID Program Officer (b)(6)
ESCORT INFORMATION (If different from Hosting Official) **Name** **IC/Organization** **Title** **Telephone Number**	same as above

HHS FOREIGN VISITOR MANAGEMENT PROGRAM

National Institute of Allergy and Infectious Diseases

Last Name	First Name	Middle Name	Gender	Visitor Title	Visitor Org/Employer	Citizenship	Place of Birth (City & Ctry)	Date of Birth	ID Type	Passport Issued By (Country)	ID Number	ID Issue Date	ID Expiration Date	Visa Type	Visa Number	Remarks	IC/Org	Sponsor/Escort	Sponsor/Escort Phone #
																	DMID/RDB	Erik Stemmy	(b)(6)
																	DMID/RDB	Erik Stemmy	

From: Peter Daszak
Sent: Wed, 24 May 2017 19:05:08 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Hongying Li; Aleksei Chmura; Alison Andre
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?
Attachments: Biosketch_Zhengli Shi_Update.doc, CV-Zhou_Peng-2016.docx, Hongying Li bio.docx, Peter Daszak Short Bio 2017.doc
Importance: High

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Monday, April 24, 2017 4:35 PM
To: Peter Daszak
Cc: Hongying Li; Aleksei Chmura; Alison Andre
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Hi Peter,

I would be happy to have you visit us in June. I am available on the 28th. If there is any flexibility in your schedule, Thursday mornings we have a division-wide seminar from 9-10am, and that would be an ideal time to have you present on your work to the larger audience. I understand if that's not possible, thought, but thought I would check to see. Please let me know.

Thanks,
Erik

From: Peter Daszak (b)(6)
Sent: Monday, April 24, 2017 4:11 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)

Cc: Hongying Li (b)(6) Aleksei Chmura (b)(6) Alison Andre (b)(6)
Subject: Potential visit to NIH by our Chinese Co-investigator in June?
Importance: High

Dear Erik,

Our Chinese Co-investigator, Zhengli Shi from the Wuhan Institute of Virology, will be visiting the US in June to give a talk at a conference here. I'd really like to come and visit you and your colleagues at NIH with her while she's here. We could have a meeting to talk about progress on the project and could even do a seminar if there is a format for these.

Zhengli's timeline is fixed, and I wondered if you and your colleagues would be available on Wednesday June 28th? If not, we can look at alternative dates…

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

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BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Zhengli	Senior scientist
FAMILY NAME	
Shi	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Department of Biology, Wuhan University, China	B.S.	07/1987	GENETICS
Wuhan Institute of Virology, Chinese Academy of Sciences, China	M.S.	07/1990	VIROLOGY
University Montpellier II, Montpellier, France	Ph.D.	05/2000	VIROLOGY
Merieux P4 Lyon	NA	10/2006	Biosafety training

Personal Statement

Dr. Shi is the director of the Center for Emerging Infectious Diseases of the Wuhan Institute of Virology. Her research focuses on viral pathogen discovery through traditional and high-throughput sequencing techniques. She has been studying the wildlife-borne viral pathogens, particularly bat-borne viruses since 2004. Her group has discovered diverse novel viruses/virus antibodies in bats, included SARS-like coronaviruses, adenoviruses, adeno-associated viruses, circoviruses, paramyxoviruses and filoviruses in China. One of her great contributions is to uncover genetically diverse SARS-like coronaviruses in bats with her international collaborators and provide unequivocal evidence that bats are natural reservoir of SARS-CoV by isolation of one strain that is closely related to the SARS-CoV in 2002-3. She has coauthored >80 publications on viral pathogen identification, diagnosis and epidemiology.

Positions and Employment

1990 - 1993, Research assistant, Wuhan Institute of Virology, Chinese Academy of Sciences, China
1993 - 1995, Research scientist, Wuhan Institute of Virology, Chinese Academy of Sciences, China
2000- Senior Scientist, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China

Other Experience and Professional Memberships

2008 - Member, American Society of Microbiology
2001 - Member, Chinese Society of Microbiology
2001 - Member, Chinese Society of Biochemistry and Molecular Biology
2004 - Editor Board, Chinese Journal of Virology
2004 - 2009, Editor Board, Virologica Sinica
2010 - Associate Editor, Virologica Sinica
2015 - 2017, Editor Board, Journal of Medical Virology
2016 - 2018, Associate Editor, Virology Journal

C. Selected peer-reviewed publications

1. Li, W., Shi Z., Yu M., Ren W., Smith C., Epstein H. J., Zhang S., Wang H., Crameri G., Hu Z., Zhang H., Zhang J., Mceachern J., Field H., Daszak P., Eaton T.B. and Wang L. F. (2005) Bats are natural reservoirs of SARS-like coronaviruses. Science 310(5748): 676-679. (co-corresponding author).
2. Ren, W., Qu, X., Li, W., Han, Z., Yu, M., Zhang, S., Wang, L. F., Deng, H., Shi, Z. (2008) Difference in receptor usage between SARS coronavirus and SARS-like coronavirus of bat origin. J Virol 82(4): 1899–1907.

3. Li, Y., Wang, J., Hickey, A. C., Zhang, Y., Li, Y., Wu, Y., Zhang, H., Yuan, J., Han, Z., McEachern, J., Broder, C. C., Wang, L. F. and Shi, Z. (2008) Potential nipah virus infection in Chinese bats. Emerg Infect Dis 14(12):1974-1976.
4. Li, Y., Ge X., Zhang H., Zhou P., Zhu Y., Zhang Y., Yuan J., Wang L-F., Shi Z. (2010). Host Range, Prevalence and Genetic Diversity of Adenoviruses in Bats. J. Virol. 84 (8):3889–3897.
5. Ge, X., Li, Y., Yang, X., Zhang, H., Zhou, P., Zhang, Y. & Shi, Z. (2012). Metagenomic analysis of viruses from bat fecal samples reveals many novel viruses in insectivorous bats in china. J Virol 86, 4620-4630
6. Ge X-Y., Li J-L., Yang X-L., Chmura A, Epstein J. H., Hu B., Zhang W., Peng C., Zhang Y-J., Luo C-M, Tang B., Wang N., Zhu Y., Crameri G., Zhang S-Y., Wang L-F, Daszak P., Shi Z-L. (2013). First isolation and characterization of bat SARS-like Coronaviruses that use the ACE2 receptor. Nature 503(7477):535-538. (co-corresponding author).
7. Zhang, G., Cowled, C., Shi, Z., Huang, Z., Bishop-Lilly, K. A., Fang, X., Wynne, J. W., Xiong, Z., Baker, M. L., Zhao, W., Tachedjian, M., Zhu, Y., Zhou, P., Jiang, X., Ng, J., Yang, L., Wu, L., Xiao, J., Feng, Y., Chen, Y., Sun, X., Zhang, Y., Marsh, G. A., Crameri, G., Broder, C. C., Frey, K. G., Wang, L. F. & Wang, J. (2013). Comparative Analysis of Bat Genomes Provides Insight into the Evolution of Flight and Immunity. Science (New York, N.Y). Science 339 (6118):456-460. (co-first author).
8. Zeng, L. P., Gao, Y. T., Ge, X. Y., Zhang, Q., Peng, C., Yang, X. L., Tan, B., Chen, J., Chmura, A. A., Daszak, P. & Shi, Z. L. (2016). Bat Severe Acute Respiratory Syndrome-Like Coronavirus WIV1 Encodes an Extra Accessory Protein, ORFX, Involved in Modulation of the Host Immune Response. J Virol 90, 6573-6582.
9. Yang, X.-L., Hu, B., Wang, B., Wang, M.-N., Zhang, Q., Zhang, W., Wu, L.-J., Ge, X.-Y., Zhang, Y.-Z., Daszak, P., Wang, L.-F. & Shi, Z.-L. (2016). Isolation and Characterization of a Novel Bat Coronavirus Closely Related to the Direct Progenitor of Severe Acute Respiratory Syndrome Coronavirus. J Virol 90, 3253-3256.
10. Yang, X. L., Zhang, Y. Z., Jiang, R. D., Guo, H., Zhang, W., Li, B., Wang, N., Wang, L., Waruhiu, C., Zhou, J. H., Li, S. Y., Daszak, P., Wang, L. F. & Shi, Z. L. (2017). Genetically Diverse Filoviruses in Rousettus and Eonycteris spp. Bats, China, 2009 and 2015. Emerg Infect Dis 23, 482-486.

Research Support
Ongoing Research Support

1. 81290341 National Natural Science Foundation of China
Genetic diversity, identification and pathogenesis of bat viruses 01/01/2013 - 12/31/2017
Role: PI

2. R01AI110964 The ecology of bat coronaviruses and the risk of future coronavirus emergence. National Institutes of Health NIAID. 01/06/2014 - 31/05/2019
Role: Participant.

Scientist

- **Name**: ZHOU Peng
- **Office Mailing Address**: 44 Xiaohongshan, Wuhan, China 430071
- **Email:** (b)(6)
- **Contact No**: (b)(6)
- **Current Position**:
  Professor, Center for Emerging Infectious Diseases of the Wuhan Institute of Virology, Chinese Academy of Sciences, China
- **Academic qualifications**:
  PhD of molecular biology, Wuhan Institute of Virology (WIV), Chinese Academy of Sciences (CAS), Wuhan, China, 2010;
  Bachelor of bioengineering, Henan University, Kaifeng, China, 2004.
- **Research interests**: Bat innate immunity; virus host interface; pathogens discovery
- **Publication track record**: 25 refereed publications in journals including *Science, PNAS, Journal of Virology and Journal of Immunology* covering virology and immunology. Publications from last five years are listed below:

1. **Peng Zhou**, Yok Teng Chionh, Sergio Erdal Irac, Matae Ahn, Justin Han Jia Ng, Even Fossum, Bjarne Bogen, Florent Ginhoux, Aaron T Irving, Charles-Antoine Dutertre & Lin-Fa Wang. 2016. Unlocking bat immunology: establishment of Pteropus alecto bone marrow-derived dendritic cells and macrophages. ***Scientific Reports***, Article number: 38597 (2016) doi:10.1038/srep38597
2. **Zhou P**, Tachedjian M, Wynne JW, Boyd V, Cui J, Smith I, Cowled C, Ng JH, Mok L, Michalski WP, Mendenhall IH, Tachedjian G, Wang LF, Baker ML. 2016. Contraction of the type I IFN locus and unusual constitutive expression of IFN-α in bats. **Proc Natl Acad Sci U S A**. 2016 Feb 22. pii: 201518240.
3. Wynne JW, Shiell BJ, Marsh GA, Boyd V, Harper JA, Heesom K, Monaghan P, **Zhou P**, Payne J, Klein R, Todd S, Mok L, Green D, Bingham J, Tachedjian M, Baker ML, Matthews D, Wang LF. 2014. Proteomics informed by transcriptomics reveals Hendra virus sensitizes bat cells to TRAIL-mediated apoptosis. ***Genome Biol***. 15:532.
4. **Zhou P**, Cowled C, Mansell A, Monaghan P, Green D, Wu L, Shi Z, Wang LF, Baker ML. 2014. IRF7 in the Australian black flying fox, Pteropus alecto: evidence for a unique expression pattern and functional conservation. ***PLoS One*** 9:e103875.
5. Cowled C, Stewart CR, Likic VA, Friedlander MR, Tachedjian M, Jenkins KA, Tizard ML, Cottee P, Marsh GA, **Zhou P**, Baker ML, Bean AG, Wang LF. 2014. Characterisation of novel microRNAs in the Black flying fox (Pteropus alecto) by deep sequencing. ***BMC Genomics*** 15:682.
6. **Zhou P**, Cowled C, Wang LF, Baker ML (2013) Bat Mx1 and Oas1, but not Pkr are highly induced by bat interferon and viral infection. ***Dev Comp Immunol***. 40: 240-7
7. Zhang G, Cowled C, Shi Z, Huang Z, Bishop-Lilly KA, Fang X, Wynne JW, Xiong Z, Baker ML, Zhao W, Tachedjian M, Zhu Y, **Zhou P**, Jiang X, Ng J, Yang L, Wu L, Xiao J, Feng Y, Chen Y, Sun X, Zhang Y, Marsh GA, Crameri G, Broder CC, Frey KG, Wang LF, Wang J (2013) Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. ***Science*** 339: 456-60
8. **Zhou, P.**, Han, Z., Wang, L.F., Shi, Z., 2013. Identification of immunogenic determinants of the spike protein of SARS-like coronavirus. ***Virologica Sinica*** 28, 92-96
9. Lijun Wu, **Peng Zhou (co-first),** Xinyi Ge, Lin-Fa Wang, Michelle L Baker, Zhengli Shi. Deep RNA Sequencing Reveals Complex Transcriptional Landscape of a Bat Adenovirus. ***Journal of Virology***, Jan. 2013, p. 503-511
10. Ge, X., Li, Y., Yang, X., Zhang, H., **Zhou, P.**, Zhang, Y., Shi, Z., 2012. Metagenomic analysis of viruses from bat fecal samples reveals many novel viruses in insectivorous bats in China. ***Journal of virology***, 86, 4620-4630
11. **Zhou, P.,** Li, H., Wang, H., Wang, L.F., Shi, Z., 2012. Bat severe acute respiratory syndrome-like coronavirus ORF3b homologues display different interferon antagonist activities. ***Journal of General***

Virology, 93, 275-281

12. Cowled C, Baker ML, **Zhou P**, Tachedjian M, Wang LF (2012) Molecular characterisation of RIG-I-like helicases in the black flying fox, Pteropus alecto. ***Dev Comp Immunol***. 36: 657-64
13. **Zhou P**, Cowled C, Marsh GA, Shi Z, Wang L-F, et al. (2011) Type III IFN Receptor Expression and Functional Characterisation in the Pteropid Bat, Pteropus alecto. ***PLoS ONE*** 6(9): e25385. doi:10.1371/journal.pone.0025385
14. **Zhou P**, Cowled C, Todd S, Crameri G, Virtue ER, Marsh GA, Shi ZL, Wang LF, and Baker ML. Type III Interferons in pteropid bats: differential expression patterns provide evidence for distinct roles in antiviral immunity. ***Journal of Immunology*** 2011;186;3138-3147
15. Cowled C, Baker M, Tachedjian M, **Zhou P**, Bulach D, Wang LF (2011) Molecular characterisation of Toll-like receptors in the black flying fox Pteropus alecto. ***Dev Comp Immunol***. 35: 7-18

Book chapter:

Baker, M. L. and **Zhou, P**. (2015) Bat Immunology, in Bats and Viruses: A New Frontier of Emerging Infectious Diseases (eds L.-F. Wang and C. Cowled), John Wiley & Sons, Inc, Hoboken, NJ. doi: 10.1002/9781118818824.ch14

Hongying Li, China Programs Coordinator at EcoHealth Alliance, combines her expertise in conservation and public health to communicate EcoHealth Alliance's science on the health implications of the wildlife trade to stakeholders. She aims to bring positive changes in human's behavior and policy in order to stop the illegal wildlife trade and prevent pandemics.

Hongying's overall efforts involve building network with stakeholders, designing and implementing educational and outreach projects, as well as assisting the management of key projects, with special preference to China and Southeast Asia.

Hongying is currently communicating with many NGOs and government departments in China to develop content-based advocacy strategies and educational projects to protect public health and stop illegal wildlife by leveraging the health implications of wildlife trade. Her academic training and previous experience at UNESCO and CARE in human development afford her a strong appreciation of working and communicating with different groups of people to find new solutions for sustainable social and ecosystem development.

Dr. Peter Daszak, Ph.D

Dr. Peter Daszak is President of EcoHealth Alliance, a US-based organization that conducts research and outreach programs on global health, conservation and international development. Dr. Daszak's research has been instrumental in identifying and predicting the impact of emerging diseases across the globe. His achievements include identifying the bat origin of SARS, identifying the underlying drivers of Nipah and Hendra virus emergence, producing the first ever global emerging disease 'hotspots' map, developing a strategy to find out how many unknown viruses exist that could threaten to become pandemic, identifying the first case of a species extinction due to disease, and discovering the disease chytridiomycosis as the cause global amphibian declines.

Dr Daszak is a member and Chair-elect of the National Academy of Sciences, Engineering and Medicine's Forum on Microbial Threats. He is a member of the NRC Advisory Committee to the US Global Change Research Program, the Supervisory Board of the One Health Platform, the One Health Commission Council of Advisors, the CEEZAD External Advisory Board, the Cosmos Club, the Advisory Council of the Bridge Collaborative; has served on the IOM Committee on global surveillance for emerging zoonoses, the NRC committee on the future of veterinary research, the International Standing Advisory Board of the Australian Biosecurity CRC; and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr. Daszak is a regular advisor to WHO, OIE and FAO, and is actively involved in the WHO Expert group on Public Health Emergency Disease Prioritization.

Dr Daszak won the 2000 CSIRO medal for collaborative research on the discovery of amphibian chytridiomycosis, is the EHA institutional lead for USAID-EPT-PREDICT, is on the Editorial Board of *Conservation Biology*, *One Health*, and *Transactions of the Royal Society of Tropical Medicine & Hygiene*, and is Editor-in-Chief of the journal *Ecohealth*. He has authored over 300 scientific papers, and his work has been the focus of extensive media coverage, ranging from press articles in The New York Times, The Wall Street Journal, The Economist, The Washington Post, US News & World Report and broadcast appearances on 60 Minutes, CNN, ABC, NPR's Talk of the Nation, Morning Edition, and Fresh Air with Terry Gross.

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Tue, 23 May 2017 11:26:43 +0000
To: Alison Andre; Peter Daszak
Cc: Hongying Li; Aleksei Chmura
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Hi Alison,
Thanks for the update.

Erik

From: Alison Andre (b)(6)
Sent: Monday, May 22, 2017 4:41 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Peter Daszak (b)(6)
Cc: Hongying Li (b)(6) Aleksei Chmura (b)(6)
Subject: Re: Potential visit to NIH by our Chinese Co-investigator in June?

Dear Erik,

Apologies for the delay in response – Peter has been traveling last week and has just returned today. He will get back to you today or tomorrow with the information requested.

Thank you,
Alison

Alison Andre
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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From: "Stemmy, Erik (NIH/NIAID) [E]" (b)(6)
Date: Thursday, May 18, 2017 at 8:25 AM
To: Peter Daszak (b)(6)
Cc: Hongying Li (b)(6) Aleksei Chmura (b)(6) Alison Andre (b)(6)
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Hi Peter,
We've got you on the calendar for June 29th. Can you send me a title for the talk, short summary, and brief bios for the presenters?

Thank you!
Erik

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Monday, April 24, 2017 4:47 PM
To: Peter Daszak (b)(6)
Cc: Hongying Li (b)(6) Aleksei Chmura (b)(6) Alison Andre (b)(6)
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Ok! I'll see about scheduling you for the slot on June 29th. Can you send me a title and short synopsis? Since our whole division would be attending it would be great if you could cover some of the collaborative work with PREDICT and not solely focus on the MERS work.

Erik

From: Peter Daszak (b)(6)
Sent: Monday, April 24, 2017 4:44 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Hongying Li (b)(6) Aleksei Chmura (b)(6) Alison Andre (b)(6)
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

That would be perfect. The conference that Zhengli's attending starts on the evening of the 29th in Colorado so she could get a midday plane and still make it.

We'll plan to come to DC the afternoon or evening before and then do the symposium and meet with you.

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance
460 West 34th Street – 17th Floor
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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Monday, April 24, 2017 4:35 PM
To: Peter Daszak
Cc: Hongying Li; Aleksei Chmura; Alison Andre
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

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Thanks,
Erik

From: Peter Daszak (b)(6)
Sent: Monday, April 24, 2017 4:11 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Hongying Li (b)(6) Aleksei Chmura (b)(6) Alison Andre (b)(6)
Subject: Potential visit to NIH by our Chinese Co-investigator in June?
Importance: High

Dear Erik,

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Zhengli's timeline is fixed, and I wondered if you and your colleagues would be available on Wednesday June 28th? If not, we can look at alternative dates…

Cheers,

Peter

Peter Daszak
President

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From: Aleksei Chmura
Sent: Tue, 18 Apr 2017 15:28:14 +0100
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Smith, Philip (NIH/NIAID) [E]
Subject: Re: eRA Commons: RPPR for Grant 5R01AI110964-04 Submitted to NIH with a Non-Compliance warning

Thanks, Erik!

-Aleksei

On Apr 18, 2017, at 12:53, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Thanks Aleksei. I've passed your response along and will let you know if there are any follow up questions.

Best,
Erik

From: Aleksei Chmura (b)(6)
Sent: Monday, April 17, 2017 1:38 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Normil, Carine (NIH/NIAID) [C] (b)(6) Dr. Peter Daszak (b)(6) Smith, Philip (NIH/NIAID) [E] (b)(6) 李泓萤 (b)(6)
Subject: Re: eRA Commons: RPPR for Grant 5R01AI110964-04 Submitted to NIH with a Non-Compliance warning

Dear Erik,

As per Peter, the work is planned to supplement that done by PREDICT and hopefully to collaborate with the PREDICT team if possible. The aim is for the Co-investigator (Zhengli Shi) and her field team to coordinate with the PREDICT Myanmar field team and co-leads to ensure that there is no duplication of effort (the NIAID group will not use the PREDICT protocols), and that there is the opportunity for cross-training. Samples will be collected from bats and tested by PCR for SARS-like Coronaviruses, then for positive samples, to do a series of further characterization of the viruses using the techniques Zhengli has developed in her lab (spike protein binding assays etc.).

Samples collected will also be made available to the Myanmar lab so that the PREDICT protocols can be run in-country.

Please let me know, if you have any further questions.

Cheers,

Aleksei Chmura
Senior Coordinator of Operations

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On 13 Apr 2017, at 12:26, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Thanks Aleksei!

One additional item. In processing the foreign clearance for Myanmar, the State Department requested a little bit more information on how the project relates to the PREDICT work. Specifically, they've asked:

"could you ask the PI to clarify how they are working with the USAID funded PREDICT Project – it is our understanding that ECO-Health is a partner in PREDICT and the sampling methods, etc. described are similar to activities in PREDICT (it may be that the PR is going to be doing additional testing on already collected samples, but that is not clear from the information provided)."

It sounds like they just want to clarify whether the sampling work is in addition to the PREDICT work. Will this be specific sampling for MERS beyond what is already being done?

Best,
Erik

From: Aleksei Chmura (b)(6)
Sent: Wednesday, April 12, 2017 7:42 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Normil, Carine (NIH/NIAID) [C] (b)(6) Peter Daszak
(b)(6) Smith, Philip (NIH/NIAID) [E] (b)(6)
Subject: Re: eRA Commons: RPPR for Grant 5R01AI110964-04 Submitted to NIH with a Non-Compliance warning

Dear Erik,

The non-compliant paper referenced above has been uploaded in NIHMS and should be updated in Peter's My NCBI as soon as NIHMS approves it.

Many thanks!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

EcoHealth Alliance
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On Thu, Apr 13, 2017 at 12:10 AM, <era-notify@mail.nih.gov> wrote:
*** This is an automated notification - Please do not reply to this message. ***

Dear Grantee,

The progress report for the above-reference award includes citation(s) that are out of compliance with the NIH Public Access Policy. Compliance with the NIH Public Access Policy is a legal requirement and a term and condition of all NIH awards. This award will be delayed until all publications arising from it are in compliance with the policy.
The Authorized Organization Representative (AOR) or PD/PI with delegated Progress Report Submit Authority must provide verification that all publications are in compliance with the NIH Public Access Policy, to the Grants Management Specialist (GMS). The Public Access compliance verification may be submitted either using the new Progress Report Additional Material (PRAM) link on the eRA Commons Status page or via email.

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- Include a My NCBI PDF report demonstrating all the formerly non-compliant public access citations are now compliant. To process your award, every citation in the report should be either complete, in process or exempt N/A). Please see http://publicaccess.nih.gov/citation_methods.htm for more information about acceptable compliance statuses for public access papers. We have more information about My NCBI athttp://publicaccess.nih.gov/communications.htm.
- If unable to provide verification, provide a justification for why the specific publication(s) cannot be brought into compliance.

NIH awardees are responsible for ensuring that evidence of compliance is included in all NIH applications, proposals and reports. If you have questions about the Policy, please check the NIH Public Access Website or send an email to PublicAccess@nih.gov.
For any further questions about this email, call the eRA Help Desk at 1-866-504-9552 or refer to http://grants.nih.gov/support for additional methods of contact. Please access Commons at http://public.era.nih.gov/commons/.
For more information please visit http://era.nih.gov/

From: Aleksei Chmura
Sent: Sat, 15 Apr 2017 17:37:08 +0100
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Normil, Carine (NIH/NIAID) [C]; Peter Daszak; Smith, Philip (NIH/NIAID) [E]; Andre, Alison
Subject: Re: eRA Commons: RPPR for Grant 5R01AI110964-04 Submitted to NIH with a Non-Compliance warning

Dear Erik,

I will confirm with Peter on Tuesday and get back to you as soon as possible.

Cheers,

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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On Thu, Apr 13, 2017 at 12:26 PM, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Thanks Aleksei!

One additional item. In processing the foreign clearance for Myanmar, the State Department requested a little bit more information on how the project relates to the PREDICT work. Specifically, they've asked:

"could you ask the PI to clarify how they are working with the USAID funded PREDICT Project – it is our understanding that ECO-Health is a partner in PREDICT and the sampling methods, etc. described

are similar to activities in PREDICT (it may be that the PR is going to be doing additional testing on already collected samples, but that is not clear from the information provided)."

It sounds like they just want to clarify whether the sampling work is in addition to the PREDICT work. Will this be specific sampling for MERS beyond what is already being done?

Best,

Erik

From: Aleksei Chmura (b)(6)
Sent: Wednesday, April 12, 2017 7:42 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Normil, Carine (NIH/NIAID) [C] (b)(6) Peter Daszak
(b)(6) Smith, Philip (NIH/NIAID) [E] (b)(6)
Subject: Re: eRA Commons: RPPR for Grant 5R01AI110964-04 Submitted to NIH with a Non-Compliance warning

Dear Erik,

The non-compliant paper referenced above has been uploaded in NIHMS and should be updated in Peter's My NCBI as soon as NIHMS approves it.

Many thanks!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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On Thu, Apr 13, 2017 at 12:10 AM, <era-notify@mail.nih.gov> wrote:

*** This is an automated notification - Please do not reply to this message. ***

Dear Grantee,

The progress report for the above-reference award includes citation(s) that are out of compliance with the NIH Public Access Policy. Compliance with the NIH Public Access Policy is a legal requirement and a term and condition of all NIH awards. This award will be delayed until all publications arising from it are in compliance with the policy.
The Authorized Organization Representative (AOR) or PD/PI with delegated Progress Report Submit Authority must provide verification that all publications are in compliance with the NIH Public Access Policy, to the Grants Management Specialist (GMS). The Public Access compliance verification may be submitted either using the new Progress Report Additional Material (PRAM) link on the eRA Commons Status page or via email.

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- If unable to provide verification, provide a justification for why the specific publication(s) cannot be brought into compliance.

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For more information please visit http://era.nih.gov/

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Tue, 11 Apr 2017 18:52:02 +0000
To: Peter Daszak
Cc: Aleksei Chmura; Alison Andre; Hongying Li
Subject: RE: Year 3 report for 5R01AI110964 - Bat Coronaviruses in China and SE Asia.

Hi Peter,
Thanks for sending this along. The USG is currently in the process of implementing a replacement to the GoF policy, called Potential Pandemic Pathogen Care and Oversight. So we are in a bit of limbo between policies. I'll review what you've sent and get back to you with any questions.

Best,
Erik

From: Peter Daszak (b)(6)
Sent: Sunday, April 09, 2017 8:06 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Aleksei Chmura (b)(6) Alison Andre (b)(6)
Hongying Li (b)(6)
Subject: Year 3 report for 5R01AI110964 - Bat Coronaviruses in China and SE Asia.

Hi Erik,

I hope all's well with you. I wanted to let you know that Aleksei will be signing off on our Year 3 report for the NIAID grant 5R01AI110964 on Bat CoVs in China and SE Asia. I've attached the word doc here so you can see it ahead of time.

Like last year, Zhengli Shi is proposing some work in Year 4 on bat coronaviruses that will involve developing chimeras between MERS-CoV and some bat CoVs we've discovered. I've included this in the report that you'll get next week, as well as the same text that explains why we believe this is not gain-of-function work. It's on the penultimate page of the report. Last year, we wrote to you and your colleagues and this work was given the go-ahead. I'm hoping that putting this paragraph in the report will make it move through quicker, but just wanted to give you a heads up…

Please let us know if you need us to write in with explanations separately.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

From: Peter Daszak
Sent: Mon, 10 Apr 2017 00:05:45 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura; Alison Andre; Hongying Li
Subject: Year 3 report for 5R01AI110964 - Bat Coronaviruses in China and SE Asia.
Attachments: Year 3 NIAID CoV Report Final.docx

Hi Erik,

I hope all's well with you. I wanted to let you know that Aleksei will be signing off on our Year 3 report for the NIAID grant 5R01AI110964 on Bat CoVs in China and SE Asia. I've attached the word doc here so you can see it ahead of time.

Like last year, Zhengli Shi is proposing some work in Year 4 on bat coronaviruses that will involve developing chimeras between MERS-CoV and some bat CoVs we've discovered. I've included this in the report that you'll get next week, as well as the same text that explains why we believe this is not gain-of-function work. It's on the penultimate page of the report. Last year, we wrote to you and your colleagues and this work was given the go-ahead. I'm hoping that putting this paragraph in the report will make it move through quicker, but just wanted to give you a heads up…

Please let us know if you need us to write in with explanations separately.

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Year 3 Report: Understanding the Risk of Bat Coronavirus Emergence

Award Number: 1R01AI110964-02

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Section B: Accomplishments

B.1 What are the Major Goals of the Project

Zoonotic coronaviruses are a significant threat to global health, as demonstrated with the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, and the recent emergence Middle East Respiratory Syndrome (MERS-CoV). Bats were identified by our group as the wildlife reservoirs of SARS-CoV, and **since then hundreds of novel bat-CoVs have been discovered (including >260 by our group)**. These, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a large scale human-wildlife interface, and potential high risk for the future emergence of novel CoVs.

To understand the risk of zoonotic CoV emergence, our work aims to examine: **1)** *The transmission dynamics of bat-CoVs across the human-wildlife interface*, and **2)** *How this process is affected by CoV evolutionary potential, and how it might force CoV evolution*. This includes assessment of the nature and frequency of contact among animals and people in two critical human-animal interfaces: live animal markets in China, and people who are highly exposed to bats in rural China. In the markets we hypothesize that viral emergence is accelerated by heightened mixing of host species leading to viral evolution, and high potential for contact with humans. Our work involves screening free ranging and captive bats in China for known and novel coronaviruses; screening people who have high occupational exposure to bats and other wildlife; and examining the genetics and receptor binding properties of novel bat-CoVs and those already discovered. The goal is to use ecological and evolutionary analyses and predictive mathematical models to examine the risk of future bat-CoV spillover to humans. This work follows 3 specific aims laid out in our proposal:

Specific Aim 1: *Assessment of CoV spillover potential at high risk human-wildlife interfaces.* We will examine if: 1) wildlife markets in China provide enhanced capacity for bat-CoVs to infect other hosts, either via evolutionary adaptation or recombination; 2) the import of animals from throughout Southeast Asia introduces a higher genetic diversity of mammalian CoVs in market systems compared to within intact ecosystems of China and Southeast Asia; We will interview people about the nature and frequency of contact with bats and other wildlife; collect blood samples from people highly exposed to wildlife; and collect a full range of clinical samples from bats and other mammals in the wild and in wet markets; and screen these for CoVs using serological and molecular assays.

Specific Aim 2: *Receptor evolution, host range and predictive modeling of bat-CoV emergence risk.* We propose two competing hypotheses: 1) CoV host-range in bats and other mammals is limited by the phylogenetic relatedness of bats and evolutionary conservation of CoV receptors; 2) CoV host-range is limited by geographic and ecological opportunity for contact between

species so that the wildlife trade disrupts the 'natural' co-phylogeny, facilitates spillover and promotes viral evolution. We will develop CoV phylogenies from sequence data collected previously by our group, and in the proposed study, as well as from Genbank. We will examine co-evolutionary congruence of bat-CoVs and their hosts using both functional (receptor) and neutral genes. We will predict host-range in unsampled species using a generalizable model of host and viral ecological and phylogenetic traits to explain patterns of viral sharing between data to parameterize mathematical models that predict CoV evolutionary and transmission dynamics. We will then examine scenarios of how CoVs with different transmissibility would likely emerge in wildlife markets.

Specific Aim 3: Testing predictions of CoV inter-species transmission. We will test our models of host range (i.e. emergence potential) experimentally using reverse genetics, pseudovirus and receptor binding assays, and virus infection experiments in cell culture and humanized mice. With bat-CoVs that we've isolated or sequenced, and using live virus or pseudovirus infection in cells of different origin or expressing different receptor molecules, we will assess potential for each isolated virus and those with receptor binding site sequence, to spill over. We will do this by sequencing the spike (or other receptor binding/fusion) protein genes from all our bat-CoVs, creating mutants to identify how significantly each would need to evolve to use ACE2, CD26/DPP4 (MERS-CoV receptor) or other potential CoV receptors. We will then use receptor-mutant pseudovirus binding assays, in vitro studies in bat, primate, human and other species' cell lines, and with humanized mice where particularly interesting viruses are identified phylogenetically, or isolated. These tests will provide public health-relevant data, and also iteratively improve our predictive model to better target bat species and CoVs during our field studies to obtain bat-CoV strains of the greatest interest for understanding the mechanisms of cross-species transmission.

B.1a Have the major goals changed since the initial competing award or previous report?
No.

B.2 What was accomplished under these goals?

SUMMARY

The results of the 3rd year of our R01 work are detailed below. They include:

- Initial analysis of behavioral risk qualitative research in Yunnan and Guangxi, and wildlife market observational data, that suggests a reduction in wildlife hunting, trade and consumption may be underway in southern China.
- Results from a behavioral risk survey of over 1,000 people in two provinces of southern China that assesses exposure to wildlife and prior bouts of unusual illness, with concurrent taking of samples to test for evidence of exposure to SL-CoVs.
- The finding of serological evidence of spillover of bat SARS-like CoVs in 6 people in Yunnan province
- Testing of over 1,000 bat samples to identify diverse alpha- and betacoronaviruses
- Full genome characterization of 26 alphacoronaviruses.
- Receptor binding domain sequences from 37 new bat SL-CoVs that shows S proteins re more diverse than previously thought.

- Host-virus co-phylogeographic analysis of a diverse group of >1,300 bat CoVs showing that these viruses have a larger host range, weaker host specificity and higher frequency of cross-genera transmission than previously thought.
- Use of our reverse genetics system to identify 3 more novel SL-CoVs with potential to directly infect people.

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces

During Year 3 we began analyzing the qualitative research that was conducted in Year 2. In addition, we developed a digital application for a community-based integrated biological behavioral surveillance system and rolled this out in two provinces. The tool aims to identify specific animal exposure risk factors associated with biological evidence of exposure to SARS-like CoV (i.e. seropositive status).

Qualitative Research

Interviews conducted in two provinces (Yunnan and Guangxi) during Year 2 were transcribed and translated into English. A total of 47 individuals (18 women; 29 men) were interviewed in rural regions where wildlife trade routes have been documented. Yunnan and Guangxi provinces were specifically selected for study because they have large wildlife populations, a diversity of wildlife species and numerous live animal markets. Individuals who were 18 years of age or older and who were able to provide informed consent were eligible to participate. Twenty-three (49%) in-depth interviews were conducted in Yunnan province at nine different sites, 24 (51%) in Guangxi province at six different sites. In addition, one focus group was conducted in Guangxi. The study was approved by the Institutional Review Boards of the School of Public Health at Wuhan University and Hummingbird IRB #2014-23.

Participants were recruited primarily through local contacts that have been developed as part of wildlife conservation and health research that has been ongoing in these regions in China for the past decade. Contacts including wildlife conservationists and researchers, local government health outreach workers and wildlife farmers facilitated introductions and provided referrals. To achieve a sample with sufficient representation of categories of interest, participants were recruited using purposive sampling, which provides minimum quotas in terms of sex, age and wildlife exposure setting (e.g., live animal market, forest preserve).

Educational attainment varied widely in the population; however, the majority of study participants reported limited schooling, primary education or less. This was further reflected in the occupational distribution of study participants (*Fig. 1*), while there was two respondents who reported more professional occupations, a doctor and an accountant, over half (52%) were unskilled laborers or farmers, either agricultural or animal. There were four individuals who self-identified as animal farmers, farming wildlife, bamboo rat, civet, or nutria.

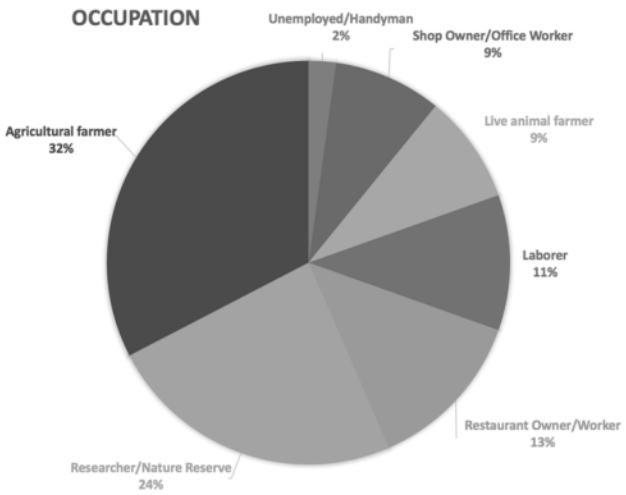

Figure 1. Occupation of Qualitative Research Participants (n=47) in Yunnan and Guangxi Provinces

Thematic analysis provided the framework with which to code and analyze data from the ethnographic interviews and focus group. Five core themes were identified to form the basis for this: (1) human-animal contact, (2) unusual illness experience and response, (3) socioeconomics and daily living, (4) biosafety and (5) human environments and movement/travel. Individual interviews and field notes were studied to ensure familiarity with the data set in its entirety and to confirm narrative consistency within individual interviews prior to coding. Using these themes and a coding keyword guide allowed for a directed and consistent coverage of the domains that were the focus of the actual interviews. Qualitative data were re-examined to develop additional theoretical categories or typologies. This analysis aims to assess perceptions, knowledge and participation in the wildlife trade, as well as barriers to participation and observed changes over time. The data were coded for factors associated with wildlife consumption, socioeconomic drivers of wildlife trade, conservation and legal efforts, the prevalence and types of wildlife observed, and wildlife exposures that could transmit disease to humans (*Table 1*).

Table 1. Topics covered in Ethnographic Interviews

Theme Discussed in Ethnographic Interview	No. of Respondents (n = 47)	%
Work	46	97.9%
Home Life	45	95.7%
Sanitation	45	95.7%
Hygiene	45	95.7%
Water & Food	44	93.6%
Direct Animal Contact	42	89.4%
Animal Responsibilities	41	87.2%
Perceptions/Knowledge	41	87.2%
Medical Care Treatment	40	85.1%
Household Illness	38	80.9%

Illness from Animals	38	80.9%
Indirect Animal Contact	38	80.9%
Daily Routine	36	76.6%
Education	36	76.6%
Animal Health	35	74.5%
Family Economics	34	72.3%
Observed Environment	33	70.2%
Animal Products/Rituals	32	68.1%
Travel	29	61.7%
Death	26	55.3%

The data coding and analytic strategy was designed to avoid the need for expensive analytic software programs and to use standard word processing and spreadsheet programs readily available to in-country teams. These teams received training on qualitative data analysis, and they initiated the first phase of analyses.

Analysis focused on wildlife trade and consumption in these two provinces, specifically on how respondents perceive and contact wildlife through the changing landscape around them. The aim was to identify motivations around animal consumption and practices. A number of participants reported that wildlife are purchased as a means to impress others as a symbol of wealth. Participants routinely reported that the cost of wildlife is double or triple that of regular livestock meat. Ironically, others reported that poorer individuals in these communities who continue to eat wildlife are sometimes scorned for their poverty, because this is a habit from an older time within China. Though there is a stigma to this habit, individuals did report opportunistically capturing and consuming wildlife when convenient.

Participants also noted a decrease in wildlife over time: that in their childhood the forests would be full of the sounds of animals and birds, but this occurs no longer. This decrease was attributed to many factors, most commonly infrastructure development. Respondents discussed the government investing resources to build new roads and renovate local infrastructure with the intention of increasing tourism, and that this has had the impact of reducing forested habitat for wildlife. Hunting and selling of wildlife was not reported by any participant as a cause of observed wildlife depletion. However, participants did attribute a reduction in wildlife hunting and consumption to an increased enforcement of conservation laws. In particular, the story of one ill-fated hunter who killed a monkey—and was caught—was reported by a number of participants from the same village.

Participants observed that the observed decrease in wildlife abundance and increased conservation law enforcement has made it more difficult to make a living from the wildlife trade. Participants reported choosing alternative forms of money making, indicating that only those people who belong to low socioeconomic classes continue to hunt secretly. The cost-benefit analysis that pits the threat of punitive consequences against the profits to be made through wildlife hunting are only feasible for those ‘who have nothing to lose.’

Observations by research staff in live animal markets in Guangzhou found wildlife to be plentiful (*Table 2*), although no bats were seen for sale during the observation period. In contrast, wildlife was not found in live animal markets at the sites we visited in either Yunnan or Guangxi. This is a change from previous research visits to the same or similar communities, when bats, rodents and wild boar could be found. Locals in Yunnan and Guangxi attribute the change to conservation law enforcement. The success of conservation enforcement may have moved hunting and trapping underground and made the capture of local wildlife less economically feasible than other income generating activities.

Table 2: Species Observed in Wet Markets in Guangdong Province from 2015 - 2016

Genus species	Common Name
Prionailurus bengalensis	Leopard Cat
Nyctereutes procyonoides	Raccoon Dog
Sus scrofa	Wild Boar
Lepus sinensis	Chinese Hare
Arctonyx collaris	Hog Badger
Hystrix brachyura	Porcupine
Marmota sp.	Marmot
Rhizomes sinensis	Bamboo Rat
Erinaceus sp.	Hedgehog
Mustela putorius	Ferrets
Muridae	Rat (species unknown)
Myocastor coypus	Nutria
Vulpes sp.	Fox
Mustela sibirica	Siberian weasel
Paguma larvata	Masked Palm Civet
Felis catus	Domestic Cat
Canis lupus familiaris	Domestic Dog
Cervinae	Sambar Deer
Ovis aries	Sheep
Capra sp.	Domestic Goat
Rattus norvegicus	Common Rat

Integrated Biological Behavioral Surveillance in Yunnan and Guangdong Provinces

To better assess the mechanisms of zoonotic viral spillover, and build on data acquired via ethnographic interview (above) we have designed a structured behavioral questionnaire to measure both exposure and outcome data. This behavioral risk survey assesses exposure to wildlife and bouts of unusual illness over a respondent's lifetime and in the past 12 months. In addition, participants were requested to provide serum to test for previous exposure to SARS-

like CoV. The integrated surveillance was pilot-tested in October 2015 among residents living near bat caves or roosts where SL-CoVs have been previously detected in the bat population in Jinning County, Yunnan. After the questionnaire was pilot tested and optimized to fit the research aim, the survey was developed as a digital application (https://www.dropbox.com/s/(b)(6) This allows standardization across all field teams and quality control. Four field team leads were trained on behavioral survey data collection, data collection technologies (the digital application) and analysis. The questionnaire was then administered in a follow-up survey in Yunnan province and then in Guangdong province. Surveillance in Guangxi is currently underway.

Of 1089 participants who completed the behavioral questionnaire, 660 (61%) were women and 424 (39%) were men (5 missing for this variable), with a mean age of 50 (range: 10-99). Most reported being farmers (79%) (*Fig. 2*), a majority were long term residents (97%) and 41% had a family income under 3000 RMB annually ($430). Almost three quarters (72%) of the respondents have had only primary level education or less.

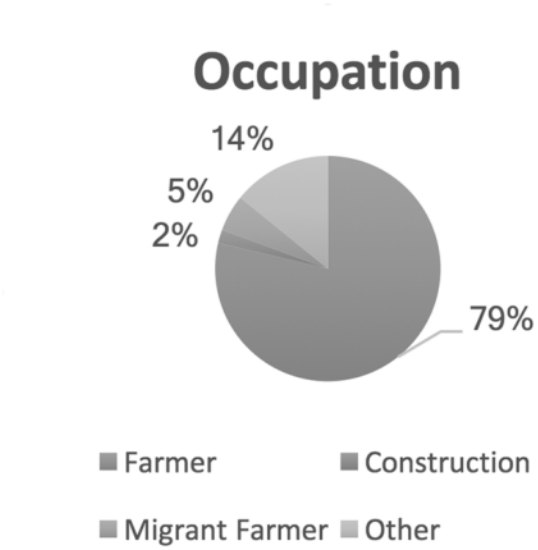

Figure 2. Occupation of Integrated Biological Behavioral Surveillance Participants in Yunnan and Guangdong Provinces

Standardized syndromic case definitions informed questions concerning unusual illness experience (e.g. severe acute respiratory infections [SARI], influenza-like illness [ILI], febrile symptoms [Encephalitis]). Lifetime, 12 month, and unusual illnesses experienced in the family for the past 12 months were assessed for all participants. In the past year, SARI was reported by 55 (5.1%) respondents and 14 of those respondents also responded SARI symptoms in family members (*Table 3*).

Table 3. Unusual Illness Experience In Respondents Lifetime, Past 12 months, Family members

Symptoms	Ever	Past 12 months	Family Past 12 months
Severe Acute Respiratory Infections (SARI)	118 (10.8%)	55 (5.1%)	40 (3.7%)
Influenza Like Illness (ILI)	305 (28.0%)	128 (11.8%)	142 (13.0%)
Encephalitis	98 (9.0%)	52 (4.8%)	30 (2.8%)

Hemorrhagic Fever	2 (0.2%)	2 (0.2%)	0 (0.0%)
Fever with Diarrhea /Vomiting	58 (5.3%)	25 (2.3%)	21 (1.9%)
Fever with Rash	10 (0.9%)	7 (0.6%)	7 (0.6%)

Type of exposure and species exposed to are shown below (*Table 4*). Poultry was the most commonly contacted animal in almost all categories. Three quarters of respondents reported rodents or shrews entering their home in the past 12 months.

Table 4: Animal Species Contact by Type of Contact

	Pets	Handled	Raised	In house	Cooked/ handled	Eaten raw/ under-cooked	Found dead collected	Scratched/ bitten	Slaughtered	Hunted/ trapped
Rodents/Shrews	0	33	5	834	38	2	1	1	28	26
Bats	0	5	0	180	8	0	0	1	5	5
Non-human primates	0	1	3	7	4	0	0	0	1	1
Birds	3	19	8	497	39	3	0	0	12	12
Carnivores	1	16	7	100	36	0	0	0	19	10
Ungulates	0	5	12	23	50	0	0	0	8	1
Poultry	5	514	843	134	719	5	8	6	425	7
Goats/Sheep	0	16	38	4	80	1	0	0	17	0
Swine	3	210	494	43	533	47	1	1	147	2
Cattle/Buffalo	0	12	77	10	102	5	1	0	11	1
Dogs	342	40	303	252	62	0	0	22	16	2
Cats	163	10	137	275	18	0	0	11	1	0

Animal exposures among those who reported unusual illness experiences in the past 12 months were evaluated, focusing on three high interest syndromes: SARI, ILI, and encephalitis. Of the 55 respondents who reported SARI symptoms, 49 reported: raising animals; animals in the home; preparing recently killed animals and buying live animals; 50% reported slaughter. Among the 16 respondents who reported ILI symptoms, 12 (75%) reported handling/preparing recently killed animals, 11 (69%) handling live animals or having animals in the home, 10 (63%) reported slaughtering/killing animals or buying live animals at wet market, 9 (56%) raised live animals, 7 (44%) reported a pet, and 1 (6%) reported animal feces near food or eating animal touched or damaged food, hunting, or eating raw/undercooked animal products. Among the four respondents who reported encephalitis symptoms, 3 (75%) reported hunting, handling or raising animals, 2 (50%) reported animals in the home, 1 (25%) reported having animals as pets, slaughtering/killing animals, or having bought live animals at a wet market.

We examined the sociodemographic attributes and the types of contacts that were reported in those who reported SARI, ILI, or encephalitis-like symptoms in the past year (see *Table 5*). Over

65% of respondents these syndromes and also reported raising animals, animals coming in the home, or preparing meat or organs from a recently killed animal. A quarter of those who reported symptoms consistent with that of encephalitis were under the age of 35.

Table 5. Self-Reporting Symptoms of Syndromes of interest and Sociodemographic and Animal Contact

	SARI Positive *n=55*		ILI Positive *n=128*		Encephalitis Positive *n=52*	
Sociodemographics	**n**	**%**	**n**	**%**	**n**	**%**
Mother Primary education or less	54	98.2%	121	94.5%	50	96.2%
Primary education or less	45	81.8%	94	73.4%	38	73.1%
Female	32	58.2%	74	57.8%	29	55.8%
Income <3000RMB	30	54.5%	45	35.2%	23	44.2%
Travel (past 12m)	30	54.5%	69	53.9%	34	65.4%
Children < 5 yo in Household	15	27.3%	38	29.7%	17	32.7%
Household member with same syndrome	14	25.5%	46	35.9%	10	19.2%
Respondent age <35	6	10.9%	24	18.8%	14	26.9%
Animal Exposures	**n**	**%**	**n**	**%**	**n**	**%**
Come in home	50	90.9%	117	91.4%	50	96.2%
Raise animals	49	89.1%	113	88.3%	48	92.3%
Prepare/cook recently killed	37	67.3%	95	74.2%	35	67.3%
Handle live	36	65.5%	72	56.3%	38	73.1%
Slaughtered	31	56.4%	57	44.5%	34	65.4%
Animals as Pets	23	41.8%	55	43.0%	28	53.8%
Buy Animals at Wet Market	16	29.1%	49	38.3%	4	7.7%
Shared water source	9	16.4%	13	10.2%	12	23.1%
Feces in/near food	8	14.5%	9	7.0%	8	15.4%
Consume raw/undercooked	7	12.7%	10	7.8%	9	17.3%
Scratch/bite	4	7.3%	2	1.6%	4	7.7%
Consume food damaged by animals	3	5.5%	5	3.9%	2	3.8%
Hunt or Trap	2	3.6%	4	3.1%	7	13.5%
Collect dead wildlife	1	1.8%	1	0.8%	1	1.9%
Consume sick animals	0	0.0%	1	0.8%	0	0.0%

Respondents were asked about the source of their unusual illnesses. None reported any kind of animal exposure as a potential source of infection and 11% did not have any idea what may

have caused their previous infection, despite the fact that a majority of respondents who reported SARI, ILI, or encephalitis symptoms also reported animal exposures (Table 5). Just over 30% of respondents reported purchasing live animals from a wet market in the past year. Over half (582; 53%) of respondents were worried about disease or disease outbreaks in animals at wet markets and 56% of people believe that animals spread disease. However, those who had purchased animals from markets in the last 12 months reported a great deal of behavior change being undertaken. In particular, respondents reported buying live animals less often 33%, only buying farmed wildlife 32% or buying meat at the supermarket 30% (*Table 6*). For those who participated in animal slaughter or were scratched or bitten in the past year, only 48 respondents (9.9%) reported visiting a doctor.

Table 6: Behavior Change at Wet Market in the last 12 months

Behavior	n	%
Wash hands	119	33.4%
Buy live animals less often	119	33.4%
Buy only farmed wildlife	113	31.7%
Sometimes shop for meat at supermarket	107	30.1%
Wear gloves	7	1.9%
Wear a mask	5	1.4%

Serological Evidence of Bat SARS-Like CoV Infection in Humans

Along with the behavioral survey questionnaire, a subset of respondents were also asked to provide a biological sample to assess SARS CoV spillover at the high-risk location where the questionnaire has been implemented.

A sensitive and specific ELISA method was developed using the recombinant bat SL-CoV Rp3 NP protein to detect SL-CoV IgG antibodies. Six (2.8%) serum samples from 218 village residents who lived closely to the bat colonies in Yunnan where we isolated SL-CoV WIV1 and WIV16 were were positive for SARS-like CoV antibodies (*Fig. 3*). The 6 ELISA positive samples were further confirmed as anti-SL-CoV NP IgG positive by western blot using recombinant Rp3-NP as antigen (*Fig. 4*).

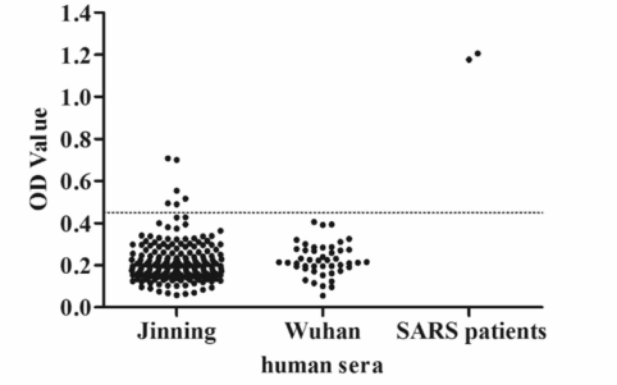

Figure 3. Serum samples from Jinning, Wuhan and SARS patients were screened for reactivity of Rp3-NP. Bar in the diagram indicates cutoff value (0.45) based on healthy blood donors in Wuhan.

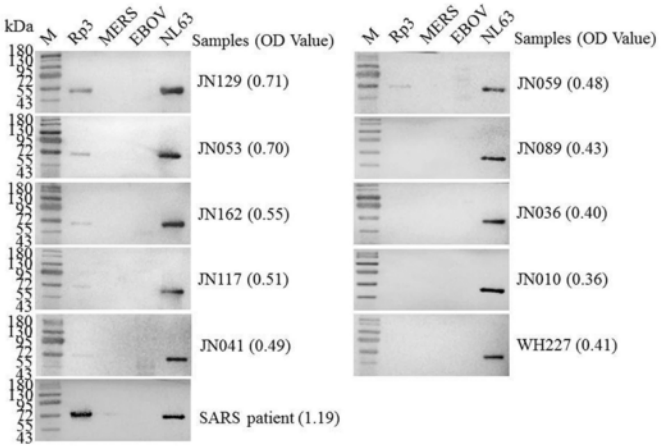

Figure 4. Western blot analysis of reactivity of human sera to Rp3-NP.

Linking serological findings with respondent questionnaire data

Of the 6 respondents in Jinning, Yunnan with serological evidence of SL-CoV infection, 4 had handled animals, 3 had raised or cooked meat from recently killed animals, 2 found animal feces near food stuffs, and 1 slaughtered or hunted an animal. Three of the individuals had contact with poultry in the past twelve months and 2 had contact with either birds, swine or buffalo. One individual reported having contact with a bat. Responses to the questionnaire show that in the last twelve months all of the respondents who have positive testing results, had animals in their dwelling and had contact with rodents or shrews. All 6 of the respondents had reported purchasing an animal from a wet market in the past twelve months.

In addition, 215 oral swabs and 212 rectal swabs collected from human participants in Jinning and Yunnan province were tested for CoV RNA, and no positive results were found. 534 oral swabs, 526 rectalswabs from Xishuangbanna, Yunnan province; and 419 oral swabs, 412 rectalswabs from Ruyuan and Zengcheng, Guangdong province are being tested for CoV.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV spillover risk

Bat CoV PCR Detection and Sequencing from Live-Sampled Bat Populations

We collected 893 rectal swab samples, 167 fecal samples and 33 blood samples from at least 17 bat genera in Yunnan, Guangdong, Guangxi, Hubei and Tibet provinces (*Table 7*) in Year 3.

During this year, overall 1060 samples were tested for CoV RNA and 130 (12.3%) were positive (*Table 8*).

Date of Sampling	Sampling Locations	rectal swab	Fecal pellet	Blood specimen
May 11th 2016	Mengla, Yunnan	32	--	9
May 16th 2016	Jingna, Yunnan	16	114	13
May 22nd 2016	Lufeng, Yunnan		53	--
June-July, 2016	Shixing country, Shaoguan, Guangdong	113	--	--
July 2016	Qingzhangshan, Shaoguan, Guangdong	101	--	--
July 10th 2016	Ruyuan, Guangdong	16	--	--
July 11th 2016	Chengjia, Nanling, Guangdong	26	--	--
July 2016	Huadu, Guangzhou, Guangdong	29	--	--
August 6th 2016	Lengshuitang village, Guilin, Guangxi	135	--	--
August 6th 2016	Nanxishan Park, Guilin, Guangxi	31	--	--
August 9th 2016	Lanwu village, Ruyuan, Guangdong	53	--	--
August 10th 2016	Liangkou twon, Conghhua, Guangdong	32	--	--
August 13th 2016	Jinning, Yunnan	34	--	--
August 14th 2016	Lufeng, Yunnan	25	--	--
August 16th 2016	Jingna, Yunnan	33	--	--
August, 2016	Menghai, Yunnan	125	--	--
August 21st 2016	Yaoqu village, Mengla, Yunnan	30	--	--
September, 2016	Wuhan, Hubei	36	--	--
September, 2016	Motuo, Tibet	26	--	11
Total		893	167	33

Table 7. Bat samples collected for CoV surveillance in Year 3

Species	Yunna	Guangdon	Guangx	Hubei	Tibet	Total
Rousettus spp.	1/34				6	1/40
Aselliscus stoliczkanus	31					31
Rhinoluphus spp.	16/41	11/136	6/60		5	33/242
Hipposideros spp.	17	1/126	6		8	1/157
Myotis spp.	7	6/34	7/69	1		13/111
Chaerephon spp.	8					8
Megaderma spp.	2				1	3

Ia io	1					1
Tylonycteris spp.	32/124	8				32/132
Pipistrellus spp.	1	45		5/35	2	5/83
Eonycteris spelaea	1/29					1/29
Nyctalus velutinus		2				2
Coelops spp.		2				2
Miniopterus spp.		9/17				9/17
Taphozous melanogopon			31			31
Cynopterus sphinx					3	3
Murina spp.					1	1
Fecal pellets	35/167					35/167
Sub-total	85/462	27/370	13/166	5/36	0/26	130/1060

Table 8. CoV testing results for bat samples collected in Year 3

Genetically diverse alphacoronaviruses related to bat coronavirus 1A/1B, HKU7, HKU6 and HKU2 were identified in *Miniopterus*, *Myotis* and *Rhinolophus* bats, respectively. A novel alphacoronavirus related to human coronavirus NL63 was detected in *Tylonycteris robustula* in Yunnan. SARS-like coronaviruses were detected in 14 Chinese horseshoe bats (*Rhinolophus sinicus*) in Yunnan and Guangdong. Betacoronaviruses related to HKU5 were found in *Pipistrellus abramus* from Hubei, while two lineages of HKU4-related viruses were identified in two species of *Tylonycteris* bats in Yunnan (*Fig. 5*).

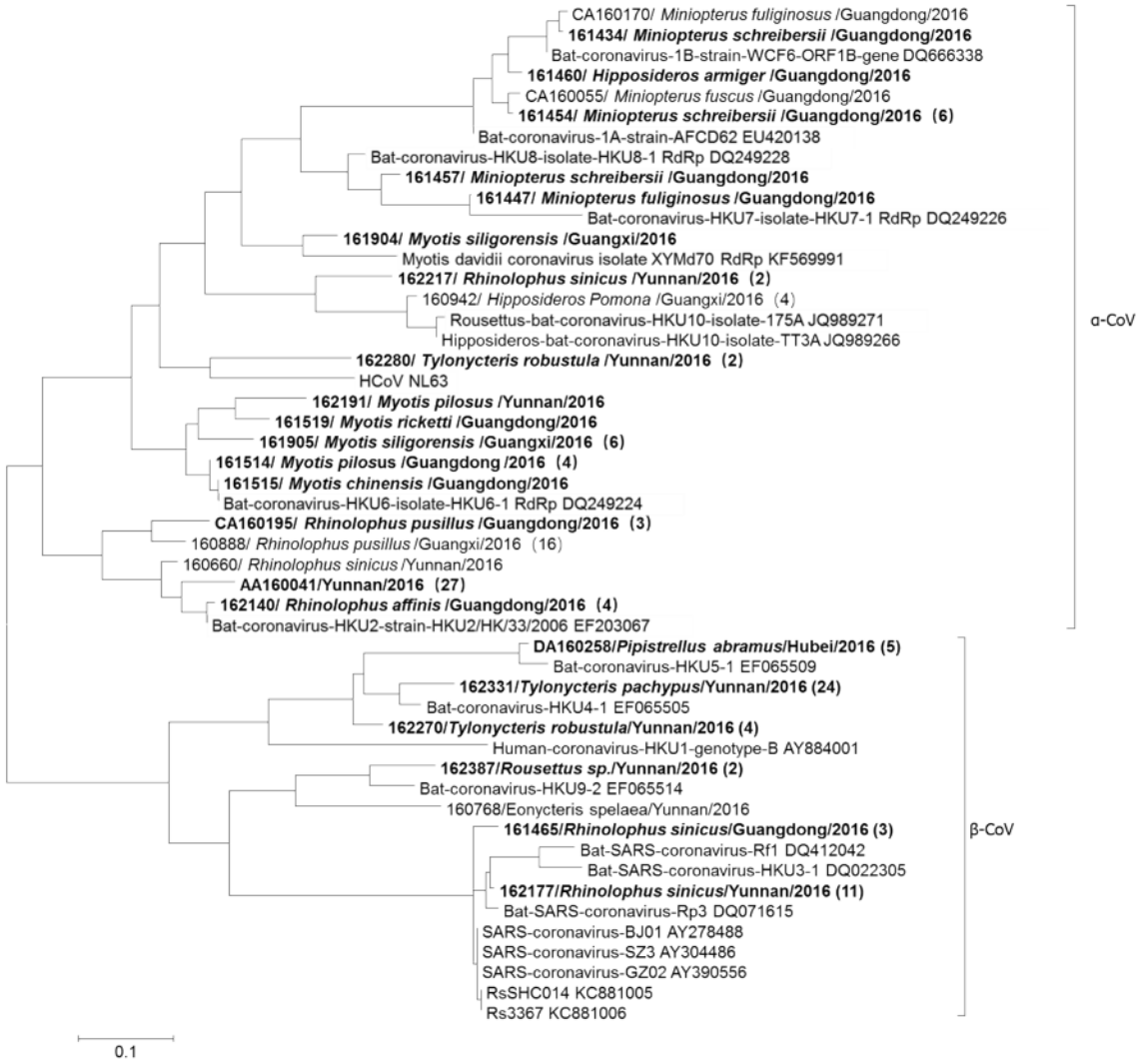

Figure 5. Phylogenetic analysis of partial RdRp gene of CoV (440-nt partial sequence).

Genomic Characterization of Novel Bat Alpha- Coronaviruses

We generated full-length genome sequences of 26 novel alphacoronaviruses from multiple *Hipposidoeros*, *Rhinolophus* and *Hypsugo* bat species. These alphacoronaviruses grouped into 4 different lineages, including HKU10-like CoVs and 3 novel species according to criteria generated by the International committee of Taxonomy of Viruses (ICTV) (*Fig. 6*). Strains belonging to the novel lineage from *Rhinolophus* share highly similar genome structures with each other but are distinct from all previously sequenced alphacoronaviruses. Putative 3b and 3c genes were identified at the upstream of the E gene, and a 7b gene at the downstream of the N gene was a homologue to *Rhinolophus* bat SARS-like CoV 7a gene. These results expand the understanding of genetic diversity of bat alphacoronaviruses.

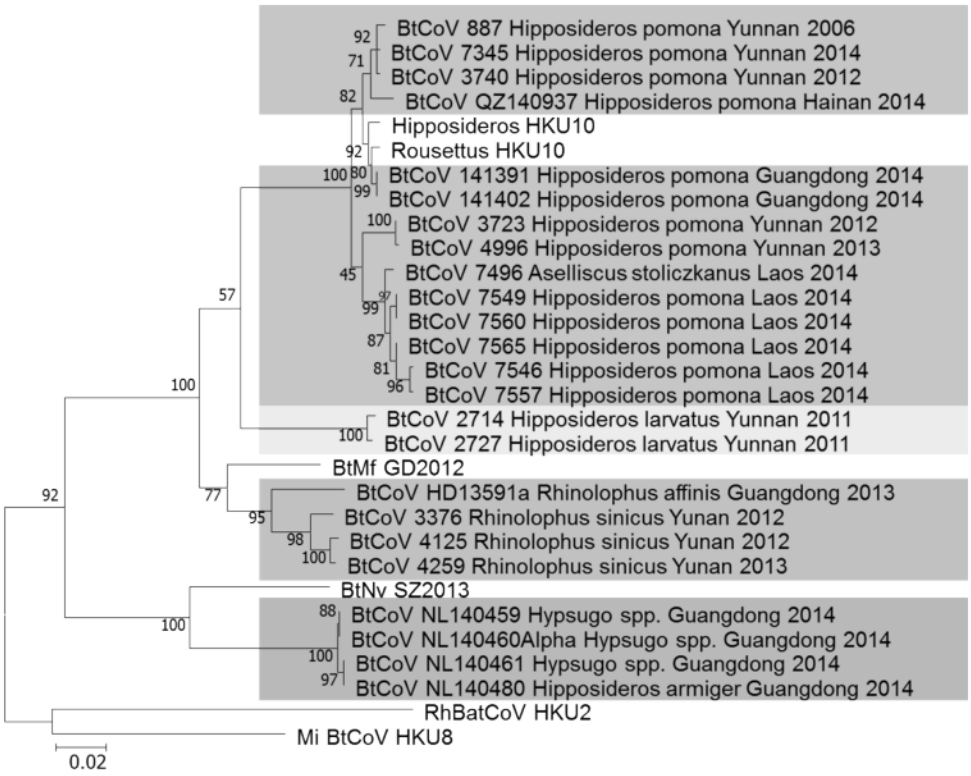

Figure 6. Phylogenetic analysis based on full-length RdRp gene sequence of alpha-CoVs

Genetic Diversity of Receptor-Binding Domain (RBD) of SARS-Like Coronavirus in Chinese Bats

RBD sequences from 37 newly identified SL-CoV from various horseshoe bat species and *Hipposideros* bat species in Yunnan, Guangdong, Guangxi, Hubei and Hunan provinces were amplified and sequenced in Year 3. Phylogenetic analysis revealed that SL-CoV circulating in bat populations in China are highly diverse in the RBD region (*Fi.g 7*). Some strains possessed an RBD sequence distinct from all currently known bat SL-CoVs and formed a new cluster in the phylogenetic tree. However, except for a few strains from Yunnan, most of these SL-CoVs contained nucleotide deletions and were relatively distant to SARS-CoV in the RBD region. These findings suggest that the S gene of SL-CoVs in Chinese bats is even more genetically diverse than expected.

The genomic characterization of SL-CoVs in Year 3 was focused on *Rhinolophus sinicus* in Yunnan, our plan for Year 4 is to obtain complete S gene, RdRp gene or full-length genome sequences of more SL-CoVs from a broader range of bat species identified all over China and conduct a more comprehensive study of the evolution of SL-CoVs in bats.

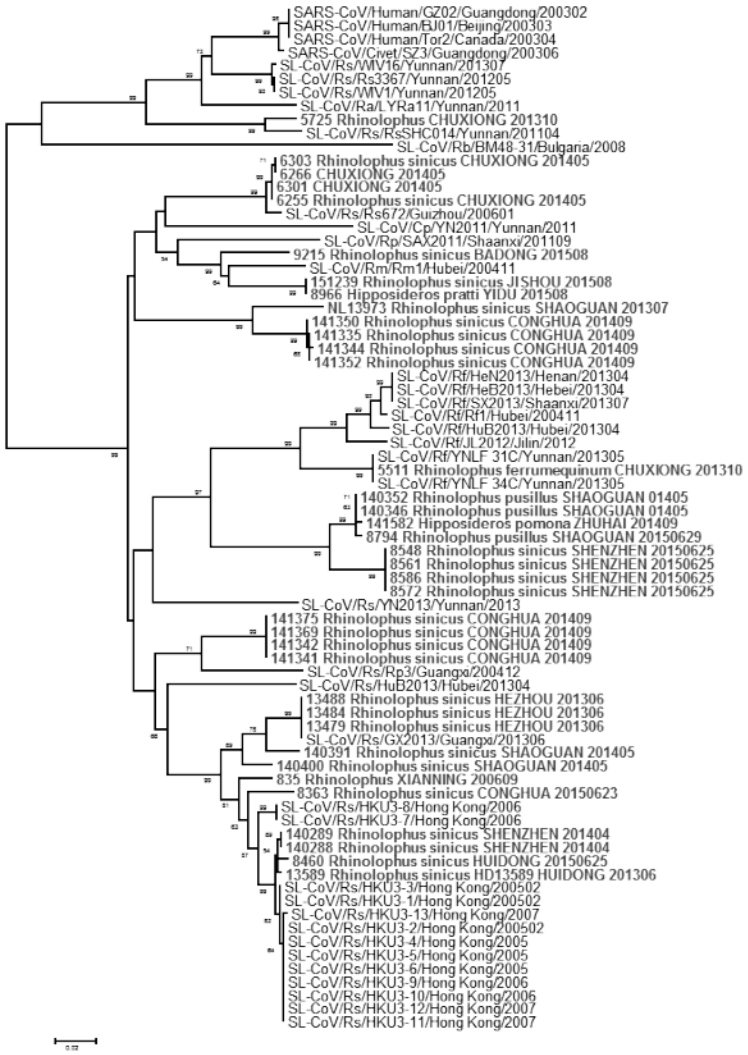

Figure 7. Phylogenetic analysis of the RBD region of the S gene of bat SL-CoVs detected in China (newly identified sequences were marked in red).

Bat Coronavirus Host-virus Phylogeography in China

To analyze the extent to which different bat species and genera are host to similar bat-CoVs, we reconstructed viral phylogenetic relationships and mapped host-species associations onto these phylogenies. Our dataset includes all CoV RdRp sequences isolated from bat specimens collected by our team from 2008-2015 (Alpha-CoVs: n = 491 – Beta-CoVs: n = 326), including those collected under prior NIAID funding (1 R01 AI079231), and funding from Chinese Federal Agencies. All Chinese bat CoV RdRp sequences available in GenBank were also added to our dataset (Alpha-CoVs: n = 226 – Beta-CoVs: n = 206). Phylogenetic trees were reconstructed for Alpha- and Beta-CoVs separately using Bayesian inference and Maximum Likelihood (ML) approaches. RAxML was used to perform ML analysis and Bayesian analyses were performed with MrBayes 3.2.6.

Beta-CoV sequences clustered into four main genetic lineages: B (SARS-CoV and SARS-like CoVs), C (MERS-CoV), D and a potential new lineage related to lineage B (*Fig. 8*). An important phylogenetic structure is observed within lineages C and D. Alpha-CoV sequences clustered into numerous closely related and less-differentiated lineages (*Fig. 9*).

We observed significant CoV lineage sharing among bat genera in our phylogenetic trees. Importantly SARS-like CoVs (SL-CoVs in lineage B) have been detected in Hipposideridae bats in addition to Rhinolophidae bats which were thought to be the putative natural host taxa of SL-CoV (*Fig. 8*). We found additional bat genera that also hosted CoVs in this clade (*Fig. 8*), expanding potential host targets for novel SL-CoV discovery. CoVs closely related to Bat coronavirus HKU9 (lineage D), which were thought to be specific to pteropodid bats, have also been detected in hipposiderid and vespertilionid bats (*Fig. 8*). Important lineage sharing across several bat families has also been observed among most Alpha-CoV lineages (*Fig. 9*). We used host DNA barcoding to confirm these findings - host mitochondrial sequences were generated to confirm the host species identity for most samples.

These results indicate a larger host range, weaker host specificity and higher frequency of cross-genera transmission for most bat CoV lineages than previously thought. These findings will have important implication in our understanding of bat CoV emergence and spillover risk in China. In Year 4 we will expand these analyses to include more explicit co-evolutionary analyses to identify the frequency and timing of host switching events for each major clade.

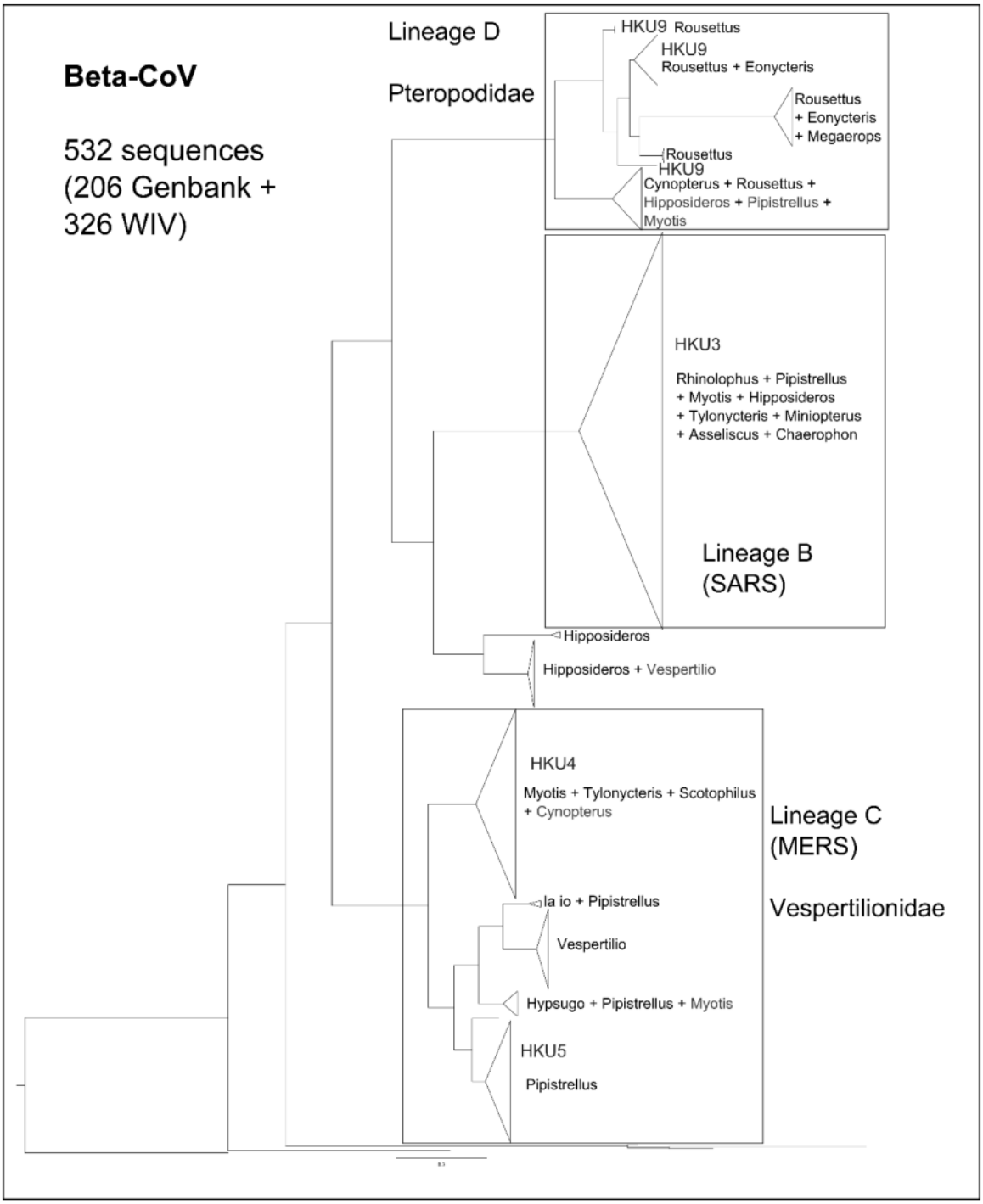

Figure 8. Maximum Likelihood tree of partial RdRp gene sequences of Beta-CoVs. Bat host genera are indicated along each lineage. Bat genera listed in red correspond to minor and potential new bat hosts and may represent cross-genera/family transmission events.

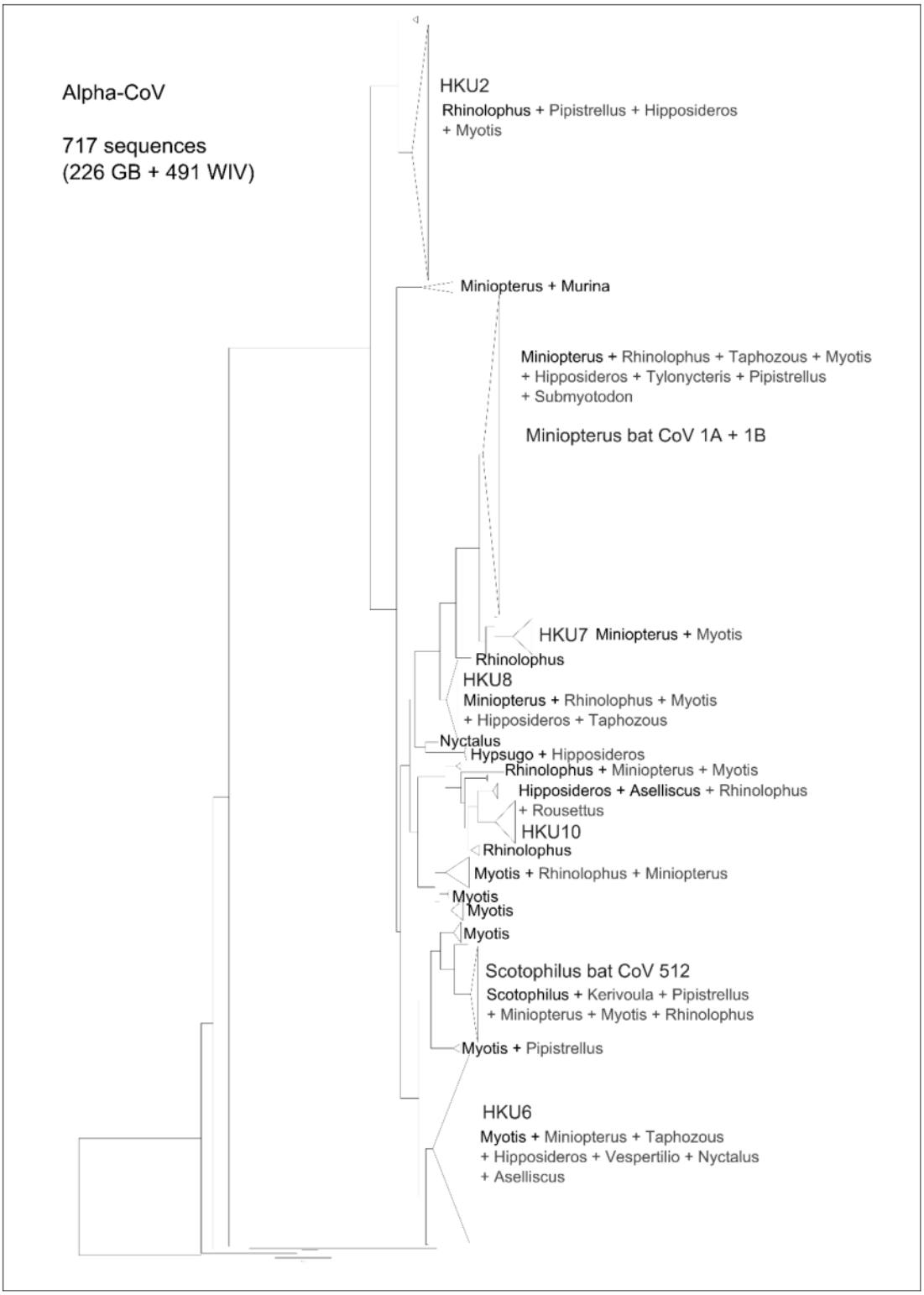

Figure 9. Maximum Likelihood tree of partial RdRp gene sequences of Alpha-CoVs. Bat host genera are indicated along each lineage. Bat genera listed in red correspond to minor and potential new bat hosts and may represent cross-genera/family transmission events.

Global analysis of bat viral sharing to identify key host species

We curated and analyzed a global dataset of bat host–virus associations to better understand the frequency, and connectivity of viral sharing among bats. We also used this to examine the importance of cave-roosting bats species in harboring and sharing viruses with non cave-roosting species, and to identify specific hosts that are central in the network (*Fig. 10*). Cave roosting bat species are host to most CoVs found in bats (orange). We identified global patterns of viral coinfection based on the number of connections between each virus in the network (Fig. 10). We will expand this approach to our China-CoV specific field data in Year 4.

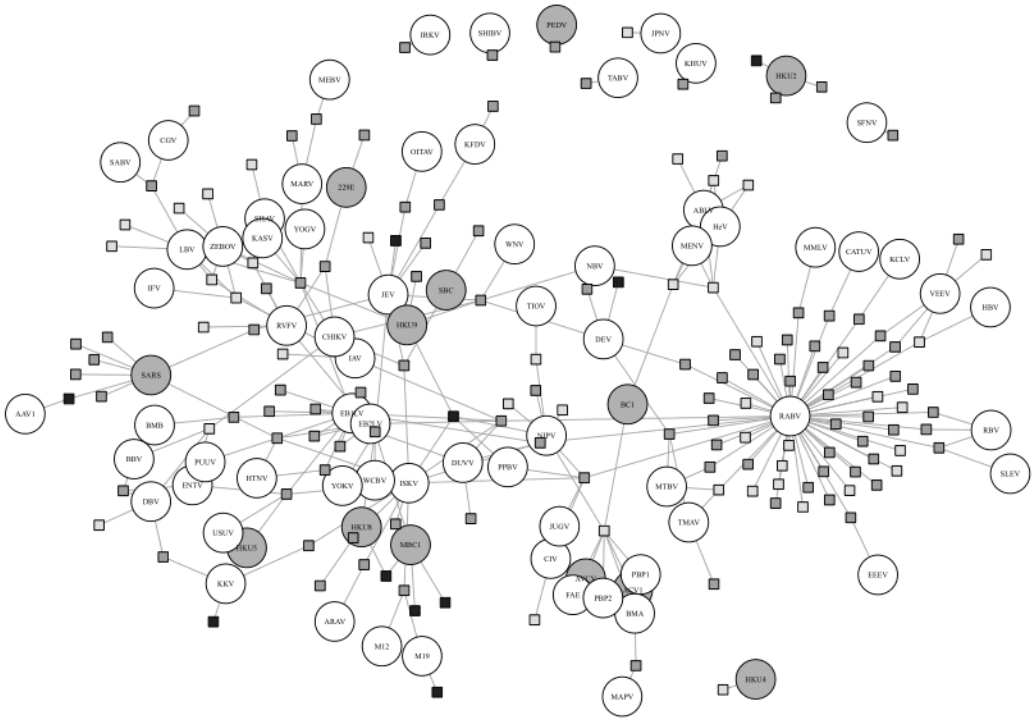

Figure 10. An analysis of global bat virus sharing using data from the published literature combined with field data. Network analysis includes 152 bat host species and 80 ICTV recognized viral species, with 273 host-viral associations. Unique viruses are represented in circles with known CoVs shown in orange, and each square represents a unique bat species. Green squares = facultative cave-roosting bat species; Blue squares = obligate cave-roosting species; Yellow squares = non cave-roosting species. Viruses are linked in the network based on host species that have been observed harboring the same virus – as detected using PCR or viral isolation.

Specific Aim 3: Testing predictions of CoV inter-species transmission

In Year 3 we established an effective and economic reverse genetics system for bat SL-CoV which can be applied to efficiently rescue SL-CoVs that are difficult to culture. This can be used to explore the functions of newly identified SL-CoV genes, as well as to assess pathogenesis of novel bat SL-CoVs. Using this system, we demonstrated that the unique ORFx in WIV1 and WIV16 is a functional gene involving modulation of the host immune response but not essential for *in vitro* viral replication (Zeng et al, 2016, J Virol).

Identification of Three Novel SL-CoVs with Potential for Direct Transmission to Humans

In Y2, we conducted full-length genome sequencing of 11 novel SL-CoVs detected in a single bat habitat in Yunnan province, which included strains highly similar to human/civet SARS-CoV in the most variable genes (N-terminal domain and RBD in the S gene, ORF8 and ORF3) (under revision). Based on recombination analysis, we hypothesized that the direct progenitor of the pandemic SARS-CoV may originated from this location after sequential recombination events at multiple genomic positions.

Among the 11 newly identified SL-CoVs, three different strains namely Rs4874, Rs7327 and Rs4231 contained no deletions in the RBD region but their RBD sequences varied from each other. Rs4874 has an S gene almost identical to that of WIV16. Rs7327's S protein varies from that of WIV1 and WIV16 at three aa residues in the receptor-binding motif, including one contact residue (aa 484) with human ACE2. Rs4231 shares similar NTD sequence with WIV1 and WIV16, but has a distinct RBD sequence. In Year 3, we successfully isolated Rs4874 from the single fecal sample. Using the reverse genetic system we previously developed, we constructed two chimeric viruses with the WIV1 backbone replaced with the S gene of Rs7327 and Rs4231, respectively. Vero E6 cells were respectively infected with Rs4874, WIV1-Rs4231S and WIV1-Rs7327S, and efficient virus replication was detected by immunofluorescence assay in all infections. To assess the usage of human ACE2 by the three novel SL-CoVs, we conducted virus infectivity studies using HeLa cells with or without the expression of human ACE2. All viruses replicated efficiently in the human ACE2-expressing cells. The results were further confirmed by quantification of viral RNA using real-time RT-PCR (*Fig.11*).

These finding suggests that diverse variants of SL-CoV S protein without deletions in their RBD are able to use human ACE2 as receptor for cell entry. Diverse SL-CoVs capable of direct transmission to humans are circulating in bats in southwestern China, which represents a potential risk of emergence given the opportunity to spillover to other animals and/or human populations.

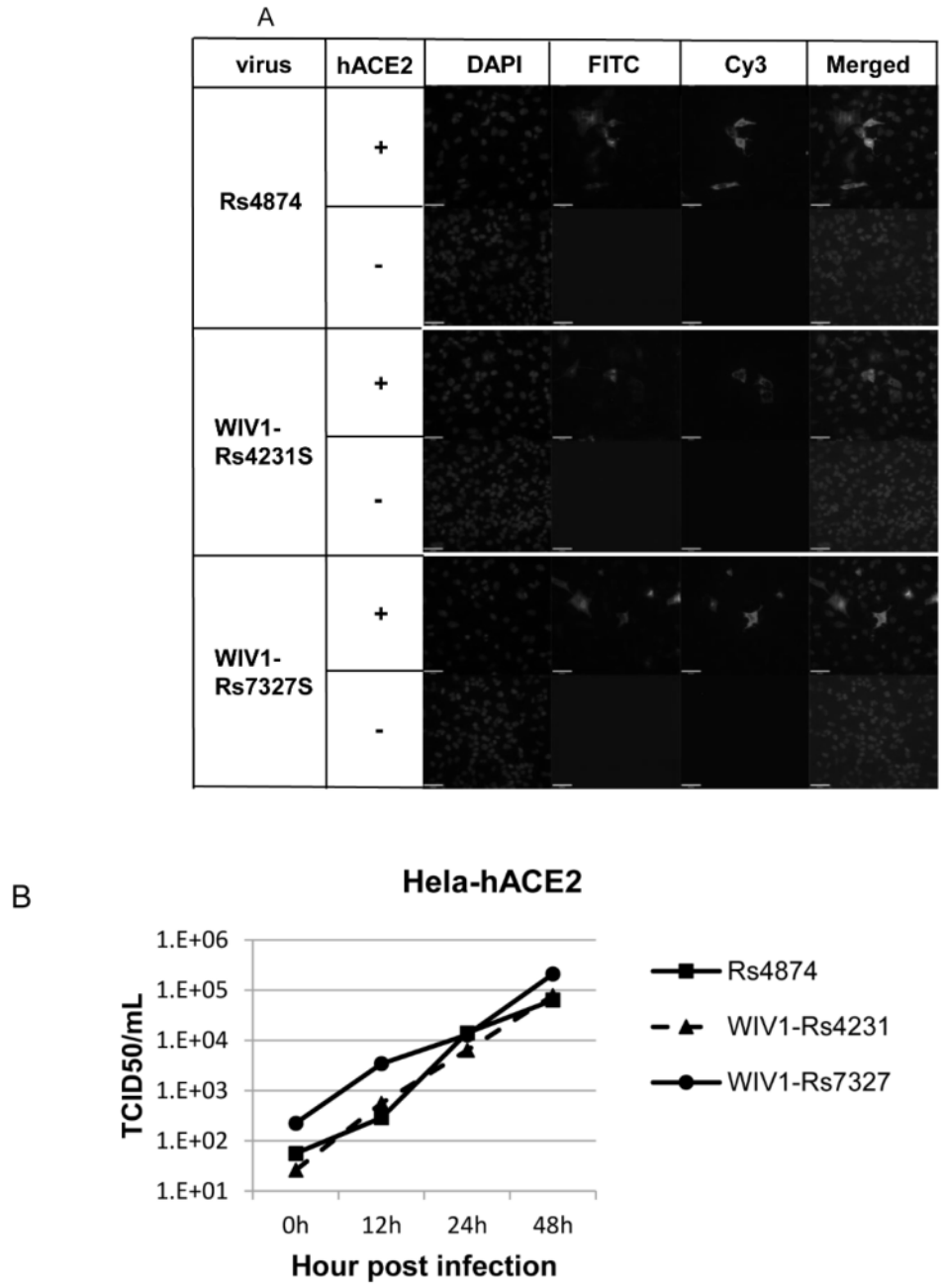

Figure 11. Analysis of receptor usage by immunofluorescence assay (A) and real-time PCR (B).

Additional Year 3 items for Specific Aim 3:

- The full-length infectious cDNA clone of MERS-CoV has been successfully constructed. The full-length S gene of 12 different novel bat MERS-related coronaviruses have been amplified and cloned into the T-vectors. In Y4, we aim to use the reverse genetic method, and construct chimeric viruses with the backbone of MERS-CoV and the S genes from

diverse newly identified bat MERS-related coronaviruses, to examine the pathogenicity of bat MERS-related coronaviruses on cell and animal levels.

Potential Gain-of-Function issues for the above proposed work: Similar experiments were proposed in our Year 2 report, and we followed up with an explanation of their potential for GoF conflicts. We have repeated this rationale below, and note that NIAID did grant us the capacity to do this work following our explanation:

Firstly, we would like to comment that this work is proposed for year 4, and none has been conducted to date. Furthermore, we will not proceed with any of this unless we are given the go-ahead by NIAID. The goal of our proposed work to construct MERS and MERS-like chimeric CoVs is to understand the potential origins of MERS-CoV in bats by studying bat MERS-like CoVs in detail. The chimeric viruses will be used to ascertain receptor usage and infectivity of bat MERS-related CoVs in vitro and in a mouse model. To achieve this purpose, our aim is to firstly construct a MERS-CoV infectious clone based on the genomic sequence of EMC2012 (GenBank no. NC_019843) and then chimeric CoVs with the replacement of the spike envelope genes from bat derived MERS-like CoVs. We have very recently discovered a small number (9 different strains) of bat MERS-like CoVs in 99 samples from bats in Guangxi, Guangdong, and Szechuan provinces. Phylogenetically, these bat viruses are not very close to MERS-CoV (only 63-66% homology to the S-protein of MERS-CoV).

We aim to test the chimeric viruses for receptor usage of DPP4 (the MERS-CoV receptor) in cells and then in DPP4 transgenic mice, to see if these bat viruses have any capacity to use the same receptor. That said, given the phylogenetic distance from MERS-CoV, we believe it is highly unlikely that these bat spike proteins attach to DPP4, and if so, that they would have any pathogenic potential. Finally, should any of these recombinants show evidence of enhanced virus growth >1 log in cells expressing the human, bat, mouse or other DPP4 receptor over wildtype parental backbone MERS-CoV strain or grow more efficiently in human airway epithelial cells, we will immediately: i) stop all experiments with the mutant, ii) inform our NIAID Program Officer and the Wuhan Institute of Virology IBC of these results and iii) participate in decision making trees to decide appropriate paths forward.

- Establishment of animal infection models for bat SL-CoV and MERS-related CoV: Mice with human ACE2 have been imported to China and have been bred for one generation in Wuhan Institute of Virology. Transgenic mice that express human DPP4 have also been constructed and are being bred. The animal infection experiments are planned to be conducted in following years to study the pathogenicity of diverse SL-CoVs and MERS-related CoV that we identified in Chinese bats.

Section C: Accomplishments: Publications

Papers published

Miller, M. and Hagan, E. "Integrated biological–behavioural surveillance in pandemic-threat warning systems." *Bulletin of the World Health Organization* 95.1 (2017): 62.

Zeng LP, Gao YT, Ge XY, Zhang Q, Peng C, Yang XL, Tan B, Chen J, Chmura AA, Daszak P, Shi ZL*, Bat SARS-like coronavirus WIV1 encodes an extra accessory protein ORFX involved in modulation of host immune response. *J. Virol*. 2016, 90(14): 6573-6582.

Hu B, Zeng LP, Yang XL, Ge XY, Zhang W, Li B, Luo DS, Zhang YZ, Wang MN, Daszak P, Wang LF, Cui J*, Shi ZL*. An Epicenter of Bat SARS-like Coronaviruses with Frequent Recombination Events Promoting the Generation of the Pandemic SARS Coronavirus. (under review).

Significant Oral Presentations

1. Daszak P. Plenary talk, One Health-EcoHealth Congress, Melbourne, Dec. 2016
2. Daszak P. 2nd annual Global Pandemic Policy Summit, Scowcroft Ctr, Texas A&M Univ.
3. Daszak P. Global Health Security Agenda side event, UN World Humanitarian Summit: FAO/WHO/USAID/Global He@lth 2030 Innovation Task Force; Istanbul, Turkey.
4. Daszak P. Symposium at École du Val-de-Grâce, Paris
5. Daszak P. Plenary, Institute of Zoology symposium on Bushmeat and disease risks, London.
6. Daszak P. Duke University Provost's Forum on Conservation and Health

From: Aleksei Chmura
Sent: Fri, 17 Feb 2017 22:18:23 -0500
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Greer, Jenny (NIH/NIAID) [E]; Smith, Philip (NIH/NIAID) [E]
Subject: Re: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER
Attachments: NIH-NIAID_5R01AI110964 Additional Site Q and A.pdf

Dear Erik,

Please find our responses in the attached PDF. If you need any additional details, please let me know.

Many thanks!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
(mobile)
(b)(6) (Skype)

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On Feb 15, 2017, at 08:52, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,
I know you said nothing will be changing from your currently approved animal studies, but it would be helpful for me in preparing the foreign clearance request if you could write a few concise sentences about the new animal work addressing the following points:

- Kind or species of animal and number to be used
- Location of the source of the animals, if known
- A brief description of the sampling (blood draw, swab, etc)
- Location from where the animals will be obtained (source)
- If possible, what will be done with the animals after the project ends (e.g., euthanized)

Let me know if you have any questions.
Thanks!
Erik

From: Aleksei Chmura (b)(6)
Sent: Monday, February 13, 2017 4:23 PM
To: Greer, Jenny (NIH/NIAID) [E] (b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Smith, Philip (NIH/NIAID) [E] (b)(6)
Subject: Re: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Super! Thanks, Jenny.

Erik and Philip - please let me know, if you have any questions or require additional details. We look forward to your responses.

Sincerely,

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
(mobile)
(b)(6) (Skype)

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On Feb 13, 2017, at 16:18, Greer, Jenny (NIH/NIAID) [E] (b)(6) wrote:

Aleksei,

Thank you for your email. I am copying Erik on this response so he can make sure he has everything needed to initiate a request for each of these foreign sites. I am also copying Philip Smith, the grants management specialist assigned to this grant for this fiscal year. Please don't hesitate to contact either of them with any questions you may have.

Please note that this response does not constitute approval and it will take at least 3 weeks for a final determination to be made.

Thanks again! And have a great afternoon!

Jenny

Jenny Greer
Grants Management Specialist
DHHS/NIH/NIAID/DEA/GMP
5601 Fishers Lane, Room 4E49, MSC 9833
Bethesda, MD 20892-9824
Phone: (b)(6)
Email: (b)(6)

***Effective January 1, 2017**, NIH closeout policy has changed (see NOT-OD-17-022). NIH is no longer accepting Final Progress Reports (FPR). Grantees must now report final project outcomes using the new F-RPPR. For instructions on how to submit the new F-RPPR please see instructions on the NIH RPPR Page.*

From: Aleksei Chmura (b)(6)
Sent: Friday, February 10, 2017 2:54 PM
To: Greer, Jenny (NIH/NIAID) [E] (b)(6)
Subject: Re: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Dear Jenny,

I am just following up with item 1 and 1a from your email below. As per Peter's email (also below), we would like to request prior approval for collecting non-human animal samples in 7 countries: Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Thailand, and Vietnam.

No new animals will be introduced nor any new field procedures, we have submitted IACUC protocol modification - for geographic locations only - and will provide approval dates as soon as they are available.

No work will be conducted until we have your approval and IACUC approval.

Testing would be conducted locally and if any samples were to be transferred to China these would be only extracted viral DNA - and not the original sample material.

Samples will be collected by either our current China field team personnel working directly with our collaborators in these countries or by respective in-country personnel and require no more

than 10% budget modification total (from already budgeted China fieldwork) for any non-China in-country work.

Here is the list of our local in-country contacts and institutions:

Cambodia
Veasna Duong
Institut Pasteur du Cambodge
No. 5 Monivong Boulevard
P.O Box. 983, Phnom Penh, Cambodia
(b)(6)

Indonesia
Joko Pamungkas
Primate Research Center at Bogor Agricultural University
JalanLodayaII/5,Bogor16151, Indonesia
(b)(6)

Lao People's Democratic Republic
Watthana Theppangna
National Animal Health Laboratory
Department of Livestock and Fisheries
Ministry of Agriculture and Forestry, Vientiane, Lao PDR
(b)(6)

Malaysia
Tom J. Hughes
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Suite 4A, Level 4, Main Office Tower
Financial Park Complex, Jalan Merdeka, 87000
Federal Territory of Labuan, Malaysia
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Aung Than Toe
San Pya Clinic
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(b)(6)

Thailand

Supaporn Wacharapluesadee
Neuroscience Center for Research and Development
King Chulalongkorn Memorial Hospital
Rama 4 Road
Patumwan, Bangkok, Thailand 10330
(b)(6)

Vietnam
Nguyen Huu Nam
Faculty of Animal and Veterinary Science
Hanoi Agricultural University
Trauquy, Gialâm, Hanoi, Vietnam
(b)(6)

If it will be easier to have a quick chat about this, I am happy to call anytime. Also, if this request should be sent more formally as a letter attachment, we can do that rapidly as well.

I hope you and yours had a lovely Holiday and are surviving the blizzard!

Cheers,

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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On Aug 1, 2016, at 12:39, Greer, Jenny (NIH/NIAID) [E] (b)(6) wrote:

Thank you for your email. To answer your questions:

1. To do any work in countries other than China, you will need to request prior approval from NIH. To do so, submit a formal request, including the names, institutions, and full contact information of any institutions with which you will collaborate for such activities. Be sure to indicate whether

animal or human research will be conducted and what funds, if any, will be going into these countries. The approval process for new foreign sites takes at least 3 weeks.

1a . If you are introducing new animals into the project, then there may be additional requirements from the Office of Laboratory Animal Welfare (OLAW). Again, you would need to submit a formal request, providing a scientific justification for the inclusion of new species on the project, and, if appropriate, a new Vertebrate Animal Section. If additional IACUC approvals are required, you will need to provide us with the IACUC approval dates (but **not** a copy of the actual approval).

2. These individuals are not listed in the Notice of Award as key personnel, so, from a grants management perspective, you do not need to get prior approval for this change. That said, if this change or other such personnel changes would have a significant impact on the scope of the project or the science itself, you would need to at least run it by your Program Officer. And if it is determined that personnel changes would cause a scope change, then you would need grants management approval as well.
3. I do not know what you are asking here. It looks like we have approved both the Wuhan University and ECNU for work on this project. Therefore, no additional prior approval is required for changes unless otherwise specified in the NIH Grants Policy Statement (eg, a change of scope).

Please don't hesitate to contact me with any additional questions. I will be available until 2:30 eastern and then again on Wednesday.

All the best,

Jenny

Jenny Greer
Grants Management Specialist
DHHS/NIH/NIAID/DEA/GMP
5601 Fishers Lane, Room 4E49, MSC 9833
Bethesda, MD 20892-9824
Phone: (b)(6)
Email: (b)(6)

From: Aleksei MacDurian (b)(6)
Sent: Sunday, July 31, 2016 6:06 AM
To: Greer, Jenny (NIH/NIAID) [E] (b)(6)
Subject: Re: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Dear Jenny,

Since you were not cc'ed on the original email, I wanted to follow up with you on three things from Dr. Daszak's email to Erik (included below):

1) Do we need to formally request permission to sample species of bats and other high-risk [rodents and carnivore] hosts in countries that neighbor China (Myanmar, Vietnam, Cambodia, Lao PDR) and others that supply wildlife to the international trade to China (Thailand, Malaysia, Indonesia). Under this award our current US and China IACUC approved protocol via Tufts University and Wuhan Institute of Virology permits us to sample these species in these regions.

2) We provided Dr. Noam Ross' CV with our Year 2 Report. Dr. Ross has replaced Dr. Hosseini who is no longer working on this project. Do we need to do anything else for this? I have attached his Biosketch here for reference.

3) Our Human surveillance work and local IRB approval have all been through the Wuhan University School of Public Health (WUSPH) in China (DUNS No. 529049295). We would like now - in Years 3 - 5 of our award to subcontract directly with them rather than with the institution on our current budget: East China Normal University (ECNU) School of Life Sciences. The Wuhan University School of Public Health budget amount would be the same annual amount as currently budgeted for East China Normal University in these same years.

It may be easier to briefly chat about these questions via telephone. If so, you may reach me at (b)(6) anytime.

Many thanks!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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On Fri, May 13, 2016 at 5:55 PM, Peter Daszak (b)(6) wrote:
Dear Erik,

I just wanted to let you know that we submitted our Year 2 Report yesterday (attached as a pdf).

It's been a pretty productive year, and some of the highlights include: collecting samples from 15 bat genera in southern China with 280 (12%) testing positive for coronaviruses; SARS-like coronaviruses

being detected in*Rhinolophus* spp. bats in both Yunnan and Guangdong provinces; 7 published papers from work under our award (including one in *J. Virol.* and one in press at *J. Virol*); 218 quantitative interviews with samples and 47 qualitative coded interviews conducted transcribed and translated.

In the report, I highlight the reduced amount of wildlife in the local markets within Southern China compared to that we've seen before, as well as the continued expansion of the Chinese wildlife trade within SE Asia so that it is now a largescale international activity. It means that SL-CoVs we find in the wildlife trade would likely have an origin in adjacent countries. Given that our collaborators and field team in China have great contacts in these countries, and EHA also has field teams in many of them, we would like to conduct short field trips to assess markets, identify wildlife in them, and sample species of bats and other high-risk hosts in countries that neighbor China (Myanmar, Vietnam, Cambodia, Lao PDR) and others that supply wildlife to the international trade to China (Thailand, Malaysia, Indonesia). All samples collected would still be tested at the Wuhan Institute of Virology in China. Is there a formal process to ask for permission for this, or is the report and this email appropriate?

I also wanted to let you know about a recent personnel change. Since Dr. Parviez Hosseini has moved to the US Department of State as an Information Advisor earlier this year, we hired another senior researcher Noam Ross to conduct data analysis and spatial mapping. Our Year 2 report includes his CV. Noam has great enthusiasm and I am eager to see his work on our data collected to date. He has already been out to China is hitting the ground running!

We have had great successes this past year and I'd be happy to discuss any of them with you, if you'd like.

Cheers,

Peter

Peter Daszak
President

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1. **Kind or species of animal and number to be used:**

Species taxa	Family, Genus or Species Name	Target numbers
Fruit bats	*e.g.: Cynopterus, Rousettus, Eonycterus spp.*	900 individuals (30 individuals from 30 different species)
Insectivorous bats	*e.g.: Rhinilophidae, Hipposideridae, Emballonuridae, Vespertillionidae, Mollsidae, Miniopteridae spp.*	
Rodents	e.g: Chinese bamboo rat (*Rhizomys sinensis*), Malayan porcupine (*Hystrix brachyura*), bandicoot (*Bandicota indica*)	900 individuals
Small Carnivores	e.g.: Raccoon dog (*Nyctereutes procyonoides*), Asian Palm civet (*Paradoxurus hemaphroditus*), ferret badger (*Melogale moschata*)	500 individuals

2. **Location of the source of the animals, if known:**

Free-ranging bat surveys and bats in wet markets: China, Malaysia, Thailand, Cambodia, Lao PDR, Myanmar, Vietnam, and Indonesia.

Other mammals: We will opportunistically sample the other aforementioned taxa that are also sold in live animal markets, trading locations or bred on farms to supply markets throughout southeast Asia. Species and numbers of animals sampled from markets will be based on animal availability.

3. **A brief description of the sampling (blood draw, swab, etc)**

Bat capture. Free-ranging bats will be captured using either a mist net or harp trap and bats are removed from the net as soon as they become entangled to minimize stress and prevent injury. Bats will be manually restrained during sampling. Bats that are fractious may be anesthetized for restraint purposes in order to maximize safety for the bat and handler. Depending on the species and size of bat, swabs will be taken from the oropharynx, urogenital tract, and rectum. Fresh feces will be collected if available, in which case a rectal swab will not be collected. Blood will be collected from either from the cephalic vein or from the radial artery or vein using a 25-gauge needle. Bats are held for a maximum of six hours and then released following sample collection. We will euthanize 2 individuals per bat species for organ tissue banking.

Wild and captive bred rodent capture. Free-ranging rodents will be captured using box traps. Captive bred rodents (e.g. at rodent farms) will be manually captured and restrained. Traps for free-ranging rodents will be checked a minimum of every 12 hours, including once in the morning. Captive bred and wild rodent sampling procedures (including anesthesia, if necessary), will involve manual restraint, venipuncture, mucosal swabs, fecal, and urine sample collection.

Other small mammals: Anesthesia will be used to restrain small mammals such as civets and ferret badgers. Animals will be monitored continuously while recovering from anesthesia and will only be released once fully recovered from anesthesia. Animals that are sourced from markets and that may potentially be consumed, will be manually restrained without anesthesia, if possible, so that they may be returned to the vendor. Otherwise, the animal will be sampled and then euthanized via exsanguination

(cardiac puncture) while under anesthesia, then disposed of using biohazard protocols in order to prevent subsequent human or animal consumption.

4. **Location from where the animals will be obtained (source):**

Markets and surrounding caves/forest: sites will be identified along value chain routes linking southern China to southeast Asian countries that serve as sources for the Chinese market system. Specific field sites have not yet been determined.

5. **If possible, what will be done with the animals after the project ends (e.g., euthanized)**

All wild animals will be released unharmed after sampling at the capture location. While we do not anticipate any severe adverse events related to the capture or sampling of free ranging wildlife, we will observe all animals caught in traps and nets for injuries. Veterinary care of wildlife in the field is limited. Any animal with an injury that is deemed life-threatening, or significant enough to prevent survival upon release, will be humanely euthanized in accordance with the AVMA guidelines for euthanasia (2013). Any animal that is injured in the course of restraint or sampling such that it is deemed unable to survive if released or if appears to be in severe pain due to injury, will be humanely euthanized. Animals that are caught and moribund (depressed mentation, non-responsive to stimuli, emaciated and weak or exhibiting neurological signs), will be humanely euthanized.

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Tue, 12 Jul 2016 14:43:40 +0000
To: Peter Daszak
Cc: Aleksei Chmura; Chen, Ping (NIH/NIAID) [E]
Subject: RE: Visit to NIAID office in Beijing

Hi Peter,
That's great, I'm glad you had a productive visit. It's been about a year since I was last in China, but if I do make it back there I'll be sure to let you know. I'd love to visit one of your field sites.

Erik

From: Peter Daszak (b)(6)
Sent: Monday, July 11, 2016 10:16 AM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Aleksei Chmura (b)(6) Chen, Ping (NIH/NIAID) [E]
(b)(6)
Subject: Visit to NIAID office in Beijing

Dear Erik,

I just wanted to update you on our meeting last week with Dr. Ping Chen at the NIAID Office in Beijing. We had a very good, informal chat at the US Embassy (a very impressive building by the way). We talked about our work on SARS-like viruses under the R01, as well as other work we're doing in China. She mentioned that you were in China recently and I suggested that next time we could set up a visit to one of our field sites to see the bat caves that harbor SL-CoVs, and the people who live nearby. Let me know when you're next planning a trip here and I'll set it up…

Cheers,

Peter

Peter Daszak
President

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From: Aleksei Chmura
Sent: Tue, 28 Jun 2016 23:58:05 -0400
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Dr. Peter Daszak; Greer, Jenny (NIH/NIAID) [E]
Subject: Re: Grant Number: 5R01AI110964 - 03 PI Name: DASZAK, PETER
Attachments: Response to GoF letter, 5R01AI110964 - 03 DASZAK, PETER.pdf

Dear Erik,

Prof. Zhengli Shi has confirmed that the Wuhan Institute of Virology Institutional Biosafety Committee would be immediately notified as per Peter's comments below. Please find the updated letter attached.

If you require further details, let us know anytime.

Sincerely,

-Aleksei

Aleksei Chmura
Authorized Organizational Representative &
Senior Coordinator of Operations

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On Jun 28, 2016, at 11:22, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Thanks Peter! Please have Aleksei send us an updated letter once you have one.

Erik

Sent with Good (www.good.com)

-----Original Message-----
From: Peter Daszak (b)(6)
Sent: Tuesday, June 28, 2016 08:02 AM Eastern Standard Time

To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Greer, Jenny (NIH/NIAID) [E]; Aleksei Chmura
Subject: RE: Grant Number: 5R01AI110964 - 03 PI Name: DASZAK, PETER

Sorry for not responding more quickly Erik – I've been at meetings for the last couple of weeks. You are correct to identify a mistake in our letter. UNC has no oversight of the chimera work, all of which will be conducted at the Wuhan Institute of Virology. This was a clerical error because we used some language that I asked Ralph Baric to give me because I wanted to make sure we followed an approach that has some precedence.

We will clarify tonight with Prof. Zhengli Shi exactly who will be notified if we see enhanced replication, and then amend and re-send the letter to you so it is clear. I will also confirm with Zhengli the make-up of the Wuhan Institute of Virology's Institutional Biosafety Committee. However, my understanding is that I will be notified straight away, as PI, and that I can then notify you at NIAID.

Apologies for the error!

Cheers,

Peter

Peter Daszak
President

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Monday, June 27, 2016 3:49 PM
To: Peter Daszak
Cc: Greer, Jenny (NIH/NIAID) [E]; Aleksei Chmura
Subject: RE: Grant Number: 5R01AI110964 - 03 PI Name: DASZAK, PETER

Hi Peter,
Just wanted to follow up with you to see if you had a chance to look in to the IBC question I sent

earlier this month. Please let us know.

Thanks,
Erik

Sent with Good (www.good.com)

-----Original Message-----
From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Friday, June 17, 2016 03:38 PM Eastern Standard Time
To: Dr. Peter Daszak
Cc: Greer, Jenny (NIH/NIAID) [E]; Aleksei Chmura
Subject: RE: Grant Number: 5R01AI110964 - 03 PI Name: DASZAK, PETER

Hi Peter,
Thanks very much for providing the additional information. I did have a couple of follow up questions for you. Can you clarify where the work with the chimeric viruses will actually be performed? Your original application described the BSL3 facilities at the Wuhan Institute of Virology, but your response letter indicated that you would notify the UNC IBC if you observed enhanced replication with any of the proposed chimeras. Therefore it's not clear where the studies are being performed. Please also clarify whether EcoHealth Alliance has its own IBC, and how the UNC IBC would be involved in the oversight of this work.

Many thanks,
Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email: (b)(6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.
****

or any other action based on the contents of this material. If you have received this communication in error, please permanently delete this from your system immediately. Thank you.

From: Greer, Jenny (NIH/NIAID) [E]
Sent: Thursday, June 09, 2016 5:56 PM
To: Aleksei Chmura (b)(6)
Cc: Dr. Peter Daszak (b)(6) Stemmy, Erik (NIH/NIAID) [E]
(b)(6)
Subject: RE: Grant Number: 5R01AI110964 - 03 PI Name: DASZAK, PETER

Thank you for your quick response!

Jenny

Jenny Greer
Grants Management Specialist
DHHS/NIH/NIAID/DEA/GMP
5601 Fishers Lane, Room 4E49, MSC 9833
Bethesda, MD 20892-9824
Phone: (b)(6)
Email: (b)(6)

From: Aleksei Chmura (b)(6)
Sent: Thursday, June 09, 2016 5:43 PM
To: Greer, Jenny (NIH/NIAID) [E] (b)(6)
Cc: Dr. Peter Daszak (b)(6) Stemmy, Erik (NIH/NIAID) [E]
(b)(6) Kirker, Mary (NIH/NIAID) [E] (b)(6) Glowinski, Irene
(NIH/NIAID) [E] (b)(6) Ford, Andrew (NIH/NIAID) [E] (b)(6)
Subject: Re: Grant Number: 5R01AI110964 - 03 PI Name: DASZAK, PETER

Dear Jenny,

I concur with the detailed response that Dr. Daszak just sent to you in response to the Gain of Function questions in your email from 28th May. Please let me know anytime, if you require any further information.

Many thanks!

Aleksei Chmura
Authorized Organizational Representative &
Senior Coordinator of Operations

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On Jun 9, 2016, at 17:37, Greer, Jenny (NIH/NIAID) [E] (b)(6) wrote:

Peter,

Thank you for providing this response. We will review it shortly. In the meantime, I look forward to receiving concurrence from your authorized business official.

Thanks again!

Jenny

Jenny Greer
Grants Management Specialist
DHHS/NIH/NIAID/DEA/GMP
5601 Fishers Lane, Room 4E49, MSC 9833
Bethesda, MD 20892-9824
Phone: (b)(6)
Email: (b)(6)

From: Peter Daszak (b)(6)
Sent: Thursday, June 09, 2016 5:23 PM
To: Greer, Jenny (NIH/NIAID) [E] (b)(6) Aleksei Chmura (b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Kirker, Mary (NIH/NIAID) [E] (b)(6) Glowinski, Irene (NIH/NIAID) [E] (b)(6) Ford, Andrew (NIH/NIAID) [E] (b)(6)

Subject: RE: Grant Number: 5R01AI110964 - 03 PI Name: DASZAK, PETER
Importance: High

Dear Jenny and Erik,

Please find our response letter to your email below, attached. I really appreciate you giving us the chance to clarify these details and look forward to your decision on our proposed work. As stated clearly in the letter, we will not (of course) move forward with any of the proposed work in Specific Aim #3 until we hear back from you with directions.

Cheers,

Peter

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President

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From: Greer, Jenny (NIH/NIAID) [E] (b)(6)
Sent: Saturday, May 28, 2016 5:15 PM
To: Aleksei Chmura
Cc: Stemmy, Erik (NIH/NIAID) [E]; Peter Daszak; Kirker, Mary (NIH/NIAID) [E]; Glowinski, Irene (NIH/NIAID) [E]; Ford, Andrew (NIH/NIAID) [E]
Subject: Grant Number: 5R01AI110964 - 03 PI Name: DASZAK, PETER

Dear Mr. Chmura,

Please find attached an important message about this grant. Your immediate response will be much appreciated.

All the best,

Jenny

Jenny Greer
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"Effective October 1, 2014, NIH closeout policy has changed (see NOT-OD-14-084). In order to avoid unilateral closeout, final reports must be submitted in a timely manner. Failure to submit accurate final reports could result in enforcement actions such as revisions to NOA funding levels, or delay in future funding."

EcoHealth Alliance

Dear Drs. Greer and Stemmy,

June 8, 2016

We appreciate your rapid review of our proposed work for year 3 of our R01 (5R01AI110964-03). We have provided the details you requested, below, including alternative strategies if we remove work that could be deemed gain of function. We look forward to your response and will modify our workplan accordingly. In the meantime, please rest assured that none of the proposed work for Specific Aim #3 that you have requested information about will begin.

Determination as to whether the above research does or does not include GoF work subject to the funding pause. Please provide a detailed explanation for this determination, including, but not limited to, descriptions of the MERS and MERS-like chimeric CoVs that you propose to create, and detailed descriptions of the experiments you plan to conduct. Your determination should also include whether each chimeric virus is reasonably anticipated to exhibit enhanced pathogenicity and/or transmissibility in mammals via the respiratory route compared to wild type MERS-CoV.

Firstly, we would like to reiterate that this work is *proposed* for year 3, and none has been conducted to date. Furthermore, we will not proceed with any of this unless we are given the go-ahead by NIAID. The goal of our proposed work to construct MERS and MERS-like chimeric CoVs is to understand the potential origins of MERS-CoV in bats by studying bat MERS-like CoVs in detail. The chimeric viruses will be used to ascertain receptor usage and infectivity of bat MERS-related CoVs *in vitro* and in a mouse model. To achieve this purpose, our aim is to firstly construct a MERS-CoV infectious clone based on the genomic sequence of EMC2012 (GenBank no. NC_019843) and then chimeric CoVs with the replacement of the spike envelope genes from bat derived MERS-like CoVs. We have very recently discovered a small number (9 different strains) of bat MERS-like CoVs in 99 samples from bats in Guangxi, Guangdong, and Szechuan provinces. Phylogenetically, these bat viruses are not very close to MERS-CoV (only 63-66% homology to the S-protein of MERS-CoV).

We aim to test the chimeric viruses for receptor usage of DPP4 (the MERS-CoV receptor) in cells and then in DPP4 transgenic mice, to see if these bat viruses have any capacity to use the same receptor. That said, given the phylogenetic distance from MERS-CoV, we believe it is *highly unlikely* that these bat spike proteins attach to DPP4, and if so, that they would have any pathogenic potential. Finally, should any of these recombinants show evidence of enhanced virus growth >1 log in cells expressing the human, bat, mouse or other DPP4 receptor over wildtype parental backbone MERS-CoV strain or grow more efficiently in human airway epithelial cells, we will immediately: i) stop all experiments with the mutant, ii) inform our NIAID Program Officer and the Wuhan Institute of Virology IBC of these results and iii) participate in decision making trees to decide appropriate paths forward.

In addition, your progress report makes reference to two chimeric bat SARS-like CoVs constructed on a WIV-1 backbone.

NIAID requests additional information on these strains of SARS-like CoVs, including: the dates the strains were created; whether the chimeric viruses exhibit enhanced pathogenicity and/or transmissibility in

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mammals via the respiratory route compared to wild type SARS-CoV; and what research plans you have for these chimeric viruses.

These two chimeric bat-like CoVs were constructed on September 24, 2015. They use the backbone of a group 2b SARS-like bat CoV WIV1 and the spike proteins of two newly discovered bat SL-CoVs (Rs7327 and RsSHC014). The construction of these chimeric viruses aims to understand the receptor usage and infectivity of bat SL-CoVs that may be progenitors of SARS-CoV. We have not yet tested the pathogenicity of these viruses in animals.

We believe that this work would not be considered GoF because the pause specifically targeted experiments that altered the pathogenicity or transmissibility of SARS-CoV, MERS-CoV and any influenza virus. Our molecular clone is WIV1, which is a group 2b SARS-like bat coronavirus that has never been demonstrated to infect humans or cause human disease. It is about 10% different from SARS-CoV. Thus, we feel that introducing other group 2b SARS-like bat coronavirus spike glycoproteins into WIV1 is not subject to the pause. Moreover, we are introducing progressively more distant S glycoproteins into WIV1 (The RBD of Rs7327 differs from WIV1 in several amino acid residues while RsSHC014 is even more distantly related phylogenetically), so it seems progressively less likely that any of these viruses would be more pathogenic or transmissible than the SARS-CoV. This is further supported by the fact that Prof. Ralph Baric's group (Menacherya *et al*., 2015, Nature Medicine, 21 (12):1508-1512; Menacherya *et al*., 2016, PNAS, 113 (11): 3048-3053) took WIV1 spike and inserted it onto a SARS-CoV backbone and showed reduced pathogenicity in mice with human ACE-2 relative to SARS-CoV (mortality rates were much lower, therefore this is *loss-of-function*). This strongly suggests that the chimeric bat spike/bat backbone viruses should not have enhanced pathogenicity in animals.

Finally, as proposed above for the MERS-like viruses, should any of these recombinants show evidence of enhanced virus growth >1 log in cells expressing the human, bat, mouse or civet receptor over wildtype parental backbone SARS-CoV strain or grow more efficiently in human airway epithelial cells, we will immediately: i) stop all experiments with the mutant, ii) inform our NIAID Program Officer and the Wuhan Institute of Virology IBC of these results and iii) participate in decision making trees to decide appropriate paths forward.

If it is determined that the above research DOES include GoF work subject to the funding pause, provide detailed information on what research will remain viable with the removal of the GoF work and appropriate budget adjustments. Options include:

- For the specific aims that propose GoF work, provide a detailed description of changes that can be made to remove the GoF work but maintain the specific aim(s); or
- Remove the specific aims and experiments that are subject to the pause from the Research Plan and request to have the award budget renegotiated.

If these proposed activities within Specific Aim #3 are considered gain of function, we would propose changing them as follows:

1) Instead of the proposed work on MERS-like chimeric CoVs, we would
    a. model the 3-D structure of bat MERS-like CoV spike to assess its potential to bond to DPP4; and
    b. build pseudoviruses with MERS-like CoV spike to conduct experiments for DPP4 binding.

2) Instead of the proposed work on SARS-like chimeric bat CoVs, we would build pseudoviruses with the spike proteins from these viruses and assess receptor binding *in vitro*.

We look forward to your response to our letter and will not conduct any of this proposed work until we hear back from you.

Yours sincerely,

Dr. Peter Daszak

PI
President and Chief Scientist
EcoHealth Alliance

Tel: (b)(6)
e-mail: (b)(6)

From: Chen, Ping (NIH/NIAID) [E]
Sent: Sun, 12 Jun 2016 21:29:16 -0400
To: Peter Daszak
Cc: Aleksei Chmura; Hongying Li; Stemmy, Erik (NIH/NIAID) [E]; Hume Field; Hume Field - EcoHealth Alliance (b)(6) Guangjian Zhu
Subject: RE: Meeting re. coronavirus research in China funded by NIAID

Hi Peter,

Yes. July 7 should be fine. I am flexible through the day. Just let me know when you plan to come.

Since my office is inside US embassy, I will have to request access for all of you. I will need your passport information or Chinese ID in order to make the access request. Just for your information, no any electronics can be brought into embassy including your computer, cell phone, iPad, flash drive, etc.

If you don't want to go through the hassle, we can meet somewhere else.

Ping

Ping Chen, PhD
Director of NIAID Office in China
Office of Global Research, NIAID, NIH
Bethesda Office: (b)(6)
BB: (b)(6)
Beijing Office: (b)(6)
Cell: (b)(6)
U.S. Embassy Beijing
#55 An Jia Lou Road
ChaoYang District, 100600
Beijing, China
(b)(6)

From: Peter Daszak (b)(6)
Sent: Sunday, June 12, 2016 23:49
To: Chen, Ping (NIH/NIAID) [E]
Cc: Aleksei Chmura; Hongying Li; Stemmy, Erik (NIH/NIAID) [E]; Hume Field; Hume Field - EcoHealth Alliance (b)(6) Guangjian Zhu
Subject: RE: Meeting re. coronavirus research in China funded by NIAID

Hi Ping,

I'll be in Beijing on Thursday the 7th July – could we meet sometime that day? I'll bring Aleksei Chmura, Guangjian, and Hume Field – all working on the project. Hume is based in Australia and has been working with us in China for the last 10 years – he was originally part of the WHO SARS investigation team during the outbreak.

It would be great to meet with you eitehr in the morning or afternoon of the 7th to talk about our work under the NIAID project and let you know about some of the other work we're doing in China.

If you can't do the 7th, I can rearrange things and do either the 5th or 6th.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

(b)(6) (direct)
+1.212.380.4465 (fax)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

From: Chen, Ping (NIH/NIAID) [E] (b)(6)
Sent: Friday, June 3, 2016 1:21 AM
To: Peter Daszak
Cc: Aleksei Chmura; Hongying Li; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Meeting re. coronavirus research in China funded by NIAID

Yes, I am around in July. Let me know the date and time when getting closer to July.
Thank you
Ping

Sent from my iPhone

On Jun 3, 2016, at 5:08 AM, Peter Daszak (b)(6) wrote:

Dear Ping,

I am sorry that we were unable to meet in April. I will be back to Beijing early next month for a few days. If you are available, I would be happy to meet with you to tell you more about our successful workshop

last month, our current work in China, including our Chinese scientist partners, and learn more about the IVLP program and ESTH programs in Asia from you.

Please let me know if you are available on either Wednesday the 6th July or Thursday the 7th of July.

Cheers,

Peter

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President

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From: Chen, Ping (NIH/NIAID) [E] (b)(6)
Sent: Monday, April 4, 2016 8:41 PM
To: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura; Hongying Li
Subject: RE: Meeting re. coronavirus research in China funded by NIAID

Hi Peter and Erik,

Thank you for reaching out to me and for the invitation. It sounds like a very interesting workshop. I know Dr. Zhengli Shi. Last year I recommended her as the only member from China to join IVLP (the International Visitor Leadership Program, a program run by the Department of States) to visit US for GHSA. She was accepted, but could not go because of schedule conflict. I am glad to learn that you work with her. She is great.

Unfortunately I won't be in Beijing during your visit later in April. I am leaving for US this Sunday and will stay to complete my obligated home leave for 5 weeks. So I will miss you. But I would want to learn about your program. Recently I went to a training course organized by DoS on environment, science and technology, health. GHSA, One Health, emerging IDs, and AMR were the topics for health. I learned that DoS has small grants to support ESTH programs in Asia. Typical NIH basic research projects do not quite fit DoS emphasis in health but what you are doing, seems to me, does fit. In addition, the IVLP program is an opportunity to send mid-career Chinese scientists to visit US on special topics. The program runs annually. Each year there will be a list of topics, which can be country specific or regional. DoS will

support the travel and expenses in US. I would like to get to know some of your Chinese partners. I can recommend people when there is a match in topics.

Erik, I will be in and out Fishers Lane from 4/11-4/15. Hope to see you then.

Peter, not sure if you have time to meet during my stay in Maryland. I realize you are in NYC. I will be in Bethesda for the above mentioned time and move to Baltimore for my home leave. I am flexible.

Again I am sorry to miss you in Beijing. Hope you have a good visit in Beijing.

Ping

Ping Chen, PhD
Director of NIAID Office in China
Office of Global Research, NIAID, NIH
Bethesda Office: (b)(6)
BB: (b)(6)
Beijing Office: (b)(6)
Cell: (b)(6)
U.S. Embassy Beijing
#55 An Jia Lou Road
ChaoYang District, 100600
Beijing, China
(b)(6)

From: Peter Daszak (b)(6)
Sent: Monday, April 04, 2016 23:22
To: Stemmy, Erik (NIH/NIAID) [E]; Chen, Ping (NIH/NIAID) [E]
Cc: Aleksei Chmura; Hongying Li
Subject: Meeting re. coronavirus research in China funded by NIAID

Dear Dr. Chen,

I'm following up on the email from Erik Stemmy a few months ago (below). As Erik mentioned, we have been collaborating with local partners in China since 2004 on SARS CoV virus (and other new viruses) that could cause emerging infectious diseases, in collaboration with Dr. Zhengli Shi at the Wuhan Institute of Virology and others.

I will be in Beijing during April 19-21 to host a workshop on wildlife and public health with the Forestry Administration and China CDC/CAS, so I would love to visit you sometime during these days, if possible, to talk to you more about our work in China. Are you available on either 4/20 Wednesday or 4/21 Thursday?

As well as this, I've attached an invitation to the Wildlife and Public Health Workshop on April 19, please feel free to register if you are able to join in us for the discussions. We will be talking about our work funded by NIAID, and it might be interesting for you or some of your staff to attend.

Thank you very much, and I hope we're able to meet this month. If not, I will be back in Beijing and June, which would give a longer lead in to arrange a meeting.

Cheers,

Peter

Peter Daszak
President

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, May 26, 2015 8:37 AM
To: Chen, Ping (NIH/NIAID) [E]
Cc: Peter Daszak
Subject: CoV Research in China

Hi Ping,
Hope things are going well in Beijing! One of the investigators in my coronavirus portfolio, Peter Daszak from EcoHealth Alliance (copied here), asked me to put him in touch with you. In one of his projects Peter is looking at the emergence of CoVs from bats, and he collaborates with several sites in China on the project so we thought it would be good idea for him to have your contact info.

Peter, as I mentioned when we spoke Ping is based out of the US Embassy in Beijing and helps facilitate NIAID research and collaborations in China and the vicinity. I'd encourage you to reach out and tell her a bit about some of your other projects, particularly if you'll be visiting China or Beijing any time.

Best,
Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Hongying Li
Sent: Fri, 3 Jun 2016 09:10:15 -0400
To: Chen, Ping (NIH/NIAID) [E]
Cc: Peter Daszak; Aleksei Chmura; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Meeting re. coronavirus research in China funded by NIAID

Dear Dr. Chen,

Thank you very much for your quick reply and availability to meet, I will confirm the date and time with you in the last week of June. Thank you!

Best,
Hongying
On Jun 3, 2016, at 1:21 AM, Chen, Ping (NIH/NIAID) [E] (b)(6) wrote:

Yes, I am around in July. Let me know the date and time when getting closer to July.
Thank you
Ping

Sent from my iPhone

On Jun 3, 2016, at 5:08 AM, Peter Daszak (b)(6) wrote:

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Please let me know if you are available on either Wednesday the 6th July or Thursday the 7th of July.

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Peter

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To: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura; Hongying Li
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Beijing, China
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Peter

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Cc: Peter Daszak
Subject: CoV Research in China

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Peter, as I mentioned when we spoke Ping is based out of the US Embassy in Beijing and helps facilitate NIAID research and collaborations in China and the vicinity. I'd encourage you to reach out and tell her a bit about some of your other projects, particularly if you'll be visiting China or Beijing any time.

Best,
Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

Hongying Li, MPH 李泓萤
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor

New York, NY 10001

(b)(6) (U.S. mobile)
(b)(6) (China mobile)
(b)(6) (Skype)
(b)(6) (WeChat)

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

From: Aleksei Chmura
Sent: Tue, 31 May 2016 10:36:31 -0400
To: Greer, Jenny (NIH/NIAID) [E]
Cc: Stemmy, Erik (NIH/NIAID) [E]; Dr. Peter Daszak; Kirker, Mary (NIH/NIAID) [E]; Glowinski, Irene (NIH/NIAID) [E]; Ford, Andrew (NIH/NIAID) [E]
Subject: Re: Grant Number: 5R01AI110964 - 03 PI Name: DASZAK, PETER

Dear Jenny,

Thank you for the notice. Dr. Daszak (PI) or I will respond to your letter as rapidly as possible.

Sincerely,

Aleksei Chmura
Senior Coordinator of Operations

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
(mobile)
(b)(6) (Skype)

www.ecohealthalliance.org

Visit our blog: www.ecohealthalliance.org/blog

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On May 28, 2016, at 17:14, Greer, Jenny (NIH/NIAID) [E] (b)(6) wrote:

Dear Mr. Chmura,

Please find attached an important message about this grant. Your immediate response will be much appreciated.

All the best,

Jenny

Jenny Greer
Grants Management Specialist
DHHS/NIH/NIAID/DEA/GMP
5601 Fishers Lane, Room 4E49, MSC 9833
Bethesda, MD 20892-9824
Phone (b)(6)
Email:

"Effective October 1, 2014, NIH closeout policy has changed (see NOT-OD-14-084). In order to avoid unilateral closeout, final reports must be submitted in a timely manner. Failure to submit accurate final reports could result in enforcement actions such as revisions to NOA funding levels, or delay in future funding."

<110964 Daszak GoF Letter 5-28-2016-signed.pdf>

From: Aleksei Chmura
Sent: Tue, 10 May 2016 08:33:56 +0800
To: Normil, Carine (NIH/NIAID) [C]
Cc: Dr. Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]; Pone, Laura (NIH/NIAID) [E]
Subject: Re: Grant Number: 5R01AI110964 - 03 PI Name: DASZAK, PETER
Importance: High

Dear Carine,

Apologies for our delayed Year 2 Report. We had notified Laura Pone previously and will definitely submit our Report on the 12th of May.

If you have any questions or require additional details, please call (b)(6) or email me anytime.

Many thanks most,

Sincerely,

Aleksei Chmura
Senior Coordinator of Operations

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
(mobile)
(b)(6) (Skype)

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Visit our blog: www.ecohealthalliance.org/blog

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On May 10, 2016, at 05:26, Normil, Carine (NIH/NIAID) [C] (b)(6) wrote:

Dear Dr. Daszak,
This is the second communication from NIAID requesting that you file the progress report for the above-referenced grant that was due no later than April 15, 2016. Please submit the delinquent report by May 12, 2016.
If you experience any difficulties meeting the submission deadline, please contact me immediately. Otherwise, please be advised that continued late submission of your non-competing grant progress report and any subsequently requested documentation will result in a reduction of time and/or funds for this grant.
Thank you,
Carine

Carine Normil

Grants Management Specialist (Contractor)

Grants Management Program, DEA, NIAID, NIH, HHS
5601 fishers Lane, Rm 4G46, Bethesda , Maryland 20892

Phone: (b)(6)

Fax: (301)-493-0597
Email: (b)(6)

<image001.jpg>

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Wed, 27 Jan 2016 18:16:56 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Bcc: (b)(6) (b)(6) Denison, Mark (NIH); (b)(6) (b)(6) Wayne Marasco; Dale Barnard; 张林琦; (b)(6) Morrey, John
Subject: MERS-CoV Animal Model Workshop at NIAID

Dear Colleagues,
NIAID is organizing a MERS-CoV animal model workshop, which will be held in Bethesda, Maryland on February 29th and March 1st, 2016. The goals of the workshop will be to evaluate the current status of model development, and to begin to define a path forward to advance MERS-CoV medical countermeasures to clinical trials. If you are interested in attending I encourage you to register via the website linked below, which also contains a brief draft agenda.

Please feel free to forward this message to others who may be interested.

Best Regards,
Erik

MERS-CoV Model Workshop: https://respond.niaid.nih.gov/conferences/MERS-CoV_Workshop2016/Pages/default.aspx

Erik J. Stemmy, Ph.D.
Program Officer for Human Coronaviruses
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone (b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Munster, Vincent (NIH/NIAID) [E]
Sent: Tue, 15 Dec 2015 10:38:29 -0500
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Hensley, Lisa (NIH/NIAID) [E]; Erlandson, Karl (OS/ASPR); Subbarao, Kanta (NIH/NIAID) [E]; Baric, Ralph; Dreier, Thomas (OS/ASPR/BARDA); Spiro, David (NIH/NIAID) [E]
Subject: Re: MERS Animal Model SAG

Sounds good to me,

Greetings from Congo

Sent from my iPhone

On Dec 14, 2015, at 20:46, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Hi Everyone,
David and I have been considering the speakers list with the funds we have to work with for the workshop. Unfortunately due to the cost it appears we will be limited to supporting three international speakers. Based on our earlier discussions with the group we feel it is important to invite someone from both the Kingdom of Saudi Arabia and South Korea for their perspectives and potential case descriptions. Our last version of the agenda included several other foreign speakers on topics of epidemiology, lessons learned from SARS, as well as NHP and mice model work from China.

In looking at the agenda David and I propose the final international speak we support cover the lessons learned from SARS model and MCM development, and we were thinking of inviting Luis Enjuanes for that role. We will attempt to have the other topics covered by others already attending the workshop.

We'd appreciate it if you could let us know if you agree with this plan. If not, please let us know who you think would be a better choice for the final speaker slot we can support.

Many thanks!
Erik

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Monday, November 9, 2015 10:33 AM
To: Hensley, Lisa (NIH/NIAID) [E] (b)(6) Erlandson, Karl (OS/ASPR) (b)(6) Munster, Vincent (NIH/NIAID) [E] (b)(6) Subbarao, Kanta (NIH/NIAID) [E] (b)(6) Baric, Ralph (b)(6) Dreier, Thomas (OS/ASPR/BARDA) (b)(6) Spiro, David (NIH/NIAID) [E] (b)(6)
Subject: RE: MERS Animal Model SAG

Hi Everyone,
Thank you again for your time last week. I have attached an updated version of the agenda that I think incorporates the discussion and speaker suggestions. I would appreciate it if you could please review

and send me any updates by Monday Nov 16th. In particular there are a few highlighted areas that we still need to identify a potential speaker.

Please let me know if you have any questions.
Erik

From: Hensley, Lisa (NIH/NIAID) [E]
Sent: Monday, November 2, 2015 9:30 AM
To: Erlandson, Karl (OS/ASPR) (b)(6) Munster, Vincent (NIH/NIAID) [E] (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) Subbarao, Kanta (NIH/NIAID) [E] (b)(6) Baric, Ralph (b)(6) Dreier, Thomas (OS/ASPR/BARDA) (b)(6) Spiro, David (NIH/NIAID) [E] (b)(6)
Cc: (b)(6)
Subject: Re: MERS Animal Model SAG

Waiting for the leader to admit
Lisa Hensley

Sent from b berry

From: Erlandson, Karl (OS/ASPR)
Sent: Monday, November 02, 2015 09:21 AM
To: Munster, Vincent (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Subbarao, Kanta (NIH/NIAID) [E]; Baric, Ralph; Munster, Vincent (NIH/NIAID) [E]; Dreier, Thomas (OS/ASPR/BARDA); Hensley, Lisa (NIH/NIAID) [E]; Spiro, David (NIH/NIAID) [E]
Cc: 'Baric, Toni C' (b)(6)
Subject: RE: MERS Animal Model SAG

(b)(6) (NIAID)
Code: (b)(6)

From: Munster, Vincent (NIH/NIAID) [E] (b)(6)
Sent: Monday, November 02, 2015 9:11 AM
To: Stemmy, Erik (NIH/NIAID) [E]; Erlandson, Karl (OS/ASPR); Subbarao, Kanta (NIH/NIAID) [E]; Baric, Ralph; Munster, Vincent (NIH/NIAID) [E]; Dreier, Thomas (OS/ASPR/BARDA); Hensley, Lisa (NIH/NIAID) [E]; Spiro, David (NIH/NIAID) [E]
Cc: 'Baric, Toni C'
Subject: Re: MERS Animal Model SAG

Trying to get onto the call?

Any ideas?

From: "Stemmy, Erik (NIH/NIAID) [E]" (b)(6)
Date: Wednesday, October 28, 2015 at 12:01 PM
To: "Erlandson, Karl (OS/ASPR)" (b)(6) "Subbarao, Kanta (NIH/NIAID) [E]" (b)(6) "Baric, Ralph" (b)(6) "Munster, Vincent (NIH/NIAID)

[E]" (b)(6) "Dreier, Thomas (OS/ASPR/BARDA)" (b)(6)
"Hensley, Lisa (NIH/NIAID) [E]" (b)(6) David Spiro (b)(6)
Cc: "'Baric, Toni C'" (b)(6)
Subject: RE: MERS Animal Model SAG

Hi Everyone,
I've chosen some times over the next week for our next call. Please see the Doodle poll link below.

Thanks!
Erik

http://doodle.com/poll/8ubsbcih7kdpp2z5

From: Erlandson, Karl (OS/ASPR)
Sent: Tuesday, October 27, 2015 11:18 AM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Subbarao, Kanta (NIH/NIAID) [E]
(b)(6) Baric, Ralph (b)(6) Munster, Vincent (NIH/NIAID) [E]
(b)(6) Dreier, Thomas (OS/ASPR/BARDA) (b)(6) Hensley,
Lisa (NIH/NIAID) [E] (b)(6) Spiro, David (NIH/NIAID) [E] (b)(6)
Cc: 'Baric, Toni C' (b)(6)
Subject: RE: MERS Animal Model SAG

Hi Erik,

I also think it would be good to talk this over. I've made a few comments that could be used in the discussion.

Karl

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Friday, October 23, 2015 8:15 AM
To: Subbarao, Kanta (NIH/NIAID) [E]; Baric, Ralph; Munster, Vincent (NIH/NIAID) [E]; Dreier, Thomas (OS/ASPR/BARDA); Erlandson, Karl (OS/ASPR); Hensley, Lisa (NIH/NIAID) [E]; Spiro, David (NIH/NIAID) [E]
Cc: 'Baric, Toni C'
Subject: RE: MERS Animal Model SAG

Hi Everyone,
I haven't received any comments back on the updated agenda. If it is easier for the group I can have a shot at suggesting organizers for the sessions and we can discuss the agenda by phone. I would appreciate it if you could either send me your feedback, or let me know if scheduling a phone call would be easier, by Tuesday 10/27.

Thanks!
Erik

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Tuesday, October 13, 2015 8:58 AM
To: Subbarao, Kanta (NIH/NIAID) [E] (b)(6) Baric, Ralph (b)(6) Munster, Vincent (NIH/NIAID) [E] (b)(6) Dreier, Thomas (OS/ASPR/BARDA) (b)(6) Erlandson, Karl (OS/ASPR) (b)(6) Hensley, Lisa (NIH/NIAID) [E] (b)(6) Spiro, David (NIH/NIAID) [E] (b)(6)
Cc: 'Baric, Toni C' (b)(6)
Subject: RE: MERS Animal Model SAG

Hi Everyone,
Just a friendly reminder soliciting your feedback on the updated agenda draft attached again here. Also, I have looked into availability of the large conference room in our Fishers Lane building and come up with some potentials dates (listed below.) Could you please let me know if there are any that should be off the table due to conflicts, any that might be good to use to piggy back on other meetings, or any other preferences you have? I would ideally like to reserve the room in the next week.

Thanks!
Erik

Current Room Availability:
January: 18-19, 20-21
February: 10-11, 15-18, 22-23, 22-25, 29-March 1
March: 9-10, 28-31

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Friday, October 02, 2015 12:55 PM
To: Subbarao, Kanta (NIH/NIAID) [E] (b)(6) Baric, Ralph (b)(6) Munster, Vincent (NIH/NIAID) [E] (b)(6) Dreier, Thomas (OS/ASPR/BARDA) (b)(6) Erlandson, Karl (OS/ASPR) (b)(6) Hensley, Lisa (NIH/NIAID) [E] (b)(6) Spiro, David (NIH/NIAID) [E] (b)(6)
Cc: 'Baric, Toni C' (b)(6)
Subject: MERS Animal Model SAG

Hi Everyone,

Thank you for your insightful discussion during our call on the 21st. David and I have incorporated your comments in the attached document. In particular we have expanded the agenda to a rough outline of a two day workshop, and would appreciate any feedback you have on the proposed session organization and topics. One other thing we'd like to ask is for volunteers to choose a session to chair. We anticipate the session chairs will take the lead in setting the format for the session, suggesting speakers, and leading the session during the workshop.

If possible we'd like to ask for your feedback on this draft agenda and deliverables on or before Oct 14th. Please let me know If you have any questions.

Many thanks!

Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

****

From: Aleksei Chmura
Sent: Mon, 8 Jun 2015 20:33:46 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Grant Number: 5R01AI110964 - 02 PI Name: DASZAK, PETER

Dear Laura,

Apologies for our delay in response. Our PIs were out-of-office last week and I wanted to confirm the details in the response. Here, below, are answers to the questions.

The PI mentioned recruiting participants at bat caves, wet markets etc., would the PI please discuss the method of recruitment on how participants would be approached to be involved in the study?

Our study will include adults living or working in the study sites selected as priority surveillance sites with high risk for viral spillover, evolution, amplification, and spread (i.e., 'hotspots'). Study sites are prioritized by identifying areas considered high-risk for contact with wildlife known to be associated with zoonotic viral diversity and with ecological and epidemiological conditions associated with disease emergence. Locations of the one-on-one interviews and focus groups will be in targeted 'hotspot' areas and determined ahead of time based upon our previous research and substantiated by observational research conducted by our research team. Sites will be selected to ensure inclusion of individuals that have contact with live, wild, and farmed animals through either direct contact (raising, hunting, selling, trading, or purchasing) or indirect contact (animals living in or entering dwellings, buildings, caves, or gardens/crops).

In order to participate in one-on-one interviews for the qualitative study, an individual must have direct or indirect contact with live animals, which includes raising, hunting, selling, trading, and/or purchasing live animals. Indirect contact includes living in or entering dwellings, buildings or gardens/crops e.g., bat roosts along roofs, rats or other animals invading stored food or crops. Our research team will use existing, local contacts for introductions to qualifying individuals who may be eligible and interested in participating. Efforts will be made to include a large variety of people with exposure to wildlife especially and initially targeting people who have more power or influence (e.g. farm owners, market leaders, restaurant owners, work-group leaders) as well as those with less (e.g. market vendors and cleaners, rat catchers, individual shoppers).

Our team will recruit adults living at the site or working or visiting the site by asking individuals if they would like to participate. Our study is completely voluntary. Our team will be thoroughly trained on communicating the research objectives and will be able to address any questions that potential subjects may have. As part of the informed consent process, both written and oral descriptions of the study will be provided in Chinese and via an interpreter if participants are not fluent in Mandarin and speak a local dialect. Contact details of our trained field-team coordinator will be provided to all subjects. All personnel on our research team will be available on site to answer questions from the study subjects.

In our previous set of questions we asked about maintaining the privacy of subjects, specifically we would like the PI to discuss how will he ensure and maintain privacy of participants during the one-on-one interviews, e.g., what is the location of the interviews?

One-on-one interviews for our qualitative survey locations will be identified prior to the interviews and will be performed in quiet and private areas where there are no other individuals present within a 10-foot distance. Specific sites for interviews will depend on the type of targeted "hotspot" area and may be in farming or rural areas, inside wildlife restaurants, behind animal storage sheds, in private rooms of dwellings, or in offices of business owners, hotel meeting rooms, etc. If necessary, a barrier will be

created so that no other individuals may view the participant while interviews are conducted in order to maintain confidentiality. Research procedures will not include accessing personal health information.

To ensure compliance with informed consent procedures, all potential one-on-one interviewees will be given a consent form prior to being asked to participate. The participant will review the consent form with our research staff and will be given time to ask questions. When reviewing the consent form with participants, our research staff will explain details of the study including why each participant was selected, potential risks to participation, how participation is beneficial, that participation is completely voluntary, and that s/he may withdraw participation at any time. It will be explained that the researchers will not share responses. A small token or gift equivalent to no more than $10 USD will be provided to each participant upon completion of the one-on-one interview.

Measures will be taken to assure the respect, dignity, and freedom of each participant. Each participant's identity will remain anonymous. All responses recorded from participants (of either one-on-one or focus group interviews) will not have names or any identifying details included with recoded responses. Results will be transcribed and/or translated into English and reports will be in aggregate form only. No individual names will ever be reported or published. For the purposes of achieving the aims of our study, data derived from interviews will be analyzed in aggregate by region within a province, without revealing any names of individuals and names/locations of specific markets. This will serve to minimize the legal and economic risks to specific markets or vendors that may provide information about potentially unlawful actions. Dr. Daszak the PI has entered into a confidentiality agreement with NIH to further protect study subjects from the release of any personally identifying information.

There is mentioned of focus group interviews, would the PI please explain which groups of participants are included and the location of the focus groups?

Focus group interview locations for the qualitative study will be identified prior to the focus group sessions and will be performed in quiet and private areas where there are no other individuals present. Specific sites for interviews depend on the type of targeted "hotspot" area and may be in farming or rural areas, inside wildlife restaurants, in private rooms of dwellings, or in offices of business owners, hotel meeting rooms, etc. If necessary, a barrier will be created so that no individuals other than those in the focus group may view or otherwise interfere with the focus group in order to maintain confidentiality. Research procedures in the current study will not include accessing personal health information.

To ensure compliance with informed consent procedures, all potential focus group participants will be given a consent form prior to being asked to participate. The participant will review the consent form with our research staff and will be given time to ask questions. When reviewing the consent form with participants, our research staff will explain details of the study including why each participant was selected, potential risks to participation, how participation is beneficial, that participation is completely voluntary, and that s/he may withdraw participation at any time. It will be explained that the researchers will not share responses. A small token or gift equivalent to no more than $10 USD will be provided to each participant upon completion of the focus group interview.

For both focus group and one-on-one participants, our research team will use existing, local contacts for introductions to individuals who are eligible and interested in being interviewed. Efforts will be made to include a large variety of people with exposure to wildlife especially targeting people who have more power or influence (e.g. farm owners, market leaders) as well as those with less (e.g. market cleaners, rat catchers, individual vendors or shoppers).

Our team will recruit adults living at the site or working or visiting the site by asking individuals if they would like to participate. The study is completely voluntary. Our team will be thoroughly trained on communicating the research objectives and will be able to address any questions that potential subjects may have. As part of the informed consent process, both written and oral descriptions of the study will be provided in Chinese and via an interpreter if a local dialect is required. Contact details of our trained field-team coordinator will be provided to all subjects and all personnel on our research team will be available on site to answer questions from the study subjects.

Lastly, in addition to following up with participants who test positive for coronavirus in 6 months, what is the PI's plan for linking positive participants to treatment?

The test we will use is not a diagnostic test for SARS-like Coronaviruses. We will be identifying SARS-like Coronavirus from genetic fragments using consensus PCR. We will also be conducting serological assays, which represent exposure, but not active virus. There is no treatment for Coronavirus unless there is acute illness in which case treatment would be supportive care. If a participant tests antibody positive for SARS-like Coronaviruses, this would be a measure of past exposure and no treatment would be necessary. If SARS-like Coronavirus RNA is found, we will inform the participant that SARS-like Coronavirus was identified and that she or he should seek medical attention if respiratory symptoms occur and inform doctor of possible SARS-like Coronavirus infection.

******

Please note that our study has an initial qualitative component with the one-on-one interviews and focus groups as detailed above and a separate survey component with questionnaires and biological specimen collection. No clinical specimens will be collected in the initial qualitative component. For clarity, here are details on participant and site selection for our survey component:

A site-specific approach to 'hotspot' identification has been widely used in infectious disease research. Specific well-defined sites are referred to as 'clusters.' Cluster sampling is a standardized sampling methodology that is used when it is either impossible or impractical to compile an exhaustive list of the elements that make up the target population. Usually, as is the case in our study, the population elements are already grouped into subpopulations, e.g., wildlife market vendors, hunters, people who live in caves that have bats. To conduct a cluster sample, clusters (i.e., hotspot settings) are identified and selected for inclusion. If the cluster is small enough, the entire cluster of respondents may be approached to be included in the final sample. This is considered to be a one-stage cluster sample. However, if the cluster is large, then a two-stage cluster sample must be obtained; that is, only a subset of respondents from the cluster will be included in the final sample.

In order to obtain a subset of respondents from large clusters, systematic random sampling will be used. The procedure involved in systematic random sampling is easy, can be done manually and is a commonly used method in two-stage cluster sampling. A random starting point is selected to begin the study. From that point the study staff will move X units (e.g., market stalls, dwellings, houses near a cave) and select that unit for study participation. For example, in a large wildlife market, the first vendor would be selected for study participation. Upon completion of the study requirements, study staff would move 3 stalls down and select a stall on the right for study participation. Upon completion the staff would move another 3 stalls down and select a stall on the right for participation and so on. Only one person per unit (e.g., household, market stall) will be interviewed.

In order to improve recruitment within target communities, introductory visits will be made to each of the selected study site. These visits will be advertised through word of mouth or letter to town leaders depending on the size of the community/site. The letter will inform the community that a research team will be coming on a particular day(s) to discuss health related to animal contact. The letter would not be for advertising recruitment purposes. It would only be used to inform the community of the research visit(s).

During these visits, discussions and meetings will be held to educate, sensitize, and inform people about infections animals may carry, which may then be transferred to humans and cause disease and potential pathways for disease spread/emergence. When appropriate and following approval from local representatives, the research team will post flyers to inform the community of when the team will be coming back to speak to them about enrollment. This "town hall" meeting is completely voluntary, and those interested would likely attend. Although local representatives may be present to introduce the study team members, he/she will not be involved in the recruitment of the participants for the study. Once initial group meetings have been completed, and the type of research to be performed introduced, individual sessions with trained counselors, nurses, and phlebotomists (as appropriate) will be set up for interested persons. Every effort will be made to minimize any form of coercion in this protocol. Local representatives will not

play a role in the recruitment of participants. During the consent process, local representatives will not be present when the consent is discussed with the participant. If research visits or enrollment will be held at a workplace, subjects shall be clearly informed during the recruitment process that their participation in the study will not impact their employment. Translators will be provided, if participants are not fluent in Mandarin.

To ensure compliance with informed consent procedures, all potential participants will be given a consent form prior to being asked to participate. The participant will review the consent form with our research staff and will be given time to ask questions. When reviewing the consent form with participants, our research staff will explain details of the study including why each participant was selected, potential risks to participation, how participation is beneficial, that participation is completely voluntary, and that s/he may withdraw participation at any time. It will be explained that the researchers will not share responses. A small token or gift equivalent to no more than $10 USD will be provided to each participant following his/her time spent in the study.

Measures will be taken to assure the respect, dignity, and freedom of each participant. Each participant's identity will remain anonymous. Each participant will be assigned a coded identification number that will link his or her responses to their clinical specimens, but any identifying information will be kept separate from these data and held in a secure, locked cabinet by the local investigator on-site. Researchers and investigators handling the data will not have access to participant names. The participants' identifiable data and contact information will be kept until the end of the study and then destroyed. Results will be translated into English and reports will be in aggregate form only; no individual names will ever be reported or published. For the purposes of achieving the aims of our study, data derived from questionnaires will be analyzed in aggregate by region within a province, without revealing the names of individuals and names/locations of specific markets. This will serve to minimize the legal and economic risks to specific markets or vendors that may provide information about potentially unlawful actions. Dr. Daszak the PI has entered into a confidentiality agreement with NIH to further protect study subjects from the release of any personally identifying information.

Please let me know, if you have further questions.

Many thanks most,

Sincerely,

Aleksei Chmura
Senior Coordinator of Operations

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
(mobile)
(b)(6) (Skype)

www.ecohealthalliance.org

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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

On Jun 1, 2015, at 08:38, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,

Thank you for providing additional information. However, there are still some concerns related to the protections of human subjects proposed in this project that we ask that the PI address. Would the PI please address the following by **close of business Tuesday, June 2nd**:

• The PI mentioned recruiting participants at bat caves, wet markets etc., would the PI please discuss the method of recruitment on how participants would be approached to be involved in the study?
• In our previous set of questions we asked about maintaining the privacy of subjects, specifically we would like the PI to discuss how will he ensure and maintain privacy of participants during the one-on-one interviews, e.g., what is the location of the interviews?
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• Lastly, in addition to following up with participants who test positive for coronavirus in 6 months, what is the PI's plan for linking positive participants to treatment?

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
5601 Fishers Lane, Room 4E29, MSC 9824
Bethesda, MD 20892-9824
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)
please consider the environment before printing this e-mail.

From: Aleksei Chmura
Sent: Wed, 3 Jun 2015 20:46:02 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Grant Number: 5R01AI110964 - 02 PI Name: DASZAK, PETER

Dear Laura,

Apologies for just responding. I was out of the office on Monday and Tuesday and just saw this email. We will send a response as soon as possible by tomorrow morning.

Many thanks!

Aleksei Chmura
Senior Coordinator of Operations

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New York, NY 10001

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Laura Pone
Grants Management Specialist

DHHS/NIH/NIAID/GMP
5601 Fishers Lane, Room 4E29, MSC 9824
Bethesda, MD 20892-9824
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

From: Chen, Ping (NIH/NIAID) [E]
Sent: Wed, 27 May 2015 05:12:08 -0400
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Daszak, Peter
Subject: RE: CoV Research in China

Thanks Erik.

I am doing just fine. Dennis Dixon and Lanling Zou were visiting China last week. We traveled to Shanghai, Hangzhou, Suzhou to visit hospitals and companies to get a sence of AMR situationas in China.

Dr. Daszak, nice meeting you online. I am based in Beijing, China. Please feel free to contact me for anything I can help you with in China.

Erik, I had planned to visit Dr. Zhengli Shi at the Wuhan Institute of Virology early May while I would visit the new BSL4 lab at WIV. But the institute canceled the visit to BSL4. I plan to go to Wuhan sometime in the summer to visit several NIAID PIs there. Just to give you a head up.

Take care,

Ping

Ping Chen, PhD
Director of NIAID Office in China
Office of Global Research, NIAID, NIH
Bethesda Office: (b)(6)
BB: (b)(6)
Beijing Office: (b)(6)
Cell: (b)(6)
U.S. Embassy Beijing
#55 An Jia Lou Road
ChaoYang District, 100600
Beijing, China
(b)(6)

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Tuesday, May 26, 2015 20:37
To: Chen, Ping (NIH/NIAID) [E]
Cc: Daszak, Peter
Subject: CoV Research in China

Hi Ping,
Hope things are going well in Beijing! One of the investigators in my coronavirus portfolio, Peter Daszak from EcoHealth Alliance (copied here), asked me to put him in touch with you. In one of his projects Peter is looking at the emergence of CoVs from bats, and he collaborates with several sites in China on the project so we thought it would be good idea for him to have your contact info.

Peter, as I mentioned when we spoke Ping is based out of the US Embassy in Beijing and helps facilitate NIAID research and collaborations in China and the vicinity. I'd encourage you to reach out and tell her a bit about some of your other projects, particularly if you'll be visiting China or Beijing any time.

Best,
Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Aleksei Chmura
Sent: Tue, 26 May 2015 17:46:28 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Stemmy, Erik (NIH/NIAID) [E]; Peter Daszak
Subject: Fwd: (HD-43100) Unable to delete an My NCBI entry
Attachments: bib.pdf, ATT00001.htm

Dear Laura,

We have heard back from NCBI - see email response, below.

Please contact the My NCBI Helpdesk (PublicAccess@nih.gov) to confirm removal of the paper in review: Brierley, L., Vonhof, M., Jones, K. E., Olival, K. J. & Daszak, P. Quantifying global drivers of zoonotic bat viruses: a process-based perspective. The American Naturalist, In Review).

We sent the revised NCBI Award Compliance Report previously on 5th May; it is attached here for reference. Let me know, if you require any further details.

Many thanks!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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From: tkt-helpdesk@ncbi.nlm.nih.gov <tkt-helpdesk@ncbi.nlm.nih.gov>
Sent: Wednesday, May 13, 2015 11:04 AM
To: Peter Daszak
Subject: (HD-43100) Unable to delete an My NCBI entry

Dear Colleague,

The silver lock on the grant association is preventing you from removing the grant from the citation, or the citation from your Bibliography. You will need to have the lock removed before you can do either of those things.

The silver padlock indicates that the paper has been reported to NIH on a progress report. To remove the lock, you have to effectively revise the progress report that listed the paper. Please contact your NIH program officer to let them know you wish to revise the report and have the paper removed. They can explain what documentation they will need from your institution to make the revision. Your program officer can then contact our help desk (PublicAccess@nih.gov) to confirm that NIH can remove the papers.

Please let me know if you have any questions.

Thank you!
David Brodsky

Summary: Unable to delete an My NCBI entry

Description:
Dear Help Desk, I uploaded the following reference to My NCBI (Brierley L, Vonhof M, Jones KE, Olival KJ, Daszak P. Quantifying global drivers of zoonotic bat viruses: a process-based perspective. The American naturalist. Forthcoming;), but it is currently IN REVIEW and not yet accepted for publication. This was an error on my part and I would like to delete this entry. It is associated with an award (R01 AI110964), but I am unable to disassociate this via the "add or delete award" link. Also, when I select the journal article and click on the "delete" button under Display Settings, I receive a notice that the action cannot be undone and when I click "delete" the item remains in My Bibliography. I appreciate your help with this. Sincerely, - Peter Daszak
(b)(6)

Please do not change the subject line when replying to this message.

Regards,
NCBI Help Desk

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
PMC Journal In Process	Young CC, Olival KJ. Optimizing Viral Discovery in Bats. PloS one. Forthcoming;
Not applicable	Olival KJ, Weekley CC, Daszak P. Bats and Viruses. Wang L, editor. New York: John Wiley & Sons, Inc.; 2015. What we know and need to know

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Tue, 26 May 2015 12:16:56 +0000
To: 'Peter Daszak'
Subject: RE: ATS

Hi Peter,
Just wanted to thank you again for taking the time to come out to Denver for the ATS session. I thought it all turned out pretty well in the end! You asked me to send you the website for NIAID's advisory council clearance. Link is pasted below, along with the link to subscribe to the NIAID listserv. NIAID sends notices to the listserv when concepts are cleared, new funding opportunities, along with other news and policy changes.

You also asked me to connect you to our NIAID representative in China, Ping Chen. I'll email her shortly copying you as a way of introduction. Hopefully she'll be able to assist with some of your work in China.

Thanks again for coming to Denver. It was great to finally meet you in person!

Best,
Erik

NIAID council clearance:
http://www.niaid.nih.gov/researchfunding/council/concepts/pages/default.aspx
NIAID listserv: http://www.niaid.nih.gov/researchfunding/newsletter/pages/subscribe.aspx

---

From: Peter Daszak (b)(6)
Sent: Tuesday, May 19, 2015 3:58 PM
To: Stemmy, Erik (NIH/NIAID) [E]; 'Matthew Frieman'
Cc: Alison Andre
Subject: RE: ATS
Importance: High

Great – will meet you around 10am

I've attached a short bio here.

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance
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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, May 19, 2015 3:34 PM
To: Peter Daszak; 'Matthew Frieman'
Subject: RE: ATS

One other thing I forgot to ask is if you both have short bios I can use when introducing you tomorrow. If not I can also get a couple of bullets when we meet tomorrow. Let me know.

Thanks!
Erik

Sent with Good (www.good.com)

-----Original Message-----
From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Tuesday, May 19, 2015 12:03 PM Eastern Standard Time
To: 'Peter Daszak'; 'Matthew Frieman'
Subject: ATS

Hi Peter and Matt,
Since you're both arriving later this evening, let's plan to meet tomorrow morning for coffee before the session. Our session starts at 12:15, and we should check in and load our talks by around 2 hours before. We could meet around 10 at the visitor desk inside the 14th street entrance, then upload our talks, and then get coffee and chat until the session starts.

Let me know if that works for you.

Thanks!
Erik

Sent with Good (www.good.com)

From: Aleksei Chmura
Sent: Wed, 20 May 2015 19:03:20 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Grant Number: 5R01AI110964 - 02 PI Name: DASZAK, PETER
Attachments: R01AI110964 IRB Approval Letter.pdf, ATT00001.htm

Dear Laura,

Attached is our IRB approval notice, which includes both our IRB protocol and our informed consent forms. The text (pages 4-5 in the PDF) details how we plan to recruit participants and ensure their privacy (consent forms in English and Chinese on pages 7-13 of the PDF).

We do not have formal plans to provide participants with information about minimizing risks of exposure to Coronavirus infection, but test-retest studies have shown that participants in surveys similar to ours do increase their knowledge about the survey topics. All participants will be allowed to ask questions and discuss any related topics.

Please call or email me anytime, if further information is required. We are still waiting on the FWA number from Wuhan University and I will keep you updated early next week with any progress.

Many thanks!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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New York, NY 10001

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(b)(6) (mobile)
Aleksei MacDurian (Skype)

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hummingbirdIRB

November 17, 2014

Peter Daszak Ph.D.
EcoHealth Alliance
460 West 34th St., 17th Floor
New York, NY 10001-2320

Protocol Title:	Understanding the Risk of Bat Coronavirus Emergence
Hummingbird IRB #:	2014-23
Grant Number:	1R01AI110964-01
Sponsor:	EcoHealth Alliance
Approval Period:	November 14, 2014 – November 13, 2015

Dear Dr. Daszak:

At the convened board meeting of November 14, 2014, Hummingbird IRB approved the above referenced study for one year.

The following document was approved:

Protocol Date: May 27, 2014

We wish to acknowledge the approval from Wuhan University's IRB which approved the portion of the study for which there was human subject intervention. Hummingbird IRB's approval extends only to the data analysis which will take place for anonymized data transferred to Dr. Daszak.

Any changes made to the protocol must be submitted to the Hummingbird IRB. Approval from Hummingbird IRB must be secured prior to initiation of the revision(s). You will receive a reminder to renew approval of the study approximately 3 months prior to the end of the approval period.

Attached, you will find a summary of investigator commitments with which the Board requires each investigator to adhere to during the approval period.

Sincerely,

(b)(6)

Isaac M. Colbert, Ph.D.
Chairman, Hummingbird IRB

Attachment
cc: Maureen Miller, EcoHealth Alliance
Hummingbird IRB File

Investigator Commitments

All Hummingbird IRB (HIRB) approved investigators are required to fulfill these commitments.

In granting approval to the investigator for the conduct of an investigational study, Hummingbird IRB requires the investigator to understand and agree to these commitments:

1. The investigator will conduct the study(ies) in accordance with the relevant, current protocol(s) and will only make changes in a protocol when necessary to protect the safety, rights, or welfare of subjects.

2. The investigator will personally conduct or supervise the described investigation(s).

3. The investigator will delegate tasks to only trained, experienced and appropriately credentialed individuals who are familiar with the protocol and understand the tasks required to conduct the study and protect human subjects during screening and while enrolled.

4. The investigator is obligated to inform Hummingbird IRB of any financial conflicts of interest which may exist through submitting appropriate forms on an annual basis. Should a conflict arise during the course of the study, this conflict will be promptly reported to the IRB.

5. The investigator will inform any patients involved in a study involving drugs, devices or biologics, or any persons used as controls, that the drugs, devices or biologics are being used for investigational purposes and will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.

6. The investigator will report to the sponsor and Hummingbird IRB (when applicable) adverse and unanticipated problems that occur in the course of the investigation(s). If after the study has concluded, new information is made available that is relevant to ongoing health or safety, the investigator will inform subjects of these results.

7. When applicable, the investigator will read and understand the information in the investigator's brochure, device manual and other scientific background that describes the potential risks and side effects of the drug, procedure or device.

8. The investigator will ensure that all associates, colleagues, and employees assisting in the conduct of the study(ies) are informed about their obligations in meeting the commitments outlined in this document.

9. The investigator will maintain adequate and accurate records and make those records available for inspection.

10. The investigator will promptly report Hummingbird IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, the investigator will not make any changes in the research without Hummingbird IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.
11. The investigator will have in place at his or her site, a process by which the HIRB approved consent form is compared to the executed contract to ensure that consistency exists between documents in terms of procedures, study visits, payment to subjects and compensation for injury as well as other conditions effecting human subjects. The investigator and sponsor will resolve any difference and notify HIRB of any changes impacting the consent.

12. The investigator will provide referrals to any subject for whom a condition or potentially adverse information is uncovered during the study. This may include, for example, learning of suicidality or a previously unknown disease. This does not pertain to results of genetic testing unless sharing this information is part of the protocol.

PROTOCOL: Understanding the Risk of Bat Coronavirus Emergence

Protocol #: R01AI110964

Version Date and Number: 6/5/13 updated 10/21/14 Version #1

The behavioral component of this multidisciplinary study has been designed directly in concert with the novel work of zoonotic viral detection and the identification and characterization of spillover and further transmission risk from wildlife. The approach is iterative and begins with rapid and focused qualitative research conducted in natural settings at biological and ecological surveillance sites. The research includes observation and mapping of public spaces, as well as focus groups and ethnographic interviews conducted with two groups of individuals: those involved with the wildlife value chain (from hunter through market to consumer) and those highly exposed exposure to wildlife, particularly bats (eg, cave dwellers). The focus is on the type and frequency of animal contact, as well as the range of wildlife observed. Participants will also be asked about observed environmental/ecological changes and impact; travel with animals, animal responsibilities and how these are divided by age and gender, and animal taboo; daily life, seasonal changes, times of shortage and other socioeconomic factors; and finally the frequency, types, causes and understanding of illness. This information provides a framework to gain rapid understanding of human-animal interactions and the actions/meanings surrounding these interactions, as well as for the exploration of unanticipated knowledge, such as the presence and rationale for taboos on certain human-animal interactions. These data will directly inform the development of detailed behavioral surveys. Alignment of the behavioral studies will coincide with animal biological surveillance to maximize the understanding of risk and reconcile information gathered on transmission risk with the actual presence of potentially zoonotic pathogens.

Consistent with the original proposal, we will recruit volunteers for the qualitative research by word of mouth or by referral from key informants or other participants from the two target groups (ie, wildlife value chain participants and those highly exposed to wildlife, particularly bats) in Guangdong, Guangxi, Yunnan, and Fujian provinces in cooperation with local Bureaus of Public Health and CDCs. To recruit participants, we will identify local individuals influential with the target population, introduce the study in public community fora and identify volunteers through these mechanisms. We will identify three sites in each province for a total of 12 sites representing the range of settings where the target population may be found (eg, bat caves, wet markets; formal and informal wildlife trade posts; animal transport/travel routes and mechanisms including transport storage and exchange centers, and wildlife value chain supporting industries such as guesthouses, restaurants, medicinal/magical/material animal parts and animal by-product preparers, vendors and purchasers). It is anticipated that eight focus groups (two per province) of approximately 8-10 individuals each (ie, a total of 48-80) and 144 ethnographic interviews (12 per site) will be conducted. Therefore, a total of 192 to 224 individuals will participate in qualitative research. With participant permission, qualitative interviews and focus groups will be recorded.

For the behavioral survey, in each of the four provinces in southern China we will aim to include 10 markets and survey 20 vendors per market; an additional 420 individuals will be selected based on the results of qualitative data analysis. In each province, 620 people will be surveyed for a total of 2480 individuals. A sampling frame and recruitment materials for this quantitative research will be developed in Year 2. Participants in the survey will be asked to provide blood (no more than 550ml), sputum, and stool samples. We will screen sera for antibodies to SARS-CoV, other alpha & beta coronaviruses including MERS-CoV,

and novel bat-CoVs. We will screen stool from CoV seropositive participants for CoV nucleic acid. We will also develop specific bat-CoV serological assays and share these with our Chinese collaborators.

In recognition of the time and expertise offered by study participants, each person will be offered a small token of practical, emotional or social significance. The token will not cost a lot of money, nor will it be money.

Only adults 18 years or older will be invited to participate. At least one of the focus groups and an estimated 35-40% of the interviews and surveys will be conducted with women. Subjects will be enrolled in this study without regard to ethnicity. The primary enrollment criteria are related to occupational exposure to wildlife and residence near wildlife.

We currently have no plans to pursue the substudy in Shanghai mentioned in the text. There are also no current plans for follow up of any study participants. In addition, if SARS virus is identified in any human sample, it will be immediately reported to public health authorities because we will have identified an outbreak.

The original sources of this information are on p112 section C1b and p120 Human subjects in the grant proposal.

On May 18, 2015, at 13:49, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,

OER has reviewed the original response to the summary statement concern and requested the following additional information. Please provide a response no later than **Wednesday, May 20th.**

-

- Please have the PI discuss the recruitment of participants.
- Please have the PI discuss how privacy is ensured, particularly with face-to-face interviews.
- Will participants be provided any information regarding minimizing their risks of Coronovirus infection?

NIH Instructions for Grant Applications may be found at:
http://grants.nih.gov/grants/funding/424/SF424_RR_Guide_General_Adobe_VerB.pdf, Part II onwards for requirements on the Protection of Human Subjects
Please also visit our public website for information on "Research Involving Human Subjects":
http://grants.nih.gov/grants/policy/hs/index.htm

Thank you,

Laura

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Tue, 19 May 2015 20:00:02 +0000
To: Peter Daszak
Subject: RE: ATS

Thanks! See you tomorrow morning.

Please note my updated contact information below:

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-7630
Phone: (b)(6)
Email:

From: Peter Daszak (b)(6)
Sent: Tuesday, May 19, 2015 3:57 PM
To: Stemmy, Erik (NIH/NIAID) [E]; 'Matthew Frieman'
Cc: Alison Andre
Subject: RE: ATS

Great – will meet you around 10am

I've attached a short bio here.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance

460 West 34th Street – 17th Floor
New York, NY 10001

(b)(6) (direct)
+1.212.380.4465 (fax)
www.ecohealthalliance.org

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, May 19, 2015 3:34 PM
To: Peter Daszak; 'Matthew Frieman'
Subject: RE: ATS

One other thing I forgot to ask is if you both have short bios I can use when introducing you tomorrow. If not I can also get a couple of bullets when we meet tomorrow. Let me know.

Thanks!
Erik

Sent with Good (www.good.com)

-----Original Message-----
From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Tuesday, May 19, 2015 12:03 PM Eastern Standard Time
To: 'Peter Daszak'; 'Matthew Frieman'
Subject: ATS

Hi Peter and Matt,
Since you're both arriving later this evening, let's plan to meet tomorrow morning for coffee before the session. Our session starts at 12:15, and we should check in and load our talks by around 2 hours before. We could meet around 10 at the visitor desk inside the 14th street entrance, then upload our talks, and then get coffee and chat until the session starts.

Let me know if that works for you.

Thanks!
Erik

Sent with Good (www.good.com)

From: Peter Daszak
Sent: Tue, 19 May 2015 19:57:50 +0000
To: Stemmy, Erik (NIH/NIAID) [E]; 'Matthew Frieman'
Cc: Alison Andre
Subject: RE: ATS
Attachments: Peter Daszak Short Bio 2015.doc
Importance: High

Great – will meet you around 10am

I've attached a short bio here.

Cheers,

Peter

Peter Daszak
President

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Subject: RE: ATS

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Thanks!

Erik

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Sent: Tuesday, May 19, 2015 12:03 PM Eastern Standard Time
To: 'Peter Daszak'; 'Matthew Frieman'
Subject: ATS

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Let me know if that works for you.

Thanks!
Erik

Sent with Good (www.good.com)

Dr. Peter Daszak, Ph.D

Dr. Peter Daszak is President of EcoHealth Alliance, a US-based organization which conducts research and outreach programs on global health, conservation and international development. Dr. Daszak's research has been instrumental in identifying and predicting the impact of emerging diseases across the globe. His achievements include identifyng the bat origin of SARS, identifying the underlying drivers of Nipah and Hendra virus emergence, producing the first ever global emerging disease 'hotspots' map, identifying the first case of a species extinction due to disease, coining the term 'pathogen pollution', and discovering the disease chytridiomycosis as the cause global amphibian declines.

Dr Daszak is a member of the IOM's Forum on Microbial Threats, the NRC Advisory Committee to the USGCRP, the Supervisory Board of the One Health Platform, the One Health Commission Council of Advisors, the CEEZAD External Advisory Board; has served on the IOM Committee on global surveillance for emerging zoonoses, the NRC committee on the future of veterinary research, the International Standing Advisory Board of the Australian Biosecurity CRC; and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues.

Dr Daszak won the 2000 CSIRO medal for collaborative research on the discovery of amphibian chytridiomycosis, is the EHA institutional lead for USAID-EPT-PREDICT and PREDICT-2, is on the Editorial Board of *Conservation Biology*, *One Health*, and *Transactions of the Royal Society of Tropical Medicine & Hygiene*, and is Editor-in-Chief of the journal *Ecohealth*. He has authored over 200 scientific papers, and his work has been the focus of extensive media coverage, ranging from popular press articles to television appearances.

From: Peter Daszak
Sent: Tue, 19 May 2015 12:05:52 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Alison Andre
Subject: RE: ATS

Hi Erik,

I'm getting into Denver late tonight and unfortunately have to fly back to NY on Wednesday evening to make sure I'm in town for meetings Thursday.

Could we meet in the morning before the session, or straight afterwards?

Let me know what works best. I'm staying at the Hyatt Regency and my cellphone number is (b)(6)

I'll be checking email as well...look forward to meeting up..

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

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www.ecohealthalliance.org

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-----Original Message-----
From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Sunday, May 17, 2015 9:21 PM
To: Peter Daszak
Subject: ATS

Hi Peter,
Just wanted to see when you're arriving at ATS. I was thinking of trying to get together with you and Matt Frieman at some point outside of the session Wednesday. Let me know when you'll be around and gave some time. I'm looking forward to hear about how things are going!

Erik

Sent with Good (www.good.com)

From: Aleksei Chmura
Sent: Tue, 19 May 2015 09:50:56 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Peter Daszak; Alison Andre
Subject: Re: Year 1 Report for Bat CoV Emergence Award (1R01AI110964-01)

Dear Erik,

Peter will be arriving in Denver late this evening and he wanted to know if you would be available to meet either before or after your session on Wednesday.

Many thanks!

Aleksei Chmura
Senior Coordinator of Operations

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
(mobile)
(b)(6) (Skype)

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On May 5, 2015, at 08:32, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Thanks Peter! I'm looking forward to our session at ATS as well.

Best,
Erik

From: Peter Daszak (b)(6)
Sent: Friday, May 01, 2015 2:57 PM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Pone, Laura (NIH/NIAID) [E]; Aleksei Chmura
Subject: Year 1 Report for Bat CoV Emergence Award (1R01AI110964-01)

Dear Erik,

We just uploaded our Y1 Report for our Understanding the Risk of Bat Coronavirus Emergence award (1R01AI110964-01). I wanted to send you a copy of the report as well.

We have already some exciting results and I look forward to talking with you more at the ATS Meeting in Colorado in a few weeks!

Cheers,

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

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From: Aleksei Chmura
Sent: Mon, 18 May 2015 20:02:12 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Grant Number: 5R01AI110964 - 02 PI Name: DASZAK, PETER

Dear Laura,

We will get back to you with these details by Wednesday or tomorrow.

Many thanks!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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On May 18, 2015, at 13:49, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,

OER has reviewed the original response to the summary statement concern and requested the following additional information. Please provide a response no later than **Wednesday, May 20th.**

-

- Please have the PI discuss the recruitment of participants.
- Please have the PI discuss how privacy is ensured, particularly with face-to-face interviews.
- Will participants be provided any information regarding minimizing their risks of Coronovirus infection?

NIH Instructions for Grant Applications may be found at:
http://grants.nih.gov/grants/funding/424/SF424_RR_Guide_General_Adobe_VerB.pdf, Part II onwards for requirements on the Protection of Human Subjects

Please also visit our public website for information on "Research Involving Human Subjects":
http://grants.nih.gov/grants/policy/hs/index.htm

Thank you,

Laura

From: Aleksei Chmura
Sent: Thu, 14 May 2015 20:11:41 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Grant Number: 5R01AI110964 - 02 PI Name: DASZAK, PETER
Attachments: Wuhan IRB_fin.pdf, ATT00001.htm

Dear Laura,

That is very good to hear!

Here is another update: attached is our IRB approval from Wuhan University.

In summary, we have IRB approval from US and China and are waiting on the FWA number for Wuhan University. I will let you know about the FWA as soon as possible.

Thanks again!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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New York, NY 10001

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WUHAN UNIVERSITY

299 Bayi Rd., Wuhan 430072, Hubei, P.R. China

Wuhan University Ethics Approval Board

Research Study US NIAID R01AI110964: Understanding the Risk of Bat Coronavirus Emergence

This multidisciplinary study will include human subjects research. The human subjects research is both qualitative and quantitative. The focus is on the type and frequency of animal contact, as well as the range of wildlife observed. The research provides a framework to gain rapid understanding of human-animal interactions. Alignment of the human subjects research will coincide with animal biological surveillance to maximize the understanding of transmission risk with the potentially zoonotic pathogens identified in animal populations.

Volunteers will be recruited by word of mouth or by referral from key informants or other participants from the two target groups (ie, wildlife value chain participants and those highly exposed to wildlife, particularly bats) in Guangdong, Guangxi, Yunnan, and Fujian provinces in cooperation with local Bureaus of Public Health and CDCs. We will identify three sites in each province for a total of 12 sites representing the range of settings where the target population may be found (eg, bat caves, wet markets; formal and informal wildlife trade posts; animal transport/travel routes and mechanisms including transport storage and exchange centers, and wildlife value chain supporting industries such as guesthouses, restaurants, medicinal/magical/material animal parts and animal by-product preparers, vendors and purchasers). It is anticipated that eight focus groups (two per province) of approximately 8-10 individuals each (ie, a total of 48-80) and 144 ethnographic interviews (12 per site) will be conducted. Therefore, a total of 192 to 224 individuals will participate in qualitative research. With participant permission, qualitative interviews and focus groups will be recorded.

For the behavioral survey, a sampling frame and recruitment materials for this quantitative research will be developed in Year 2. It is anticipated that approximately 2500 individuals will be interviewed and asked to provide blood (no more than 550ml), sputum, and stool samples. We will screen sera for antibodies to SARS-CoV, other alpha & beta coronaviruses including MERS-CoV, and novel bat-CoVs.

Only adults 18 years or older will be invited to participate. At least one of the focus groups and an estimated 35-40% of the interviews and surveys will be conducted with women. Subjects will be enrolled in this study without regard to ethnicity. The primary enrollment criteria are related to occupational exposure to wildlife and residence near wildlife. All participants will sign an informed consent approved by the Wuhan Ethics Approval Board. In recognition of the time and expertise offered by study participants, each person will be offered a small token of practical, emotional or social significance. The token will not

WUHAN UNIVERSITY

299 Bayi Rd., Wuhan 430072, Hubei, P.R. China

cost a lot of money, nor will it be money.

All data, including notes, recordings, questionnaires, and computer files will be will be coded to strictly preserve confidentiality. Paper files will be scanned electronically and then shredded. Biological samples will be coded to maintain anonymity of sample results. Identifying information such as consent forms and test results will be kept under lock and key in a file cabinet. All electronic data will be encrypted. Data access will be limited to investigators conducting analyses; data will have protections with data access codes required. Data collection is cross sectional and master list data will not be required for the analysis of data. Data will be presented in the aggregate. Original data will be stored for five years after the completion of the study. At that time, electronic files will be permanently deleted.

(b)(6)

Chuanhua Yu, Ph.D
Director of Medical Ethics Committee
School of Public Health
Wuhan University
115 Donghu Rd.
Wuhan, Hubei 430071
Tel: (b)(6)
Fax: (+8627)68758648
Email: (b)(6)

Nov. 11, 2014

On May 14, 2015, at 16:00, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,

I apologize for the confusion. I see that the summary statement concern was responded to and have submitted that for review. Please let me know once Wuhan has the FWA.

Thank you!

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
5601 Fishers Lane, Room 4E29, MSC 9824
Bethesda, MD 20892-9824
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

From: Aleksei Chmura (b)(6)
Sent: Wednesday, May 13, 2015 4:36 PM
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Grant Number: 5R01AI110964 - 02 PI Name: DASZAK, PETER
Importance: High

Dear Laura,

Apologies for any delays or confusion on my part, but I am not certain what summary statement concerns you are requesting. We provided details about protection of human subjects via our Just in Time Report in May of last year and sent our US IRB approval for our human research protocol under our award last month (attached here for reference). We expect to have an FWA for Wuhan University before the end of the month, but I will update you on our progress in the next week.

Can we have a quick chat about the summary statement concerns anytime that is good for you. Once we are clear on what is required, we will provide the requested details immediately.

Is the deleted reference with updated My NCBI report for Dr. Daszak ok as well? I have not yet had a response from the NCBI support re. removing and/or disassociating the reference.

Please call me anytime day/night at (b)(6)

Many thanks!

Aleksei Chmura
Senior Coordinator of Operations

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New York, NY 10001

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From: Aleksei Chmura
Sent: Tue, 12 May 2015 18:23:54 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: eRA Commons: RPPR for Grant 5R01AI110964-02 Submitted to NIH with a Non-Compliance warning

Laura,

The problem here may be that the paper has not been accepted for publication and is still in review with the journal, so there is no way to provide a date accepted for publication - at least not presently.

We have tried to 'disassociate' the reference with our award as well as delete the reference/paper from My NCBI, but neither options are effective. We are waiting for the NCBI support to get back to us to explain how we may do either. I have emailed them again today.

Can we have a quick chat about this sometime today or tomorrow? Call me anytime on (b)(6) I will also try your direct number.

Many thanks!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
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On 12 May 2015, at 13:56:56, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,

I received the clarification below on the non-compliant publication from the RPPR for grant AI110964. Please register the publication in NIHMS and provide a response as soon as possible.

The public access policy states that the final peer reviewed manuscript should be placed in Pub Med Central (PMS) when it's accepted for publication, not when it is published. If it was an A or B submission journal (journals types that submit to PMC for them) and the journals don't do it – the Grantee/PI is still responsible. Only Journals can place papers on Pub Med, not authors. Authors do this indirectly thru the NIH Manuscript Submission System (NIHMS). If an author (or the PI, if he is the author or co-author) does place the final peer reviewed manuscript on the NIHMS when it was accepted for publication, they would have gotten the provisional compliance status of "In-Process at NIHMS". Since this did not happen the manuscript correctly coded by eRA as non-compliant.

They still have the option to move forward with placing the final peer-reviewed manuscript on the NIHMS. Or they can wait on the publisher to do this but we would not be able to award the grant until done.

If they provide us with the NIHMS ID number and the date it was accepted for publication by the journal and confirm whether it has been published yet – we might accept it depending on the date of acceptance and the RPPR submitted date.

Thank you,
Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
5601 Fishers Lane, Room 4E29, MSC 9824
Bethesda, MD 20892-9824
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)
 please consider the environment before printing this e-mail.

-----Original Message-----
From: Aleksei Chmura (b)(6)
Sent: Tuesday, May 05, 2015 1:10 PM
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: eRA Commons: RPPR for Grant 5R01AI110964-02 Submitted to NIH with a Non-Compliance warning

Laura,

The Brierly et al paper (Brierley, L., Vonhof, M., Jones, K. E., Olival, K. J. & Daszak, P. Quantifying global drivers of zoonotic bat viruses: a process-based perspective. The American Naturalist, In Review) is in review and as per the guidelines you sent may not be included in Dr. Daszak's Report.

We have removed this paper and a revised NCBI Award Compliance Report is attached (PDF). As soon as this paper is accepted (in press, etc.), I will let you know and send a revised NCBI Report.

Many thanks!

Aleksei Chmura
Senior Coordinator of Operations

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
(mobile)
(b)(6) (Skype)

www.ecohealthalliance.org

Visit our blog: www.ecohealthalliance.org/blog

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

From: Aleksei Chmura
Sent: Tue, 5 May 2015 17:10:12 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: eRA Commons: RPPR for Grant 5R01AI110964-02 Submitted to NIH with a Non-Compliance warning
Attachments: bib.pdf, ATT00001.txt

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Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
PMC Journal In Process	Young CC, Olival KJ. Optimizing Viral Discovery in Bats. PloS one. Forthcoming;
Not applicable	Olival KJ, Weekley CC, Daszak P. Bats and Viruses. Wang L, editor. New York: John Wiley & Sons, Inc.; 2015. What we know and need to know

> On 05 May 2015, at 10:07:03, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:
>
> Hi Aleksei,
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> You may add the citation manually to My NCBI by clicking the Add Citation button in My Bibliography, and selecting "manual citation'. Enter the details, including the journal, and specify the paper as 'in press'. This will make the paper available in the RPPR selection window.
> My NCBI will continually look for the paper in PubMed, and seek your permission to update the "in press" record with the publication details once the paper is published.
> Please note papers in press still need to deposited to PubMed Central, or be published in a journal that will automatically deposit the final published article. See http://publicaccess.nih.gov/citation_methods.htm for more details.
>
> http://grants.nih.gov/grants/rppr/faqs.htm#3567
>
> Thank you,
>
> Laura Pone
> Grants Management Specialist
> DHHS/NIH/NIAID/GMP
> 5601 Fishers Lane, Room 4E29, MSC 9824
> Bethesda, MD 20892-9824
> Phone: (b)(6)
> e-Fax: 301-493-0597
> Email: (b)(6)
> P please consider the environment before printing this e-mail.
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> Disclaimer:
> The information in this e-mail and any of its attachments is confidential and may contain sensitive information. It should not be used by anyone who is not the intended recipient. If you have received this e-

mail in error please inform the sender and delete it from your mailbox or any other storage devices. The National Institutes of Allergy and Infectious Diseases (NIAID) shall not accept liability for any statement made that are the sender's own and not expressly made on behalf of the NIAID by one of its representatives
>
> From: Aleksei Chmura (b)(6)
> Sent: Monday, May 04, 2015 5:59 PM
> To: Pone, Laura (NIH/NIAID) [E]
> Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
> Subject: Re: eRA Commons: RPPR for Grant 5R01AI110964-02 Submitted to NIH with a Non-Compliance warning
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> Laura,
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> -Aleksei
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> On May 4, 2015, at 17:08, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:
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> Phone: (b)(6)
> e-Fax: 301-493-0597
> Email: (b)(6)
> P please consider the environment before printing this e-mail.
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> The two journal articles (#1 & #2) are in review currently with their respective journals; we would expect review decisions in the next 1.5 months at the very latest.
>
> The book chapter (#3) is in press and should be published sometime over the summer.
>
> When we have any update on these three publications, we will notify you immediately.
>
> Many thanks!
>
> -Aleksei
>
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> Aleksei Chmura
> Senior Coordinator of Operations
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> EcoHealth Alliance
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> New York, NY 10001
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> On 04 May 2015, at 15:53:39, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:
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> Mr. Chmura,
>
> The report showed one listed as n/a: not journal and one as PMC Journal - in Procress (these are acceptable). The one that was non-compliant must be brought into compliance. I believe after the initial submission it will take 2-3 weeks for it to be processed and sent back to you for final review, then a PMCID can be assigned. Please let me know where you are in the process so I know when to expect the final PMCID.
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> Thank you,
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> Laura Pone
> Phone: (b)(6)
> e-Fax: 301-493-0597
> Email: (b)(6)
> P please consider the environment before printing this e-mail.
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> From: Aleksei Chmura (b)(6)
> Sent: Friday, May 01, 2015 3:19 PM
> To: Pone, Laura (NIH/NIAID) [E]
> Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
> Subject: Re: eRA Commons: RPPR for Grant 5R01AI110964-02 Submitted to NIH with a Non-Compliance warning
>
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>
> We received this error message (below) regarding non compliant publications. I understand that these three publications are not in compliance since they are currently in press or review and DOIs are not yet issued nor are these publications posted in NIHMS/PMC. How should we proceed at this point - remove these references from our Year 1 Report or email you with updated details upon acceptance of the articles?
>
> Many thanks most,
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> Sincerely,
>
> Aleksei Chmura
> Senior Coordinator of Operations
>
> EcoHealth Alliance
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> On 01 May 2015, at 14:49:08, era-notify@mail.nih.gov wrote:
>
> *** This is an automated notification - Please do not reply to this message. ***
>
> Dear Grantee,
>
> The progress report for the above-reference award includes citation(s) that are out of compliance with the NIH Public Access Policy. Compliance with the NIH Public Access Policy is a legal requirement and a term and condition of all NIH awards. This award will be delayed until all publications arising from it are in compliance with the policy.
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>
> Instructions for SO/AOR verification:
> • Verify that the PD/PI has used My NCBI to enter publications and/or update compliance status.

> • Include a My NCBI PDF report demonstrating all the formerly non-compliant public access citations are now compliant. To process your award, every citation in the report should be either complete, in process or exempt N/A). Please see http://publicaccess.nih.gov/citation_methods.htm for more information about acceptable compliance statuses for public access papers. We have more information about My NCBI athttp://publicaccess.nih.gov/communications.htm.
> • If unable to provide verification, provide a justification for why the specific publication(s) cannot be brought into compliance.
> NIH awardees are responsible for ensuring that evidence of compliance is included in all NIH applications, proposals and reports. If you have questions about the Policy, please check the NIH Public Access Website or send an email toPublicAccess@nih.gov.
> For any further questions about this email, call the eRA Help Desk at 1-866-504-9552 or refer tohttp://grants.nih.gov/support for additional methods of contact. Please access Commons athttp://public.era.nih.gov/commons/.
> For more information please visit http://era.nih.gov/

From: Pone, Laura (NIH/NIAID) [E]
Sent: Tue, 5 May 2015 10:07:03 -0400
To: Aleksei Chmura
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My NCBI will continually look for the paper in PubMed, and seek your permission to update the "in press" record with the publication details once the paper is published.
Please note papers in press still need to deposited to PubMed Central, or be published in a journal that will automatically deposit the final published article. See http://publicaccess.nih.gov/citation_methods.htm for more details.

http://grants.nih.gov/grants/rppr/faqs.htm#3567

Thank you,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
5601 Fishers Lane, Room 4E29, MSC 9824
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On 01 May 2015, at 14:49:08, era-notify@mail.nih.gov wrote:

*** This is an automated notification - Please do not reply to this message. ***

Dear Grantee,

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- If unable to provide verification, provide a justification for why the specific publication(s) cannot be brought into compliance.

NIH awardees are responsible for ensuring that evidence of compliance is included in all NIH applications, proposals and reports. If you have questions about the Policy, please check the NIH Public Access Website or send an email toPublicAccess@nih.gov.

For any further questions about this email, call the eRA Help Desk at 1-866-504-9552 or refer tohttp://grants.nih.gov/support for additional methods of contact. Please access Commons athttp://public.era.nih.gov/commons/.

For more information please visit http://era.nih.gov/

From: Aleksei Chmura
Sent: Mon, 4 May 2015 20:48:04 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Grant Number: 5R01AI110964 - 02 PI Name: DASZAK, PETER

Dear Laura,

Only one of the institutions - Wuhan University - will be involved in the Human Subject Work. None of those listed below will be involved with human subject work. We will work on getting the FWA as soon as possible and update you.

Many thanks!

-Aleksei

On 04 May 2015, at 15:35:50, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Dear Mr. Chmura,

Please work immediately to resolve the below non-compliant publication as the award cannot be made until this publication is brought into compliance.

Non-Compliant Brierley L, Vonhof M, Jones KE, Olival KJ, Daszak P. Quantifying global drivers of zoonotic bat viruses: a process-based perspective. The American naturalist. Forthcoming;

I will also need the FWA for the following foreign institutes that will be involved in human subject work.

- Guangdong Entomological Institute
- Wuhan Institute of Virology
- East China Normal University
- Center for Disease Control and Prevention of Guangdong

Thank you,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
5601 Fishers Lane, 4E29, MCS 9824
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From: Peter Daszak
Sent: Fri, 1 May 2015 18:56:52 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Pone, Laura (NIH/NIAID) [E]; Aleksei Chmura
Subject: Year 1 Report for Bat CoV Emergence Award (1R01AI110964-01)
Attachments: NIAID CoV Year 1 report 1R01AI110964-01.pdf

Dear Erik,

We just uploaded our Y1 Report for our Understanding the Risk of Bat Coronavirus Emergence award (1R01AI110964-01). I wanted to send you a copy of the report as well.

We have already some exciting results and I look forward to talking with you more at the ATS Meeting in Colorado in a few weeks!

Cheers,

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

(b)(6) (direct)
+1.212.380.4465 (fax)
www.ecohealthalliance.org

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Year 1 Report for Understanding the Risk of Bat Coronavirus Emergence

Award Number: 1R01AI110964-01

B1: Major Goals of Project

Zoonotic coronaviruses are a significant threat to global health, as demonstrated with the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, and the recent emergence Middle East Respiratory Syndrome (MERS-CoV). The wildlife reservoirs of SARS-CoV were identified by our group as bat species, and since then hundreds of novel bat-CoVs have been discovered (including >260 by our group). These, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a largescale human-wildlife interface, and high risk of future emergence of novel CoVs.
To understand the risk of zoonotic CoV emergence, we propose to examine 1) the transmission dynamics of bat-CoVs across the human-wildlife interface, and 2) how this process is affected by CoV evolutionary potential, and how it might force CoV evolution. We will assess the nature and frequency of contact among animals and people in two critical human-animal interfaces: live animal markets in China and people who are highly exposed to bats in rural China. In the markets we hypothesize that viral emergence may be accelerated by heightened mixing of host species leading to viral evolution, and high potential for contact with humans. In this study, we propose three specific aims and will screen free ranging and captive bats in China for known and novel coronaviruses; screen people who have high occupational exposure to bats and other wildlife; and examine the genetics and receptor binding properties of novel bat-CoVs we have already identified and those we will discover. We will then use ecological and evolutionary analyses and predictive mathematical models to examine the risk of future bat-CoV spillover to humans. This work will follow 3 specific aims:

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces. We will examine if: 1) wildlife markets in China provide enhanced capacity for bat-CoVs to infect other hosts, either via evolutionary adaptation or recombination; 2) the import of animals from throughout Southeast Asia introduces a higher genetic diversity of mammalian CoVs in market systems compared to within intact ecosystems of China and Southeast Asia; We will interview people about the nature and frequency of contact with bats and other wildlife; collect blood samples from people highly exposed to wildlife; and collect a full range of clinical samples from bats and other mammals in the wild and in wetmarkets; and screen these for CoVs using serological and molecular assays.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk. We propose two competing hypotheses: 1) CoV host-range in bats and other mammals is limited by the
phylogenetic relatedness of bats and evolutionary conservation of CoV receptors; 2) CoV host-range is limited by geographic and ecological opportunity for contact between species so that the wildlife trade disrupts the ‘natural’ co-phylogeny, facilitates spillover and promotes viral evolution. We will develop CoV phylogenies from sequence data collected previously by our group, and in the proposed study, as well as from Genbank. We will examine co-evolutionary congruence of bat-CoVs and their hosts using both functional (receptor) and neutral genes. We will predict host-range in unsampled species using a generalizable model of host and viral ecological and phylogenetic traits to explain patterns of viral sharing between species. We will test for positive selection in market vs. wild-sampled viruses, and use data to parameterize mathematical models that predict CoV evolutionary and transmission dynamics. We will then

examine scenarios of how CoVs with different transmissibility would likely emerge in wildlife markets.

Specific Aim 3: Testing predictions of CoV inter-species transmission. We will test our models of host range (i.e. emergence potential) experimentally using reverse genetics, pseudovirus and receptor binding assays, and virus infection experiments in cell culture and humanized mice. With bat-CoVs that we've isolated or sequenced, and using live virus or pseudovirus infection in cells of different origin or expressing different receptor molecules, we will assess potential for each isolated virus and those with receptor binding site sequence, to spill over. We will do this by sequencing the spike (or other receptor binding/fusion) protein genes from all our bat-CoVs, creating mutants to identify how significantly each would need to evolve to use ACE2, CD26/DPP4 (MERS-CoV receptor) or other potential CoV receptors. We will then use receptor-mutant pseudovirus binding assays, in vitro studies in bat, primate, human and other species' cell lines, and with humanized mice where particularly interesting viruses are identified phylogenetically, or isolated. These tests will provide public health-relevant data, and also iteratively improve our predictive model to better target bat species and CoVs during our field studies to obtain bat-CoV strains of the greatest interest for understanding the mechanisms of cross-species transmission.

B2: What was accomplished under these goals?

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces.

In the first year of this R01, we have:

1) Designed a behavioral risk study using an iterative approach that begins with rapid and focused qualitative research at or near biological surveillance sites in China where bats have previously been captured, sampled and found to contain novel CoVs. The study design includes: 1) structured observation and mapping of public spaces, 2) focus groups and 3) ethnographic interviews. The primary enrollment criteria are related to occupational exposure to bats and residence near bats. This research is conducted with two groups of individuals: those involved in the bat value chain (from hunter through market to consumer) and those highly exposed to bats (e.g., cave dwellers). The qualitative data will be used to inform a behavioral risk survey, as well as to contextualize findings from behavioral surveillance analyses.

2) Conducted observational research and mapping in: ***Yunnan***: In and around Xiang Yun village (two clinics and one wildlife restaurant); in and around the remote Lu Feng village (1 wildlife farm, 1 wildlife butcher and 1 wildlife restaurant) and at the An Ning communicable disease hospital complex; ***Guangxi***: In and around LiPu, (two markets, 3 wildlife farms, 1 wildlife restaurant); and ***Guangdong***: Guangzhou wildlife market, Foshon wildlife market (this market is where the first cases of SARS were traced back to in 2003).

3) Secured local IRB approval in November 2014 from Wuhan University School of Public Health, Hubei Province, to conduct qualitative research, to administer behavioral surveys and to collect biological data including blood (no more than 550ml), sputum, and stool samples from humans. We secured US IRB approval through Hummingbird IRB (2014-23 approval letter sent to NIH) in November 2014 for qualitative, quantitative and biological specimen data collection.

4) Drafted protocols, guides, and training modules for Observational Research, Focus Groups, and Ethnographic Interviews and pilot tested these. The Observational Guide and Ethnographic Interview materials were pilot tested in live animal markets in Queens, New York City. Consistent with the original proposal, we have trained interviewers and identified key informants. Key informants include community health workers from three different administrative level CDCs, Barefoot Doctors, public health clinicians, local wildlife farmers and wildlife restaurant owners, as well as market vendors and workers. Ethnographic and Focus Group Interviews to be conducted pending NIH approval of IRB approval letter.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk.

1) *Collation and preliminary analysis of published bat Coronavirus data to optimized specimen collection and taxonomic targets for surveillance.*
Over the last decade a large number of bat viral discovery studies have been published globally (including a large number focused on CoVs). In year 1, we conducted the first ever systematic analysis of these data. We collated literature from over 100 viral discovery studies in bats, to examine patterns of host range and known viral diversity in different bat taxa (Young and Olival, In Review). We found that Coronavirus diversity has been most thoroughly characterized in a few bat families, including the Vespertillionidae and 5 other families, but several bat taxa remain under-represented in global virus surveillance efforts (**Fig 1**). Identification of these surveillance gaps allows us to better target our field surveillance towards bat taxa where CoV diversity is largely unknown (blue and light colored cells, **Fig 1**). These analyses were completed at various taxonomic levels, including by bat subfamily and genera (Family level analysis only shown).

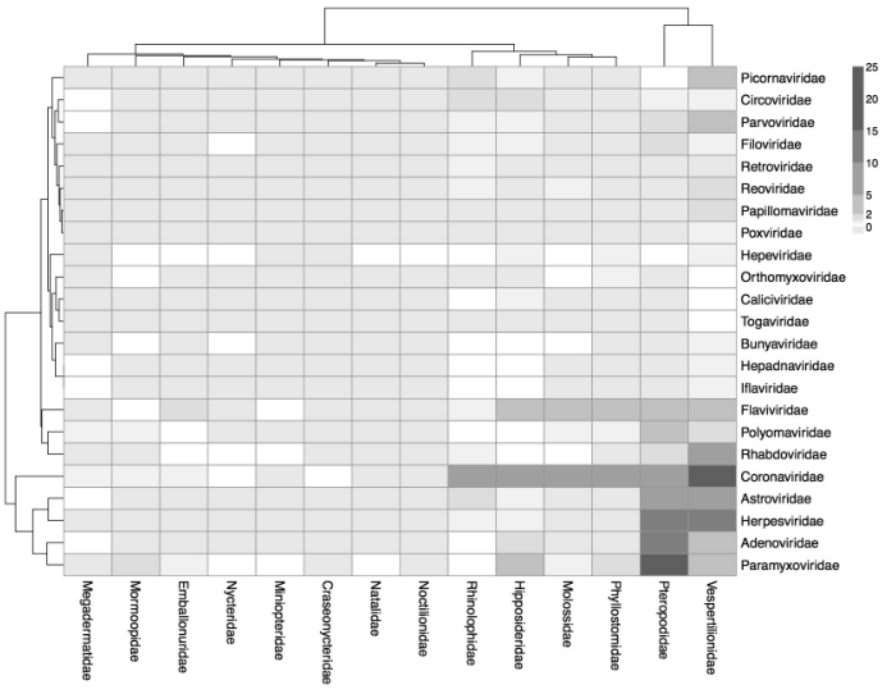


Figure 1. Heat map of viral richness by bat host and viral family, clustered by similarity in viral richness across host and viral families.

To maximize our chances of discovering CoVs, we need to define the number of specimens required for our bat surveillance work and the bat taxonomic groups on which to focus our surveillance. We used generalized linear mixed models (GLMM) and applied this to a subset of our collated data for CoVs alone. We found that sample type screened (feces), collection methods, and the number of specimens tested best explains the probability of finding an individual CoV positive sample. We will now use these approaches to increase the likelihood of getting positive samples in our fieldwork in China.

2) *Preliminary 'What-if' Model: Role of species diversity in CoV emergence risk.* We built a mathematical model to analyze different scenarios of CoV spillover. We began with an assessment of how the diversity of wildlife (and other factors) in wet markets may affect the probability of CoV zoonotic spillover. We modeled evolution of CoVs within wildlife in a market following the initial introduction of a novel virus in one specific host. We assume this initial virus is a single genotype that does not yet have a great enough rate of spread to create an epidemic, but has a rate of spread close to this threshold. When this virus infects a new host, a new genotype is generated, based on random drift from the infecting genotype. We use Neutral Theory of Species Diversity to specify the species distribution in the market, for a given total number of species and total abundance of animals. We assume 500 animals in the market, and alter the species diversity from 3 to over 40. These numbers are easily attained in a small to medium market in Southern China (and in year 2 we will groundtruth these assumptions)

As the number of species present in a market increases from 3 to 20, the percent of simulations where zoonotic spillover occurred from any of the animals into humans increases (**Fig 2**). However, the risk remains fairly level if wildlife biodiversity increases above that level. The probability of epidemic failure is inverse to the probability of a zoonotic spillover taking hold and decreases with increasing species diversity (**Fig 2**). Therefore our null model shows that reducing the diversity of species in live animal markets could reduce the risk of zoonotic spillover, including of potentially pandemic CoVs.

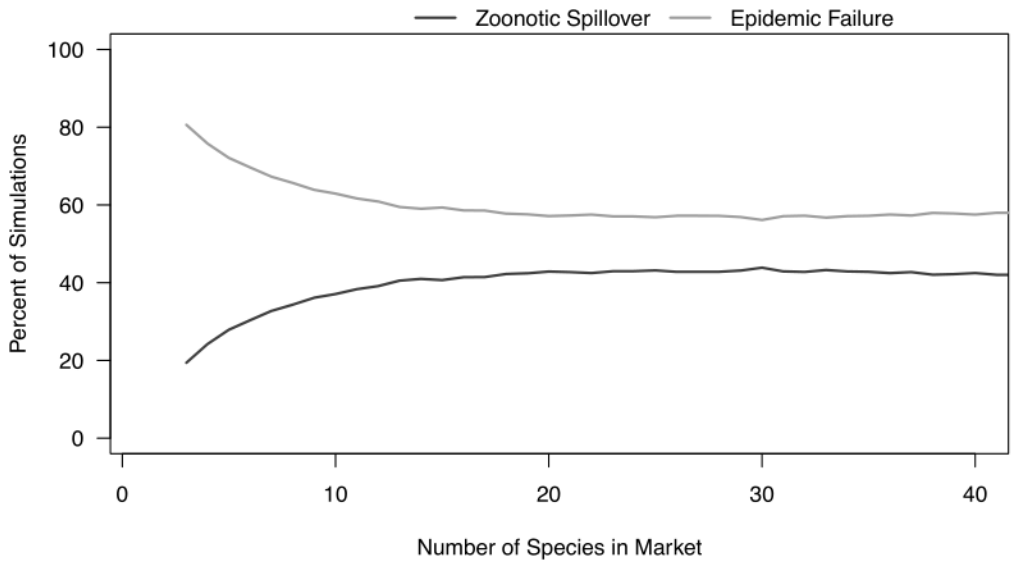


Figure 2. 'What-if' scenario model based on the Neutral Theory of Species Diversity to examine the role of wildlife species diversity for CoV spillover in markets.

Specific Aim 3: Testing predictions of CoV inter-species transmission.

1) Bat Coronavirus Surveillance in 2014

We collected 1555 anal swab samples, 1357 fecal samples, 461 blood samples, 469 serum samples and 24 tissue samples from > 14 bat genera in 5 provinces and in Laos (**Table 1**).

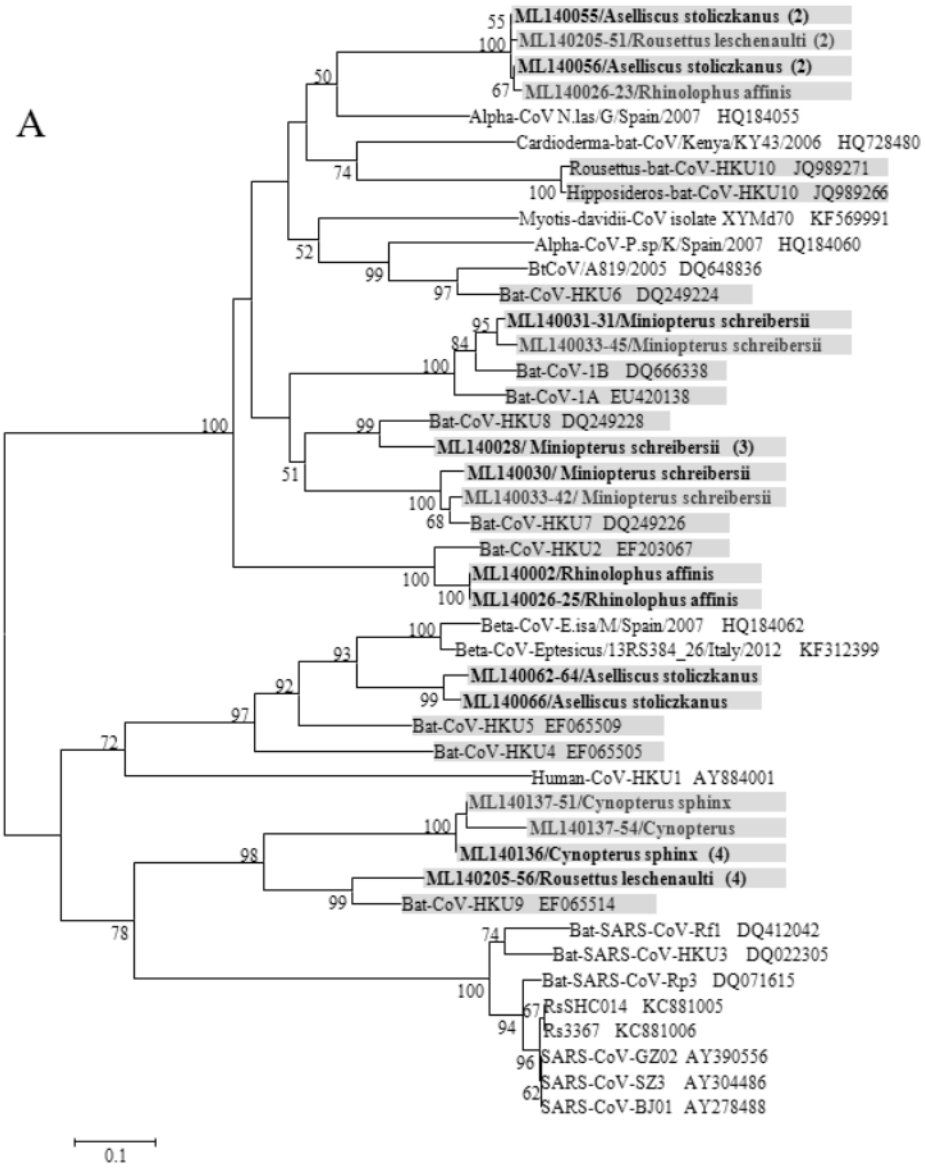
Table 1 Bat Samples collected for CoV surveillance in 2014

		Anal	Oral	Fecal	Blood	Serum	tissue
Jan. 2014	Mengla, Yunnan	164	--	--	--	--	--

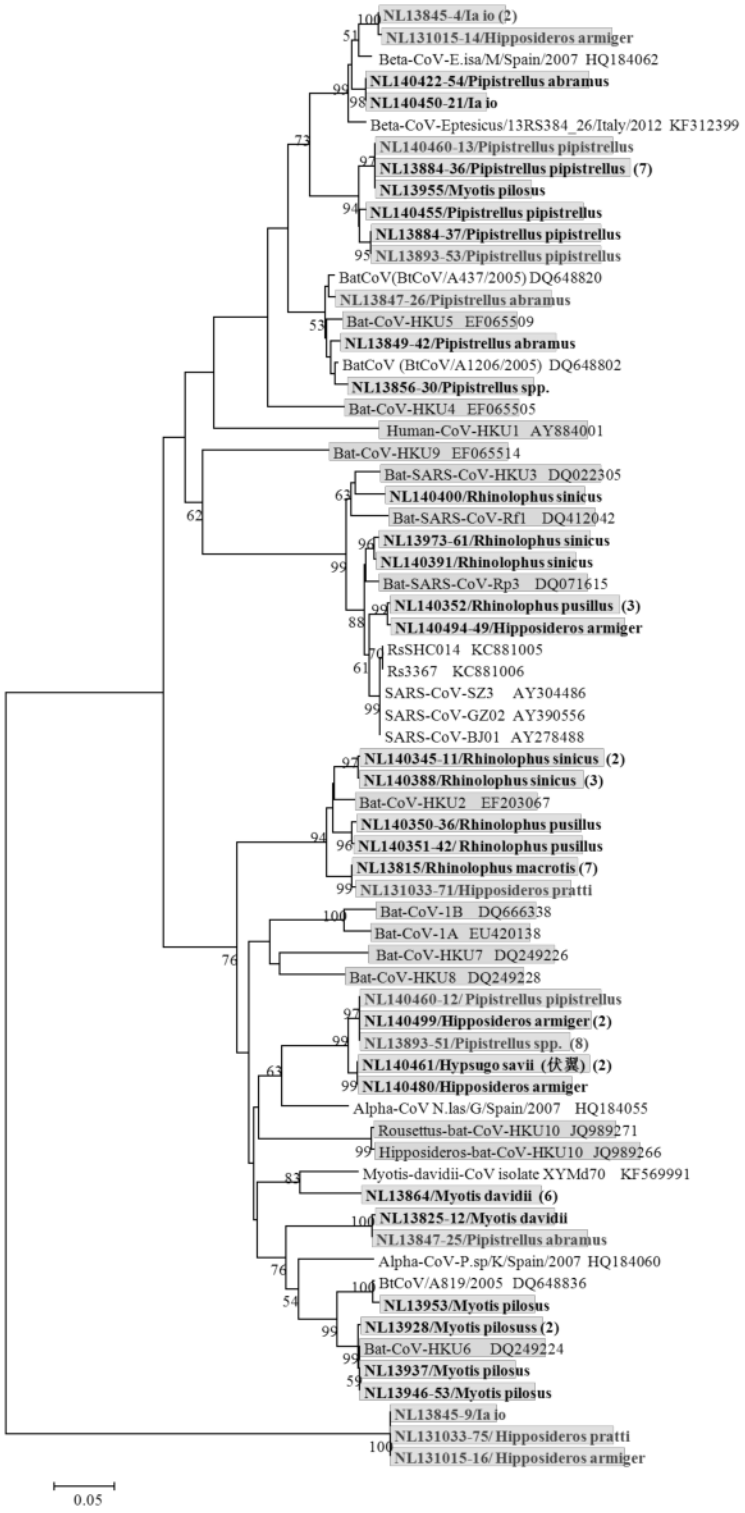
Mar. 2014	Beihai, Guangxi	30	--	--	--	--	--
Apirl 2014	Shenzhen	77	--	--	--	--	--
May 2014	Ruyuan, Guangdong	167	--	--	--	--	--
	Chuxiong, Yunnan	52	52	103	--	8	16
	Jinning, Yunnan	--	--	131	--	--	--
	Mojiang, Yunnan	25	25	103	--	--	3
May-Sep. 2014	Xianning, Hubei	--	--	583	--	--	--
Jun. 2014	Guangdong	77	--	--	--	--	--
Jul. 2014	Hainan	460	--	--	--	--	--
Aug. 2014	Yichang, Hubei	--	--	114	--	--	--
Sep. 2014	Guilin,Guangxi	121	122	--	122	122	--
	Guangdong	335	337	--	335	335	--
Jul.--Sep. 2014	Mojiang, Yunan	--	--	96	--	--	--
Oct. 2014	Jinning, Yunan	13	13	6	3	3	4
	Mojiang, Yunan	34	34	100	1	1	1
	Laos			121			
	Total	1555	583	1357	461	469	24

CoV was detected in 14% (336/2329) samples (**Table 2**). Diverse alphacoronaviruses were identified, including isolates closely related to Bat CoV 1A, 1B, HKU2, HKU6, HKU7, HKU8 and HKU10. Groups of novel alphacoronaviruses were discovered in a variety of bat species (**Fig 3**). **Novel SARS-like coronaviruses were detected in**

***Rhinolophus* bats collected in different regions of Guangdong province.** Diverse novel betacoronaviruses related to HKU5 were detected in *Pipistrellus* bats and *Ia io* in Guangdong and in *Aselliscus stoliczkanus* in Mengla, Yunnan. Novel coronaviruses related to HKU9 were found in *Cynopterus sphinx* and *Rousettus leschenaulti* in Mengla (**Fig 3A**). In addition, sequences significantly divergent to other CoV were obtained from three samples of *Ia io* and *Hipposideros* bats.



B


NL13845-4/Ia io (2)
NL131015-14/Hipposideros armiger
Beta-CoV-E.isa/M/Spain/2007 HQ184062
NL140422-54/Pipistrellus abramus
NL140450-21/Ia io
Beta-CoV-Eptesicus/13RS384_26/Italy/2012 KF312399
NL140460-13/Pipistrellus pipistrellus
NL13884-36/Pipistrellus pipistrellus (7)
NL13955/Myotis pilosus
NL140455/Pipistrellus pipistrellus
NL13884-37/Pipistrellus pipistrellus
NL13893-53/Pipistrellus pipistrellus
BatCoV(BtCoV/A437/2005) DQ648820
NL13847-26/Pipistrellus abramus
Bat-CoV-HKU5 EF065509
NL13849-42/Pipistrellus abramus
BatCoV (BtCoV/A1206/2005) DQ648802
NL13856-30/Pipistrellus spp.
Bat-CoV-HKU4 EF065505
Human-CoV-HKU1 AY884001
Bat-CoV-HKU9 EF065514
Bat-SARS-CoV-HKU3 DQ022305
NL140400/Rhinolophus sinicus
Bat-SARS-CoV-Rf1 DQ412042
NL13973-61/Rhinolophus sinicus
NL140391/Rhinolophus sinicus
Bat-SARS-CoV-Rp3 DQ071615
NL140352/Rhinolophus pusillus (3)
NL140494-49/Hipposideros armiger
RsSHC014 KC881005
Rs3367 KC881006
SARS-CoV-SZ3 AY304486
SARS-CoV-GZ02 AY390556
SARS-CoV-BJ01 AY278488
NL140345-11/Rhinolophus sinicus (2)
NL140388/Rhinolophus sinicus (3)
Bat-CoV-HKU2 EF203067
NL140350-36/Rhinolophus pusillus
NL140351-42/ Rhinolophus pusillus
NL13815/Rhinolophus macrotis (7)
NL131033-71/Hipposideros pratti
Bat-CoV-1B DQ666338
Bat-CoV-1A EU420138
Bat-CoV-HKU7 DQ249226
Bat-CoV-HKU8 DQ249228
NL140460-12/ Pipistrellus pipistrellus
NL140499/Hipposideros armiger (2)
NL13893-51/Pipistrellus spp. (8)
NL140461/Hypsugo savii (伏翼) (2)
NL140480/Hipposideros armiger
Alpha-CoV N.las/G/Spain/2007 HQ184055
Rousettus-bat-CoV-HKU10 JQ989271
Hipposideros-bat-CoV-HKU10 JQ989266
Myotis-davidii-CoV isolate XYMd70 KF569991
NL13864/Myotis davidii (6)
NL13825-12/Myotis davidii
NL13847-25/Pipistrellus abramus
Alpha-CoV-P.sp/K/Spain/2007 HQ184060
BtCoV/A819/2005 DQ648836
NL13953/Myotis pilosus
NL13928/Myotis pilosuss (2)
Bat-CoV-HKU6 DQ249224
NL13937/Myotis pilosus
NL13946-53/Myotis pilosus
NL13845-9/Ia io
NL131033-75/ Hipposideros pratti
NL131015-16/ Hipposideros armiger
0.05

C

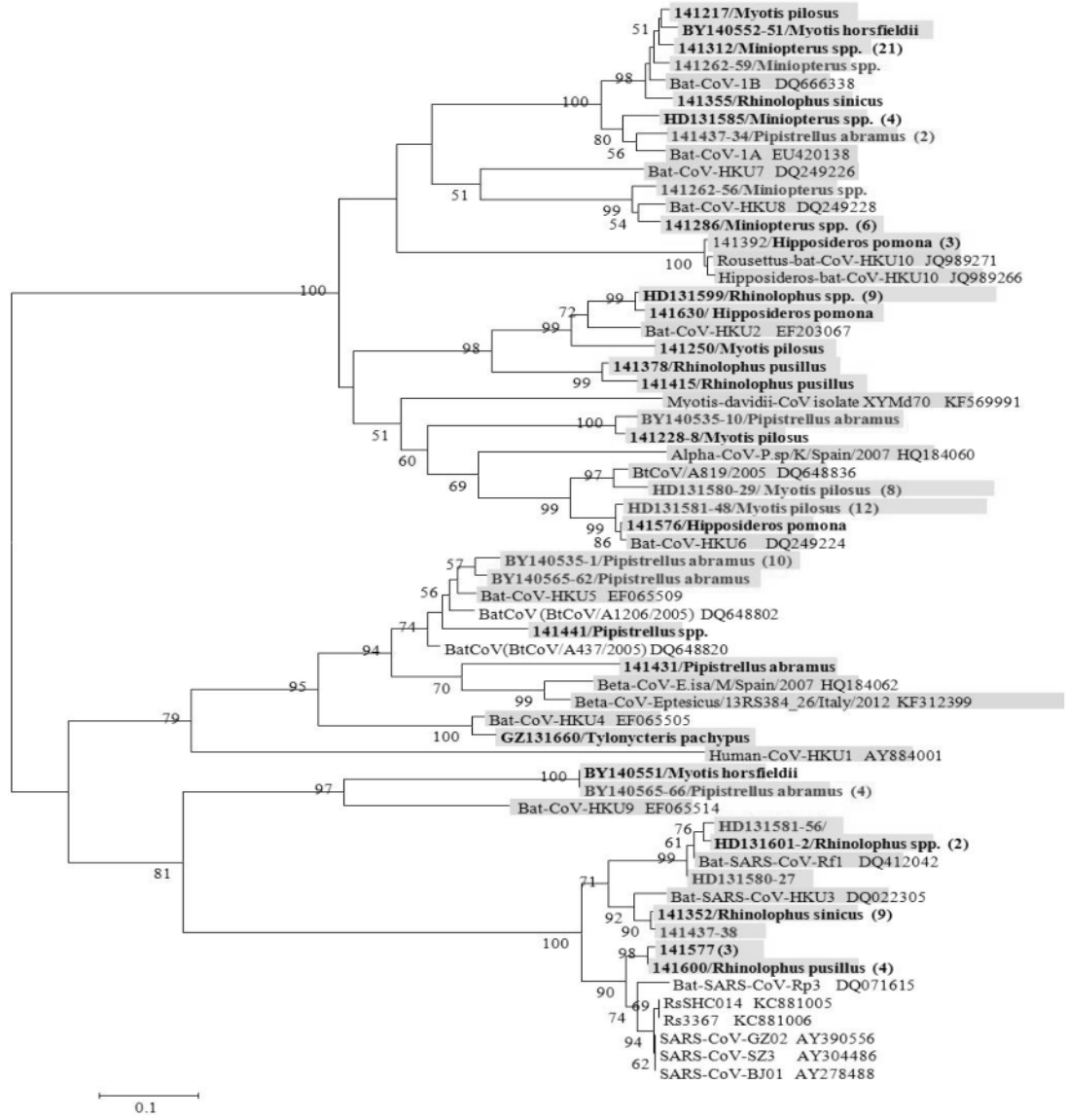


Figure 3: Phylogenetic analysis of partial RdRp gene of CoV. CoVs identified in this study are in bold and named by the sample numbers. Sequence amplified from samples co-infected with two CoV strains are indicated in red. (A) CoVs detected in Mengla, Yunnan. (B) CoVs detected in Ruyuan, Guangdong. (C) CoVs detected in other regions in Guangdong.

2) Complete S gene sequencing and recombination analysis of novel SARS-like CoV

We amplified the full-length S gene of the novel SL-CoV detected in a *Rhinolophus sinicus* colony in Yunnan Province. In addition to our previously reported Rs3367 and RsSHC014, we now have 24 new full-length S gene sequences from 22 samples. Phylogenetic analysis showed that these SL-CoV are diverse, **and identified two strains of novel SL-CoV more closely related to SARS-CoV than Rs3367** (**Fig 4A**). Our new strains named Rs4841 and Rs4874 share the highest homology to SARS-CoV than any other known SL-CoV, including those we published previously in *Nature*. These viruses are highly similar to SARS-CoV in receptor-binding domain (RBD) sequence but also in N-terminal domain (NTD) (**Figure 4B**). Analysis of the complete S protein shows > 97% amino acid identify to that of SARS-CoV isolates.

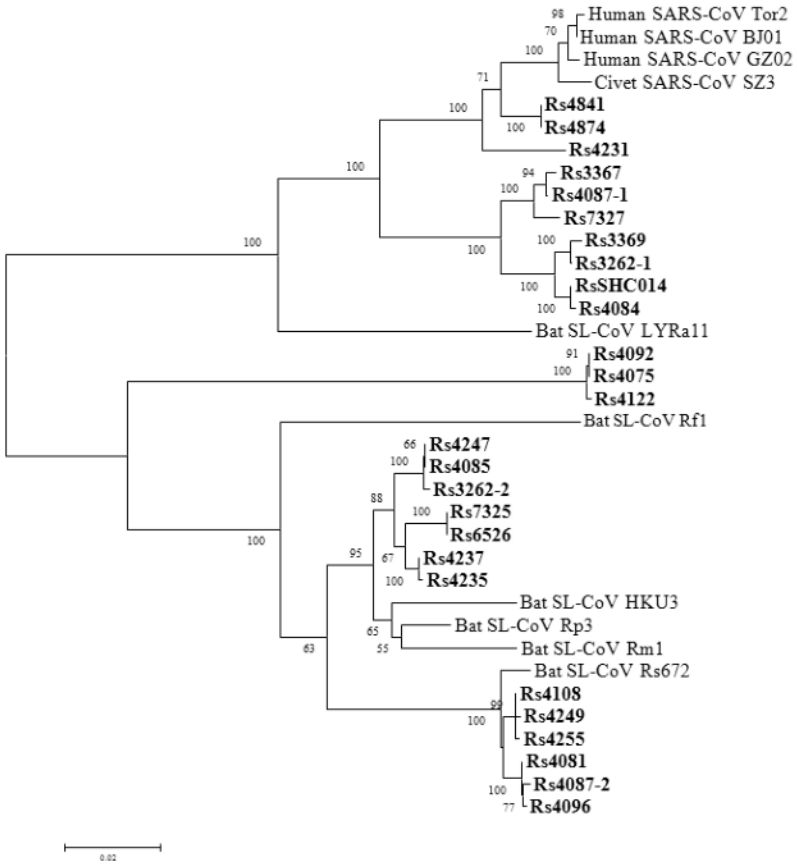


Figure 4A Phylogenetic analysis of novel SL-CoVs discovered in Year 1 of this project (**Bold**), based on amino acid sequences of complete S gene.

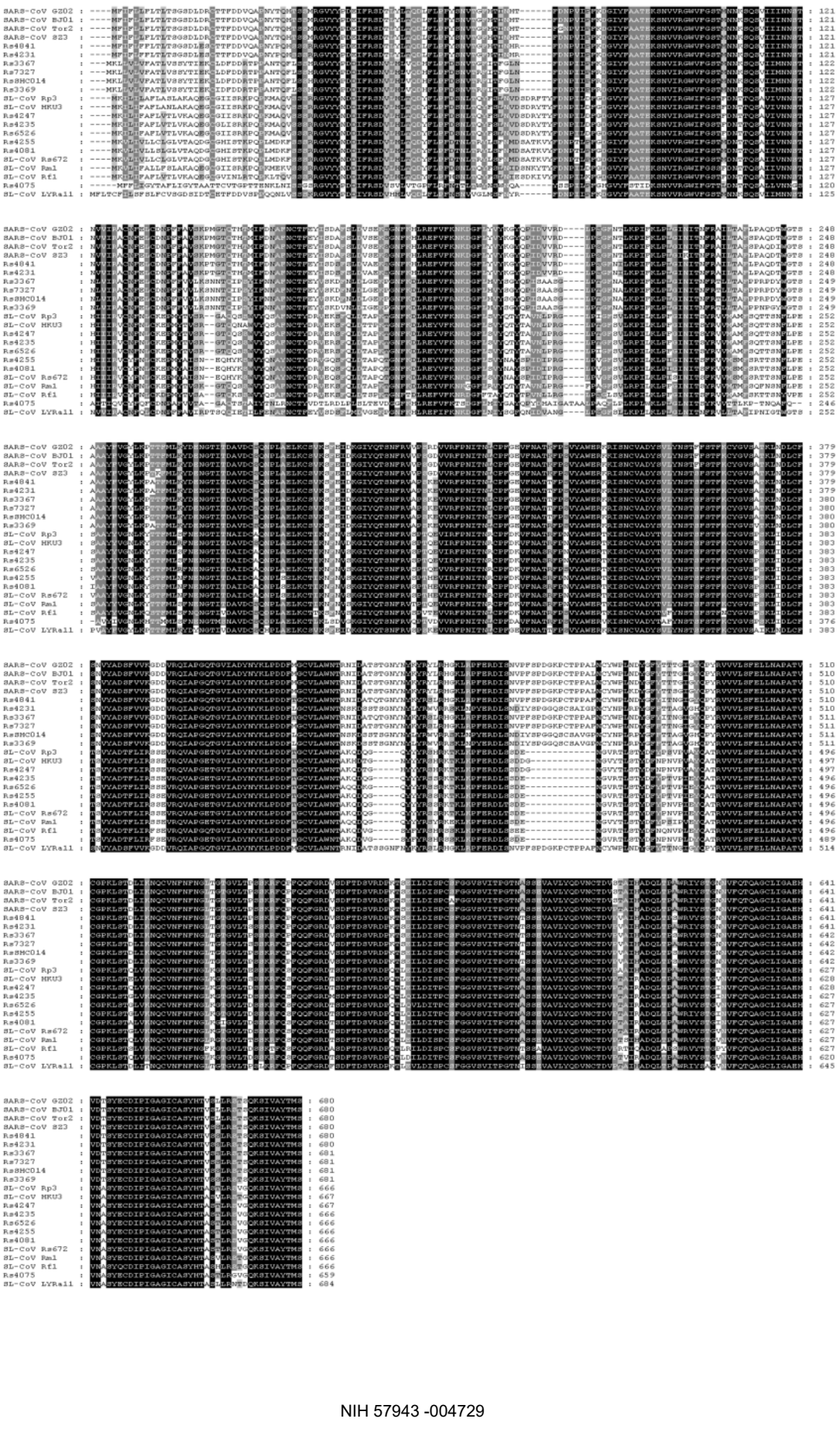


Figure 4B Alignment of amino acid sequences of S1 (aa1-680) of SARS-CoV and bat SL-CoVs.

We performed recombination analysis and detected potential recombination events in S genes of multiple SL-CoV strains suggesting that that the region around nt1000 in RBD is a recombination hotspot. In addition, a novel SL-CoV strain (Rs4075) with an NTD sequence distinct from all other SL-CoVs was identified (**Figure 4**). The results suggest that the high genetic diversity of SL-CoV in this colony is related to the frequent recombination.

3) Virus isolation and characterization

Isolation on Vero E6 cells was conducted on all CoV PCR-positive samples using an optimized protocol. Repoducible CPE was observed for Rs4841 (the strain closely related to SARS-CoV in both the RBD and NTD region of the S protein). Purified virions displayed typical coronavirus morphology under electron microscopy, and this novel isolate was named SL-CoV-WIV16.

We conducted virus infectivity studies (using HeLa cells expressing or not expressing ACE2 from humans, civets or Chinese horseshoe bats) to determine whether SL-CoV-WIV16 can use ACE2 as a cellular entry receptor **(Figure 5).** We found that WIV16 is able to use ACE2 of different origins as an entry receptor.

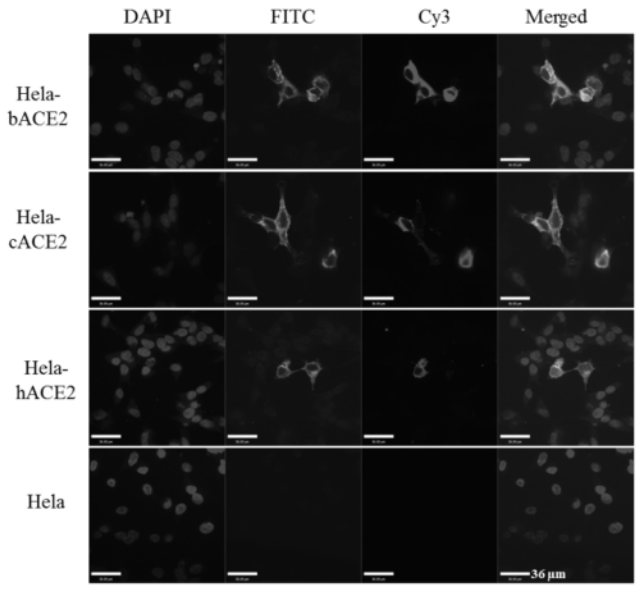


Figure 5. Analysis of receptor usage of SL-WIV16 determined by immunofluorescence assay. Determination of virus infectivity in Hela cells without the expression of ACE2. b, bat; c, civet; h, human. Nuclei are stained with DAPI. The columns (from left to right) show staining of nuclei (blue), ACE2 expression (green), virus replication (red) and merged triple-stained images.

To assess its cross-species transmission potential, we conducted infectivity assays in cell lines from a range of species. Our results (**Figure 6)** show that SL-CoV-WIV16 can grow in human alveolar basal epithelial (A549), pig kidney-15 (PK15), *Rhinolophus sinicus* kidney (RSKT), *Macaca mulatta* Kidney cell lines (MK2) and human lung carcinoma (NCI-H292), but not in human cervix (HeLa), Syrian golden hamster kidney (BHK21), *Myotis davidii* kidney (BK), *Myotis davidii* intestine (MDI), *Rousettus leschenaulti* kidney (RLK), *Rhinolophus sinicus* brain (RSBT), *Rhinolophus sinicus* heart (RSHT), *Rhinolophus sinicus* Lung (RSLuT), *Rhinolophus sinicus* intestine (RSI) or *Pteropus alecto* kidney (PaKi) lines.

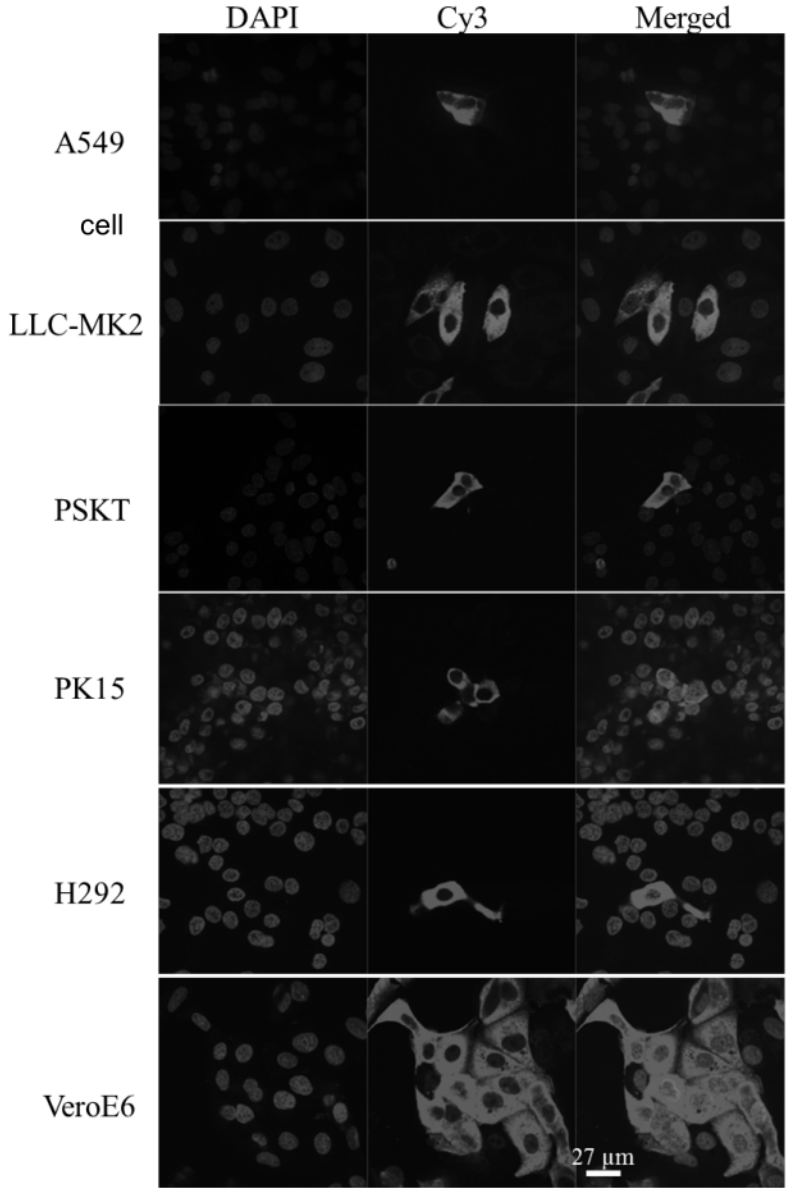


Figure 6 Cell infection with SL-CoV WIV16 determined by immunofluorescence assay with antibody against SARS-like coronavirus nucleocapsid protein. The columns (from left to right) show staining of nuclei (blue), virus replication (red) and merged double-stained images.

B3: Competitive revisions, supplements?

N/A

B4: Opportunities for training/professional development?

In year 1 of this work, we have trained undergraduate interns from Columbia University in modeling approaches to understand bat risk of harboring zoonotic CoVs. In the behavioral risk work, we used standardized training materials for all three qualitative behavioral risk data collection methodologies have been created. Materials were used to train six people in New York City and 12 people in Yunnan, China, of which 11 were from three different administrative levels of local government Centers for Disease Control (CDC). The trainees include the Chinese EcoHealth Alliance Field Coordinator, six workers from Xiangyun County CDC (4 women, 2 men), two from Yunnan Institute for Endemic Diseases (Yunnan Provincial CDC; 2 men), and three from Lu Feng village CDC (3 men).

B5: How have the results been disseminated?

1) *Conference and University lectures*
   - *PI Daszak, and Co-investigators Olival and Shi gave >10 invited University lectures that included specific discussion of the current project and results.*

2) *Agency and other USG briefings*
   - NRC, 2015: Invited speaker, IOM Forum on public health preparedness, Interagency meeting on Medical Countermeasures. PI Daszak specifically reported on the findings from Year 1 of this project and the risk of SARS-like viruses causing future pandemics
   - World Health Summit, Berlin 2014: PI Daszak was an invited panelist at a session on pandemic risk, and specifically reported the results and aims of this project
   - International bat virus conference, Colorado, 2014: PI Daszak and Co-investigator Olival presented results from this study
   - National Academies, Division of Earth & Life Studies, Spring Advisory Committee Meeting, DC. PI Daszak presented results from this study as part of an invited talk.
   - Consortium of Universities for Global Health Conf., Washington DC, 2014. PI Daszak presented data from this study in a session on disease ecology

3) *Public outreach*
   - PI Daszak reported on this project at an EcoHealth Alliance meeting hosted by the Cosmos Club, 2014

B6: Plan for next reporting period

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces. Early in Year 2 of the study, it is anticipated that all of the qualitative research (i.e, 5-7 focus groups and ~100 ethnographic interviews) will be completed, transcribed and translated. It is anticipated that a total of approximately 100 ethnographic interviews and five to seven focus groups will be conducted in targeted areas with known bat populations in Yunnan, Guangxi, Guangdong and Fujian over the next few months. At least one of the focus groups and an estimated 35-40% of the interviews and surveys will be conducted with women. Subjects will be enrolled in this study without regard to ethnicity.

Preliminary analyses will be conducted and will focus on the factors least understood, but crucial to the development of a behavioral risk survey that captures relevant behaviors and practices. Factors include specific human-animal interactions, experiences of unusual illness in both humans and animals, and an assessment of the context within which these activities occur. Because of the unique dataset and the expected richness of the data, additional research questions will be developed and explored using grounded theory, as well as more recently developed methods such as narrative analysis and case oriented understanding.

Results from preliminary analyses will contribute to the development of the behavioral risk survey. A behavioral survey sampling frame and recruitment materials are currently being developed. After pilot testing the behavioral survey, we will begin concurrent biologic specimen collection from bats, other wildlife and humans to compare circulating CoV strains in the bat population with serological exposure in human populations. The behavioral risk survey will facilitate the identification of explicit behavioral risks and practices that are found among study participants seropositive for SARS-like corona virus. These findings will be used to develop better risk mitigation policies and targeted intervention strategies.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk.

Future steps to optimize the model of role of species diversity in CoV emergence risk will include:

1. Parameterizing with actual data on species diversity and abundance of animals from Southern China markets.
2. Parameterizing with species-specific data on CoV prevalence and strain variation in different bat species from field surveillance, e.g. if Rhinolopus spp. represent the highest risk for SARS-related CoV emergence, these species will be given a higher weight.
3. Incorporation of CoV lineage specific probabilities for inter-host spillover based on receptor binding data.

We will also conduct further modeling activities, including:

1. Comparative cophylogenetic analyses of bat host and CoV RdRp and Spike gene phylogenies, to assess patterns of evolutionary congruence and frequency of cross-species transmission.
    a. Using previously published data from literature and Genbank
    b. Using sequence data from our S. China surveillance
2. Calculate CoV divergence times using Spike RBD sequences for S. China.

3. Construct initial generalized linear mixed model to predict CoV diversity using S. China data and bat host-specific trait data. Update model regularly with new data from CoV screening in different bat species.

Specific Aim 3: Testing predictions of CoV inter-species transmission.

The following experiments will be undertaken in Year 2:

1. *Animal infection experiment with SARS-like CoV*
   Option 1. Virus infection through ACE2 humanized mouse. Human ACE2 promotor (9-10 kb) and ACE2 will be inserted into a expressing vector and sent to a commercial company to generate transgenic mice. The stably expressed human ACE2 mice will be used for virus infection.
   Option 2. Virus infection through SARS-CoV susceptible animals such as ferrets.
   All above animal infection experiment will be performed under the containment of BSL3.
2. Continued surveillances of SARS-like CoVs in Yunnan and Guangdong provinces and isolation of novel virus strains.
3. Surveillance of infection in human populations by SARS-like CoVs. This work will be performed at two locations, one each in Yunnan and Guangdong provinces. PCR and ELISA will be used, respectively, for detection of viral replicase gene and antibody against the viral nucleocapsid protein.

Publications

Publications from Year 1:

1 Young, C. C. & **Olival, K. J.** Optimizing Viral Discovery in Bats. *Plos One*, (In Review).

2 Brierley, L., Vonhof, M., Jones, K. E., Olival, K. J. & **Daszak, P**. Quantifying global drivers of zoonotic bat viruses: a process-based perspective. *The American Naturalist*, (In Review).

3 **Olival, K. J.,** Weekley, C. C. & **Daszak, P.** Are bats really “special” as viral reservoirs?: What we know and need to know. in *Bats and Viruses: From Pathogen Discovery to Host Genomics* (ed Linfa Wang) (John Wiley & Sons, Inc., In Press).

From: Peter Daszak
Sent: Thu, 23 Apr 2015 15:00:32 +0000
To: Pone, Laura (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Aleksei Chmura
Subject: RE: Grant Number: 5R01AI110964 - 02 PI Name: DASZAK , PETER

Dear Laura,

I just want to thank you very much for allowing us this extra time! I'll make sure the report is finalized, uploaded and approved by our SRO before May 1st. This extra timing will allow us to include some very exciting new findings from China that are very recent, so it is much appreciated.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

From: Pone, Laura (NIH/NIAID) [E] (b)(6)
Sent: Thursday, April 23, 2015 10:37 AM
To: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]; Aleksei Chmura
Subject: RE: Grant Number: 5R01AI110964 - 02 PI Name: DASZAK , PETER

Dr. Daszak,

We our having a system shutdown and must award this grant before May 15th so please provide the RPPR **no later than May 1st.** If not received by that date the award may go out a month late and be adjusted for time and funding.

Thank you,

Laura Pone
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)
please consider the environment before printing this e-mail.

Effective October 1, 2014, NIH closeout policy has changed (see NOT-OD-14-084). In order to avoid unilateral closeout, final reports must be submitted in a timely manner. Failure to submit accurate final reports could result in enforcement actions such as revisions to NOA funding levels, or delay in future funding.

From: Peter Daszak (b)(6)
Sent: Wednesday, April 22, 2015 1:54 PM
To: Pone, Laura (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Aleksei Chmura
Subject: RE: Grant Number: 5R01AI110964 - 02 PI Name: DASZAK , PETER

Dear Laura and Erik,

I just want to check if you've read my email below and are able to grant me the 2 week extension with a revised due date of May 5th.

Again, apologies for this delay, due to my visit with our collaborators in China, and the need to include a lot of new information from their work on this award.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor

New York, NY 10001

(b)(6) (direct)
+1.212.380.4465 (fax)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

From: Peter Daszak
Sent: Monday, April 20, 2015 2:45 PM
To: 'Pone, Laura (NIH/NIAID) [E]'; Stemmy, Erik (NIH/NIAID) [E]; Aleksei Chmura
Subject: RE: Grant Number: 5R01AI110964 - 02 PI Name: DASZAK , PETER

Dear Laura and Erik,

I'm emailing to respectfully request a 2-week extension to this report. I've just returned from a long trip to China, working on the project and completing analyses with our collaborators on the grant.

I would like to use the extension to draft a complete, up-to-date report, with the plan of having this uploaded before 9am on the 5th May.

Please advise if this is possible, and I do appreciate your support so that I can make this report far more valuable and useful to you.

Cheers,

Peter

Peter Daszak
President

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From: Pone, Laura (NIH/NIAID) [E] (b)(6)
Sent: Monday, April 20, 2015 2:09 PM
To: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]; Aleksei Chmura
Subject: RE: Grant Number: 5R01AI110964 - 02 PI Name: DASZAK , PETER
Importance: High

Mr. Stemmy,

This is a friendly reminder that the above referenced progress report is delinquent. Per the email below **please submit no later than tomorrow.**

Thank you,

Laura Pone
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)
please consider the environment before printing this e-mail.

Effective October 1, 2014, NIH closeout policy has changed (see NOT-OD-14-084). In order to avoid unilateral closeout, final reports must be submitted in a timely manner. Failure to submit accurate final reports could result in enforcement actions such as revisions to NOA funding levels, or delay in future funding.

From: Pone, Laura (NIH/NIAID) [E]
Sent: Thursday, April 16, 2015 10:23 AM
To: (b)(6) Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
Subject: Grant Number: 5R01AI110964 - 02 PI Name: DASZAK , PETER

Good Morning,

Per NIH Grants Policy, noncompeting continuation progress reports must be submitted directly to the NIH Institute 45 days before the beginning date of the next budget period, unless instructed otherwise. The progress report for the non-competing continuation grant referenced-above was due at the NIAID on or before April 15th. As of the date of this e-mail, the progress report has **not yet been received**.

If you are unable to submit this progress report within three (3) working days, please reply to this message, explaining the reason for the delay and state the date by which NIAID will receive the

delinquent report. If you have already submitted the progress report or have any questions, please reply to this message.

Thank you in advance, for your prompt attention to this matter.

Please let me know if you have any questions.

Sincerely,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
5601 Fishers Lane, Room 4E29, MSC 9824
Bethesda, MD 20892-9824
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

From: Peter Daszak
Sent: Fri, 27 Mar 2015 17:58:16 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura; Alison Andre
Subject: RE: Speaking at a joint NAS/IOM meeting about the corona virus work

Great - will do Erik. The session went very well. I gave a plug for the NIAID funding, and presented results, with the basic message that some of these bat SL-CoVs are so similar to SARS-CoV that the pandemic risk is clear and present. Fred Cassells was on the panel and the room had some high impact people present, including Richard Hatchett, head of BARDA.

I'll set up time to visit with you next time I'm in DC, or if not, will give you an update at the ATS.

Cheers,

Peter

Peter Daszak
President

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New York, NY 10001

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-----Original Message-----
From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Friday, March 27, 2015 9:48 AM
To: Peter Daszak
Cc: Aleksei Chmura; Alison Andre
Subject: RE: Speaking at a joint NAS/IOM meeting about the corona virus work

Hi Peter,
That's great that you'll be presenting at the IOM! Unfortunately I wasn't able to make it over there for this meeting. Let me know the next time you're in DC and maybe we can have you stop by and give a short talk. Otherwise we'll catch up at ATS in May. I'm looking forward to that meeting as well!

Best,
Erik

-----Original Message-----

From: Peter Daszak (b)(6)
Sent: Thursday, March 26, 2015 1:33 PM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura; Alison Andre
Subject: Speaking at a joint NAS/IOM meeting about the corona virus work

Hi Erik,

Just wanted to let you know that I'm part of a panel tomorrow at the NAS on coronaviruses and will be giving them a brief update on our CoV work that you're funding. Things are going well on the work. We have a team set up in China doing human behavioral risk work and in going out again in a couple of weeks to keep that moving. We've also got some interesting new CoVs from bats there that are even closer to SARS. I look forward to giving you a proper update, perhaps when I'm next in dc or at the latest at the ATS meeting in May.

Cheers,

Peter

Peter Daszak
(Sent from my iPhone)

President
EcoHealth Alliance

460 West 34th Street, New York, NY10001, USA

www.EcoHealthAlliance.org

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Tue, 3 Feb 2015 18:08:40 +0000
To: 'Peter Daszak'
Subject: RE: EHA event at the Cosmos Club in DC tonight

Hi Peter,
Thanks very much for the invite, but I can't make it tonight. Look forward to catching up soon, though!

Erik

From: Peter Daszak (b)(6)
Sent: Tuesday, February 03, 2015 12:51 PM
To: Repik, Patricia (NIH/NIAID) [E]; Cassetti, Cristina (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]
Cc: Alison Andre; Blakely Larrabee; Anthony Ramos
Subject: RE: EHA event at the Cosmos Club in DC tonight
Importance: High

Here is the original invite, and the title of Dennis' talk will be:

"Emerging Pandemic Threats in the Age of Ebola"

You're Invited

Dr. Peter Daszak and the scientists of EcoHealth Alliance cordially invite you a special presentation.

Emerging Pandemic Threats:

A Report on the PREDICT program

The Emerging Pandemic Threats (EPT) program strengthens capacities in developing countries to prevent, detect, and control infectious diseases in animals and

When

Tuesday,
February 3,2015
at 6:00 pm

Where

The Cosmos Club
2121
Massachusetts
Avenue NW
Washington, DC

people with an emphasis on early identification of, and response to, dangerous pathogens from animals before they can become significant threats to human health.

Presenters:

Dr. Peter Daszak
President and Disease Ecologist,
EcoHealth Alliance

Special Guest:

Dr. Dennis Carroll
Special Representative for Global Health Security
Director, Global Health Security and Development
Bureau for Global Health
U.S. Agency for International Development

Cocktail Reception
6:00 pm to 7:00 pm

Panel Discussion
7:00 pm to 8:00 pm

Q&A will follow discussion

****Jacket and tie required for gentlemen***

RSVP NOW

Map & Directions

RSVP

Please RSVP by February 2, 2015 to Blakely Larrabee at (b)(6) (b)(6) or email RSVP@EcoHealthAlliance.org

About EcoHealth Alliance

Building on over 40 years of groundbreaking science, EcoHealth Alliance is a global, nonprofit organization dedicated to protecting wildlife and safeguarding human health from the emergence of disease. The organization develops ways to combat the effects of damaged ecosystems on human and wildlife health. Using environmental and health data covering the past 60 years, EcoHealth Alliance

Please RSVP by Monday, February 2, 2015 by contacting Blakely Larrabee at (b)(6) or email RSVP@EcoHealthAlliance.org

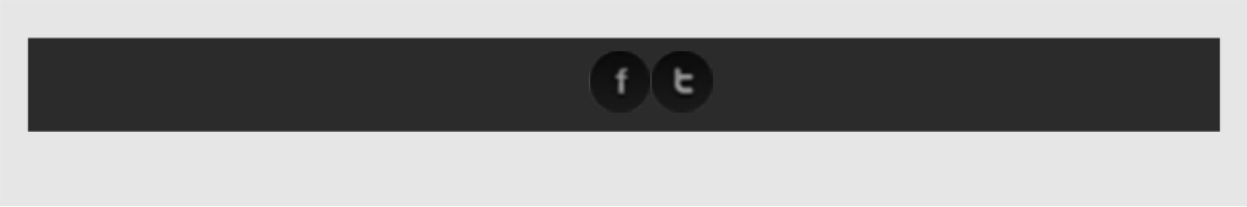
scientists created the first-ever, global disease hotspots map that identified at-risk regions, to help predict and prevent the next pandemic crisis. That work is the foundation of EcoHealth Alliance's rigorous, science-based approach, focused at the intersection of the environment, health and capacity building. Working in the U.S. and more than 20 countries worldwide, EcoHealth Alliance's strength is founded on innovations in research, training, global partnerships and policy initiatives.

TO LEARN MORE ABOUT ECOHEALTH ALLIANCE PLEASE VISIT www.EcoHealthAlliance.org

EcoHealth Alliance
460 West 34th Street - 17th Floor
New York, NY 10001-2320

212.380.4460 contact privacy

Local conservation. Global health.



Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
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From: Peter Daszak
Sent: Tuesday, February 3, 2015 12:44 PM
To: Pat Repik; (b)(6) Stemmy, Erik (NIH/NIAID) [E]
Cc: Alison Andre; Blakely Larrabee; Anthony Ramos
Subject: EHA event at the Cosmos Club in DC tonight
Importance: High

Dear Pat, Cristina and Erik,

Not sure if you get these invites, but just want you to know that we're hosting a public lecture at the Cosmos Club tonight (starts 6pm ish). Dennis Carroll will be talking about Ebola and USAID's EPT-2 program, and there will be cheese and cocktails beforehand. Come along if you can – we usually get a good group of people from diverse agencies in DC and it would be great to see you and catch up.

Cheers,

Peter

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From: Repik, Patricia (NIH/NIAID) [E]
Sent: Tue, 3 Feb 2015 13:01:28 -0500
To: Cassetti, Cristina (NIH/NIAID) [E]; 'Peter Daszak'; Stemmy, Erik (NIH/NIAID) [E]
Cc: Alison Andre; Blakely Larrabee; Anthony Ramos
Subject: RE: EHA event at the Cosmos Club in DC tonight

Hi Peter,
I will be attending so will see you then.

Pat

From: Cassetti, Cristina (NIH/NIAID) [E]
Sent: Tuesday, February 03, 2015 12:58 PM
To: 'Peter Daszak'; Repik, Patricia (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]
Cc: Alison Andre; Blakely Larrabee; Anthony Ramos
Subject: RE: EHA event at the Cosmos Club in DC tonight

Thank you Peter. I would love to attend but I have another commitment tonight.
I hope to catch up with you soon.
Cheers,
Cristina

From: Peter Daszak (b)(6)
Sent: Tuesday, February 03, 2015 12:51 PM
To: Repik, Patricia (NIH/NIAID) [E]; Cassetti, Cristina (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]
Cc: Alison Andre; Blakely Larrabee; Anthony Ramos
Subject: RE: EHA event at the Cosmos Club in DC tonight
Importance: High

Here is the original invite, and the title of Dennis' talk will be:

"Emerging Pandemic Threats in the Age of Ebola"

EcoHealth Alliance

You're Invited

Dr. Peter Daszak and the scientists of EcoHealth Alliance cordially invite you a special presentation.

When

Emerging Pandemic Threats:

A Report on the PREDICT program

The Emerging Pandemic Threats (EPT) program strengthens capacities in developing countries to prevent, detect, and control infectious diseases in animals and people with an emphasis on early identification of, and response to, dangerous pathogens from animals before they can become significant threats to human health.

[redacted]

Presenters:

Dr. Peter Daszak
President and Disease Ecologist,
EcoHealth Alliance

Special Guest:

Dr. Dennis Carroll
Special Representative for Global Health Security
Director, Global Health Security and Development
Bureau for Global Health
U.S. Agency for International Development

Cocktail Reception
6:00 pm to 7:00 pm

Panel Discussion

Tuesday, February 3,2015 at 6:00 pm

Where

The Cosmos Club
2121 Massachusetts Avenue NW
Washington, DC
Map & Directions

RSVP

Please RSVP by February 2, 2015 to Blakely Larrabee at (b)(6) **or email RSVP@EcoHealthAlliance.org**

[redacted]

About EcoHealth Alliance

Building on over 40 years of groundbreaking science, EcoHealth Alliance is a global, nonprofit organization dedicated to protecting wildlife and safeguarding human health from the emergence of disease. The organization develops ways to combat the effects of damaged ecosystems on human and wildlife health. Using environmental and health

7:00 pm to 8:00 pm

Q&A will follow discussion

**Jacket and tie required for gentlemen*

RSVP NOW

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data covering the past 60 years, EcoHealth Alliance scientists created the first-ever, global disease hotspots map that identified at-risk regions, to help predict and prevent the next pandemic crisis. That work is the foundation of EcoHealth Alliance's rigorous, science-based approach, focused at the intersection of the environment, health and capacity building. Working in the U.S. and more than 20 countries worldwide, EcoHealth Alliance's strength is founded on innovations in research, training, global partnerships and policy initiatives.

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212.380.4460 contact privacy

Local conservation. Global health.

Cheers,

Peter

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From: Peter Daszak
Sent: Tuesday, February 3, 2015 12:44 PM
To: Pat Repik; (b)(6) Stemmy, Erik (NIH/NIAID) [E]
Cc: Alison Andre; Blakely Larrabee; Anthony Ramos
Subject: EHA event at the Cosmos Club in DC tonight
Importance: High

Dear Pat, Cristina and Erik,

Not sure if you get these invites, but just want you to know that we're hosting a public lecture at the Cosmos Club tonight (starts 6pm ish). Dennis Carroll will be talking about Ebola and USAID's EPT-2 program, and there will be cheese and cocktails beforehand. Come along if you can – we usually get a good group of people from diverse agencies in DC and it would be great to see you and catch up.

Cheers,

Peter

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From: Kevin Olival, PhD
Sent: Wed, 29 Oct 2014 16:11:04 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Summary Statement available for grant (b)(4); (b)(6)

Thanks Erik, and thanks for your time today.

Kevin

On Oct 29, 2014, at 11:52 AM, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Hi Kevin,
It was nice speaking with you this morning. Please find below a link to NIH's revised resubmission policy. Let me know if you have any other questions.

Good luck making your revisions!
Erik

http://grants.nih.gov/grants/policy/amendedapps.htm

Please note my updated contact information below:
Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-7630
Phone: (b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Kevin Olival, PhD (b)(6)
Sent: Thursday, October 23, 2014 12:53 PM
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Summary Statement available for grant (b)(4); (b)(6)

No problem, I'm free at 11am.

Cheers,
Kevin

On Oct 23, 2014, at 12:48 PM, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Hi Kevin,
Looks like I spoke too soon… Could we bump the call to 11am on 10/29 instead? I've had something come up from 9:30-10:30 that day.

Erik

From: Kevin Olival, PhD (b)(6)
Sent: Thursday, October 23, 2014 9:52 AM
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Summary Statement available for grant (b)(4); (b)(6)

Erik,

Great. That works. I can call your direct line then.

(b)(4); (b)(6) EHA now has a federal wide assurance number and IRB of record, and I've completed human subjects training (b)(4); (b)(6) (b)(4); (b)(6) We have new current funding to update (our R01 on CoVs in China now funded) (b)(4); (b)(6) (b)(4); (b)(6) (b)(4); (b)(6) but we can discuss further on Weds.

Thank you,
Kevin

On Oct 23, 2014, at 8:24 AM, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Hi Kevin,
Let's plan for Wednesday 10/29 at 10am. Does that work for you?

Erik

From: Kevin Olival, PhD (b)(6)
Sent: Tuesday, October 21, 2014 2:06 PM
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Summary Statement available for grant (b)(4); (b)(6)

Thanks Erik.

If anytime this weeks works for you, I can easily be reached on my mobile, just need to confirm the time. Otherwise next week Weds would be great.

Cheers,
Kevin

Kevin J. Olival, PhD

Senior Research Scientist

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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On Oct 21, 2014, at 1:20 PM, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Hi Kevin,
Thanks for your message. I've been a bit swamped this week with some other issues, so my apologies for missing you. (b)(4); (b)(6)
(b)(4); (b)(6) Let's set up a time to talk once you're back in the office next week. Tuesday I'll be attending an offsite meeting, but I can chat anytime on Wednesday before noon. Please let me know if that works for you.

Best,
Erik

From: Kevin Olival, PhD (b)(6)
Sent: Monday, October 20, 2014 11:47 AM
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: Fwd: Summary Statement available for grant (b)(4); (b)(6)

Dear Erik,

Please let me know if you have any time today or tomorrow to discuss my application and next steps. I'm in meetings today until about 130pm, but otherwise can make myself available.

Best,
Kevin

Kevin J. Olival, PhD

Senior Research Scientist

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Begin forwarded message:

From: <era-notify@mail.nih.gov>
Subject: Summary Statement available for grant (b)(4); (b)(6)
Date: October 18, 2014 at 10:09:04 PM EDT
To: (b)(6)
Reply-To: <eraNotifications@mail.nih.gov>

Dr. Kevin Olival,

The summary statement for the grant you recently submitted to NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES, (b)(4); (b)(6) (b)(4); (b)(6) is now available in the eRA Commons. Please use your account ID, (b)(6) and password to access the Commons at https://commons.era.nih.gov/commons/. If you do not remember your password, you can click on the "Forgot Password" link below the "Login" button. Enter your email address in the area provided, click "Submit" and your password will be reset and sent to you via email.

If you are able to log into the eRA Commons and cannot see your summary statement, there are two likely causes. First is that your account has been established to use the Internet Assisted Review (IAR) module, but has not been affiliated with your institution. In this case, contact your Office of Sponsored Research or similar office to have your account affiliated. The other probable cause is that your account has been recently created and is currently in a "Pending" status. In this case, please allow another day or two for your account to become active and then try again.

If you have any questions about this email, please contact your Program Officer, Erik Stemmy at (b)(6) or by phone at (b)(6)

For any further questions about this email, please contact the eRA Help Desk at our preferred method of contact http://ithelpdesk.nih.gov/eRA/ or call 1-866-504-9552 (tty: 301-451-5939) orcommons@od.nih.gov . Please access the Commons at http://public.era.nih.gov/commons/ For more information please visit http://era.nih.gov/

*** This is an automated notification - Please do not reply to this message. ***

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Thu, 23 Oct 2014 16:55:26 +0000
To: 'Kevin Olival, PhD'
Subject: RE: Summary Statement available for grant (b)(4); (b)(6)

Great. I should be working remotely that day so you can reach me on my blackberry: (b)(6)

Thanks!
Erik

From: Kevin Olival, PhD (b)(6)
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(b)(4); (b)(6)

(b)(4); (b)(6) but we can discuss further on Weds.

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Kevin J. Olival, PhD

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Kevin J. Olival, PhD

Senior Research Scientist

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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1.212.380.4465 (fax)
www.ecohealthalliance.org

EcoHealth Alliance integrates innovative science-based solutions and partnerships that increase capacity to achieve two interrelated goals: protecting global health by preventing the outbreak of emerging diseases and safeguarding ecosystems by promoting conservation.

Begin forwarded message:

From: <era-notify@mail.nih.gov>
Subject: Summary Statement available for grant (b)(4); (b)(6)
Date: October 18, 2014 at 10:09:04 PM EDT
To: (b)(6)
Reply-To: <eraNotifications@mail.nih.gov>

Dr. Kevin Olival,

The summary statement for the grant you recently submitted to NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES, (b)(4); (b)(6) (b)(4); (b)(6) is now available in the eRA Commons. Please use your account ID, (b)(6) and password to access the Commons at https://commons.era.nih.gov/commons/. If you do not remember your password, you can click on the "Forgot Password" link below the "Login" button. Enter your email address in the area provided, click "Submit" and your password will be reset and sent to you via email.

If you are able to log into the eRA Commons and cannot see your summary statement, there are two likely causes. First is that your account has been established to use the Internet Assisted Review (IAR) module, but has not been affiliated with your institution. In this case, contact your Office of Sponsored Research or similar office to have your account affiliated. The other probable cause is that your account has been recently created and is currently in a "Pending" status. In this case, please allow another day or two for your account to become active and then try again.

If you have any questions about this email, please contact your Program Officer, Erik Stemmy at (b)(6) or by phone at (b)(6)

For any further questions about this email, please contact the eRA Help Desk at our preferred method of contact http://ithelpdesk.nih.gov/eRA/ or call 1-866-504-9552 (tty: 301-451-5939) orcommons@od.nih.gov . Please access the Commons at http://public.era.nih.gov/commons/ For more information please visit http://era.nih.gov/

*** This is an automated notification - Please do not reply to this message. ***

From: Kevin Olival, PhD
Sent: Thu, 23 Oct 2014 12:24:54 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: Automatic reply: Summary Statement available for grant (b)(4)

I'm away from the office until Monday, Oct 27th.

If you need immediate assistance, please call EcoHealth Alliance at (b)(6) and they can get in touch with me. Otherwise, I will respond as soon as possible after I return.

Cheers,
Kevin

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Thu, 16 Oct 2014 18:21:17 +0000
To: 'Peter Daszak'
Cc: Alison Andre
Subject: RE: Invitation to Participate in a MERS-CoV Session at ATS 2015

Hi Peter,
That's great, thanks very much. We'll be in touch with additional details as we get them.

Best,
Erik

From: Peter Daszak (b)(6)
Sent: Thursday, October 16, 2014 11:56 AM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Alison Andre
Subject: Re: Invitation to Participate in a MERS-CoV Session at ATS 2015

Hi Erik,

I'd be delighted to give a talk and will book it in my diary right now.

Cheers,

Peter

Peter Daszak
(Sent from my iPhone)

President
EcoHealth Alliance

460 West 34th Street, New York, NY10001, USA

www.EcoHealthAlliance.org

On Oct 14, 2014, at 3:32 PM, "Stemmy, Erik (NIH/NIAID) [E]" (b)(6) wrote:

Dear Peter,
NIAID has been invited to organize a session on MERS-CoV at the 2015 annual meeting of the American Thoracic Society in Denver, CO. We are in the process of putting together a 1-hour session titled "Research Response to MERS-CoV: Epidemiology, Pathogenesis, and Medical Countermeasures", and we would like to ask you to present a talk on the epidemiology and pathogenesis of MERS CoV. The session will be held from 12:15-1:15 on Wednesday May 20th, 2015 and we envision dividing the time

between 3 speakers. I will provide a short introduction to NIAID Research Resources, and then there will be two 20-minute scientific talks: one potentially by you on MERS-CoV epidemiology/pathogenesis and one on medical countermeasures. The audience is expected to be a mix of clinicians, researchers, and students with broad interests in infectious diseases of the lung.

The meeting will be held from May 15th – 20th, 2015 at the Colorado Convention Center in Denver. Additional details about the meeting can be found on the ATS website (http://conference.thoracic.org/2015/). We would be very happy if you're able to participate, however due to recent limits on government travel it may not be possible for us to support your travel costs. If possible we would greatly appreciate you letting us know your availability by this Friday October 17th. Please let me know if you have any questions.

Many thanks,
Erik

Please note my updated contact information below:

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Fri, 19 Sep 2014 14:56:11 +0000
To: 'Peter Daszak'
Cc: Aleksei Chmura
Subject: RE: Update on 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hi Peter,
It's good to hear how much you're getting done in China. Please do keep me posted as the IRB approval progresses. If I recall correctly we made a restricted award for the first year to give time for the approval. We'll need to be sure to remove the bar before the second year so there's no delay for you starting the work.

Thanks for keeping me in the loop, and let me know if there's anything I can do to assist.

Best,
Erik

Please note my updated contact information below:

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
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Phone: (b)(6)
Email:

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From: Peter Daszak (b)(6)
Sent: Sunday, September 14, 2014 6:22 PM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura
Subject: Update on 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hi Erik,

I've just returned from another trip to SE Asia and wanted to give you a brief update.

The first piece of news is that we've hired a social epidemiologist to design behavioral surveys and set up two cohorts in Southern China – the first a group of people involved in the wildlife trade in Southern China and the second a group of people highly exposed to bats in rural China. The epidemiologist's name is Dr Maureen Miller. She has a BA in Anthropology and a Ph.D in Social Epidemiology, both from Columbia University. She's worked internationally mainly on HIV projects and is eager to get started on the fieldwork. Maureen will be funded part time on the NIAID CoV grant, and part time on other funds at EHA, but her work right now it focused on this project so we can get the IRBs up and running quickly.

The second main item is that I've set up a meeting in China at the end of October with representation by Directors, Deputy Directors and Heads of Depts from CDCs in 5 provinces across Southern China. This will be in Wuhan, where our collaborators and Senior/Key Personnel on the grant Dr Zhengli Shi and XingYi Ge are based. We'll also have the Deputy Director of the China CDC (the main Federal agency in China) Dr Gao Fu – he's mainly a flu specialist, but will help a great deal in getting the human sampling off the ground. The main goal of the meeting will be to find out what sampling the Provincial CDCs are doing that we can leverage for this project, to reduce costs and maximize the results, as well as to involve them in the planning and direction so we can get their support.

We've also begun the process of setting up the IRBs for sample collection and behavioral surveys. EHA doesn't have its own IRB, so we have two options for these – Tufts University and Columbia University. I'm an adjunct faculty member at both organizations and we have inter-institutional agreements with them. We've run IRBs through Tufts before, but Columbia is closer and more efficient, so we'll be trying that out also. I'll let you know how the process goes, but we don't expect any major issues, and all being well we should have approval within a few months.

Wildlife sampling has begun in S. China, and we're also working with samples collected under previous contracts and grants. We've not got testing results in, but I'll let you know when we do.

Cheers,

Peter

Peter Daszak
President

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Thursday, August 21, 2014 8:17 AM
To: Peter Daszak
Cc: Aleksei Chmura
Subject: RE: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hi Peter,
My pleasure. Hopefully we can get something worked out for next year. It will entirely depend on NIAID's budget situation at the time, so unfortunately there are no guarantees.

I'm very eager to hear how the work is progressing and would appreciate an update when you get the time.

Thanks again!
Erik

Please note my updated contact information below:

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email:

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From: Peter Daszak (b)(6)
Sent: Wednesday, August 20, 2014 5:26 PM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura
Subject: RE: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hi Erik,

I'm belatedly responding to your email after travel in China and Borneo, partly to set up some of the work on this grant. I really appreciate you looking into this. As we get near to the beginning of year 2, we'll assess our needs and get in touch with you to see if this is possible. In the meantime I look forward to filling you in on progress with the project.

Cheers,

Peter

Peter Daszak
President

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, July 22, 2014 8:35 AM
To: Peter Daszak
Cc: Aleksei Chmura
Subject: RE: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hi Peter,
I've looked into this a bit more, and the only way additional funds can be added to an award is via an administrative supplement. In this instance since you're only asking for the additional in years 4 and 5, we would have to consider a supplement during those years of the award. That being said, there is no way to predict what funding levels will be in years 4 and 5 of your award, so I can't comment on how likely it would be for us to be able to supplement you.

I'm a little confused about your question about the Modeler/Statistician. You'd said in your January 23rd email that you could increase the effort of the modeler without increasing the overall budget. Are you now saying that you'll need an additional (b)(4); (b)(6) Unfortunately we aren't able to add additional funds to awards, so that would also need to be considered as a separate administrative supplement. We are not able to consider any more supplements for FY2014, so at the very least it wouldn't be something we'd be able to consider until the second half of FY2014.

Erik

Please note my updated contact information below:

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email:

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From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Tuesday, July 15, 2014 8:51 AM
To: 'Peter Daszak'
Cc: Aleksei Chmura
Subject: RE: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hi Peter,
Apologies for my delayed response. I'd been out of the office and am still catching up! I'll have to check with our grant folks and get back to you on your request. I'll let you know as soon as possible.

Thanks!
Erik

From: Peter Daszak (b)(6)
Sent: Saturday, July 05, 2014 2:51 AM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura
Subject: FW: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER
Importance: High

Dear Eric,

Thanks for the quick reply! I understand the strategy within NIAID, but want to make a plea to you for an increase to the award to cover the cut.

The reason is that because salary allocations won't increase in our budget, this effectively reduces the time originally requested for each of our funded personnel to allocate to this project work in years 2 through 5. Despite the NIH cap, the actual salary of EcoHealth Alliance personnel increases ~5% per year and our fringe rate increases by 1% per year both as per our negotiated DHHS fringe rate. In order to compensate for this, we would like to request permission to add an Assistant Research Scientist (tbd) at a fixed salary of (b)(4); (b)(6) only in years 4 and 5. This would not modify year 1 budgeted funding as per our Notice of Award nor increase us beyond the total award cap for direct costs. The Assistant Research Scientist would support the work done by the Research Scientist and Modeler/Statistician.

The attached budget outlines this request as per our proposal as well as increases the salary allocation for our Modeler/Statistician (Dr. Hosseini) from the 2 months in our proposal budget to 3 months as per our email exchange on 24th January. If you approve this total increase of (b)(4); (b)(6) (b)(4); to our budget, then our AOR Aleksei Chmura (cc'ed here) will follow up with you or Laura to provide the budget line item details and update the budget narrative accordingly. I am available any time to discuss this.

Thanks for all your support and I look forward to hearing back from you

Cheers,

Peter

Peter Daszak
President

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, June 25, 2014 8:06 AM

To: Peter Daszak
Cc: Aleksei Chmura
Subject: RE: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hi Peter,
Sincere apologies for my delayed response! I'd checked with our grant folks, but neglected to forward you their response. Thank you for reminding me. This policy took effect in FY2012, and NIAID Grants Management can't waive it. The NIH guide notice describing the policy can be found at the link below. Sorry I can't be more helpful!

Best,
Erik

NIH Guide Notice: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-12-036.html

Please note my updated contact information below:
Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-7630
Phone: (b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Peter Daszak (b)(6)
Sent: Tuesday, June 24, 2014 3:35 PM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura
Subject: RE: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Erik,

I just wanted to check in with you and see if you were able to speak to Laura Pone about this issue.

Cheers,

Peter

Peter Daszak
President

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Friday, June 6, 2014 1:05 PM
To: Peter Daszak
Subject: RE: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hi Peter,
Thanks for your message. I'll check in to the issue and get back to you as soon as possible.

Best,
Erik

Please note my updated contact information below:

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Peter Daszak (b)(6)
Sent: Friday, June 06, 2014 12:56 PM
To: Stemmy, Erik (NIH/NIAID) [E]; Aleksei Chmura
Subject: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER
Importance: High

Dear Eric,

We are very pleased and excited to receive our Coronavirus award (R01 AI110964-01) and have spent the last few months preparing so that we are now hitting the ground rolling on this!

I want to check in with you on one issue. Our proposal budget was cut by $275,604.30 (8%) over the five years (see attached excel file comparing the NoA budget and the proposal budget). My AOR (Mr Chmura, cc'd here) has communicated with Laura Pone who is handling the grant. Laura said this is due to an NIH/NIAID policy of not permitting annual cost-of-living escalation of salaries and fringe rates. Laura has stated that there now are no exceptions for cost-of-living increase despite our institutional policy on salary escalation and prior NIH escalation factors for recurring costs.
At the same time, our Grants and Contracting Office claim that NIH guidelines permit 3% escalation plus federally negotiated institutional annual fringe increases. Can you please confirm whether this is correct or not and direct us to a website with the corresponding language, so I can use it as guidance for this and all future NIH proposals.

This cut does affect us, and I'd like, if possible, either to reinstate the increase, or submit a revised budget with funds for extra support during the 'out years' so we can still maintain this work.

Any advice you can give on this would be greatly appreciated!

Cheers,

Peter

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President

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, February 11, 2014 10:09 AM
To: Peter Daszak; Aleksei Chmura
Cc: Pone, Laura (NIH/NIAID) [E]
Subject: RE: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hi Peter and Aleksei,
One other question about your application. There were a couple of human subjects concerns noted by the study section. I know your IRB approval is still pending, but were you able to address the other human subjects questions? Unless I missed it I didn't see anything in the JIT documents you uploaded.

Thanks,
Erik

Erik J. Stemmy, Ph.D.
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
6610 Rockledge Drive, Room 3210
Bethesda, MD 20892-7630
Phone: (b)(6)
Fax: 301-496-8030
Email: (b)(6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Pone, Laura (NIH/NIAID) [E]
Sent: Monday, February 10, 2014 3:56 PM
To: (b)(6) Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
(b)(6)
Subject: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Institute of Allergy
and Infectious Diseases
Bethesda, Maryland 20892

Dear Mr. Chmura,

For applications well received by study section during peer review, we attempt to obtain documentation that must be submitted to the National Institute of Allergy and Infectious Diseases should an application subsequently be identified for funding. Since your application is among those favorably received, we request that you submit the information listed below: Please submit this information by close of business **Thursday, February 13th.**

- Human Subjects Assurance documentation. **Include grant specific IRB approval date**. Grant specific IRB approvals must include either the project title or grant number.
- Documentation of the Required Education in the Protection of Human Subject Research Participants for all personnel involved.
- IACUC verification statement/letter with approval date.
- Response to Summary Statement Concern Regarding:
  - Protection of Human Subjects
  - Overlap
- Copy of EcoHealth Alliance's most recent F&A rate agreement.

Timely submission of the above information will enable us to expedite the issuance of an award should an application be identified for funding. Please submit this information by 02/13/14.

JIT information should be submitted using the Just-In-Time feature of the eRA Commons found in the Commons Status section. Submit **all** information at one time. For information on the Commons, go to the Commons Web site: https://commons.era.nih.gov/commons/index.jsp. If not submitting through the Commons ***or*** for information unable to be submitted through the Commons, please email the requested information signed by an authorized institutional business official**. Emailed documents not endorsed by an Institution Business Official will not be accepted as valid.**

Please feel free to contact me with any questions or concerns.

Thanks and have a nice day!

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

 **please consider the environment before printing this e-mail.**

From: Peter Daszak
Sent: Tue, 22 Jul 2014 12:35:31 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: Automatic reply: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hello,

I am in Malaysia, then China until August 1st with limited access to internet. Please cc Aleksei (b)(6) and Alison (b)(6) on all emails and I will respond when I'm back in the office in early August.

Cheers,

Peter

From: Peter Daszak
Sent: Tue, 15 Jul 2014 12:51:32 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: Automatic reply: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER

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Cheers,

Peter

From: Peter Daszak
Sent: Fri, 6 Jun 2014 17:11:38 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER

Thank you Erik!

Cheers,

Peter

Peter Daszak
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Sent: Friday, June 6, 2014 1:05 PM
To: Peter Daszak
Subject: RE: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER

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Best,
Erik

Please note my updated contact information below:

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases

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Phone: (b)(6)
Email:

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To: Stemmy, Erik (NIH/NIAID) [E]; Aleksei Chmura
Subject: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER
Importance: High

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Peter

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, February 11, 2014 10:09 AM
To: Peter Daszak; Aleksei Chmura
Cc: Pone, Laura (NIH/NIAID) [E]
Subject: RE: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

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Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
6610 Rockledge Drive, Room 3210
Bethesda, MD 20892-7630
Phone: (b)(6)
Fax: 301-496-8030
Email: (b)(6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Pone, Laura (NIH/NIAID) [E]
Sent: Monday, February 10, 2014 3:56 PM
To: (b)(6) Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
(b)(6)
Subject: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Institute of Allergy
and Infectious Diseases
Bethesda, Maryland 20892

Dear Mr. Chmura,

For applications well received by study section during peer review, we attempt to obtain documentation that must be submitted to the National Institute of Allergy and Infectious Diseases should an application subsequently be identified for funding. Since your application is among those favorably received, we request that you submit the information listed below:
Please submit this information by close of business **Thursday, February 13th.**

- Human Subjects Assurance documentation. **Include grant specific IRB approval date**. Grant specific IRB approvals must include either the project title or grant number.
- Documentation of the Required Education in the Protection of Human Subject Research Participants for all personnel involved.
- IACUC verification statement/letter with approval date.
- Response to Summary Statement Concern Regarding:
    - Protection of Human Subjects
    - Overlap
- Copy of EcoHealth Alliance's most recent F&A rate agreement.

Timely submission of the above information will enable us to expedite the issuance of an award should an application be identified for funding. Please submit this information by 02/13/14.

JIT information should be submitted using the Just-In-Time feature of the eRA Commons found in the Commons Status section. Submit **all** information at one time. For information on the Commons, go to the Commons Web site: https://commons.era.nih.gov/commons/index.jsp. If not submitting through the Commons ***or*** for information unable to be submitted through the Commons, please email the requested information signed by an authorized institutional business official. **Emailed documents not endorsed by an Institution Business Official will not be accepted as valid.**

Please feel free to contact me with any questions or concerns.

Thanks and have a nice day!

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

From: Aleksei Chmura
Sent: Wed, 7 May 2014 18:45:35 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Jon Epstein; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: UPDATE RE: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Laura,

The IACUC review date is today, so we would not have their decision until this evening at the earliest or later this week at the latest. Upon receipt of their decision, we will inform you immediately.

I will also let you know when we submit our Tufts IIA to OLAW.

Many thanks!

Aleksei Chmura
Program Coordinator & AOR
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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(mobile)
(b)(6) (China)
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EcoHealth Alliance integrates innovative science-based solutions and partnerships that increase capacity to achieve two interrelated goals: protecting global health by preventing the outbreak of emerging diseases and safeguarding ecosystems by promoting conservation.

On 07 May 2014, at 14:37:51, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,

Please let me know the status of IACUC approval from Tufts and submission of the IIA.

Thank you,

Laura Pone
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)
please consider the environment before printing this e-mail.

From: Aleksei Chmura (b)(6)
Sent: Tuesday, May 06, 2014 11:01 AM
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Jon Epstein; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: UPDATE RE: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Laura,

Our colleagues in China were out-of-office for the Labor/May Day Holiday and just sent us the IIA last night. We just sent it to Barbara Williams and Division of Assurances at OLAW.

Many thanks!

Aleksei Chmura
Program Coordinator
EcoHealth Alliance
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New York, NY 10001

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On 05 May 2014, at 16:07:09, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,

I just wanted to follow up on the email below.

Thank you,

Laura Pone
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)
please consider the environment before printing this e-mail.

From: Aleksei Chmura (b)(6)
Sent: Tuesday, April 29, 2014 11:46 AM
To: Peter Daszak
Cc: Pone, Laura (NIH/NIAID) [E]; Jon Epstein; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: UPDATE RE: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Thanks, Peter!

We sent the Interinstitutional Assurance form to Wuhan Institute of Virology and have now followed up to check the status. We also had been waiting on Wuhan IACUC approval, which we now have. We are hoping to have the Interinstitutional Assurance completed this week. As soon as we have the form, we will submit it to OLAW and let Laura know.

Many thanks!

Aleksei Chmura
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On 29 Apr 2014, at 10:52:29, Peter Daszak (b)(6) wrote:

Dear Laura,

Unfortunately, I'm in a meeting in DC all day, but I've just spoken with Aleksei and Jon and asked them to clarify where we are with this. We spoke with our collaborators at the Wuhan Inst. Virol. which has a FWA, but we weren't clear from that conversation whether they should contact you, or OLAW, or whether this should come from OLAW to them.

Jon and Aleksei are speaking with Valerie Parkison at OLAW today to clarify, and we should then have some progress.

We will keep you updated on this and any other outstanding issues at each step.

sincerely,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

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+1.212.380.4465 (fax)
www.ecohealthalliance.org

EcoHealth Alliance builds innovative science-based solutions and partnerships that increase our global capacity to achieve two interrelated goals: protecting global health by preventing pandemics; and safeguarding ecosystems by promoting conservation.

From: Pone, Laura (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, April 29, 2014 10:11 AM
To: Aleksei Chmura
Cc: Jon Epstein; Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: UPDATE RE: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hi Aleksei,

While we await the full review at Tufts can you please let me know the status of submitting the IIA between EcoHealth and Wuhan?

Thank you,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)


please consider the environment before printing this e-mail.

From: Aleksei Chmura (b)(6)
Sent: Tuesday, April 22, 2014 9:40 AM
To: Pone, Laura (NIH/NIAID) [E]
Cc: Jon Epstein; Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: UPDATE RE: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Laura,

We just had confirmation this morning that the Committee Decision is to conduct a full review - not an expedited review. This means we must wait until the next review date, which will be on the 7th of May. We will update you immediately thereafter with the Committee results.

Many thanks!

-Aleksei

Aleksei Chmura
Program Coordinator & AOR
EcoHealth Alliance
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On 22 Apr 2014, at 09:32:32, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,

I just wanted to follow up and ask when the review date will be for this grant's IACUC?

Thank you,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

From: Aleksei Chmura (b)(6)
Sent: Wednesday, April 09, 2014 5:20 PM
To: Pone, Laura (NIH/NIAID) [E]
Cc: Jon Epstein; Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: UPDATE RE: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Laura,

Just an update: Tufts has just confirmed that we are in queue for expedited IACUC review.

This is good news, but no review-date will be confirmed until next Thursday. Though unlikely, if there will be a call for a full committee meeting, this may delay us until the 7th of May at the

latest - which would be the next full committee meeting review-date. We will let you know as soon as we receive an update, next week.

Many thanks,

Aleksei Chmura
Program Coordinator
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From: Aleksei Chmura
Sent: Tue, 8 Apr 2014 21:50:43 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]; Jon Epstein
Subject: Re: URGENT - RE: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER
Importance: High

Dear Laura,

Our application has already passed through pre-review. The committee has some comments, which have all been addressed, but this took us past the deadline for the April 2 review. Tufts has just informed us that we may be eligible for an expedited review and once we have a confirmed date, we will let you know.

Many thanks!

Aleksei Chmura
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New York, NY 10001

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On 08 Apr 2014, at 17:00:12, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,

Can you please elaborate. Did the review occur on the 2nd? Was it returned for modifications? If so, when do you expect the revisions to be submitted and reviewed? If it wasn't reviewed on the 2nd please explain why and when you expect it to be reviewed.

Thank you,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)

Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

From: Aleksei Chmura (b)(6)
Sent: Tuesday, April 08, 2014 4:50 PM
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]; Jon Epstein
Subject: Re: URGENT - RE: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER
Importance: High

Dear Laura,

We are still waiting on the approval and will update you immediately upon receipt of confirmation from Tufts and as well as when we will submit documentation to OLAW.

Many thanks!

Aleksei Chmura
Program Coordinator
EcoHealth Alliance
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On 08 Apr 2014, at 13:27:15, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,

I am writing to follow up on the status of the IACUC review you noted was on April 2nd. Please let me know when we may expect to receive a copy of the approval. Also, a request from OLAW to establish an IIA with Tufts was sent on 4/1, please let me know the status and when you expect to submit the finalized documentation to OLAW.

Thank you,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

From: Aleksei Chmura (b)(6)
Sent: Monday, March 31, 2014 5:20 PM
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]; Jon Epstein
Subject: Re: URGENT - RE: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER
Importance: High

Dear Laura,

The next meeting date of the Tufts IACUC review committee will be on the 2nd of April. We will follow up with Tufts immediately thereafter and notify you.

Many thanks most,

Sincerely,

Aleksei Chmura
Program Coordinator & AOR
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460 West 34th Street – 17th floor
New York, NY 10001

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EcoHealth Alliance integrates innovative science-based solutions and partnerships that increase capacity to achieve two interrelated goals: protecting global health by preventing the outbreak of emerging diseases and safeguarding ecosystems by promoting conservation.

On 31 Mar 2014, at 16:17:37, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Dear Aleksei,

I am writing to follow up on the email below. Please confirm the date that the IACUC review is scheduled for.

Thank you,

Laura Pone
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)
please consider the environment before printing this e-mail.

From: Aleksei Chmura (b)(6)
Sent: Friday, March 21, 2014 9:52 AM
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]; Jon Epstein
Subject: Re: URGENT - RE: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER
Importance: High

Dear Laura,

The techniques are the same across the board. There is no animal work being conducted at Guangdong CDC, East China Normal University, or Guangdong CDC. Members of the field team will be composed of personnel from EcoHealth Alliance Headquarters, Guangdong Entomological Institution, and Yunnan CDC. All animal work will occur in the field - excepting the experimental laboratory work done at the Wuhan Institute of Virology in China.

We have submitted two separate IACUC protocols: one at the Wuhan Institute of Virology which will cover the experimental work and the other via Tufts University for our fieldwork. We are waiting on committee review dates. These should be sent to us very soon and we will update you immediately.

If you have any further questions, please let me know anytime.

Sincerely,

-Aleksei

Aleksei Chmura
Program Coordinator & AOR
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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(b)(6) (Skype)

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Visit our blog: www.ecohealthalliance.org/blog

EcoHealth Alliance integrates innovative science-based solutions and partnerships that increase capacity to achieve two interrelated goals: protecting global health by preventing the outbreak of emerging diseases and safeguarding ecosystems by promoting conservation.

From: Pone, Laura (NIH/NIAID) [E]
Sent: Monday, March 10, 2014 11:01 AM
To: (b)(6) Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
Subject: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Good Morning Aleksei,

Please provide a revised VAS including each of the sites listed below.

- Guangdong Entomological Institute (ECNU)
  Zhongshanbei Rd

Room 1707
Building 622 3663
Shanghai Putuo
CHINA

- East China Normal University
  3663 Zhongshan
  Beilu Shanghai
  CHINA

- Center for Disease Control and Prevention of Guangdong
  176 Xigang Xilu
  Guangzhou
  CHINA

- Yunnan Institute of Endemic Diseases Control and Prevention
  33 Wenhua Rd
  Dali
  CHINA

Thank you,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

From: Pone, Laura (NIH/NIAID) [E]
Sent: Mon, 17 Mar 2014 13:36:36 -0400
To: (b)(6) Stemmy, Erik (NIH/NIAID) [E];
(b)(6)
Subject: URGENT - RE: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER
Importance: High

Dear Aleksei,

I am writing to follow up on the email below. Please provide this information no later than close of business **Tuesday, March 18th.**

Thank you,
Laura Pone
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)
please consider the environment before printing this e-mail.

From: Pone, Laura (NIH/NIAID) [E]
Sent: Monday, March 10, 2014 11:01 AM
To: (b)(6) Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
Subject: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Good Morning Aleksei,

Please provide a revised VAS including each of the sites listed below.

- Guangdong Entomological Institute (ECNU)
  Zhongshanbei Rd
  Room 1707
  Building 622 3663
  Shanghai Putuo
  CHINA

- East China Normal University
  3663 Zhongshan
  Beilu Shanghai

CHINA

- Center for Disease Control and Prevention of Guangdong
  176 Xigang Xilu
  Guangzhou
  CHINA

- Yunnan Institute of Endemic Diseases Control and Prevention
  33 Wenhua Rd
  Dali
  CHINA

Thank you,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

From: Kevin Olival, PhD
Sent: Mon, 17 Mar 2014 14:02:40 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Question on status or review: (b)(4); (b)(6)
(b)(4); (b)(6)

Thanks Erik, will call in 30 minutes.

Cheers,
Kevin

On Mar 14, 2014, at 1:26 PM, Stemmy, Erik (NIH/NIAID) [E] wrote:

10:30 is great. But I'll be working remotely that day, so you can reach me at (b)(6)

Erik

From: Kevin Olival, PhD (b)(6)
Sent: Friday, March 14, 2014 1:16 PM
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Question on status or review: (b)(4); (b)(6)
(b)(4); (b)(6)

Thanks Erik. How about 1030am Monday? If this works, I can call you directly to the phone number in your email signature.

Appreciate your time in advance,
Kevin

On Mar 14, 2014, at 1:06 PM, Stemmy, Erik (NIH/NIAID) [E] wrote:

Hi Kevin,
Let's set a time to talk on Monday. I can do any time before 11:30am, or between 2-3pm. Please let me know when you're free.

Erik

Erik J. Stemmy, Ph.D.
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
6610 Rockledge Drive, Room 3210
Bethesda, MD 20892-7630
Phone: (b)(6)

Fax: 301-496-8030
Email: (b)(6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Kevin Olival, PhD (b)(6)
Sent: Friday, March 14, 2014 11:48 AM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Peter Daszak
Subject: Re: Question on status or review: (b)(4); (b)(6)
(b)(4); (b)(6)

Dear Erik,

I have gone through the SRG reviews, and was wondering if you would have time (perhaps later today or Monday, March 17) to speak on the phone about this grant proposal and next steps? Would appreciate your advise on our resubmission.

I'll be presenting on MERS at the IOM meeting in DC on March 18-19th, so won't be available those days. Were you planning to attend the IOM meeting by any chance?

Best,
Kevin

On Mar 10, 2014, at 9:52 AM, Stemmy, Erik (NIH/NIAID) [E] wrote:

Hi Kevin,
Generally the summary statements from the SRG reviews are posted by a week or two after the meeting. You certainly should have them before the council date in May. (b)(4); (b)(6)
(b)(4); (b)(6)
(b)(4); (b)(6) Once the summary statement comes out I'll be happy to set a time to talk with you if you like.

Best,
Erik

Erik J. Stemmy, Ph.D.

Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
6610 Rockledge Drive, Room 3210
Bethesda, MD 20892-7630
Phone: (b)(6)
Fax: 301-496-8030
Email: (b)(6)

From: Kevin Olival, PhD (b)(6)
Sent: Thursday, March 06, 2014 2:44 PM
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: Question on status or review: (b)(4); (b)(6)
(b)(4); (b)(6)

Dear Erik,

I see in the system that my R21 grant, (b)(4); (b)(6)
(b)(4); (b)(6) has been reviewed by the SRG, and that the council review is pending (05/2014). (b)(4); (b)(6) I'm wondering when/if the SRG assessment and specific reviewer comments will be available to us? Does this wait until after the Council review? Also, am I right to assume that (b)(4); (b)(6)
(b)(4); (b)(6)

Best,
Kevin

Kevin J. Olival, PhD
Senior Research Scientist

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From: Aleksei Chmura
Sent: Mon, 10 Mar 2014 04:16:31 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Stemmy, Erik (NIH/NIAID) [E]; Peter Daszak; Jon Epstein
Subject: Re: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Laura,

We will definitely provide the date as rapidly as possible in the next few days.

Many thanks!

Aleksei Chmura
Program Coordinator
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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EcoHealth Alliance integrates innovative science-based solutions and partnerships that increase capacity to achieve two interrelated goals: protecting global health by preventing the outbreak of emerging diseases and safeguarding ecosystems by promoting conservation.

On 07 Mar 2014, at 14:25:06, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,

I am writing to follow up on the email below. Please confirm the IACUC review date as soon as possible.

Thank you,

Laura Pone
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)
please consider the environment before printing this e-mail.

any statement made that are the sender's own and not expressly made on behalf of the NIAID by one of its representatives.

From: Aleksei Chmura (b)(6)
Sent: Monday, March 03, 2014 10:46 AM
To: Pone, Laura (NIH/NIAID) [E]
Cc: Stemmy, Erik (NIH/NIAID) [E]; Peter Daszak; Jon Epstein
Subject: Re: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Laura,

We will get you the information about IACUC dates as soon as possible.

Many thanks!

-Aleksei

Aleksei Chmura
Program Coordinator
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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(mobile)
(b)(6) (China)
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EcoHealth Alliance integrates innovative science-based solutions and partnerships that increase capacity to achieve two interrelated goals: protecting global health by preventing the outbreak of emerging diseases and safeguarding ecosystems by promoting conservation.

On 26 Feb 2014, at 14:10:51, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,

Since there will be a delayed onset of human subject work I will be able to award the grant with a restriction once all of the animal requirements are met. **Please confirm the date that the IACUC review is scheduled for** at your earliest convenience. I am including a list of items that must be submitted before the award can be made.

- IACUC – please confirm date of review
- AWA for each site conducting animal work

- IIA between EcoHealth Alliance and each site conducting animal work (separate IIAs must be negotiated because EcoHealth does not have their own AWA)

Thank you,

Laura Pone
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)
please consider the environment before printing this e-mail.

From: Aleksei Chmura (b)(6)
Sent: Wednesday, February 26, 2014 12:35 PM
To: Pone, Laura (NIH/NIAID) [E]
Cc: Stemmy, Erik (NIH/NIAID) [E]; Peter Daszak
Subject: Re: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER
Importance: High

Dear Laura,

As per our proposed Timeline and Management Plan (Section D, page 119), our human sampling work would not commence until the 6th Quarter ~1.5 years after the commencement of the project, so definitely in Year 2.

Please call or email me, if you have further questions.

Many thanks!

Aleksei Chmura
Program Coordinator
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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(mobile)
(b)(6) (China)
(b)(6) (Skype)

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On 26 Feb 2014, at 11:31:19, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,

Dr. Stemmy mentioned that the human subject work begins in year 2. I could not find any correspondence from you stating that, so please let me know when the work is schedule to begin.

Thank you,

Laura Pone
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

From: Aleksei Chmura (b)(6)
Sent: Monday, February 24, 2014 9:55 PM
To: Pone, Laura (NIH/NIAID) [E]
Cc: Stemmy, Erik (NIH/NIAID) [E]; Peter Daszak
Subject: Re: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Laura,

We are in process of obtaining AWAs and FWAs for each site that does not already have these, but this process may take a month or more. Would this hold up an award or could an award be made with a block on human or animal work (until we have FWAs and/or AWAs)?

Many thanks most,

Sincerely,

Aleksei Chmura
Program Coordinator and AOR

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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(mobile)
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On 24 Feb 2014, at 08:47:26, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Dear Aleksei,

It has come to my attention that animal and human subject work will be done at Guangdong Entomological Institute, Wuhan Institute of Virology, East China Normal University, Center for Disease Control and Prevention of Guangdong, and Yunnan Institute of Endemic Diseases Control and Prevention. Please begin the process for obtaining AWA's and FWA's for each site that does not already have them.

- Confirmation of FWA for human subject work. If no FWA exists please establish one. http://ohrp.cit.nih.gov/efile/FwaStart.aspx
- Confirmation of AWA for animal subject work. If no AWA exists please establish one.http://grants.nih.gov/grants/olaw/obtain_assurance.htm

Thank you,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

From: Aleksei Chmura
Sent: Wed, 5 Mar 2014 20:43:19 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER
Attachments: JIT Other Support 1R01AI110964 Updated.pdf, ATT00001.htm

Dear Laura,

Apologies for the discrepancies between the proposal budget and the JIT Other Support file. These were the result of copy-pasting errors. The budgeted amounts are correct and we have no changes to them. Please find a corrected and updated Other Support file attached. The efforts for the following senior personnel are as follows:

Dr. ZL Shi	-	(b)(4); (b)(6)	calendar months
Dr. SY Zhang	-		calendar months
Dr. JH Epstein	-		calendar months
Dr. KJ Olival	-		calendar months
Dr. PR Hosseini	-		calendar months
Dr. XY Ge	-		calendar months
Dr. GJ Zhu	-		calendar months
Dr. CW Ke	-		calendar months
Dr. YZ Zhang	-		calendar months

Dr. Chang Wen Ke and Dr. Yun Zhi Zhang are not listed in our budget, since in order to save costs they are not taking any salary, but will be dedicating (b)(4); (b)(6) per year to liaise regularly with Co-Investigators, PD/PI, and other staff as well as collaborate on research design and papers.

Please let me know, if you have any further questions most,

Sincerely,

Aleksei Chmura
Program Coordinator and Authorized Organizational Representative
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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(mobile)
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CURRENT OTHER SUPPORT

DASZAK, PETER

ACTIVE

DEB-0955897 (Daszak) 07/01/10 – 06/30/15 (b)(4); (b)(6)
NSF $497,121
EcoHealthNet: Ecology, Environmental Science and Health Research Network
Funding for student exchange and workshops to fuse veterinary science, ecology and human medical sciences.
Role: PI

5R01GM100471 (Perrings) 09/15/11 – 06/30/15 (b)(4); (b)(6)
NIGMS $289,953
Modeling Anthropogenic Effects in the Spread of Infectious Disease
A collaborative international proposal using interdisciplinary approaches to address the links between globalization and emerging infectious disease risks.
Role: Co-Investigator

1R56TW009502 (Daszak) 09/17/12 – 04/30/14 (b)(4); (b)(6)
NIH Fogarty International Center $300,000
Comparative Spillover Dynamics of Avian Influenza in Endemic Countries
Our research will advance the understanding of the long-term dynamics of H5N1 by relaxing the assumption of homogeneous mixing implicit in classical epidemiological models through fine-scale measurements of realistic contact networks in Bangladesh, China, and Egypt.
Role: PI

Emerging Pandemic Threats (Morse) 10/01/09 – 09/30/14 (b)(4); (b)(6)
USAID $18,000,000
PREDICT
Modeling hotspots for disease emergence and conducting surveillance in wildlife in hotspots for new emerging zoonoses.
Role: PI on Subcontract from UC Davis

2R01TW005869 09/01/08 – 06/30/14 (b)(4); (b)(6)
NIH Fogarty International Center $2,498,829
The Ecology, Emergence and Pandemic Potential of Nipah virus in Bangladesh
To conduct mathematical modeling and fieldwork to understand the dynamics of Nipah virus in Bangladesh
Role: PI

PENDING

1R01AI110964 (Daszak) 07/01/2014 – 06/30/2019 (b)(4); (b)(6)
NIAID $3,362,339
Understanding the Risk of Bat Coronavirus Emergence
To examine risk of future coronavirus emergence from wildlife using in-depth field investigations across the human-wildlife interface in China, molecular characterization of novel CoVs, and host receptor binding domain genes, mathematical models of transmission and evolution, and in vitro and in vivo laboratory studies of host range.
Role: PI

OVERLAP: none

CURRENT OTHER SUPPORT

SHI, ZHENG LI

ACTIVE

2011CB504700 (Shi) 01/01/2011-12/31/2015 (b)(4); (b)(6)
National Basic Research Program, China $150,000
Mechanism of interspecies transmission of zoonotic viruses
Study of the means of transmission of zoonotic viruses.
Role: PI

81290341 (Shi) 01/01/2013-12/31/2017 (b)(4); (b)(6)
NSF China $100,000
Genetic diversity, identification, and pathogenesis of bat viruses
Molecular characterization of viruses of bats in China.
Role: PI

PENDING

1R01AI110964 (Daszak) 07/01/2014 – 06/30/2019 (b)(4); (b)(6)
NIAID $3,362,339
Understanding the Risk of Bat Coronavirus Emergence
To examine risk of future coronavirus emergence from wildlife using in-depth field investigations across the human-wildlife interface in China, molecular characterization of novel CoVs, and host receptor binding domain genes, mathematical models of transmission and evolution, and in vitro and in vivo laboratory studies of host range.
Role: Co-Investigator

OVERLAP: none

CURRENT OTHER SUPPORT

ZHANG, SHU-YI

ACTIVE

Emerging Pandemic Threats (Morse) 10/01/09 – 09/30/14 (b)(4); (b)(6)
USAID $18,000,000
PREDICT
Modeling hotspots for disease emergence and conducting surveillance in wildlife in hotspots for new emerging zoonoses.
Role: PI on Subcontract from EcoHealth Alliance

PENDING

1R01AI110964 (Daszak) 07/01/2014 – 06/30/2019 (b)(4); (b)(6)
NIAID $3,362,339
Understanding the Risk of Bat Coronavirus Emergence
To examine risk of future coronavirus emergence from wildlife using in-depth field investigations across the human-wildlife interface in China, molecular characterization of novel CoVs, and host receptor binding domain genes, mathematical models of transmission and evolution, and in vitro and in vivo laboratory studies of host range.
Role: Co-Investigator

OVERLAP: none

CURRENT OTHER SUPPORT

KE, CHANG WEN

ACTIVE

2012ZX10004213-004 (Ke) 07/01/2012 – 06/30/2015 (b)(4); (b)(6)
Ministry of Science and Technology, PRC $372,451
National Major Projects of Major Infectious Disease Control and Prevention
Investigation of Disease Outbreaks in Guangdong Province
Role: PI

PENDING

1R01AI110964 (Daszak) 07/01/2014 – 06/30/2019 (b)(4); (b)(6)
NIAID $3,362,339
Understanding the Risk of Bat Coronavirus Emergence
To examine risk of future coronavirus emergence from wildlife using in-depth field investigations across the human-wildlife interface in China, molecular characterization of novel CoVs, and host receptor binding domain genes, mathematical models of transmission and evolution, and in vitro and in vivo laboratory studies of host range.
Role: Co-Investigator

OVERLAP: none

CURRENT OTHER SUPPORT

EPSTEIN, JONATHAN H.

ACTIVE

DEB-0955897 (Daszak) 07/01/10 – 06/30/15 (b)(4); (b)(6)
NSF $497,121
EcoHealthNet: Ecology, Environmental Science and Health Research Network
Funding for student exchange and workshops to fuse veterinary science, ecology and human medical sciences.
Role: Senior Scientist

Emerging Pandemic Threats (Morse) 10/01/09 – 09/30/14 (b)(4); (b)(6)
USAID $18,000,000
PREDICT
Modeling hotspots for disease emergence and conducting surveillance in wildlife in hotspots for new emerging zoonoses.
Role: Senior Scientist

2R01TW005869 09/01/08 – 06/30/14 (b)(4); (b)(6)
NIH Fogarty International Center $2,498,829
The Ecology, Emergence and Pandemic Potential of Nipah virus in Bangladesh
To conduct mathematical modeling and fieldwork to understand the dynamics of Nipah virus in Bangladesh
Role: Senior Scientist

4500036150 (Epstein) 07/01/14-06/30/19 (b)(4); (b)(6)
NIH $ 275,000
Risk of Zoonotic Transmission of Herpes B Virus from Wild Macaques in Bangladesh
Investigate causes of encephalitis for non-Nipah non-Japanese encephalitis in Bangladesh and determine the shedding prevalence of B Virus in macaques.
Role: PI

F12AP01117 (Epstein) 09/13/12 - 09/13/14 (b)(4); (b)(6)
USFW $35,000
Development of a Great Ape Health Unit in Sabah, Malaysia.
Develop a Great Ape Health unit to evaluate the health of rescued and translocated gibbons and orangutans in Sabah, Malaysia.
Role: PI

PENDING

1R01AI110964 (Daszak) 07/01/2014 – 06/30/2019 (b)(4); (b)(6)
NIAID $3,362,339
Understanding the Risk of Bat Coronavirus Emergence
To examine risk of future coronavirus emergence from wildlife using in-depth field investigations across the human-wildlife interface in China, molecular characterization of novel CoVs, and host receptor binding domain genes, mathematical models of transmission and evolution, and in vitro and in vivo laboratory studies of host range.
Role: Co-Investigator

OVERLAP: none

CURRENT OTHER SUPPORT

OLIVAL, KEVIN J.

ACTIVE

Award GVSU 04152012 (Russell) 06/18/12 – 06/17/13 (b)(4); (b)(6)
USFWS/USGS $12,000
Genetic Approaches to Defining Taxonomic and conservation Units for the Hawaiian Hoary Bat
Using molecular tools to date the origins and divergence of the endangered Hawaiian Hoary bat.
Role: Co-PI

4500036150 (Epstein) 07/01/12-06/30/14 (b)(4); (b)(6)
USFWS $197,950
Characterization of Climatic Parameters within Bat Hibernacula, their Influence on Environmental Loads of *Geomyces destructans*, and Implications for the Migration of White-Nose Syndrome in Bats.
Role: Co-PI

PENDING

1R01AI110964 (Daszak) 07/01/2014 – 06/30/2019 (b)(4); (b)(6)
NIAID $3,362,339
Understanding the Risk of Bat Coronavirus Emergence
To examine risk of future coronavirus emergence from wildlife using in-depth field investigations across the human-wildlife interface in China, molecular characterization of novel CoVs, and host receptor binding domain genes, mathematical models of transmission and evolution, and in vitro and in vivo laboratory studies of host range.
Role: Co-Investigator

(b)(4); (b)(6)

OVERLAP: none

CURRENT OTHER SUPPORT

HOSSEINI, PARVIEZ R.

ACTIVE

EF-1015791 (Mitchell) 07/01/10 – 6/30/15 (b)(4); (b)(6)
NSF $745,295
The community ecology of viral pathogens
Causes and consequences of coinfection in hosts and vectors. To conduct mathematical modeling and fieldwork to understand implications in a wild grass, aphid-vectored disease system.
Role: Co-PI

Emerging Pandemic Threats (Morse) 10/01/09 – 09/30/14 (b)(4); (b)(6)
USAID $18,000,000
PREDICT
Modeling hotspots for disease emergence and conducting surveillance in wildlife in hotspots for new emerging zoonoses.
Role: Hotspots Modeler

1R56TW009502 (Daszak) 09/17/12 – 04/30/14 (b)(4); (b)(6)
NIH Fogarty International Center $300,000
Comparative Spillover Dynamics of Avian Influenza in Endemic Countries
Our research will advance the understanding of the long-term dynamics of H5N1 by relaxing the assumption of homogeneous mixing implicit in classical epidemiological models through fine-scale measurements of realistic contact networks in Bangladesh, China, and Egypt.
Role: Senior Scientist

PENDING

1R01AI110964 (Daszak) 07/01/2014 – 06/30/2019 (b)(4); (b)(6)
NIAID $3,362,339
Understanding the Risk of Bat Coronavirus Emergence
To examine risk of future coronavirus emergence from wildlife using in-depth field investigations across the human-wildlife interface in China, molecular characterization of novel CoVs, and host receptor binding domain genes, mathematical models of transmission and evolution, and in vitro and in vivo laboratory studies of host range.
Role: Co-Investigator

OVERLAP: none

CURRENT OTHER SUPPORT

GE, XING YI

ACTIVE: none

PENDING

1R01AI110964 (Daszak) 07/01/2014 – 06/30/2019 (b)(4); (b)(6)
NIAID $3,362,339
Understanding the Risk of Bat Coronavirus Emergence
To examine risk of future coronavirus emergence from wildlife using in-depth field investigations across the human-wildlife interface in China, molecular characterization of novel CoVs, and host receptor binding domain genes, mathematical models of transmission and evolution, and in vitro and in vivo laboratory studies of host range.
Role: Co-Investigator

OVERLAP: none

CURRENT OTHER SUPPORT

ZHU, GUANG JIAN

ACTIVE: none

PENDING

1R01AI110964 (Daszak) 07/01/2014 – 06/30/2019 (b)(4); (b)(6)
NIAID $3,362,339
Understanding the Risk of Bat Coronavirus Emergence
To examine risk of future coronavirus emergence from wildlife using in-depth field investigations across the human-wildlife interface in China, molecular characterization of novel CoVs, and host receptor binding domain genes, mathematical models of transmission and evolution, and in vitro and in vivo laboratory studies of host range.
Role: Co-Investigator

OVERLAP: none

CURRENT OTHER SUPPORT

ZHANG, YUN ZHI

ACTIVE:

(no number – Zhang) 01/01/2013- 12/01/2017 (b)(4); (b)(6)
Ministry of Science $51,277
Yunnan region is an important natural reservoir
Pathogen survey of Yunnan province
Role: PI

81260437 (Zhang) 01/01/2013 -12/01/2016 (b)(4); (b)(6)
NSF, China $108,561
Rat and mouse viral metagenome
Yunnan murine viral metagenome important viral epidemic status and related research
Role: PI

(no number – Zhang) 11/01/ 2012- 11/01/2015 (b)(4); (b)(6)
Talent Research Foundation $87,000
Health Study Ecology
Yunnan Provincial Health Hall "Ten hundred" health study of the ecology of Yunnan province.

PENDING

1R01AI110964 (Daszak) 07/01/2014 – 06/30/2019 (b)(4); (b)(6)
NIAID $3,362,339
Understanding the Risk of Bat Coronavirus Emergence
To examine risk of future coronavirus emergence from wildlife using in-depth field investigations across the human-wildlife interface in China, molecular characterization of novel CoVs, and host receptor binding domain genes, mathematical models of transmission and evolution, and in vitro and in vivo laboratory studies of host range.
Role: Co-Investigator

OVERLAP: none

On 03 Mar 2014, at 16:23:19, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,

Please provide a response to the following effort discrepancies. A response by **Wednesday, March 5th** is appreciated.

- Please confirm effort for these individuals:
  - Zheng li Shiï¿½s effort is listed as (b)(4); (b)(6) in the budget and (b)(4); (b)(6) (b)(4); (b)(6) in the other support.
  - Shu-Yi Zhangï¿½s effort is listed as (b)(4); (b)(6) in the budget and (b)(4); (b)(6) (b)(4); in the other support.
  - Jonathan Epsteinï¿½s effort is listed as (b)(4); (b)(6) in the budget and (b)(4); (b)(6) (b)(4); (b)(6) in the other support.
  - Kevin Olivalï¿½s effort is listed as (b)(4); (b)(6) in the budget and (b)(4); (b)(6) in the other support.
  - Parviez Hosseiniï¿½s effort is listed as (b)(4); (b)(6) in the budget and (b)(4); (b)(6) (b)(4); (b)(6) in the other support.
  - Xing-Yi Geï¿½s effort is listed as (b)(4); (b)(6) in the budget and (b)(4); (b)(6) in the other support.
  - Guang Jian Zhuï¿½s effort is listed as (b)(4); (b)(6) in the budget and (b)(4); (b)(6) (b)(4); in the other support.
  - Chang Wen Keï¿½s effort is not provided in the budget but is listed as (b)(4); (b)(6) in the other support.
  - Yun Zhi Zhangï¿½s effort is not provided in the budget but is listed as (b)(4); (b)(6) in the other support.

Thank you,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

From: Aleksei Chmura
Sent: Mon, 3 Mar 2014 21:21:31 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER
Attachments: response to VAS NIAID CoV.docx, ATT00001.htm

Dear Laura,

I confirm that the the vertebrate animal section as updated and highlighted in the text of Dr. Peter Daszak's email below (and in the attached word document) is correct.

If you have any further questions, please let me know.

Many thanks!

Aleksei Chmura
Program Coordinator & Authorized Organizational Representative
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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(b)(6) (China)
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EcoHealth Alliance integrates innovative science-based solutions and partnerships that increase capacity to achieve two interrelated goals: protecting global health by preventing the outbreak of emerging diseases and safeguarding ecosystems by promoting conservation.

From: Peter Daszak (b)(6)
Sent: Monday, March 03, 2014 2:52 PM
To: Pone, Laura (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura; Jon Epstein
Subject: RE: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Laura,

Thank you for your questions. I've amended the VAS so that there is clarity on each of these, and highlighted the text that I've inserted. I've attached the amended version to this email.

I've also inserted the specific responses in the body of your email below.

Please don't hesitate to email or call if there are any other questions.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

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EcoHealth Alliance builds innovative science-based solutions and partnerships that increase our global capacity to achieve two interrelated goals: protecting global health by preventing pandemics; and safeguarding ecosystems by promoting conservation.

From: Pone, Laura (NIH/NIAID) [E] (b)(6)
Sent: Thursday, February 27, 2014 2:34 PM
To: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]; Peter Daszak
Subject: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Dr. Daszak,

Please provide the revised VAS information request by OLAW below by close of business Monday, March 3rd.

I have reviewed the VAS provided for 1R01AI110964-01 and I cannot approve it as written. Points 3 and 4 were not fully answered. I will require the remaining information on points 3 and 4 to be addressed:

Performance Site: Wuhan University
Point 3 - Veterinary care

- **How does the veterinary staff communicate with the PI to provide medical care or intervention for the animals if required?**

There are two senior veterinarians (Drs. An XueFang and Zhang Fan) at the Wuhan Institute of Virology who will oversee the experiments using humanized mice. Any issues regarding animal health and welfare will be communicated directly to the onsite co-PI, Dr. Zhengli Shi and the Principal Investigator, Dr. Peter Daszak, via phone call and email

Point 4 - Provisions to minimize discomfort, distress, pain and injury

- **Methods to alleviate discomfort, distress or pain should be described. If pharmacological agents are used, the agent(s) should be specified by name or class.**

Market animals: Bats, rodents, and small mammals sampled in markets, sourced from vendors, will be manually restrained and sampled on-site, to minimize stress and discomfort. Because these animals are designated for human consumption, we will not use anesthetic agents if the animal is to be returned to the vendor following sampling. Manual restraint and sampling will be conducted by experienced members of the field team. Any animal that shows signs of distress (respiratory distress, pale mucous membranes) will be immediately released into a holding cage to recover. If the veterinarian or senior scientist in charge of sampling deems an individual animal to be fractious, or at risk for excessive stress and discomfort, anesthetic agents may be used for the safety of both animal and handler. Injectable tiletamine zolazepam (Telozol HCl) given intramuscularly, or isoflurane gas using a portable vaporizer may be used. Any animal that has been anesthetized for sampling will not be returned to the food chain due to possibility of human consumption of anesthetic drug. These animals will be purchased from the vendor and not returned to the market. Following sampling the animal will be euthanized according to AVMA standards and disposed of according to safe biohazard practices.

Experimental animals (mice): All experimental work will be conducted at Wuhan Institute of Virology under the supervision of senior veterinarians Drs. An XueFang and Zhang Fan. Animals will be observed daily for clinical signs of illness. All mice will be provided comfortable housing with regular access to water and food throughout the experiment. The experiments under this study do not include surgical procedures or use of experimental pharmacological agents. Mice will be anesthetized prior to sampling using isoflurane gas, which will reduce stress and discomfort. During experimental infections, mice will be monitored for signs of pain and discomfort. Moribund mice (e.g. mice showing depression, inappetance, respiratory distress, or severe fever) will be euthanized, according to AVMA recommendations.

- **Any additional means to avoid discomfort, distress, pain or injury should be described briefly.**

There are no other methods that will be used.

- **If procedures (e.g., pharmacological or surgical) might lead to severe discomfort, distress, pain or injury,indicators for humane endpoints and euthanasia (e.g., severe infection, respiratory distress, failure to eat, tumor size) should be described.**

The indicators for humane endpoints and euthanasia are now described above for both market and experimental animals

- **Describe the use of restraint devices, if relevant.**

No restraint devices will be used

Link to the VAS on the OLAW website:http://grants.nih.gov/grants/olaw/VAChecklist.pdf

Thank you,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

 **please consider the environment before printing this e-mail.**

VERTEBRATE ANIMALS:

1. Detailed description of animal use.

All work with vertebrate animals will be conducted in China.

Capture and sampling techniques for all wild animals described in this study have been previously approved by UC Davis IACUC (Mazet and Epstein; UC Davis 15898; current). Experimental work using humanized mice will be conducted at the Center for Animal Experiment Biosafety 3 lab of Wuhan University at the School of Medicine in Wuhan, China. The Center is AAALAC accredited and has both an Institutional Biosafety Committee and an Institutional Animal Care and Use Committee. Animals will be housed in a BSL-3 facility and will be under the care of a full-time veterinarian. We will submit our protocols for IACUC approval should this proposal be funded. Conditions for animal use are described below.

Note: The majority of wild animals captured and sampled will be done using non-destructive, techniques. In a small number of instances (~ 2 bats per species), where intestine and lung tissue is required to establish cell lines, animals will be humanely euthanized and a necropsy performed according to accepted protocols (see euthanasia section)

Bat capture. Free-ranging bats will be captured using either a mist net or harp trap. The net system is manned by two people during the entire capture period, and bats are removed from the net as soon as they become entangled to minimize stress and prevent injury. In the Co-PI's (Dr. Epstein) experience, a maximum of 20-30 bats can be safely held and processed by a team of three people per trapping period. Duration of trapping will depend on the capture rate. Bats are placed into a pillowcase or small cloth bag and hung from a branch or post until samples are collected. Bats are held for a maximum of six hours.

Wild rodent capture. Free-ranging rodents will be captured through pit traps and box traps; captive rodents, including resident free-ranging wild rats/rodents in markets, will be manually captured or captured through traps. Traps will be checked a minimum of once daily in the morning. If adverse weather (extreme heat, rain) is expected or researchers are working in areas where predation is common, traps will be checked more frequently, and closed during the adverse weather. Handling of rodents will involve morphometric measurements. Captive and wild rodent sampling procedures (including anesthesia if necessary), will involve manual restraint, venipuncture, mucosal swabs, fecal, urine, and external parasite collection. Following capture, small animals will be restrained with a fine mesh bag to minimize entanglement, taking precautions to ensure the animals are not traumatized by the hoop of the net or through net removal. Larger rodents will be restrained for sampling in specialized squeeze-cages, allowing adjustments appropriate to the size of the animal. Squeeze-cages consist of a wooden frame with a plasticized wire bottom and a Plexiglas shield used to press the animal, while ensuring visible communication between the field veterinarian and the animal. Once squeezed, a rod is inserted to keep the plastic shield in place. The box is then inverted, allowing sampling to be conducted through the open wire bottom and abdomen of the animal when the animal is safely immobilized. Anesthesia for small rodents will be conducted using plastic tubes, with the animals transferred directly from the traps to the tubes containing a cotton swab soaked in ether, isoflurane, or methoxyflurane for anesthetic induction. For larger rodents, chemical restraint and anesthesia (ketamine alone, or ketamine combined with xylazine) will be applied either through the squeeze cages by syringe if applicable.

Laboratory mice. Lab mice will be sourced commercially by the Wuhan Center for Animal Experiment at Wuhan University.

Sample Collection. Bats will be manually restrained during sampling.

Bats: Depending on the species and size of bat, swabs will be taken from the oropharynx, urogenital tract, and rectum. Fresh feces will be collected if available, in which case a rectal swab will not be collected. Blood will be collected from fruit bats either from the cephalic vein or from the radial artery or vein using a 25 gauge needle and 1cc syringe. Blood will be collected from bats weighing less than 100g according to published techniques (*126*).

Rodents: Rodents will be anesthetized prior to sampling. Once anesthetized a small blood sample will be collected using a capillary tube placed into the retro-orbital sinus. Only trained technicians will perform retro-orbital bleeding and it will only be performed on anesthetized rodents. Femoral or jugular venipuncture may be used for larger rodents (e.g. rats). In all rodents, blood volumes of no more than 1% of body weight will be withdrawn. (example 0.2 ml blood from a 20 gram rodent).

Civets and other small mammals: Anesthesia will be used to restrain small free ranging mammals according to published protocols. Animals will be monitored continuously while recovering from anesthesia. Animals that are sampled in the marketplace, and that may potentially be consumed, will not be anesthetized. Manual restraint will be used and blood will be drawn from the femoral artery or saphenous vein.

Laboratory Mice. Humanized mice will be bred at the University of Wuhan. Mice will be inoculated with a specific dose (e.g. $1x10_6$ TCID50) of virus through different routes (intranasally and intraperitoneally). Mouse body temperature will be monitored with implanted temperature sensing microchips (LifeChip Bio-thermo, Destron Fearing), and mice will be weighed daily. Animals will be observed daily for clinical signs of illness. Moribund mice will be euthanized, according to AVMA recommendations. Live animals will be euthanized at three weeks post-inoculation and organs harvested. We will collect sera on days 10, 15 and 21 to test for neutralizing antibodies against bat CoVs. We will collect nasal washes, oral swabs, and rectal swabs, and urine every two days. These are minimally invasive procedures, and will be performed by experienced lab technicians under the supervision of a full-time veterinarian.

2. Justify use of animals, choice of species, numbers to be used. Species and number used in study:
The purpose of this study is to conduct multi-regional surveillance in large populations of animals to detect coronaviruses that may pose a risk to the health of both humans and animals. The experimental work is designed to understand the ability of bat coronaviruses to bind to human receptors. Because we don't have prevalence estimates for novel strains of coronaviruses, we assume a conservative estimate of 10% prevalence. SARS-like coronaviruses have been found in between 10% and 38% of bats studied (*4, 25*). A 10% in wild populations of bats would require a sample of 30 individuals per species in order to ensure detection of an infected individual with 95% confidence.

Wild bats: We will sample 30 individuals from 30 different species in each province in China (2 per species euthanized for organ tissue); representing but not limited to the following families: *Rhinolophidae, Hipposideridae, Vespertillionidae, Mollossidae,* and *Pteropodidae*, all of which are present in Southern China and potentially in the wildlife markets.
Bats in wet markets: We will opportunistically sample a wide variety of insectivorous and frugivorous bats according to what is present in markets. In addition to bats, we will sample civets, raccoon dogs, rats, bandicoots, bamboo rats, and other rodents present in the markets

that may act as intermediate hosts. Numbers of animals sampled from markets will be limited to animal availability. In every situation, sampling of wildlife will be conducted in the most humane manner while minimizing the impacts on individual animals and their wild populations. In cases where feces are collected for testing, non-invasive techniques will be used. In all instances, the fewest number of animals will be sampled that will provide valid information and statistical inference for the pathogen and disease of interest and every effort will be made to minimize stress and discomfort for the animal.
A small number of bats (maximum 2 per species) representing each of the species in this study may be euthanized in order to collect lung and intestinal tissue required for characterizing coronavirus receptors. Voucher specimens may also be collected at the discretion of the team leader for the accurate identification of species using molecular methodology.

Humanized mice for experimental infection for Specific Aim 3: In order to understand whether bat coronaviruses that utilize receptors found in people have the potential to infect people, we will use Swiss albino mice (standard breed at Wuhan University) that have been genetically modified to have human receptors. We'll infect them with cultured bat coronaviruses and determine which organs become infected and whether these mice are capable of shedding infectious virus. Humanized mice will be genetically modified to carry human ACE2 or DPP4 gene will be used to evaluate pathogenesis of CoVs. We cannot anticipate exactly how many viruses we will find that are candidates for experimental models, however we estimate that we will use four adult mice (2 male, 2 female) per virus and that we will identify approximately 20 viruses that will be used for mouse infection experiments. This will require a total of 80 mice over the study period.

3. Provide information on veterinary care. For wild caught animals, **t**here is no specific veterinary care that is appropriate, nor will clinical veterinary facilities be available. Animals that are injured during the capture or sampling process will be assessed by an experienced team leader, and if the animal is determined to be unlikely to survive if released, it shall be euthanized humanely (see euthanasia section). Animals will be released within hours of capture. In the markets, animals will be sampled using manual restraint or anesthesia. Animals will be returned to vendors after sampling, or, if wild caught in the markets (e.g. rodents), they will be released in the area outside the marketplace.

Laboratory mice will be housed in the BSL-3 small animal facility Center for Animal Experiment at Wuhan Institute of Virology. Two senior Wuhan Institute of Virology veterinarians (Drs. An XueFang and Zhang Fan) will oversee the experiments. Experimental animals will be regularly monitored by experienced staff and a supervising veterinarian. The supervising veterinarian will have responsibility for the care and well-being of all mice used in the experimental studies. The animal facility operates 24 hours a day and has full-time veterinarians on staff. All animals will be provided with food and water ad libitum and will otherwise receive standard care. The Veterinarian in charge will notify the on-site Co-PI (Dr. Zhengli Shi) and the Principal Investigator (Dr. Daszak) by telephone and email if there are any issues regarding animal health and welfare

4. Procedures for ensuring animal comfort, lack of distress, pain, or injury:
Wild-caught animals: Animals will not be held longer than 6 hours. Co-PIs, Drs. Epstein and Olival have extensive experience in capture, anesthesia, and sampling wildlife, including bats. In our experience, bats and rodents tolerate the described procedure well. Mist nets will be attended continuously during capture periods, and bats will be extracted from the net as soon as they become entangled. This will minimize stress and injury from entanglement. Bats will be placed individually in cotton bags and hung from tree branches while awaiting processing and

during recovery. The bags are sufficiently porous as to allow for ventilation and are designed for bat capture. The enclosed environment seems to calm the bats, as they do not struggle once inside, but they hang quietly. Animals will be monitored by a veterinarian or experienced field team member during all stages of capture, processing, and release. Animals will be kept in a cool place while in the pillowcases. Rodent traps will be set overnight and all traps will be checked in the morning while it still cool outside. Rodents will be kept in a cool, shaded environment during sampling and will be released within 10 hours of capture. The procedures used in this experiment (blood draw, nasal, oral, and rectal swabs) are minimally invasive, however, mice that show signs of morbidity post-infection will be examined and euthanized according to AVMA standards (see below).

Market animals: Bats, rodents, and small mammals sampled in markets, sourced from vendors, will be manually restrained and sampled on-site, to minimize stress and discomfort. Because these animals are designated for human consumption, we will not use anesthetic agents if the animal is to be returned to the vendor following sampling. Manual restraint and sampling will be conducted by experienced members of the field team. Any animal that shows signs of distress (respiratory distress, pale mucous membranes) will be immediately released into a holding cage to recover. If the veterinarian or senior scientist in charge of sampling deems an individual animal to be fractious, or at risk for excessive stress and discomfort, anesthetic agents may be used for the safety of both animal and handler. Injectable tiletamine zolazepam (Telozol HCl) given intramuscularly, or isoflurane gas using a portable vaporizer may be used. Any animal that has been anesthetized for sampling will not be returned to the food chain due to possibility of human consumption of anesthetic drug. These animals will be purchased from the vendor and not returned to the market. Following sampling the animal will be euthanized according to AVMA standards and disposed of according to safe biohazard practices.

Experimental animals (mice): All experimental work will be conducted at Wuhan Institute of Virology under the supervision of senior veterinarians Drs. An XueFang and Zhang Fan. Animals will be observed daily for clinical signs of illness. All mice will be provided comfortable housing with regular access to water and food throughout the experiment. The experiments under this study do not include surgical procedures or use of experimental pharmacological agents. Mice will be anesthetized prior to sampling using isoflurane gas, which will reduce stress and discomfort. During experimental infections, mice will be monitored for signs of pain and discomfort. Moribund mice (e.g. mice showing depression, inappetance, respiratory distress, or severe fever) will be euthanized, according to AVMA recommendations.

5. Euthanasia: In the event of injury to an animal that results in pain and suffering, and reasonable veterinary care is unavailable, the animal will be euthanized by a veterinarian or trained field team member using ketamine injected intramuscularly 37.5mg/kg and sodium pentobarbital injected intravenously at a dose of 1.0ml per 5kg injected intravenously. This protocol is in accordance with the AVMA euthanasia report (2013). Any animal that is euthanized using a chemical agent will be disposed such that it will not be permitted to enter the food supply either through markets or hunting.

From: Pone, Laura (NIH/NIAID) [E]
Sent: Thu, 27 Feb 2014 14:34:02 -0500
To: (b)(6) Stemmy, Erik (NIH/NIAID) [E];
(b)(6)
Subject: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Dr. Daszak,

Please provide the revised VAS information request by OLAW below by close of business Monday, March 3rd.

I have reviewed the VAS provided for 1R01AI110964-01 and I cannot approve it as written. Points 3 and 4 were not fully answered. I will require the remaining information on points 3 and 4 to be addressed:

Performance Site: Wuhan University
Point 3 - Veterinary care

- **How does the veterinary staff communicate with the PI to provide medical care or intervention for the animals if required?**

Point 4 - Provisions to minimize discomfort, distress, pain and injury

- **Methods to alleviate discomfort, distress or pain should be described. If pharmacological agents are used, the agent(s) should be specified by name or class.**
- **Any additional means to avoid discomfort, distress, pain or injury should be described briefly.**
- **If procedures (e.g., pharmacological or surgical) might lead to severe discomfort, distress, pain or injury, indicators for humane endpoints and euthanasia (e.g., severe infection, respiratory distress, failure to eat, tumor size) should be described.**
- **Describe the use of restraint devices, if relevant.**

Link to the VAS on the OLAW website:
http://grants.nih.gov/grants/olaw/VASchecklist.pdf

Thank you,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

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From: Pone, Laura (NIH/NIAID) [E]
Sent: Mon, 24 Feb 2014 08:47:18 -0500
To: Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
(b)(6)
Subject: Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Aleksei,

It has come to my attention that animal and human subject work will be done at Guangdong Entomological Institute, Wuhan Institute of Virology, East China Normal University, Center for Disease Control and Prevention of Guangdong, and Yunnan Institute of Endemic Diseases Control and Prevention. Please begin the process for obtaining AWA's and FWA's for each site that does not already have them.

- Confirmation of FWA for human subject work. If no FWA exists please establish one. http://ohrp.cit.nih.gov/efile/FwaStart.aspx
- Confirmation of AWA for animal subject work. If no AWA exists please establish one. http://grants.nih.gov/grants/olaw/obtain_assurance.htm

Thank you,

Laura Pone
Grants Management Specialist
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From: Peter Daszak
Sent: Wed, 19 Feb 2014 21:00:16 +0000
To: Stemmy, Erik (NIH/NIAID) [E]; Aleksei Chmura
Subject: RE: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Erik (cc'd to our SOR, Aleksei Chmura),

Thanks for the questions: We do have a collection of banked samples collected from bats in China over the past four years under prior R01 and USAID funding and part of our work will involve further testing and analysis of these. However, these samples were not collected strategically from markets and sites where humans have a high risk of contact with bats. In the specific aims of the current proposal, we aim to generate predictions about how likely inter-species transmission of CoVs is, and whether this is heightened in markets, as well as about how likely it is for people with high risk of contact with bats to be infected. Therefore, at each site we will also collect samples from wild bats, bats within markets, and from other species within markets that may be susceptible to inter-species transmission of CoVs (e.g. insectivorous mammals, rodents, carnivores). This means that animal and human subjects work will be carried out in each of the four Provinces (Yunnan, Guangdong, Fujian and Guangxi, and at each site. Our IACUC and IRB applications in the USA provide information on these details.

In China, all work will be carried out by our staff under our IACUC and IRB (pending). Local contacts within university labs and provincial CDCs are necessary to ensure that access to sites and sampling is smooth and trouble-free, but they will not directly take the samples or interview people in most cases. We are working to clarify their FWA and IRB details and will make sure these are all in order as soon as possible so that where they do plan to take samples or interview people, they will have the correct assurances. The human work will begin in year 2, so that gives us enough time to make sure all these assurances are in order. The lab work at the Wuhan Institute of Virology will involve animals, and we are working on their IACUC right now (as per the just in time docs)

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
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www.ecohealthalliance.org

EcoHealth Alliance builds innovative science-based solutions and partnerships that increase our global capacity to achieve two interrelated goals: protecting global health by preventing pandemics; and safeguarding ecosystems by promoting conservation.

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, February 19, 2014 1:16 PM
To: Aleksei Chmura
Cc: Peter Daszak
Subject: RE: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

HI Aleksei,
Thanks very much for the additional information. I have a few other questions for you regarding the work in China. I know that you have multiple sites in China that will be conducting work on the animal and human samples, and that based on your application a large number of samples were previously collected.

Reading your application it's not immediately clear to me what work and sample collection will be performed at each site in China. Could you please clarify for me what animal and human subjects work will be conducted at each site, and whether it will involve collection of new samples or analysis of previously collected? Also, foreign collaborators that are collecting new animal samples or conducting human subjects work will need to have their own FWA and animal assurance numbers.

You can just email me this information directly. There's no need to update your JIT documents with it.

Thanks very much!
Erik

From: Aleksei Chmura (b)(6)
Sent: Wednesday, February 12, 2014 9:59 AM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Peter Daszak; Pone, Laura (NIH/NIAID) [E]
Subject: Re: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Erik,

We have uploaded a response to the questions about human subjects and I just cc'ed you on my response to Laura's earlier email.

Many thanks!

-Aleksei

Aleksei Chmura
Program Coordinator & AOR
EcoHealth Alliance

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www.ecohealthalliance.org

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EcoHealth Alliance integrates innovative science-based solutions and partnerships that increase capacity to achieve two interrelated goals: protecting global health by preventing the outbreak of emerging diseases and safeguarding ecosystems by promoting conservation.

On 11 Feb 2014, at 10:09:25, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Hi Peter and Aleksei,
One other question about your application. There were a couple of human subjects concerns noted by the study section. I know your IRB approval is still pending, but were you able to address the other human subjects questions? Unless I missed it I didn't see anything in the JIT documents you uploaded.

Thanks,
Erik

Erik J. Stemmy, Ph.D.
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
6610 Rockledge Drive, Room 3210
Bethesda, MD 20892-7630
Phone: (b)(6)
Fax: 301-496-8030
Email: (b)(6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Pone, Laura (NIH/NIAID) [E]
Sent: Monday, February 10, 2014 3:56 PM
To: (b)(6) Stemmy, Erik (NIH/NIAID) [E]; (b)(6)

(b)(6)

Subject: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

<image001.png>

Dear Mr. Chmura,

For applications well received by study section during peer review, we attempt to obtain documentation that must be submitted to the National Institute of Allergy and Infectious Diseases should an application subsequently be identified for funding. Since your application is among those favorably received, we request that you submit the information listed below:

Please submit this information by close of business **Thursday, February 13th.**

- Human Subjects Assurance documentation. **Include grant specific IRB approval date**. Grant specific IRB approvals must include either the project title or grant number.
- Documentation of the Required Education in the Protection of Human Subject Research Participants for all personnel involved.
- IACUC verification statement/letter with approval date.
- Response to Summary Statement Concern Regarding:
  - Protection of Human Subjects
  - Overlap
- Copy of EcoHealth Alliance's most recent F&A rate agreement.

Timely submission of the above information will enable us to expedite the issuance of an award should an application be identified for funding. Please submit this information by 02/13/14.

JIT information should be submitted using the Just-In-Time feature of the eRA Commons found in the Commons Status section. Submit **all** information at one time. For information on the Commons, go to the Commons Web site: https://commons.era.nih.gov/commons/index.jsp. If not submitting through the Commons ***or*** for information unable to be submitted through the Commons, please email the requested information signed by an authorized institutional business official**. Emailed documents not endorsed by an Institution Business Official will not be accepted as valid.**

Please feel free to contact me with any questions or concerns.

Thanks and have a nice day!

Laura Pone
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Email: (b)(6)

please consider the environment before printing this e-mail.

From: Aleksei Chmura
Sent: Wed, 12 Feb 2014 14:57:32 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER
Attachments: Daszak PI Understanding the risk of bat coronavirus emergence JIT requests 02.pdf, ATT00001.htm

Dear Laura,

We have uploaded responses to the five points of information requested below via the Just In Time portal on eRA Commons. I have attached a PDF of the information here as well.

Please let me know, if you have any further questions.

Many thanks most,

Sincerely,

Aleksei Chmura
Program Coordinator & AOR
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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EcoHealth Alliance integrates innovative science-based solutions and partnerships that increase capacity to achieve two interrelated goals: protecting global health by preventing the outbreak of emerging diseases and safeguarding ecosystems by promoting conservation.

EcoHealth Alliance

February 11, 2014

JUST IN TIME REQUESTED INFORMATION

1R01AI110964 *Understanding the Risk of Bat Coronavirus Emergence* (PI, Daszak)

Dear Laura Pone,

In response to your email from the 10th of February we have provided responses below to the information requested as below:

1) Human Subjects Assurance documentation. Include grant specific IRB approval date. Grant specific IRB approvals must include either the project title or grant number.
2) Documentation of the Required Education in the Protection of Human Subject Research Participants for all personnel involved.
3) IACUC verification statement/letter with approval date.
4) Response to Summary Statement Concern Regarding:
    a. Protection of Human Subjects
    b. Overlap
5) Copy of EcoHealth Alliance's most recent F&A rate agreement.

RESPONSES:

1) IRB: Our IRB with Tufts University Health Science through our inter-institutional agreement with them is in process and the FWA for this is **FWA00004517**.

2) EDUCATION IN THE PROTECTION OF HUMAN SUBJECT RESEARCH PARTICIPANTS for all personnel involved is underway and we will provide certificates for Daszak, Epstein, Ge, Shi, Zhu, Ke, Olival, Zhang, Olival, and Zhang **before the end of February**.

3) IACUC: Our IACUC approval is also pending with Tufts University through our inter-institutional agreement with them. The OLAW Assurance number listed (**A4059-01)** is correct. Once we have an IACUC date, we will inform NIH immediately.

4) A: PROTECTION OF HUMAN SUBJECTS: We have revised this and specifically included language to address the following: **(a)** the survey is totally voluntary and the subjects may withdraw at any time**, (b)** the survey is anonymous and there is no connection between the surveyed individual ID and the clinical samples, and **(c)** we have a signed confidentiality agreement with NIH that protects the PIs from having to disclose information about the study**.** The following addresses the SRG concerns about protection of human subjects and applies to both human studies described in the proposal:

a. Survey of people highly exposed to wildlife in Guangxi, Yunnan, and Fujian provinces

b. Survey of cases of respiratory illness within the Shanghai CDC influenza-like illness surveillance program

[Study description from proposal with new material highlighted and in bold] *Expanding on our work in Guangdong, we will develop a voluntary study of animal vendors and hunters in Guangxi, Yunnan, and Fujian provinces in cooperation with local Bureaus of Public Health and CDCs. We will develop a survey to identify people with high exposure to wildlife, particularly bats, and will recruit volunteers, collect blood, sputum, and stool sample from each enrolled participant. We will screen sera for antibodies to SARS-CoV, other alpha & beta coronaviruses including MERS-CoV, and novel bat-CoVs. We will screen stool from CoV seropositive participants for CoV nucleic acid. We will also develop specific bat-CoV serological assays and share these with our Chinese collaborators. In each province in southern China we will aim to include 10 markets and survey 20 vendors per market; 20 additional wildlife hunters per province (220 case subjects); 400 control subjects from the general population near the markets in each province (total of 620 people per province). For Shanghai, we will enroll 200 acute respiratory illness cases and 400 non-respiratory controls (600 total), The total number of human subjects will be 2460. The study will be conducted in Guangxi, Yunnan, Fujian and Shanghai provinces*

HUMAN SUBJECTS RESEARCH

1. Risk to subjects: This project is a study of human exposure to animal coronaviruses in southern China. Subjects will be enrolled on a voluntary basis and a single interview and sample collection will be conducted. Informed consent will be obtained. People found to be infected with an animal coronavirus will be followed up after 6 months with a secondary interview and collection of biological specimens to determine whether infection is persistent and exposure is ongoing. Primary subjects will be male or female adults who are highly exposed to wildlife through hunting, butchering, or general handling in the context of live animal markets or restaurants that prepare and serve wild animals. The study population will be selected in Shanghai, Yunnan, Fujian, and Guangxi provinces, China, and will be open to people of all ethnicities that fit the subject criteria. We will target human subjects, comprising 220 subjects (market workers and hunters) and 400 controls from the general population in Yunnan, Fujian, and Guangxi provinces plus 600 subjects in Shanghai (total enrolled: 2460). The market types are defined in Specific Aim 1, Human exposure to CoVs. There are no data to suggest an ethnic bias for coronavirus exposure or infection, therefore subjects will be enrolled based on exposure criteria, though subjects will not be excluded based on ethnicity or gender. We will endeavor to have an equal number of men and women, if the composition of animal vendors in markets allows.

Sources of Materials: Samples to be collected and screened for coronaviruses include blood, saliva and stool samples. 10 mL of blood will be collected from each subject. Subjects will also be asked to provide saliva and stool in sterile containers. An initial sample collection and interview will be performed by trained medical personnel from the local CDC under the provincial Public Health Bureau. Sample collection will be done once in years 2-4 of the study. Samples will be screened for coronaviruses using PCR and an ELISA at the appropriate CDC microbiology lab or at the Wuhan Institute of Virology. Samples that test positive for coronavirus or antibodies to coronavirus will be followed up after 6 months with a secondary interview designed to determine the current level of

exposure to wild animals, and whether exposure at the current level was consistent between the first and subsequent interview. Repeated clinical samples will also be collected and tested for coronaviruses. In all instances, volunteers will be given a medical exam and informed of their test results.

Potential risks: The potential risks to study participants resulting from study participation are minimal. ***The volume of blood being collected is within normal safety limits. The interview questions will be designed to assess exposure risk, and may ask personal questions, but surveys will be done in private and anonymized to protect privacy. Some of the questions may include information about selling or trading animal species that are prohibited by local or federal laws. The participants may be reluctant to answer questions that implicate them in criminal activity and may become nervous following participation if their answers implicate them in potentially illegal activities. Participation in the survey and study is completely voluntary, and a participant may withdraw from the study at any time, or decline to participate in any aspect of the study, including declining to answer specific questions.***

There may be information contained in the surveys that implicates an individual or place of business in illegal trade activities. This could potentially have real or perceived negative legal or financial impacts on the respondent, their place of business, or the larger marketplace from which the information was obtained.

There may be some stress to subjects who are informed that they have been exposed to an animal virus, but counseling will be available and options for medical care will be included in the discussion.

2. Adequacy of protection against risks: Recruitment and informed consent: Prospective study participants will be identified by the research team at each site in partnership with provincial CDC personnel. The team will be thoroughly trained on communicating the research objectives and will be able to address any questions that potential subjects may have. Both written and oral descriptions of the study will be provided in Chinese (in Mandarin or via an interpreter in local dialect if necessary) as part of the informed consent process. Contact details of the collaborators at local CDCs and the study PI will be provided to all subjects, and CDC personnel on the research team will be available on site to answer questions from the study subjects. Test results will be communicated to each subject and counseling offered to minimize stress.

Subjects will be informed, via written consent forms and oral explanation of the consent forms, that their participation is entirely voluntary and that they will have the right to decline to participate in any part of the study, and may decline to answer any questions in the survey. Further, the participant's identity will remain anonymous. They will be assigned a coded ID number that will link their responses to the questionnaire to their clinical specimens, but any identifying information will be kept separate from these data and held in a secure cabinet by the local investigator. For the purposes of achieving the aims of this study, data derived from questionnaires can be analyzed in aggregate by region within a province, without revealing the name or location of specific markets. This will serve to minimize the legal and economic risks to specific markets or vendors that may provide information about potentially unlawful actions.

The PI has entered into a confidentiality agreement with NIH to further protect study subjects from the release of any personally identifying information. Confidentiality for all participants will be protected to the greatest possible extent by law. Consent forms and the front page of the

questionnaire containing the name of the participants will be stored separately from the rest of the data and held by the Local Project Manager on site. Access to personal identifiers by the Project Coordinator is allowed only for the purposes of contacting the participant of their results and participation in follow-up studies if they desire. For research purposes and data analysis, test results and questionnaires will be linked by coded numbers, and only by code numbers. Researchers and investigators handling the data will not have access to participant names. The page containing identifiers will be separated from the rest of the questionnaire and stored separately in a locked facility on site. Only the Project Director and site Coordinators will have access to such information for follow-up, identification (such as photographs) and the offering of counseling services. Only unidentifiable-linked questionnaire data, accident report information, and corresponding test results will be made accessible to project investigators. The participants' identifiable data and contact information will be kept until the end of the study and then destroyed. Results given to the Ministry of Health will be reported in aggregate form only; no individual names will ever be reported or published. Results will not be included in the individual's general health record.

3. Potential benefits to Subjects and Others: There are potential benefits to the study subjects including receiving a physical exam/health check from a medical officer and the potential benefit of identifying an occupational health hazard. At the conclusion of the study, we will deliver an educational workshop for high risk individuals (open to study subjects and non-study subjects) describing the health benefits of using PPE and hand-washing during animal handling activities throughout the day.

4. The importance of knowledge to be gained. There are valuable potential benefits to the general public from the knowledge to be gained by this study, as it may identify sources of zoonotic coronaviruses in the market system or which are commonly hunted. Avoidance of these animals or extra care when handling them may substantially reduce the risk of CoV (and other zoonotic pathogen) transmission.

Inclusion of Women: This proposal will enroll men and women as study subjects. Depending on local gender composition of animal vendors, we will make every effort to have men and women equally represented in this study.

Inclusion of minorities: Subjects will be enrolled in this study without regard to ethnicity. Occupational exposure to wildlife in a market, hunting, or butchering context will be the primary criteria for identifying subjects.

*Inclusion of Children: Children **(subjects below age 18)** will not be included in this study. Children do not normally work in wildlife markets, and are not normally involved in the wildlife trade in China.*

Total planned enrollment: See enrollment table

4) B: OVERLAP: The summary statement requested that: "[Budgetary] Overlap with PREDICT and other R01 funded projects should be better defined". The first (PREDICT) is a contract from USAID with the goal of building capacity in developing countries to identify and address new pandemic threats. The work funded by this contract covers 24 countries, and aims to 1) identify regions of high risk for viral spillover from wildlife to humans, conduct preliminary surveillance of wildlife, take blood samples, and conduct RT-PCR assays to identify new viruses present in them; and 2) to work with local agencies to

build laboratory capacity for viral work within the countries. The surveillance conducted in this project was used to build preliminary data for our proposal. However, this is primarily a capacity building project and is specifically defined as a non-research project so that none of the hypotheses in our current proposal are being tested. Furthermore, fieldwork for this project has been designated by USAID to end by June 2014 and the project completely ends on September 30th 2014. Work in China is now being conducted on birds, rats and primates only. Three other R01 projects were current at the time of submission:

1) R01GM100471 ("Modeling anthropogenic effects in the spread of infectious diseases") is an economic modeling grant that uses mathematical equations to describe the economic impact of disease spread, and therefore has no overlap

2) 2R01TW005869 ("The Ecology, Emergence and Pandemic Potential of Nipah virus in Bangladesh") focuses the vast majority of its work on Nipah virus within Bangladesh, but some of the funding was used with permission from the Fogarty International Center to build collaborations with our Chinese partners by conducting bat testing within China. This grant is now in a 6th year no-cost extension to finish human survey work in Bangladesh and no further work in China is planned or budgeted. The no-cost extension year ends on June 1st 2014

3) 1R01AI079231 ("Risk of viral emergence from bats") was focused on detailed surveys of bat species in 10 countries globally and viral diversity analyses (PCR-based), as well as hotspot modeling for bat-origin viruses. The grant ended on 8/31/2013 and the final report has been filed. This award was also used to build preliminary data for our current proposal. No other grants have been applied for or awarded that have any other overlap with the current proposed work.

5) **F&A RATE AGREEMENT:** We have already uploaded the latest EcoHealth Alliance F&A rate agreement via the Just In Time interface in eRA Commons.

If you have any other questions, please contact me anytime. We are very appreciative of your consideration and look forward to further details.

Yours sincerely,

(b)(6)

Aleksei Chmura
Program Coordinator & AOR
EcoHealth Alliance
460 West 34th Street, 17th Fl.
New York, NY 10001, USA
(b)(6)
(b)(6)

On 10 Feb 2014, at 15:56:29, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

<image001.png>
Dear Mr. Chmura,

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- IACUC verification statement/letter with approval date.
- Response to Summary Statement Concern Regarding:
  - Protection of Human Subjects
  - Overlap

|↵| Copy of EcoHealth Allianceï¿½s most recent F&A rate agreement.

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JIT information should be submitted using the Just-In-Time feature of the eRA Commons found in the Commons Status section. Submit **all** information at one time. For information on the Commons, go to the Commons Web site: https://commons.era.nih.gov/commons/index.jsp. If not submitting through the Commons ***or*** for information unable to be submitted through the Commons, please email the requested information signed by an authorized institutional business official. **Emailed documents not endorsed by an Institution Business Official will not be accepted as valid.**

Please feel free to contact me with any questions or concerns.

Thanks and have a nice day!

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)
please consider the environment before printing this e-mail.

From: Pone, Laura (NIH/NIAID) [E]
Sent: Tue, 11 Feb 2014 17:00:54 -0500
To: Aleksei Chmura
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hi Aleksei,

I apologize for any inconvenience this may cause, however, it is a standard requirement that EcoHealth obtain their own FWA and I cannot issue the award until this is completed. The FWA that you establish will need to be linked to Tuft's IRB as instructed below. Establishing the FWA is a fairly quick process that can be completed online. Please work to obtain the FWA and let me know once it has been established.

Thank you,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)
please consider the environment before printing this e-mail.

From: Aleksei Chmura (b)(6)
Sent: Tuesday, February 11, 2014 3:33 PM
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Laura,

EcoHealth Alliance has an inter-institutional agreement with Tufts University that was set up specifically for IRB work under our previous NIH awards. We have used Tufts' FWA for a

number of IRB approval requests. We have provided the Tufts University FWA and will send the IRB approval letter as rapidly as possible.

Please call or email me anytime, if you have questions or require further details.

Many thanks most,

Sincerely,

Aleksei Chmura
Program Coordinator
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
(mobile)
(b)(6) (China)
(b)(6) (Skype)

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EcoHealth Alliance integrates innovative science-based solutions and partnerships that increase capacity to achieve two interrelated goals: protecting global health by preventing the outbreak of emerging diseases and safeguarding ecosystems by promoting conservation.

On 11 Feb 2014, at 14:00:34, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Dear Mr. Chmura,

I would also like to follow up and note that EcoHealth must establish its own FWA and link that FWA to the designated IRB. I have included information regarding establishing an FWA below for your reference. You will also need to fill out this form.

http://www.hhs.gov/ohrp/assurances/forms/irbauthorizpdf.pdf

What are the procedures for submitting a Federalwide Assurance (FWA)?
Institutions must submit all FWAs (including new submissions, updates, and renewals) electronically using the electronic submission system available through the OHRP website at http://ohrp.cit.nih.gov/efile/, unless an institution lacks the ability submit electronically. If an institution believes it lacks the ability to submit its FWA electronically, please contact OHRP by telephone or email (seehttp://www.hhs.gov/ohrp/assurances/contact/index.html) and explain why the institution cannot submit its FWA electronically.

The FWA application will only be considered complete by OHRP when it is completed in its entirety, signed by the Signatory Official, and dated. Additionally, the IRB(s) designated on the FWA must be registered with OHRP before the FWA can be approved.
The instructions for submitting an FWA (new submission, update, and renewal) may be found athttp://www.hhs.gov/ohrp/assurances/assurances/index.html.
http://answers.hhs.gov/ohrp/categories/1563

Thank you,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
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e-Fax: 301-493-0597
Email: (b)(6)
please consider the environment before printing this e-mail.

From: Pone, Laura (NIH/NIAID) [E]
Sent: Monday, February 10, 2014 3:56 PM
To: (b)(6) Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
(b)(6)
Subject: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

<image001.png>
Dear Mr. Chmura,

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    - Overlap
- Copy of EcoHealth Alliance's most recent F&A rate agreement.

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Please feel free to contact me with any questions or concerns.

Thanks and have a nice day!

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

From: Peter Daszak
Sent: Tue, 11 Feb 2014 20:26:50 +0000
To: Stemmy, Erik (NIH/NIAID) [E]; Aleksei Chmura
Cc: Pone, Laura (NIH/NIAID) [E]
Subject: RE: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Erik,

We will definitely be able to deal with these concerns. I'm drafting a cover letter that will respond to these as well as the potential budgetary overlap, and will ask Mr. Chmura to send these on to you once I've checked on the dates for the IACUC and IRBs etc. I should be able to send this out to you before COB today.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

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+1.212.380.4465 (fax)
www.ecohealthalliance.org

EcoHealth Alliance builds innovative science-based solutions and partnerships that increase our global capacity to achieve two interrelated goals: protecting global health by preventing pandemics; and safeguarding ecosystems by promoting conservation.

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, February 11, 2014 10:09 AM
To: Peter Daszak; Aleksei Chmura
Cc: Pone, Laura (NIH/NIAID) [E]
Subject: RE: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hi Peter and Aleksei,
One other question about your application. There were a couple of human subjects concerns noted by the study section. I know your IRB approval is still pending, but were you able to address the other human subjects questions? Unless I missed it I didn't see anything in the JIT documents you uploaded.

Thanks,
Erik

Erik J. Stemmy, Ph.D.
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
6610 Rockledge Drive, Room 3210
Bethesda, MD 20892-7630
Phone: (b)(6)
Fax: 301-496-8030
Email: (b)(6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Pone, Laura (NIH/NIAID) [E]
Sent: Monday, February 10, 2014 3:56 PM
To: (b)(6) Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
(b)(6)
Subject: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
National Institute of Allergy
and Infectious Diseases
Bethesda, Maryland 20892

Dear Mr. Chmura,

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Please feel free to contact me with any questions or concerns.

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e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

From: Aleksei Chmura
Sent: Mon, 10 Feb 2014 22:47:03 +0000
To: Steele, Lisa (NIH/CSR) [E]
Cc: Peter Daszak; Kevin Olival, PhD; Evelyn Luciano; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Review of your recent NIH grant application submission (b)(4); (b)(6)
(b)(4); (b)(6)

Dear Lisa,

We appreciate your rapid reply.

Our *mBio* paper was just accepted, so there was no way to provide notification prior to the 30-day window, but we respect the restriction.

Many thanks!

-Aleksei

On 10 Feb 2014, at 16:43:25, Steele, Lisa (NIH/CSR) [E] (b)(6) wrote:

I'm afraid tha NIH policy sets the post-submission deadline to 30 days prior to the meeting as outlined in my introductory email.
Best regards,
Lisa

From: Aleksei Chmura (b)(6)
Sent: Monday, February 10, 2014 04:36 PM
To: Steele, Lisa (NIH/CSR) [E]
Cc: Peter Daszak (b)(6) Kevin Olival, PhD (b)(6)
Evelyn Luciano (b)(6) Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Review of your recent NIH grant application submission (b)(4); (b)(6)
(b)(4); (b)(6)

Dear Dr. Steele,

I would like to let you know about a paper from our research group that has just been accepted and will be published this month in *mBio*. Our study furthers the understanding of the ecology and diversity of MERS and gives excellent background to the work detailed in our proposal (b)(4); (b)(6)
(b)(4); (b)(6)

In our *mBio* paper, we examined the historical and current prevalence of MERS coronavirus infection in dromedary camels and other livestock in the Kingdom of Saudi Arabia where the index and the majority of cases of Middle East Respiratory Syndrome have been reported and provide evidence that MERS coronaviruses have been circulating in camels since at least 1992, are distributed countrywide, and can be

phylogenetically classified into clades that correlate with outbreaks of human disease. I have pasted the abstract and citation below and attached a pre-production copy.

We hope that you will consider the publication of our paper by *mBio* as providing additional support for our capacity to conduct significant international collaborative research on MERS-CoV.

Sincerely,

Aleksei Chmura
Program Coordinator & AOR
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
(mobile)
(b)(6) (China)
(b)(6) (Skype)

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EcoHealth Alliance integrates innovative science-based solutions and partnerships that increase capacity to achieve two interrelated goals: protecting global health by preventing the outbreak of emerging diseases and safeguarding ecosystems by promoting conservation.

Citation: Briese T, Mishra N, Kapoor V, Sameroff S, de Wit E, Munster VJ, Hensley LE, Zalmout IS, Kapoor A, Epstein JH, Karesh W, Daszak P, Mohammed OB, Lipkin WI. (2014) Middle East Respiratory Syndrome coronavirus (MERS-CoV) infection in Dromedary Camels in Saudi Arabia. *mBio*

Abstract: The Middle East Respiratory Syndrome (MERS) is proposed to be a zoonotic disease; however, the reservoir and mechanism for transmission of the causative agent, the MERS coronavirus, is unknown. Dromedary camels have been implicated through reports that some victims have been exposed to camels, camels in areas where disease has emerged have antibodies to the virus, and viral sequences have been recovered from camels in association with outbreaks of human disease. Nonetheless, whether camels mediate transmission to humans is unresolved. Here, in a geographical and temporal survey of camels in the Kingdom of Saudi Arabia, we provide evidence that MERS coronaviruses have been circulating in camels since at least 1992, are distributed countrywide, and that they can be phylogenetically classified into clades that correlate with outbreaks of human disease. We found no evidence of infection in domestic sheep or domestic goats.

From: Aleksei Chmura
Sent: Mon, 10 Feb 2014 21:36:01 +0000
To: Steele, Lisa (NIH/CSR) [E]
Cc: Peter Daszak; Kevin Olival, PhD; Evelyn Luciano; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Review of your recent NIH grant application submission (b)(4); (b)(6)
(b)(4); (b)(6)
Attachments: Briese et al mbio.pdf, ATT00001.htm

Dear Dr. Steele,

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(b)(4); (b)(6)

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Title: Middle East Respiratory Syndrome coronavirus (MERS-CoV) infection in Dromedary Camels in Saudi Arabia
Running title (54 characters): MERS-CoV in Saudi Arabian camels

Authors: Abdulaziz N. Alagaili [*,1,2], Thomas Briese [*,3], Nischay Mishra[3], Vishal Kapoor[3], Stephen C. Sameroff[3], Emmie de Wit[4], Vincent J. Munster[4], Lisa E. Hensley[5], Iyad S. Zalmout[1], Amit Kapoor[3], Jonathan H. Epstein[6], William B. Karesh[6], Peter Daszak[6], Osama B. Mohammed[1], W. Ian Lipkin[3]

1. KSU Mammals Research Chair, Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia
2. Saudi Wildlife Authority, Riyadh, Saudi Arabia
3. Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, NY, USA
4. Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, MT, USA
5. Integrated Research Facility, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Frederick, MD, USA
6. EcoHealth Alliance, New York, NY, USA

* *Equal contributions and corresponding authors*

Correspondent footnote: Thomas Briese, tb2047@columbia.edu. Abdulaziz N. Alagaili, aalagaili@ksu.edu.sa.

Manuscript Word Count (incl. acknowledgements): **3,313**

Abstract: The Middle East Respiratory Syndrome (MERS) is proposed to be a zoonotic disease; however, the reservoir and mechanism for transmission of the causative agent, the MERS coronavirus, is unknown. Dromedary camels have been implicated through reports that some victims have been exposed to camels, camels in areas where disease has emerged have antibodies to the virus, and viral sequences have been recovered from camels in association with outbreaks of human disease. Nonetheless, whether camels mediate transmission to humans is unresolved. Here, in a geographical and temporal survey of camels in the Kingdom of Saudi Arabia, we provide evidence that MERS coronaviruses have been circulating in camels since at least 1992, are distributed countrywide, and that they can be phylogenetically classified into clades that correlate with outbreaks of human disease. We found no evidence of infection in domestic sheep or domestic goats.
Abstract Word Count: 141

Importance: This study was undertaken to determine the historical and current prevalence of MERS coronavirus infection in dromedary camels and other livestock in the Kingdom of Saudi Arabia, where the index and the majority of cases of Middle East Respiratory Syndrome have been reported.
Importance Word Count: 43

Introduction

One-hundred-and-eighty laboratory-confirmed cases of human infection with Middle East respiratory syndrome coronavirus (MERS-CoV), 77 of them fatal, have been reported through January 30, 2014 (1) following identification of the index case in the Kingdom of Saudi Arabia (KSA) in September 2012 (2). The majority of infections have been identified in KSA with lower numbers in Jordan, Qatar, Tunisia and United Arab Emirates. Although cases have also been reported in France, Germany, Italy and the United Kingdom, all have been linked to the Middle East either by travel of the individual through an area where MERS-CoV has been reported or by direct or indirect contact with others who have a travel history consistent with exposure in the Middle East (3).

Clusters of human infection indicate that human-to-human MERS-CoV transmission can occur (4, 5). However, the origin of infection in most cases remains unknown. Analysis of human sequences by Cotten et al. has revealed the presence of at least three circulating genotypes within KSA alone (6). Phylogenetic analyses of 13 complete and 8 partial genome sequences enabled estimates of the timing and geographic origin of individual viral clades. The authors proposed that MERS-CoV emerged in humans in 2011 and noted that sequence divergence between clades is consistent with several sporadic introductions of the virus into the human population, presumably from an animal reservoir.

Efforts to identify an animal reservoir have focused on bats and camels. Bats harbor a wide range of beta-coronaviruses (7); furthermore, bat cell lines display the MERS-CoV receptor, dipeptidyl peptidase 4 (8), and can be experimentally infected. A short

sequence fragment consistent with MERS-CoV was reported in a bat in Bisha, KSA, in close proximity to the home and workplace of the 2012 index case that resulted in the initial isolation of the virus (9). That same case owned four pet dromedary camels (DC). Serological analysis of those DC revealed the presence of antibodies reactive with MERS-CoV; however, no MERS-CoV sequences were found by PCR analysis of nasal or rectal swabs or serum. Additional human cases have been associated with exposure to DC and in some instances investigators have described both serological and genetic evidence of MERS-CoV infection in DC. Memish and coworkers reported PCR detection of MERS-CoV sequences in a DC with respiratory illness owned by an individual with MERS-CoV who had no history of contact with other human cases (10). Haagmans et al. investigated an outbreak of human disease on a Qatari farm and found MERS-CoV sequences in nasal swabs from 6 of 14 seropositive DC. Analysis of ORF1a and fragments representing ORF1b, spike and ORF4b revealed similarity but not identity to sequences obtained from the human MERS-CoV cases from the same farm. The authors provide evidence that MERS-CoV can infect DC but cautiously conclude that data are insufficient to determine whether infection spread from DC to humans, humans to DC or via another host to both species (11).

Several groups have reported serological reactivity with MERS-CoV or a closely related virus in DC in the Middle East (12-15). Reusken et al. found antibodies in 100% of 50 Omani DC, 14% of 105 Canary Island DC but no seropositive northern European DC, domestic sheep, domestic goat or domestic cattle (13). In two regions of KSA, Hemida and colleagues detected antibodies to MERS-CoV in 90% of 310 DC but not in sheep, goats, cattle or chickens. The seroprevalence was lower in DC less than 1 year of age

(72% vs. 95%) suggesting widespread infection in early life (15).

To determine the prevalence of MERS-CoV infection in DC throughout KSA we undertook a nationwide survey using both serological and molecular methods.

Results

Serum, whole blood, and rectal and nasal swabs were freshly collected from DC, sheep and goats in November and December of 2013 in the southwestern (Gizan), western (Taif), northwestern (Tabuk), eastern (Hofuf) and central regions (Unizah, Riyadh) of KSA (**Table 1**). We also collected archived serum samples obtained from DC in 1992 through 2010 (**Table 2**). Sera were initially tested for the presence of antibodies reactive with MERS-CoV by using a cell enzyme-linked immunosorbent assay (ELISA) based on Vero cells infected with MERS-CoV. Subsets of sera positive in ELISA were tested in Western blot assays that employed extracts of Vero cells infected with MERS-CoV and a luciferase immunoprecipitation system (LIPS) assay based on recombinant MERS-CoV nucleoprotein. Potential serologic cross-reactivity to bovine coronavirus (Bo-CoV) was addressed by testing for reactivity to Bo-CoV nucleocapsid protein by LIPS assay. The presence of viral nucleic acids was assayed in rectal and nasal swabs, and a subset of sera and whole blood samples by reverse transcription quantitative polymerase chain reaction (RT-qPCR) employing primers targeting the upE and ORF1a genome regions of MERS-CoV (16, 17).

One-hundred-and-fifty of 203 DC (74%) sampled countrywide in 2013 had antibodies to MERS-CoV in ELISA. The prevalence of seropositive DC was higher in adults (>2 years

of age; 93/98, 95%) than in juveniles (≤2 years of age; 57/104, 55%) ($p < 0.0001$, X^2 test). The lowest prevalence of seropositive juveniles was found in the southwestern region, in proximity to the city of Gizan (1/21, 5%) (**Fig. 1A**). A higher prevalence of antibodies to MERS-CoV in older animals was also seen in samples obtained in years prior to 2012. In 2010 the seroprevalence was 76% (16/21) in juveniles vs. 91% (21/23) in adults in the central region (Riyadh/Kharj). In 2009 the seroprevalence was 72% (40/56) in juveniles vs. 92% (24/26) in adults (Riyadh/Rumah) (**Table 2**). Seroreactivity was also found in samples from DC collected as early as the 1990s: 1/1 (100%) in 1992, 2/2 (100%) in 1993, 114/123 (93%) in 1994, 6/6 (100%) in 1996 and 6/6 (100%) in 2003. Analysis of selected seropositive DC sera by western immunoblot indicated two distinct reaction patterns that showed reactivity either to MERS-CoV spike glycoprotein alone or to spike glycoprotein and nucleocapsid protein. These results were concordant with results obtained in MERS-CoV nucleocapsid LIPS assays (**Fig. 2; Table S1**).

Potential serologic cross-reactivity to bovine coronavirus (Bo-CoV) was addressed by analyzing Bo-CoV nucleocapsid reactivity, which has been shown to be subgroup-specific in CoVs (18). Overall, 17% (35/203) of DC were positive for Bo-CoV, ranging from 3% in the southwest (Gizan) to 25% in the east (Hofuf) and 20% in adult vs. 14% in juvenile animals; 2 animals were seropositive to Bo-CoV exclusively (2 juveniles from Taif), while the remaining 16% (33/203) were reactive to Bo-CoV and MERS-CoV, and 58% (117/203) were reactive to MERS-CoV alone (see **Table S1**). In 2010, 47% (22/47) were Bo-CoV positive (57% (13/23) of adults and 38% (9/24) of juveniles, with 1 adult exclusively positive for Bo-CoV) and in 2009 20% (16/80; 8% (2/26) adults and 26% (14/54) juvenile, with 2 juveniles exclusively positive for Bo-CoV).

MERS-CoV nucleic acids were assayed by RT-qPCR in rectal and nasal swabs collected in parallel to serum samples from the same animals. Nucleic acid was most frequently detected in nasal swabs; rectal swabs were found positive in only 3 cases, in 2 of them the nasal sample was also positive. The regional distribution of PCR-positive animals is shown in **Fig. 1B**. In contrast to serology where MERS-CoV-reactive antibodies were more prevalent in adults than juveniles (95% vs. 55%), MERS-CoV nucleic acid was found more frequently in juveniles (36/104, 35%) than in adults (15/98, 15%) ($p= 0.003$, X^2 test). The 5 samples with $>10^6$ copies were all from juveniles, 4 of them from seronegative animals. The prevalence of PCR positive DC ranged from 66% in Taif in the west to 0% in Gizan in the southwest. PCR analysis of a random selection of serum and whole blood samples collected from nasal or rectal swab PCR-positive, seropositive and seronegative DC revealed no evidence of viremia (**Table S1**). These included 13 adults and 29 juveniles collected in 2009,15 adults and 14 juveniles collected in 2010, and 8 adults and 13 juveniles collected in 2013. These samples included the 5 juveniles with the highest viral genome sequence load in nasal swabs.

Sera from goats (n=36) and sheep (n=112) collected in 2013 from the central region (Unizah/Riyadh) were not immunoreactive with MERS-CoV but were immunoreactive with Bo-CoV (25% of goats, n=36; 54% of sheep, n=24). Nasal swabs of 36 goats and 78 sheep were negative in RT-qPCR assays for MERS-CoV upE.

To test the validity of RT-qPCR results and determine phylogenetic relationships of viral sequences found in KSA DC to previously reported sequences, we amplified and

sequenced longer regions of the spike, ORF1ab and nucleocapsid genes from RT-qPCR positive samples (for primer sequences see **Table S2**). Eleven of 13 swab samples with >10^5 copies in upE RT-qPCR yielded products for sequencing. No suitable products were obtained in samples with lower viral sequence load (<10^4 copies) (see **Table S1)**. Phylogenetic analysis of a 1044 nt region of the spike gene and a 2004 nt region of the ORF1ab gene indicated <1% divergence from published MERS-CoV sequences (**Fig. 3**)(GenBank accession no. KJ396766-71). Nucleocapsid sequence was identical to reported MERS-CoV sequences (GenBank accession no. KJ396756-65).

Discussion

MERS-CoV is posited to be a zoonosis. However, the evolutionary history of MERS-CoV and the reservoirs and vectors for human infection remain obscure. Early anecdotal reports that some MERS-CoV victims had exposure to DC led to serologic investigation of DC in Spain and Oman (14), Jordan (12), Egypt (13) and KSA (15) that revealed antibodies to MERS-CoV. Definitive evidence that DC can be infected with MERS-CoV was obtained when viral sequences were detected in nasal swabs from DC sampled in close proximity to outbreaks of human disease in Qatar (11) and Jeddah, KSA (10). Nonetheless, as noted by Nishiura and colleagues, current data do not fulfill the two criteria required to implicate DC as a significant reservoir species in the epidemiology of MERS-CoV (19): (1) that DC are sufficient to maintain MERS-CoV and (2) that the presence of DC is essential to continuous transmission of infection. Results presented here do not establish the latter; however, they do provide evidence for the former.

Our study is the first comprehensive countrywide survey of DC from KSA, the country with the most recorded MERS-CoV cases, using both serological and molecular diagnostic methods. Analysis of specimens from western regions of the country, from Tabuk in the northwest, Taif in the west, and Gizan in the southwest, revealed regional differences. Although the seroprevalence was high in adults throughout the country at >80%, in juveniles it ranged from 90% in the east to 5% in the southwest. The seroprevalence in DC ≤2 years of age was lower than in older animals, confirming results of Hemida et al. (15). Molecular analysis of nasal and rectal swab specimens indicated the highest prevalence of MERS-CoV sequences in DC in the west/north-west. Nasal swabs with high sequence loads (>10^5 copies) also clustered in the Taif region. A second sample collection in the west (Taif) separated from the first by an interval of two months confirmed the presence of high viral sequence loads in nasal swabs collected from juvenile animals sampled in this area (data not shown). These findings suggest that continuous, longer-term surveillance will be necessary to determine the dynamics of virus circulation in DC populations. Lower prevalence rates were evident in samples from the southwest for both MERS-CoV and Bo-CoV. This may relate in part to the enforcement of livestock movement restrictions in and out of Gizan Province due to the Rift Valley Fever (RVF) outbreak in 2000 but also to the generally lower DC population density characteristic of this region compared to other regions in the Kingdom of Saudi Arabia.

Viral nucleic acid was more commonly detected in nasal swabs than in rectal specimens and was more frequent in juvenile than in adult animals. These findings together with absence of viremia and known respiratory tract tropism of several other coronaviruses

suggest airborne transmission is the most likely mode of MERS-CoV transmission. Although nucleic acid copy numbers were commonly highest in seronegative or low antibody titer juvenile animals, positive findings were also seen with specimens from highly seropositive and adult animals.

Our findings in archived DC specimens, although restricted to serology, strongly suggest that MERS-CoV or a closely related virus has been circulating in DC in KSA for at least two decades. Complete genomic sequences of MERS-CoV found in contemporary DC in KSA are identical to sequences of viruses recovered from human MERS-CoV victims (unpublished data). Although we speculate that DC are potential reservoirs for human transmission, we cannot prove this relationship from current data. Rigorous epidemiological investigation into the potential for exposure to DC in sporadic cases of MERS-CoV (those where there is no opportunity for human-to-human transmission) will be required to test this model. If DC can be implicated, other questions will arise. Did MERS-CoV truly emerge as a human pathogen in 2012 or were cases of cryptic infection not appreciated due to lack of suitable diagnostic tests? We may be able to address this conundrum using archived human materials. If evidence of human MERS-CoV infections cannot be detected prior to 2012, we must entertain the possibility that mutation facilitated cross-species transmission. However, we see no path to address this possibility absent access to historical DC respiratory tract specimens. The only archived DC specimens we have been able to locate are DC sera; our efforts to recover MERS-CoV sequences from camel blood have been unsuccessful. What are the roles of bats, if any, as reservoirs of MERS-CoV? These limitations notwithstanding, the most urgent public health concern, raised in work we and others have reported that focuses

on DC infection, is to determine the role of these animals in sporadic human infection. The evidence is clearly sufficient to support targeted investigation of direct or indirect exposure to DC in human disease.

Materials and Methods

Sample collection. Samples included DC, sheep and goat sera, whole blood, nasal and rectal swabs freshly collected in 2013 and archived serum samples from 1992, 1993, 1994, 1996, 2004, 2009 and 2010. Two rectal and two nasal swabs were obtained from each animal. One rectal and one nasal swab was placed into RNAlater (Life Technologies, Carlsbad, CA, USA); the second was placed into viral transport media (VTM; Becton Dickinson, Franklin Lakes, NJ, USA). All were stored at -80°C.

ELISA assay. Vero (African green monkey kidney, ATCC CRL-1586) cells were maintained at the Integrated Research Facility (IRF, Frederick, MD, USA) in Dulbecco's modified Eagle's medium (DMEM; Corning Inc., Corning, NY, USA) and 10% fetal bovine serum (FBS). Cells were plated at a concentration of 4 x 10^4/well in 96-well plates (#3603, Corning). When cells were at or near confluency, they were infected with the Jordan strain of MERS-CoV (GenBank accession no. KC776174, MERS-CoV-Hu/Jordan-N3/2012 [41]), kindly provided by Kanta Subbarao (National Institutes of Health, Bethesda, MD, USA) and Gabriel Defang (Naval Medical Research Unit-3, Cairo, Egypt) at a multiplicity of infection of 1.0. At 24 hours, post-infection cells were fixed in 10% neutral-buffered formalin (NBF) or 4% paraformaldehyde (PFA) solution.

After 24 hours in fixative, plates were rinsed three times with phosphate-buffered saline (PBS) and placed in PBS for storage at 4°C. Plates were loaded with infected and non-infected cells in alternating rows to generate a differential reading for each serum tested (positive = $OD_{infected}$ >0.6 and >3x non-infected). Test sera were diluted 1:3000 in PBS/0.05% Tween 20/1% bovine serum albumin; secondary antibodies were rabbit anti-goat IgG (H+L) horseradish peroxidase conjugate (1: 3,000; Bio-Rad, Hercules, CA, USA), rabbit anti-sheep IgG (H+L) horseradish peroxidase conjugate (1: 3,000; Bio-Rad) and anti Llama IgG horseradish peroxidase conjugate (1: 10,000; Bethyl Laboratories, Montgomery, TX, USA).

Western blot. Extracts of non-infected Vero cells or Vero cells infected with MERS-CoV strain EMC were generated at Rocky Mountain Laboratories, loaded onto discontinuous 3%/7.5% SDS gels (Bio-Rad) and transferred onto nitrocellulose using iBlot Transfer Stacks (Invitrogen iBlot, Life Technologies). Lanes were loaded alternating with infected and non-infected extract and a pair cut for incubation with DC sera (1: 800 in blocking solution) after blocking of the membrane in PBS/0.05% Tween 20/5% dry milk blocking solution for 1 hour. Membranes were washed 3 times with PBS/0.05% Tween 20 after 2 hours incubation with serum and then incubated for another 1.5 hours with secondary antibody (1: 7,000 in blocking solution; anti Llama IgG horseradish peroxidase conjugate, Bethyl Laboratories). Following three more washes the membranes were developed with WesternSure premium chemiluminescent substrate (Li-Cor, Lincoln, NE, USA) and read on a C-DiGit Blot Scanner (Li-Cor).

Luciferase immunoprecipitation system (LIPS) assay. The nucleocapsid proteins of Bo-CoV and MERS-CoV were PCR-amplified with primers introducing appropriate restriction sites for cloning into vector pREN-2 fused to the C-terminus of the Renilla luciferase reporter (20). Sequence confirmed construct DNA was purified from *E. coli* cultures (Qia), and transfected into COS-1 cells (African green monkey kidney, ATCC CRL-1650; 1 ug; Lipofectamine, Invitrogen, Life Technologies). Cells were harvested 48 hours post transfection in lysis buffer (50 mM Tris, pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$, 1% Triton X-100, 50% glycerol and protease inhibitors) and the protein extract clarified by two rounds of centrifugation at 12,000g. Target concentration in relative light units (RLU) was determined and approx. 10x 10^6 RLU were incubated with test serum (1: 100) in a final volume of 100 µl buffer A (50 mM Tris, pH 7.7, 100 mM NaCl, 5 mM $MgCl_2$, 1% Triton X-100) per well in 96-well plates (#249944 Thermo/Nunc, Waltham, MA, USA). After 1 hour of incubation at RT, protein A/G beads (#53135 Pierce, Junction City, OR, USA) were added and the mixtures transferred to 96-well filter plates (#MSBVN1B50 Millipore, Billerica, MA, USA) for another hour incubation at RT with shaking. Bead-bound antigen was washed 8 times with buffer A and 3 times with PBS (Tecan Hydroflex, Maennedorf, Switzerland) and then read with coelenterazine substrate (Renilla Luciferase Assay System, Promega, Madison, WI, USA) on a Centro LB960 luminometer (Berthold, Bad Wildbad, Germany).

Nucleic acid extraction and PCR. Total nucleic acids from nasal swabs, serum, and whole blood were extracted on a QiaCube with Cador Reagent Kits (Qiagen, Hilden, Germany) or RNeasy Reagent Kits for extraction of RNA from rectal swabs. Quantitative real-time PCR used a OneStep Real-Time qPCR buffer (Invitrogen, Life Technologies)

and primer/probes upE and ORF1a (16, 17). Products for sequencing were generated by RT-PCR. cDNA was reverse transcribed using Superscript III and random hexamer primers. PCR was performed using Amplitaq Gold (Life Technologies) and primers designed to amplify a 1044 nt region of the spike gene (hemi-nested PCR), a 913 nt region of the N-gene (nested PCR) or a 2004 nt region of the ORF1ab region (hemi-nested PCR). Primer sequences are shown in **Table S2**. Products were purified by agarose gel electrophoresis and QIAquick Gel Extraction Kits (Qiagen), and subsequently sequenced on both strands by the di-deoxynucleotide chain termination method (GeneWiz, South Plainfield, NJ, USA).

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Tables

Table 1. Samples collected in 2013 by animal species, geographic location, age group and specimen type (DC= dromedary camel).

Animal species	Location	Age group*	Number	Specimens†
DC	Hofuf	juvenile	19	S, B, N, R
DC	Hofuf	adult	21	S, B, N, R
DC	Gizan	juvenile	21	S, B, N, R
DC	Gizan	adult	19	S, B, N, R
DC	Taif	juvenile	22	S, B, N, R
DC	Taif	adult	19	S, B, N, R
DC	Tabuk	juvenile	24	S, B, N, R
DC	Tabuk	adult	16	S, B, N, R
DC	Riyadh	juvenile	12	S, B, N, R
DC	Riyadh	adult	8	S, B, N, R
DC	Unizah	juvenile	6	S, B, N, R
DC	Unizah	adult	16	S, B, N, R
Goat	Unizah	unknown	31	S, B, N, R
Goat	Riyadh	unknown	5	S, B, N, R
Sheep, Barbari§	Unizah	3 mo – 2 y	29	S, B, N, R
Sheep, Harri	Unizah/Riyadh	3 mo – 2 y	10	S, B, N, R
Sheep, Najdi	Unizah	3 mo – 2 y	21	S, B, N, R
Sheep, Naimi	Unizah	3 mo – 2 y	21	S, B, N, R
Sheep, Sawakni	Unizah	3 mo – 2 y	31	S, B, N, R

* Juvenile animals were defined as ≤2 years of age; adults as >2 years of age.
† Specimens collected for each animal: S, serum; B, blood; N, nasal swab; R, rectal swab.
§ Domestic sheep were separated into the common breeds found in KSA: Barbari, Harri, Najdi, Naimi, and Sawakni.

Table 2. Analysis of archived dromedary camel sera from KSA, 1992-2010.

Year	Location	Age group	Number	Seropositivity	
1992	Riyadh	adult	1	100%	(1/1)
1993	Riyadh	adult	2	100%	(2/2)
1994	Empty Quarter	adult	123	93%	(114/123)
1996	Riyadh	adult	6	100%	(6/6)
2004	Riyadh	adult	6	100%	(6/6)
2009	Riyadh	juvenile	56	72%	(40/56)

2009	Rumah	adult	26	92%	(24/26)
2010	Riyadh	juvenile	21	76%	(16/21)
2010	Kharj	adult	23	91%	(21/23)

Figure Legends

Figure 1: Prevalence of MERS-CoV antibody reactivity in serum (**A**) and nucleic acid detection in nasal swabs (**B**) by geographic region in dromedary camels from KSA. Images were created with software from ESRI maps.

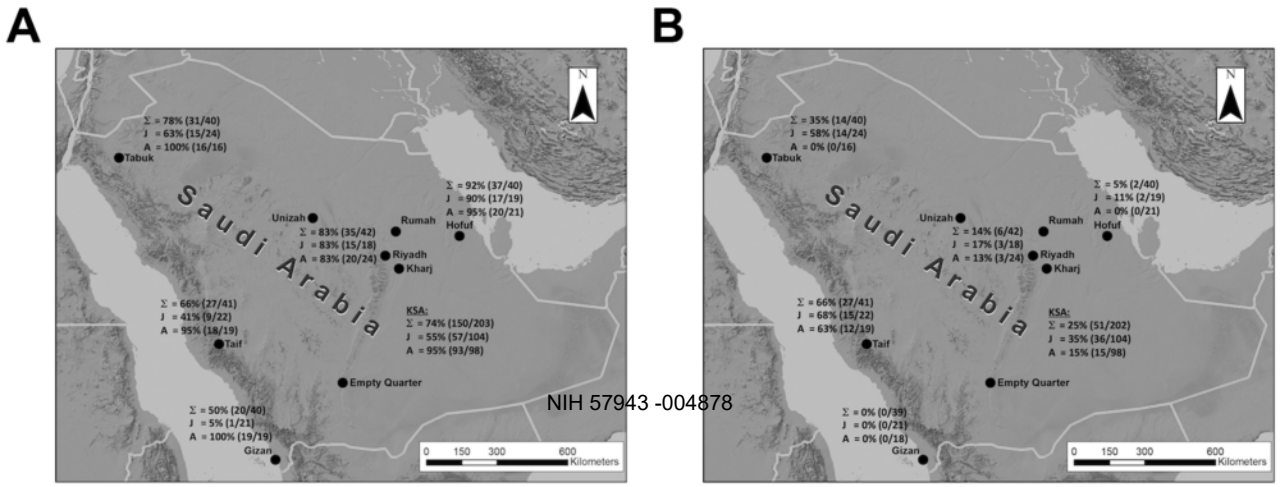
Figure 2: MERS CoV proteins detected in western blot by sera from KSA dromedary camels.

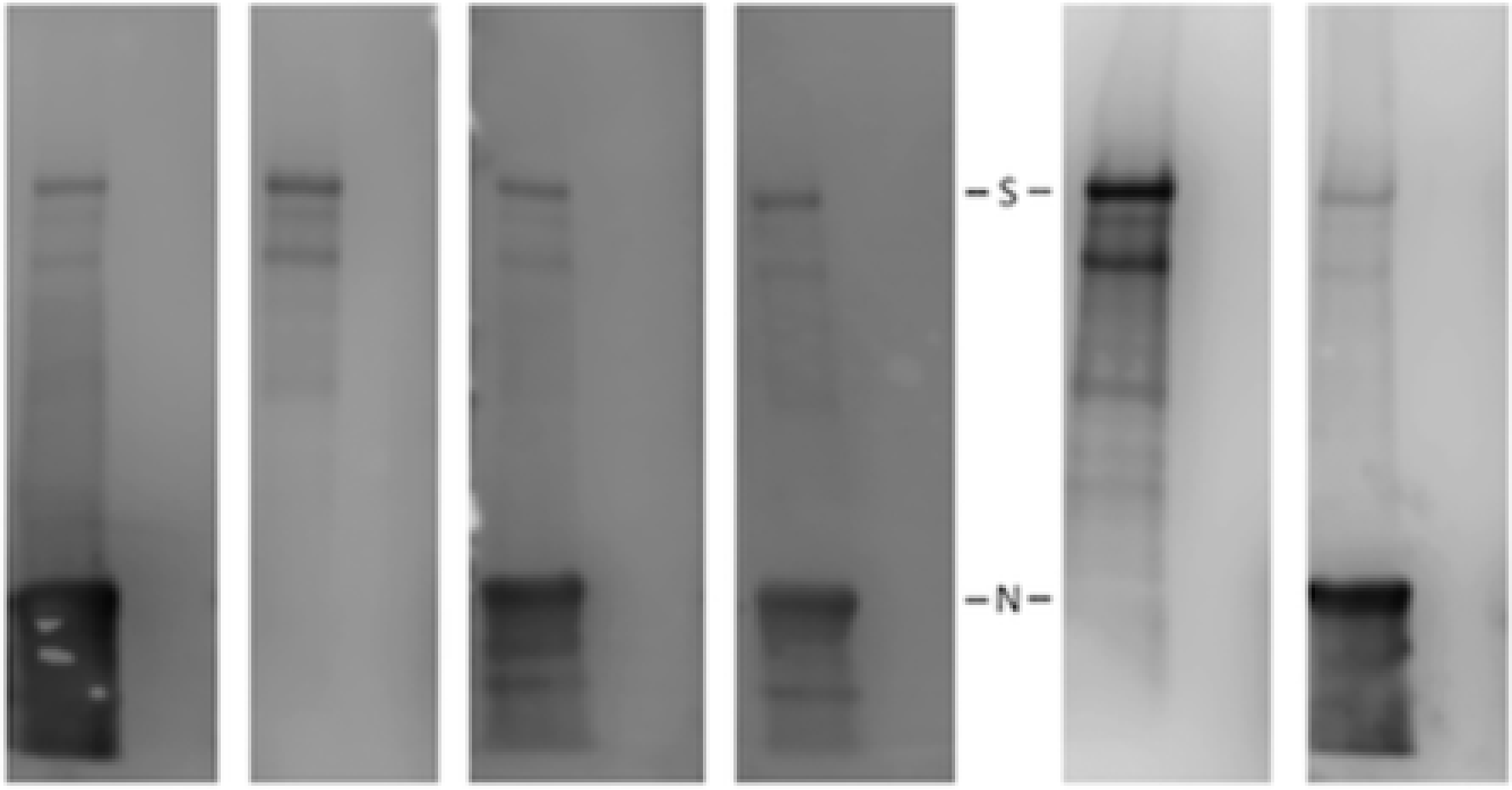
Figure 3: Relationship of partial spike (**A**, 1044 nt) and ORF1ab (**B**, 2009 nt) sequences derived from dromedary camel with selected human derived MERS-CoV sequences. Maximum likelihood trees were inferred based on p-distance; scale bar shows base difference per site. GenBank reference sequences are indicated by accession number.

Supplemental Table Legends

Table S1. MERS-CoV infection in Saudi Arabian animals in samples collected in 2013.

Table S2. PCR primer sequences.





H-9
juvenile
ELISA +++
LIPS N +++
PCR neg

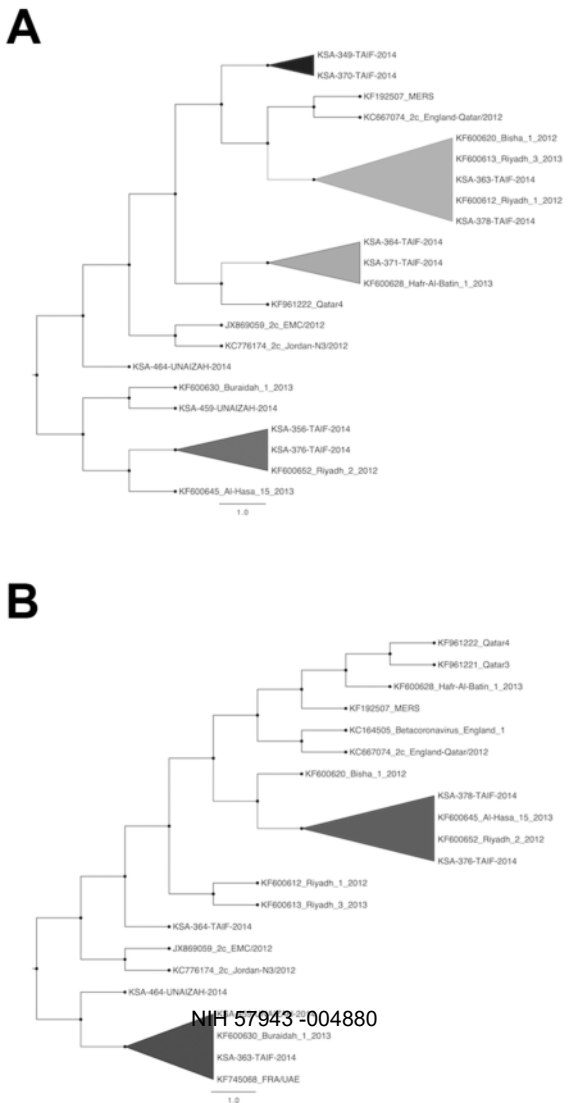
H-10
juvenile
ELISA +++
LIPS N -
PCR neg

T[illegible]
adult
ELISA +++
LIPS N ++
PCR + nasal

[illegible]2
adult
ELISA ++
LIPS N ++
PCR +++ rectal

Ctrl
Rabbit anti-
MERS CoV
spike

Ctrl
Rabbit anti-
inactivated
MERS CoV



From: "Steele, Lisa (NIH/CSR) [E]" (b)(6)
Subject: Review of your recent NIH grant application submission
Date: December 31, 2013 2:18:59 AM GMT+07:00
To: (b)(6)

Dear Dr. Olival ,

I am the Scientific Review Officer (SRO) of the Infectious Disease, Reproductive Health, Asthma and Pulmonary Conditions (IRAP) Study Section. Your grant application (b)(4); (b)(6) has been assigned to IRAP for review on **February 12-13th 2014**. I am your NIH contact between now and the time of the review. If you have questions or concerns that are not answered below you may contact me at (b)(6) .

IRAP Reviewers
The complete meeting roster will be posted 30 days before the meeting date. The link includes current membership and the roster for the upcoming meeting.

Confidentiality and Conflict of Interest
Do not contact reviewers about your application. Likewise, reviewers may not contact you. Failure to observe this policy will create a serious breach of confidentiality and conflict of interest in the peer review process. Likely actions include removal of your application(s) from immediate review, coupled with delay and deferral of evaluation to a different review group in the following review cycle. You must also avoid actions that could create the appearance of a conflict of interest with a reviewer. Reviewers recuse themselves from the review of any application with which they have a conflict of interest or the appearance of a conflict of interest. If you have concerns about a conflict please let me know in a timely manner. Please note that simply working in your scientific area (i.e., scientific competition) does not place a reviewer in conflict with your application.

Post -Submission Application Materials * New Policy*****
Please refer to the NIH Policy on Post-Submission Application Materials. NIH no longer accepts supplemental (post-submission) materials for applications submitted for the September 25, 2010 due date and thereafter. There are**EXCEPTIONs** in the case of ï¿½unforeseen administrative issuesï¿½ as defined by ï¿½the departure of a participant, natural disaster, etc.ï¿½ Forgetting to include something or seeking to include late-breaking research findings do not constitute an unforeseen administrative issue. If you need to submit appropriate materials for a qualifying exception, please submit it to me via an email attachment directly from or with explicit approval from your institutional signing official no later than **January 13TH** (**30 calendar days prior to review**).

The Review Process
The following NIH web sites provide useful information about the review process:

- Center for Scientific Review: http://cms.csr.nih.gov/
- Peer Review Polices & Practices: http://grants.nih.gov/grants/peer/peer.htm
- Office of Extramural Research: http://grants.nih.gov/grants/oer.htm

After the Study Section Meeting

Your priority score will be posted on your personal eRA Commons account within three working days after the meeting. Summary statements for initial (**A0) RO1** applications from new investigators are posted within 10 business days of the meeting. All summary statements will be posted within 30 days of the meeting (by **March 14th, 2014**). Summary statements are produced in score order starting with the lowest (i.e., best) scores and finishing with applications that were not discussed in the meeting within each category.

It is NIH policy that SROs not discuss the results of the review with investigators. Your point of contact for questions about the review and status of your application after the review meeting is your Program Officer whose name and contact information can be found in the upper left corner of your summary statement. Your program officer can help you understand your summary statement and make future plans.

Best regards,
Lisa

Lisa N. Steele, Ph.D.
Scientific Review Officer
Population Sciences and Epidemiology (PSE) Integrated Review Group
Center for Scientific Review, NIH
6701 Rockledge Drive, Room 3139, MSC 7770
Bethesda, MD 20892-7770 (20817 for Fed Ex/delivery)
PLEASE NOTE NEW PHONE: (b)(6)
e-mail: (b)(6)

From: Aleksei Chmura
Sent: Sat, 25 Jan 2014 01:24:55 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Peter Daszak
Subject: Re: Just In Time information for Proposal: 1R01AI110964 Understanding the Risk of Bat Coronavirus Emergence (PI, Daszak)

Dear Erik,

We have submitted the JIT.

Many thanks!

Aleksei Chmura
Program Coordinator
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
(mobile)
(b)(6) (China)
(b)(6) (Skype)

www.ecohealthalliance.org

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EcoHealth Alliance integrates innovative science-based solutions and partnerships that increase capacity to achieve two interrelated goals: protecting global health by preventing the outbreak of emerging diseases and safeguarding ecosystems by promoting conservation.

On 24 Jan 2014, at 13:27:08, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,
You should go ahead and submit the JIT requested info now. You can then submit the final IRB and IACUC approvals later on. The JIT document can always be revised.

Best,
Erik

Erik J. Stemmy, Ph.D.
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
6610 Rockledge Drive, Room 3210
Bethesda, MD 20892-7630
Phone: (b)(6)
Fax: 301-496-8030

Email: (b)(6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

****

From: Aleksei Chmura (b)(6)
Sent: Thursday, January 23, 2014 8:22 PM
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Just In Time information for Proposal: 1R01AI110964 Understanding the Risk of Bat Coronavirus Emergence (PI, Daszak)
Importance: High

Dear Erik,

Should we save and/or submit our Just In Time Report or wait until the IACUC and IRB dates as well as the completed Human Subjects training information are all available to input?

Many thanks!

Aleksei Chmura
Program Coordinator
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
(mobile)
(b)(6) (China)
(b)(6) (Skype)

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On 23 Jan 2014, at 18:07:37, Peter Daszak (b)(6) wrote:

Dear Erik,

The AOR for our organization has uploaded our Just In Time information. I have prepared a cover note (attached) which details our progress with other current support, IRB, IACUC. I have also made a

statement about how we would be able to address the only real criticism of the proposal, the amount of effort for the modeler/statistician, and that this would not increase the overall budget. I've copied the contents of the letter below. I'm not sure if it can be uploaded onto the NIH website, and wondered if you could make this available at the council review, or what the correct procedure should be.

Thank you for your advice on this, and please let me know if there is any other information I need to send.

JUST IN TIME REQUESTED INFORMATION

1R01AI110964 *Understanding the Risk of Bat Coronavirus Emergence* (PI, Daszak)

Dear reviewers,

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3) **IRB:** Our IRB with Tufts University Health Science, through our inter-institutional agreement with them, is in process and the FWA for this is **FWA00004517**. Human subject education for all key personnel is being completed currently and all details will be provided at each step of approval.

4) **IACUC:** Our IACUC approval is also pending with Tufts University through our inter-institutional agreement with them. The OLAW Assurance number listed (**A4059-01)** is correct. Once we have an IACUC date, we will inform NIH immediately.

If you have any other questions, please contact me anytime. We are very appreciative of your consideration and look forward to further details.

Peter Daszak

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<Daszak PI Understanding the risk of bat coronavirus emergence, JIT requests.docx>

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Fri, 24 Jan 2014 18:26:04 +0000
To: Peter Daszak
Cc: Aleksei Chmura
Subject: RE: Just In Time information for Proposal: 1R01AI110964 Understanding the Risk of Bat Coronavirus Emergence (PI, Daszak)

Hi Peter,
I think the best thing to do would be to include your letter as a page of your JIT package. You can update the JIT at any time.

Best,
Erik

Erik J. Stemmy, Ph.D.
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
6610 Rockledge Drive, Room 3210
Bethesda, MD 20892-7630
Phone: (b)(6)
Fax: 301-496-8030
Email: (b)(6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Peter Daszak (b)(6)
Sent: Thursday, January 23, 2014 6:08 PM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura
Subject: Just In Time information for Proposal: 1R01AI110964 Understanding the Risk of Bat Coronavirus Emergence (PI, Daszak)
Importance: High

Dear Erik,

The AOR for our organization has uploaded our Just In Time information. I have prepared a cover note (attached) which details our progress with other current support, IRB, IACUC. I have also made a statement about how we would be able to address the only real criticism of the proposal, the amount of effort for the modeler/statistician, and that this would not increase the overall budget. I've copied the

contents of the letter below. I'm not sure if it can be uploaded onto the NIH website, and wondered if you could make this available at the council review, or what the correct procedure should be.

Thank you for your advice on this, and please let me know if there is any other information I need to send.

JUST IN TIME REQUESTED INFORMATION

1R01AI110964 *Understanding the Risk of Bat Coronavirus Emergence* (PI, Daszak)

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If you have any other questions, please contact me anytime. We are very appreciative of your consideration and look forward to further details.

Peter Daszak

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www.ecohealthalliance.org

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From: Peter Daszak
Sent: Thu, 23 Jan 2014 23:07:37 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura
Subject: Just In Time information for Proposal: 1R01AI110964 Understanding the Risk of Bat Coronavirus Emergence (PI, Daszak)
Attachments: Daszak PI Understanding the risk of bat coronavirus emergence, JIT requests.docx
Importance: High

Dear Erik,

The AOR for our organization has uploaded our Just In Time information. I have prepared a cover note (attached) which details our progress with other current support, IRB, IACUC. I have also made a statement about how we would be able to address the only real criticism of the proposal, the amount of effort for the modeler/statistician, and that this would not increase the overall budget. I've copied the contents of the letter below. I'm not sure if it can be uploaded onto the NIH website, and wondered if you could make this available at the council review, or what the correct procedure should be.

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If you have any other questions, please contact me anytime. We are very appreciative of your consideration and look forward to further details.

Peter Daszak

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EcoHealth Alliance

January 15, 2014

JUST IN TIME REQUESTED INFORMATION

1R01AI110964 *Understanding the Risk of Bat Coronavirus Emergence* (PI, Daszak)

Dear reviewers,

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4) **IACUC:** Our IACUC approval is also pending with Tufts University through our inter-institutional agreement with them. The OLAW Assurance number listed (**A4059-01)** is correct. Once we have an IACUC date, we will inform NIH immediately.

If you have any other questions, please contact me anytime. We are very appreciative of your consideration and look forward to further details.

Yours sincerely

(b)(6)

Dr. Peter Daszak
EcoHealth Alliance
460 West 34th Street, 17th Fl.
New York, NY 10001, USA
(b)(6)

From: Peter Daszak
Sent: Fri, 10 Jan 2014 21:55:24 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura; Alison Andre
Subject: RE: Can I call you this afternoon re. our grant application 1 R01 AI110964-01

Great thanks – I'll call you then.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

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+1.212.380.4465 (fax)
www.ecohealthalliance.org

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Friday, January 10, 2014 4:47 PM
To: Peter Daszak
Cc: Aleksei Chmura; Alison Andre
Subject: Re: Can I call you this afternoon re. our grant application 1 R01 AI110964-01

10:30 should be fine. You can reach me (b)(6)

Erik

Erik J. Stemmy, Ph.D.
Health Specialist
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
6610 Rockledge Drive, Room 3210

Bethesda, MD 20892-7630
Phone: (b)(6)
Fax: 301-496-8030
Email: (b)(6)

This message was sent from my mobile device. Please excuse any errors.

****

From: Peter Daszak (b)(6)
Sent: Friday, January 10, 2014 04:02 PM Eastern Standard Time
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura (b)(6) Alison Andre (b)(6)
Subject: RE: Can I call you this afternoon re. our grant application 1 R01 AI110964-01

Thanks for the email Erik – could we talk on Monday at 10.15 or 10.30am Eastern time?

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
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New York, NY 10001

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Friday, January 10, 2014 3:35 PM

To: Peter Daszak
Cc: Aleksei Chmura; Alison Andre
Subject: RE: Can I call you this afternoon re. our grant application 1 R01 AI110964-01

Hi Peter,
Unfortunately today I'm pretty booked. I'm available to chat on Monday morning, or after 1:30pm. Please let me know when you have some time.

Best,
Erik

Erik J. Stemmy, Ph.D.
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
6610 Rockledge Drive, Room 3210
Bethesda, MD 20892-7630
Phone: (b)(6)
Fax: 301-496-8030
Email: (b)(6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Peter Daszak (b)(6)
Sent: Friday, January 10, 2014 10:42 AM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura; Alison Andre
Subject: Can I call you this afternoon re. our grant application 1 R01 AI110964-01
Importance: High

Dear Erik,

Would it be possible to talk with you for a few minutes this afternoon (anytime after 12pm) to discuss our proposal (see email below and attachment).

It's an R01 on coronavirus emergence, and will be discussed at the Jan 27th council meeting.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
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New York, NY 10001

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EcoHealth Alliance builds innovative science-based solutions and partnerships that increase our global capacity to achieve two interrelated goals: protecting global health by preventing pandemics; and safeguarding ecosystems by promoting conservation.

From: era-notify@mail.nih.gov [mailto:era-notify@mail.nih.gov]
Sent: Sunday, December 22, 2013 10:06 PM
To: Peter Daszak
Subject: Review Results available for Application 1 R01 AI110964-01

*** This is an automated notification - Please do not reply to this message. ***

Dr. PETER DASZAK,

Your application 1 R01 AI110964-01, entitled Understanding the Risk of Bat Coronavirus Emergence, has completed the first phase of review and the results are available in the eRA Commons. A summary statement, containing evaluative comments and budget recommendations, will be available within approximately eight weeks. You will receive an email notification when the summary statement is available.

NOTE: The National Institutes of Health no longer routinely sends paper copies of summary statements. You may access your summary statement through the Status Module in the eRA Commons. Please use your account ID, (b)(6) and password to access the Commons at https://commons.era.nih.gov/commons/. If you do not remember your password, you can click on the "Forgot Password" link below the "Login" button. Enter your email address in the area provided, click "Submit" and your password will be reset and sent to you via email.

If you are able to log into the eRA Commons and cannot see the status for this application, there are two likely causes. First is that your account has been established to use the Internet Assisted Review (IAR) module, but has not been affiliated with your institution. In this case, contact your Office of Sponsored Research or similar office to have your account affiliated. The other probable cause is that your account has been recently created and is currently in a "Pending" status. In this case, please allow another day or two for your account to become active and then try again.

If you have any questions about this email, please contact your Program Officer, Erik Stemmy at (b)(6) or by phone at (b)(6)

If you incur any problem while using the eRA Commons, please contact the eRA Help Desk at 1-866-504-9552 (tty: 301-451-5939) or commons@od.nih.gov.

From: Peter Daszak
Sent: Fri, 10 Jan 2014 15:41:47 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura; Alison Andre
Subject: Can I call you this afternoon re. our grant application 1 R01 AI110964-01
Attachments: R01AI110964-01 Summary Statement.PDF
Importance: High

Dear Erik,

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Cheers,

Peter

Peter Daszak
President

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From: era-notify@mail.nih.gov [mailto:era-notify@mail.nih.gov]
Sent: Sunday, December 22, 2013 10:06 PM
To: Peter Daszak
Subject: Review Results available for Application 1 R01 AI110964-01

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Dr. PETER DASZAK,

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If you are able to log into the eRA Commons and cannot see the status for this application, there are two likely causes. First is that your account has been established to use the Internet Assisted Review (IAR) module, but has not been affiliated with your institution. In this case, contact your Office of Sponsored Research or similar office to have your account affiliated. The other probable cause is that your account has been recently created and is currently in a "Pending" status. In this case, please allow another day or two for your account to become active and then try again.

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SUMMARY STATEMENT

PROGRAM CONTACT: **(Privileged Communication)** ***Release Date:*** **01/02/2014**

Erik Stemmy

(b)(6)

Application Number: **1 R01 AI110964-01**

Principal Investigator

DASZAK, PETER PHD

Applicant Organization: ECOHEALTH ALLIANCE, INC.

Review Group: **CRFS**
Clinical Research and Field Studies of Infectious Diseases Study Section

Meeting Date: **12/18/2013**
Council: **JAN 2014**
Requested Start: **10/01/2013**

RFA/PA: **PA11-260**
PCC: **M51C**

Project Title: **Understanding the Risk of Bat Coronavirus Emergence**

SRG Action: **Impact Score: 20 Percentile: 8**
Next Steps: **Visit http://grants.nih.gov/grants/next_steps.htm**
Human Subjects: **44-Human subjects involved - SRG concerns**
Animal Subjects: **30-Vertebrate animals involved - no SRG concerns noted**
Gender: **1A-Both genders, scientifically acceptable**
Minority: **5A-Only foreign subjects, scientifically acceptable**
Children: **3A-No children included, scientifically acceptable**
Clinical Research - not NIH-defined Phase III Trial

Project Year	Direct Costs Requested	Estimated Total Cost
1	499,993	672,628
2	499,469	671,924
3	499,978	672,608
4	499,953	672,575
5	499,974	672,603
TOTAL	2,499,367	3,362,338

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

1R01AI110964-01 DASZAK, PETER

BUDGETARY OVERLAP
PROTECTIONS FOR HUMAN SUBJECTS UNACCEPTABLE

RESUME AND SUMMARY OF DISCUSSION: This impressive application proposes studies to determine factors that increase the risk of zoonotic coronavirus (CoV) emergence in people by studying CoV diversity in a critical zoonotic reservoir (bats), at sites of high risk for emergence (wildlife markets) in an emerging disease hotspot (China). Given the SARS outbreak and the current emergence of MERS in the Middle East, the significance relates to advancing the knowledge of zoonotic potential of coronaviruses. Some strengths are the highly experienced and accomplished investigator and his established collaborative team. The application is also innovative in that it integrates the biology, ecology, animal-human interface, mathematical modeling and evolutionary analysis to study the risk of spillover of the CoV from bats into humans. Other strengths are the superb environment, available samples and a feasible approach. Despite these strengths, it is noted that there is a limited effort for modeling and statistics. Although the proposed multidisciplinary approach seems ambitious, given the investigators impressive track-record and the feasibility of collecting data, there is confidence in the success and the generation of important data.

DESCRIPTION (provided by applicant): This project will examine the risk of future coronavirus (CoV) emergence from wildlife using in-depth field investigations across the human-wildlife interface in China, molecular characterization of novel CoVs and host receptor binding domain genes, mathematical models of transmission and evolution, and in vitro and in vivo laboratory studies of host range. Zoonotic CoVs are a significant threat to global health, as demonstrated with the emergence of pandemic severe acute respiratory syndrome coronavirus (SARS-CoV) in China in 2002, and the recent and ongoing emergence of Middle East Respiratory Syndrome (MERS-CoV). Bats appear to be the natural reservoir of these viruses, and hundreds of novel bat-CoVs have been discovered in the last two decades. Bats, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a large scale human-wildlife interface, and high risk of future emergence of novel CoVs. This project aims to understand what factors increase the risk of the next CoV emerging in people by studying CoV diversity in a critical zoonotic reservoir (bats), at sites of high risk for emergence (wildlife markets) in an emerging disease hotspot (China). The three specific aims of this project are to: 1. Assess CoV spillover potential at high risk human-wildlife interfaces in China. This will include quantifying the nature and frequency of contact people have with bats and other wildlife; serological and molecular screening of people working in wet markets and highly exposed to wildlife; screening wild-caught and market sampled bats from 30+ species for CoVs using molecular assays; and genomic characterization and isolation of novel CoVs. 2. Develop predictive models of bat CoV emergence risk and host range. A combined modeling approach will include phylogenetic analyses of host receptors and novel CoV genes (including functional receptor binding domains); a fused ecological and evolutionary model to predict host-range and viral sharing; and mathematical matrix models to examine evolutionary and transmission dynamics. 3. Test predictions of CoV inter-species transmission. Predictive models of host range (i.e. emergence potential) will be tested experimentally using reverse genetics, pseudovirus and receptor binding assays, and virus infection experiments across a range of cell cultures from different species and humanized mice.

PUBLIC HEALTH RELEVANCE: Most emerging human viruses come from wildlife, and these represent a significant threat to global public health and biosecurity - as demonstrated by the SARS coronavirus pandemic of 2002-03 and an ongoing SARS-like epidemic in the Middle East. This project seeks to understand what factors allow animal Coronaviruses to evolve and jump into the human population by studying virus diversity in a critical group of animals (bats), at sites of high risk for emergence (wildlife markets) in an emerging disease hotspot (China).

CRITIQUE 1:

Significance: 2
Investigator(s): 2
Innovation: 2
Approach: 2
Environment: 1

Overall Impact: This is a new R01 application by an experienced investigator and an established collaborative team. The goal of the proposal is to understand the risk of zoonotic coronavirus (CoV) emergence. The proposal has three interrelated main aims. The first is to assess CoV spillover potential at high risk human-wildlife interfaces. The second is to investigate receptor evolution and host range and to conduct predictive modeling of bat-CoV emergence risk. The third is to test predictions of CoC inter-species transmission. The first aim will gather information about the human-wildlife interface in markets and by hunters and also sample numerous bat species for CoV. The information will be used to inform three types of modeling exercises, phylogenetic analysis, predictive models of CoV host-range and diversity, and modeling of the dynamics of spillover risk. In the third aim, both in vitro and in vivo (humanized mouse models) will be used to examine questions of infectivity and pathogenicity of different CoVs. The team is experienced in this type of research, and has many related projects ongoing. The research is significant because the zoonotic potential of coronaviruses should be understood much better given the SARS outbreak and the current emergence of MERS in the Middle East. The innovation of the project is largely in the combination of the different activities -- from field to models. Enthusiasm for the project is slightly dampened as it is not clear from the distribution of resources on the project how the ambitious modeling and statistical analyses will be achieved.

1. Significance:

Strengths

- Coronaviruses in animals have a potential for spillover to become emerging pathogens in humans, such as in SARS and the newly emerging MERS-CoV.
- The data proposed to be collected could inform models of spillover potential of CoV from bats to humans.
- The phylogenetic data on receptor binding domains (RBDs) could give insight into the potential for spillover of novel CoVs into other host species beyond bats.

Weaknesses

- If there is little evidence of spillover of CoV strains among bat species roosting in the same cave, as stated in the proposal, why would one expect a lot of bat-human spillover?

2. Investigator(s):

Strengths

- The PI Daszak is an experienced investigator with many years of experience in characterizing the CoV interface between bats and humans and a long-term collaboration with the Chinese investigators. Dr. Daszak is president and chief scientist of the organization EcoHealth Alliance, New York.
- Dr. Kevin Olival is a junior investigator with experience in looking at the bats and their associated pathogens and integrating phylogenetic analysis with ecological analysis.
- Dr. Hosseini works in modeling of infectious diseases, particularly emerging infectious diseases.

- Dr. Zhengli Shi is Senior Scientist at the Wuhan Institute of Virology and will be responsible for the laboratory testing of wild and domestic animals from Southern China.
- Dr. Shuyi Zhang is Dean and trained in wildlife biology. He worked with Dr. Daszak on the discovery that bats were the reservoir of SARS-like CoVs.
- Dr. Epstein has experience with SARS-like CoV and is involved with investigating the MERS-CoV.
- The team has a track record of working together in the area of the proposed research.

Weaknesses

- Although the mathematical and statistical modeling in Aim 2 are a large portion of the proposal, only two months of Hosseini's time is committed to the project. It is not clear how this portion of the work will be accomplished. It seems Aim 2 is an entire grant of its own.

3. Innovation:

Strengths

- The proposal integrates the biology, ecology, animal-human interface, mathematical modeling and evolutionary analysis to study the risk of spillover of the CoV from bats into humans.

Weaknesses

- The approach draws on well-established methods.

4. Approach:

Strengths

- The proposed research in Aim 1 builds on considerable CoV surveillance activities of the team in China in bats, wildlife and humans. Part of the proposed research would re-screen some samples to see if in fact they are SARS positive or positive to some other CoV that cross-reacts with SARS. A large part of the aim is to sample bats from areas that supply the local markets in four provinces of China. This seems sound.
- A survey of animal vendors and hunters will expand on current activities.
- The three approaches to mathematical modeling seem to be well-integrated. Although not described in detail, the group does have experience with using the methods.
- The co-phylogenetic analysis of bat genera and viral sequences can be used to discover whether some CoVs can more easily jump species. Preliminary studies have shown that phylogenetic closeness among bay species is a predictor of sharing of viruses across species.
- Aim 3 proposes a combination of in vitro testing for virus infectivity assays and infecting humanized mice for pathogenicity studies. The in vitro infection assays will be done with a number of human and animal lines.

Weaknesses

- They do not have prevalence estimates for novel strains of coronaviruses in bats, so plan to sample 30 bats from 30 different species. Some preliminary studies of this might be useful.
- The diversity of coronaviruses identified might be inadequate for the phylogenetic analyses proposed, or that other information for the mathematical modeling might not be obtained. They propose some sensitivity analyses when parameters cannot be estimated.

5. Environment:

Strengths

- The environment in China is excellent for the proposed research.
- The environment at EcoHealth Alliance seems suitable for the proposed research given its track-record in this field.

Weaknesses

- None noted.

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

- Will draw small amounts of blood. Minimal risk.

Inclusion of Women, Minorities and Children:

G1A - Both Genders, Acceptable

M5A - Only Foreign Subjects, Acceptable

C3A - No Children Included, Acceptable

- The market people and hunters are adults. Not clear if includes people over 18, which would include children.

Vertebrate Animals:

Acceptable

- Wild bats and humanized mice. Adequately addressed.

Biohazards:

Not Applicable (No Biohazards)

Select Agents:

Acceptable

- SARS-like CoV is a select agent. The biosafety has been addressed. In the field, precautions will be taken. BSL-2 and BSL-3 laboratories in Wuhan.

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2:

Significance: 1
Investigator(s): 1
Innovation: 1
Approach: 4
Environment: 1

Overall Impact: This ambitious proposal addresses the emergence of coronaviruses in bats and the potential emergence of human infectious agents via the human-bat interface. The high quality of the well-published team, their previous data collected from another funded study, the strong collaboration with Chinese colleagues all suggest that the project will be successful in generating a great deal of interesting data on this understudied but important field. The enormous scope of the project which includes not only a major field component, laboratory components and the development of novel methods to integrate phylogenetic and epidemic models leaves little room in the proposal for the kind of detail that allow a systematic appraisal of its feasibility. It also seems unlikely that all of the work proposed can be accomplished in the given time frame.

1. Significance:

Strengths

- The emergence and transmission of new coronaviruses from bats to humans and within human populations is an understudied area of major importance for global public health given that several of the most deadly emerging infectious disease have arisen from this reservoir.
- The team is well positioned to undertake the proposed research given their previous studies and grants and their existing collaborations.

Weaknesses

- The vast scale of this proposal makes it difficult to assess specific projects such as the shanghai based study of respiratory infections and non-respiratory infections, among others.

2. Investigator(s):

Strengths

- Superb well-published team.

Weaknesses

- Not entirely clear who will undertake the phylogenetic/ epidemic modeling and if the current team members have substantial experience in this challenging area. It is clear that many of the investigators have co-authored papers in this field but it is less clear that a senior experienced person with training in phylodynamics is available.
- Given the proposed interviews with vendors and other workers in China, it would be useful to see some experience or training from the team in developing, conducting and interpreting these kinds of qualitative data, especially given the possibility of non-disclosure of information given possible perceived economic consequences to the participants. For example, if illegal trade of wildlife is occurring, the investigators would need to demonstrate that they are able to elicit this information in this challenging cultural context.

3. Innovation:

Strengths

- Highly innovative mix of wildlife biology, sociology, evolutionary biology and modeling.

Weaknesses

- None noted

4. Approach:

Strengths

- Given the investigators track-record and the abundance of data available to them or being collected in this project, it's clear that they will be able to generate interesting and probably important results.
- The mathematical models are well presented and identification of specific parameters to be specified is to be commended.

Weaknesses

- The size and scope of this vast project means that many details of the many proposed sub-projects are not included for evaluation. To choose one of many, for example, there is no information for the Shanghai based clinical study on what constitutes a case definition of respiratory infection, how patients in that study will be recruited, what clinical tests they will undergo in addition to the serologics proposed, etc. Similarly, the qualitative studies that propose to measure human contact with bats are challenging and the investigators do not present any of the possible problems they may encounter in this work.

5. Environment:

Strengths

- Excellent

Weaknesses

- None noted

Protections for Human Subjects:

Unacceptable

- The human subjects section does not enumerate the various proposed human subjects projects and does not address most of the required fields.
- Given that those involved in wildlife trading in China may be in remote areas or involved in illegal activities, some description of how potential economic risks in such subjects would be addressed is needed.

Inclusion of Women, Minorities and Children:

- G1A - Both Genders, Acceptable
- M5A - Only Foreign Subjects, Acceptable
- C3A - No Children Included, Acceptable

Vertebrate Animals:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3:

Significance: 3
Investigator(s): 2
Innovation: 3
Approach: 3
Environment: 2

Overall Impact: A complex multidisciplinary approach with many moving parts addressing an important question. The proposed work may be overly ambitious. It will require very large number of viral isolates from different origins to grow in cell culture (or numerous pseudotypes) for its claims based on complex modeling to be tested. The use of receptors other than the one listed here likely required based on current knowledge.

1. Significance:

Strengths

- It is an important research question whether the risk of zoonotic virus emergence is related to host genetic relatedness (here receptors) or more ecological opportunities like physical contacts. Most likely a combination of both. Measuring actual CoV infection and diversity is important and doable.

Weaknesses

- Testing the cross over potential based on receptor interaction overlooks many other potential blocks to virus replication. Given that closely related viruses like SARS, MERS, and some SL-CoV use different receptors it should be assumed that a wide range of receptors will be used by other CoV. Goals are rather diffuse and overly reliant on complex modeling.

2. Investigator(s):

Strengths

- Peter Daszak is an accomplished researcher leading an organization experienced in this field who has partnered with very strong laboratory teams in China

Weaknesses

- None noted

3. Innovation:

Strengths

- Access to Chinese human and animal samples and expertise for collection and testing plus an interesting mixture of molecular epidemiology, modeling and laboratory work make this a strong proposal.

Weaknesses

- The complexity of this multi-pronged approach to predicting what is likely a very complex and stochastic phenomenon of coronavirus emergence risk producing lots of data leading to theoretical conclusions. Conclusion testing based on infectious viruses which may not be generated. Basing prevention measures based on models rather than in reaction to an actual outbreak or high risk virus detection requires a high degree of confidence which may be lacking when reducing a complex phenomenon to a few equations.

4. Approach:

Strengths

- The mixture of expertise and the researchers' track record are impressive.

Weaknesses

- Focus strictly limited to coronaviruses. Pan-coronavirus PCR may not work for all coronaviruses. It is not clear how serological ELISA assay will be made and whether high level of specificity can be expected.

5. Environment:

Strengths

- The US and Chines groups operate in the best of environments

Weaknesses

- None noted

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

- Necessary precaution will be taken to protect blood donors

Inclusion of Women, Minorities and Children:

G1A - Both Genders, Acceptable

M5A - Only Foreign Subjects, Acceptable

C3A - No Children Included, Acceptable

Vertebrate Animals:

Acceptable

Biohazards:

Acceptable

Budget and Period of Support:

Recommended budget modifications or possible overlap identified:

- Overlap with PREDICT and other R01 funded projects should be better defined.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS (Resume): ACCEPTABLE

PROTECTIONS FOR HUMAN SUBJECTS (Resume): UNACCEPTABLE
The potential risk to human subjects is not fully addressed and raises concerns. It is noted that the potential economic risk to subjects that may be involved in illegal wildlife trading should be described and addressed.

INCLUSION OF WOMEN PLAN (Resume): ACCEPTABLE

INCLUSION OF MINORITIES PLAN (Resume): ACCEPTABLE

INCLUSION OF CHILDREN PLAN (Resume): ACCEPTABLE

VERTEBRATE ANIMALS (Resume): ACCEPTABLE

BUDGETARY OVERLAP:
Potential overlap with other currently funded projects, e.g., PREDICT, has been noted.

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-10-080 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-10-080.html.
The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

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* Temporary Member. For grant applications, temporary members may participate in the entire meeting or may review only selected applications as needed.

Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Mon, 23 May 2016 15:24:25 +0000
To: Baric, Ralph
Cc: Lim, Jean; Leyva-Grado, Victor; Cockrell, Adam
Subject: RE: May 2016 A57 Animal Models Report
Attachments: NIAID RDB Product Development Information Sheet from OstriGen 20150814.docx

As mentioned during the call, attached is the information sheet from Ostrigen. Please keep for internal A57 use only and don't distribute further.

Glancing back quickly it looks like they provided ELISA data and EC50s from live virus inhibition assays.

Erik

Erik J. Stemmy, Ph.D.
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Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

-----Original Message-----
From: Lim, Jean (b)(6)
Sent: Monday, May 23, 2016 11:04 AM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Subject: FW: May 2016 A57 Animal Models Report

On 5/21/16, 1:02 PM, "Sims, Amy C" (b)(6) wrote:

>All,
>
>Please find attached the A57 animal models report for May 2016 from the
>Baric laboratory.
>
>The partially executed contract to extend the end date of Option Period
>1 was sent back to Mt. Sinai late on Friday, May 20, 2016.

>
>I received an email yesterday indicating that the NIH is exercising
>Option Period 2.
>
>If at all possible please copy me on the email when that agreement is
>sent to UNC so that I can follow up on it and do all I can to get it
>returned in a timely manner.
>
>Please let me know if you have any questions.
>
>Thank you, Amy
>

NIAID Respiratory Disease Branch
Product Development
Information Sheet from Requestors

PLEASE PROVIDE THE FOLLOWING INFORMATION:

NAME: Stuart Greenberg
INSTITUTION: OstriGen Inc.
ADDRESS: 303 Wyman Street, Suite 300, Waltham, MA 02451
TEL#: (b)(6)
FAX#:
E-MAIL: (b)(6)

If applicable, provide:
NIH GRANT OR CONTRACT:
GRANT OR CONTRACT NUMBER:
GRANT OR CONTRACT START DATE:
GRANT OR CONTRACT END DATE:

NIH GRANT OR CONTRACT FUNDING IS NOT A REQUIREMENT FOR ACCESS TO THESE RESOURCES

1. **SERVICE REQUESTED: Testing of ostrich polyclonal antibodies at the University of Iowa in mice that were sensitized to MERS-CoV infection by prior transduction with adenoviral vectors expressing the human host-cell receptor dipeptidyl peptidase 4.**

2. **GENERAL PRODUCT INFORMATION (this information will be kept confidential)**
Please be as complete and succinct as possible; indicate if the information is not available or not applicable to your request. References may be cited

   a. Candidate name: anti-MERS IgY

   b. Manufacturer/developer: OstriGen

   c. Product type (vaccine, adjuvant, therapeutic) and description (e.g. whole cell derived, subunit, vector based, etc.): Therapeutic polyclonal antibodies from ostriches

   d. Target: Neutralization of the MERS-CoV virus

   e. Candidate composition or active ingredient (e.g. DNA vaccine, recombinant vaccine, enzyme/neuraminidase inhibitor, etc.): Ostrich IgY derived from immunization of ostriches with recombinant MERS-CoV (HcoV-EMC/2012) Spike protein (ECD, aa 1-1297, His Tag)

f. Formulation (storage buffer, pH, salt conditions, etc.): PBS (-) (Ca2+ and Mg2+ free) with the following composition:

NaCl	137 mmol/L
KCl	2.7 mmol/L
Na2HPO4	10 mmol/L
KH2PO4	1.76 mmol/L
pH (-)	7.2~7.4

g. Is the manufacturing process fully developed? (describe the current process) IgY is derived from ostrich egg yolk in a GLP laboratory with over 99% purity

h. Is GMP material available? No

i. Amount of candidate available for the requested studies:
Two antibodies, IgY-1 and IgY-2, specific to MERS have concentrations of 17.9 and 18.7 mg/mL, respectively, and we have approximately 11 mL of each. This will provide 197mg of IgY-1 and 205 mg of IgY-2..

j. Stability of candidate; storage and handling conditions: Candidate material should be refrigerated.

k. Current other support for product development including grants, contracts and private sources of funding: Generation of the antibodies is supported by private funding; *in vitro* testing has been done unfunded by USAMRIID.

3. PROVIDE A CONCISE SCIENTIFIC JUSTIFICATION FOR USE OF THIS CANDIDATE (Include a discussion of the mode of action, if applicable):

The OstriGen anti-MERS-CoV binds with MERS-CoV spike protein to neutralize the virus.

4. PROVIDE A SUMMARY OF EXISTING DATA:

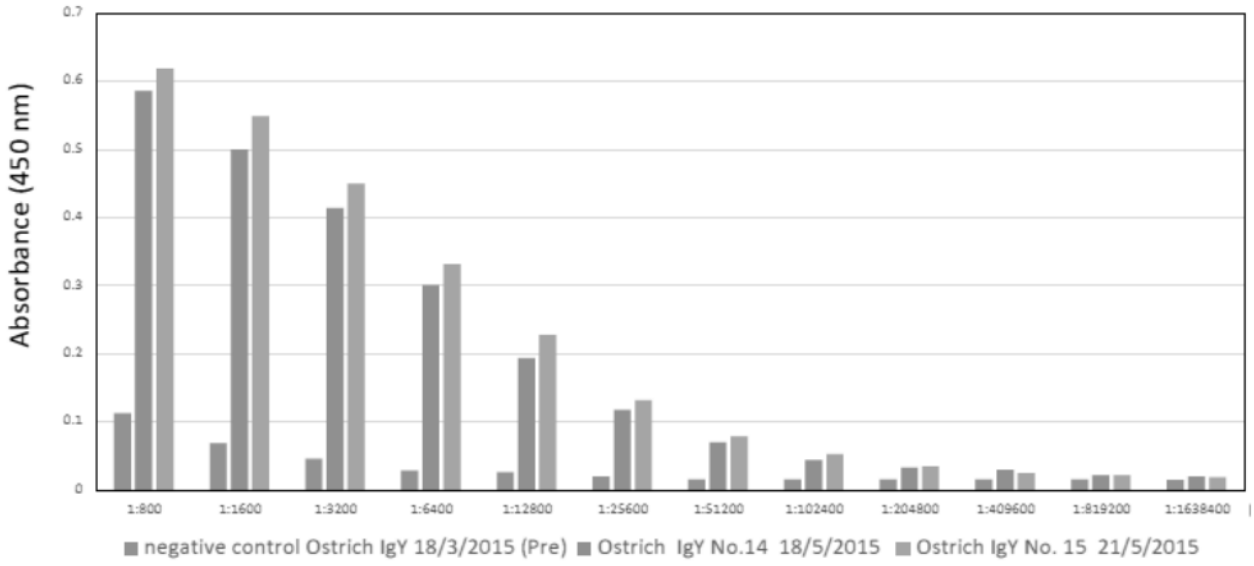
Preclinical data

Provide a brief summary of *in vitro* and *in vivo* experimental results relevant to the candidate including but not limited to safety, immunogenicity, efficacy, pharmacokinetic and pharmacodynamic studies. Indicate if data are not available.

To date, only the primary testing in spike protein bound ELISAs and live virus cell-based inhibition assays have been completed.

We have been able to demonstrate in our ELISAs that the overall signal does not decrease for several months following antigen injection into the ostrich (data not shown). Figure 1 below

demonstrates two successive purifications of the MERS-CoV antibody and the resulting binding to a spike-protein ELISA.



The live-virus inhibition assays are still under development at USAMRIID, however, these assays provide the best information possible for a candidate to be selected for *in vivo* efficacy evaluation. In this assay whole ostrich sera, as well as two purified products, have been tested to date. The purified samples demonstrated an activity of reducing the viral spread by 50% in the assay (here called EC50) by (b)(4) respectively. (Table 1)

Compound ID	Plate ID	cell line	Pathogen	EC50, ug/ml	SD	Fit Model	CC50, ug/ml
MERS IgY 1	150616MervVeroAB001	Vero	MERV	(b)(4)		4pHill (AC50,n,S0,Sinf)	(b)(4)
MERS IgY 2	150616MervVeroAB002	Vero	MERV			3pHill (AC50, n, S0)	
MERS anti-serum 6W	150616MervVeroAB002	Vero	MERV			3pHill (AC50, n, S0)	
Pre-im IgY NC	150616MervVeroAB001	Vero	MERV			4pHill (AC50,n,S0,Sinf)	

Efficacy data

Efficacy data is not yet available

Toxicity data

Provide a summary of existing toxicity data including any IND enabling studies. Identify the cell culture system(s) and animal model(s) and provide results of testing. Include such information as dose and/or formulations tested, dose schedule, concentration/titer, negative and positive controls, performance lab results, and other relevant details. Indicate if data are not available.
Toxicity data is not yet available

5. DEVELOPMENT PLAN

Please indicate how data obtained via DMID/NIAD contract resources fit into the overall development plan of your product.

The data obtained will provide:

1. *In vivo* validation of the efficacy of the product

2. Dosage guidance for planned non-human primate studies

In addition please address:

- Have you filed a patent request? Patents have not yet been filed, but they will be extensions of an existing process patent.
- Do you have IP rights? If so, please provide the U.S. patent# US 8,003,762 B2, August 23, 2011
- If results warrant, do you plan to pursue licensure of the candidate? Yes

6. REFERENCES

Please list references providing relevant background information on your product. References from peer reviewed publications are preferred.

Dr. Yasuhiro Tsukamoto, the developer of the process for generating and purifying ostrich antibodies, has successfully created antibodies for a wide range of pathogens and allergens. Listed below are publications on H5N1 and H1N1 antibodies.

Development of neutralization antibodies against highly pathogenic H5N1 avian influenza using ostrich (Struthio camelus) yolk, Kazuhide Adachi, Ekowati Handharyani, Dwi Kesuma Sari, Kentaro Takama, Keiko Fukuda, Isako Endo, Ryohei Yamamoto, Masaki Sawa, Masaru Tanaka, Itsuro Konishi and Yasuhiro Tsukamoto, *Molecular Medicine Reports*, Vol. 1, No. 2, 2008.

Ostrich produce cross-reactive neutralization antibodies against pandemic influenza virus A/H1N1 following immunization with a seasonal influenza vaccine, Kazuhide Adachi, Kentaro Takama, Masaya Tsukamoto, Marie Inai, Ekowati Handharyani, Satoshi Hiroi, and Yasuhiro Tsukamoto, *Experimental and Therapeutic Medicine*, Vol. 2, No. 1, 2011.

From: Peter Daszak
Sent: Mon, 4 Apr 2016 15:22:19 +0000
To: Stemmy, Erik (NIH/NIAID) [E]; Chen, Ping (NIH/NIAID) [E]
Cc: Aleksei Chmura; Hongying Li
Subject: Meeting re. coronavirus research in China funded by NIAID
Attachments: Invitation Letter to the Wildlife and Public Health Workshop_Signed_中英文.pdf

Dear Dr. Chen,

I'm following up on the email from Erik Stemmy a few months ago (below). As Erik mentioned, we have been collaborating with local partners in China since 2004 on SARS CoV virus (and other new viruses) that could cause emerging infectious diseases, in collaboration with Dr. Zhengli Shi at the Wuhan Institute of Virology and others.

I will be in Beijing during April 19-21 to host a workshop on wildlife and public health with the Forestry Administration and China CDC/CAS, so I would love to visit you sometime during these days, if possible, to talk to you more about our work in China. Are you available on either 4/20 Wednesday or 4/21 Thursday?

As well as this, I've attached an invitation to the Wildlife and Public Health Workshop on April 19, please feel free to register if you are able to join in us for the discussions. We will be talking about our work funded by NIAID, and it might be interesting for you or some of your staff to attend.

Thank you very much, and I hope we're able to meet this month. If not, I will be back in Beijing and June, which would give a longer lead in to arrange a meeting.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
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www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, May 26, 2015 8:37 AM
To: Chen, Ping (NIH/NIAID) [E]
Cc: Peter Daszak
Subject: CoV Research in China

Hi Ping,
Hope things are going well in Beijing! One of the investigators in my coronavirus portfolio, Peter Daszak from EcoHealth Alliance (copied here), asked me to put him in touch with you. In one of his projects Peter is looking at the emergence of CoVs from bats, and he collaborates with several sites in China on the project so we thought it would be good idea for him to have your contact info.

Peter, as I mentioned when we spoke Ping is based out of the US Embassy in Beijing and helps facilitate NIAID research and collaborations in China and the vicinity. I'd encourage you to reach out and tell her a bit about some of your other projects, particularly if you'll be visiting China or Beijing any time.

Best,
Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

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Formerly known as Wildlife Trust

This is a formal invitation to the first **Wildlife and Public Health Workshop** on **April 19, Tuesday, 2016 in Beijing, China** sponsored by EcoHealth Alliance, the Department of Wildlife Conservation and Nature Reserve Management, State Forestry Administration, and Chinese Center for Diseases Control and Prevention (CDC).

Since 2004, we have been conducting research projects in China to assess the effects of wildlife trade (hunting, trading, consuming) on human and animal health, local ecosystems, and social economy. What we've learned is the potential for another SARS-like outbreak is alarmingly high and our best defense will be working together to implement local programs and policy to prevent another disease event, and protect wildlife at the same time.

This workshop will bring together technical experts, scientists, policy-makers, and leaders from a number of organizations and agencies to build a network for solid strategies aimed at leveraging the health impacts of wildlife trade to prevent diseases and maintain ecosystem integrity. We will specifically focus on:

- Share knowledge and trends in wildlife trade and its health implications
- Share information on best practices on reducing the health threats of the wildlife trade and preventing epidemic/epizootic disease
- Co-design new strategies to reduce wildlife trade and its health risks
- Discuss the effective methods to translate related scientific findings into actions for the well-being of human and animals
- Develop collaborative relationships among government, local people, and the private and public sectors to respond to the challenges of global health and conservation

Speakers and institutes in this workshop include:

- Experts from the Department of Wildlife Conservation and Nature Reserve Management, and the Veterinary Bureau of the Ministry of Agriculture
- Conservation medicine scientists from EcoHealth Alliance
- Scientists from Chinese CDC, Chinese Academy of Science, Chinese Academy of Engineering, and Chinese Animal Health and Epidemiology
- International and local NGOs with extensive experience working on wildlife trade reduction and infectious disease/pandemics prevention
- Foundations with interests in public health and conservation in Southeast Asia
- Other key stakeholders from public media and industry

Topics for discussion at this workshop will include, but are not limited to:

- "One Health" for human and animals
- The role of wildlife trade in the ecology of emerging infectious diseases

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- Facts and data about wildlife trade and its health implications
- Research and practice to respond to the health risks from contacting wild animals
- How the work in preventing emerging infectious diseases will benefit conservation

The workshop will help develop strategies as well as information/policy recommendation papers based on the presentations and discussions. The recommendations will be widely distributed to inform policy makers nationally and internationally, and the strategies will then be implemented through EcoHealth Alliance's collaborative programs in China and other countries in Southeast Asia over a three-year period (2016-2019).

We would highly appreciate your participation and contribution at this workshop. To facilitate the discussion and interaction, we have chosen to limit the number of people for this workshop, if you or a senior staff person in your organization is unable to attend, please inform us as early as possible, so we can extend invitations to other interested organizations and individuals.

If you individually are unable to attend, please let us know who can participate on your behalf. This is an invitation only event and we are requesting to have at least one representative from your organization. Accommodation and meals will be provided on both days, a reception will be held on April 19, hosted by Dr. Peter Daszak, the President of EcoHealth Alliance.

Please kindly RSVP to Ms. Hongying Li at (b)(6) by **10st April, 2016** as soon as possible. The workshop agenda and logistics info will be sent to you upon receiving your RSVP.

If you have any questions about the workshop, please do not hesitate to contact Hongying, the coordinator for this workshop. Thank you very much. We hope to see you at the workshop!

Yours Sincerely,

(b)(6)

Peter Daszak
President
EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001
(b)(6) (direct)
+1.212.380.4465 (fax)

EcoHealth Alliance

Formerly known as Wildlife Trust

生态健康联盟（EcoHealth Alliance）真诚地邀请您参加2016年4月19日于中国北京召开的“野生动物和公共健康”研讨会，与我们分享您所在工作领域的现状和发展，以及您宝贵的观点。

自2003年非典型性肺炎爆发以来，由野生动物引起的新发传染病（如：非典型性肺炎、禽流感、中东呼吸综合征、埃博拉、艾滋病等）越来越受到包括疾病预防和控制部门、野生动物疫源疫病监测防控部门、农业动物检疫部门等国内外的各界专家学者的关注。生态健康联盟也自2004年起，开始在中国开展了多个研究项目，评估野生动物贸易给公共健康带来的影响。

多年的研究和监测结果提醒我们，在当今高度发达的全球贸易环境下，动物和人类的健康，以及我们共同赖以生存的环境时刻都在受到威胁。另一场像非典那样的疫情随时可能爆发，一个个物种正在不断地因为疾病或环境的破坏而消失，我们应该尽快采取有效的政策和措施，保护动物，也就是保护我们人类自己。

本次会议由国家疾控中心，国家林业局保护司，生态健康联盟（EcoHealth Alliance）共同举办，北京爱它动物保护公益基金会承办，将聚集来自不同领域和部门的专家、学者、政策制定者、相关机构的领导，从野生动物可能带来的公共健康影响的角度，探讨如何通过建立多部门之间的合作，制定行之有效的策略和方案，加强野生动物疫源疫病监测，减少野生动物贸易，保护野生动物和公共健康 。

本次会议中我们将：

- 分享目前野生动物贸易的相关数据及其对公众健康的影响
- 分享预防人兽共患病／新发传染病、减少与野生动物接触的健康风险的研究和经验
- 制定野生动物疫源疫病监测、减少野生动物贸易、保护动物和人类健康的新策略
- 探讨如何将目前各部门相关工作转化为有利于动物和人类健康福祉的有效行动
- 建立政府部门、研究机构、民间机构和当地居民之间的合作，共同应对全球的环境和健康挑战

参与本次会议的嘉宾和机构包括：

- 林业局保护司和农业部兽医局的专家
- 生态健康联盟来自美国、非洲、澳洲、和中国的保护生物医学专家学者
- 中国疾病预防与控制中心、中国科学院、中国工程院的专家学者
- 关注野生动物和／或公共健康的国际和本土民间组织
- 关注东亚地区公众健康和野生动物保护工作的基金会
- 来自媒体和商界的各利益相关者

Local conservation.
Global health.

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在本次会议中我们将讨论的话题包括，但不仅限于：

- 动物与人类的“同一个健康”
- 野生动物贸易在新发传染病生态学中的重要角色
- 野生动物贸易相关的数据的信息，及其对公共健康的影响
- 如何有效应对与野生动物接触带来的健康风险
- 新发传染病 / 人兽共患病预防控制工作如何能够有利于野生动物的保护

本次会议将制定新的战略计划和项目提案，并且基于会议内容发表学术和倡议报告。这些报告将被提交给相关机构，并被广泛传播。新的战略计划和项目提案将通过生态健康联盟及其当地合作伙伴在中国未来 3-5 年的项目开展实施。

您的参与将是我们至高的荣幸。为了有助于会议的讨论和互动，我们不得不限制会议的人数，如果您本人无法出席，请及时告知谁将代表您出席本次会议，如果您机构的相关人员无法出席，也请及时告诉我们，以帮助我们将参与的机会留给别的机构和个人。

会议期间的住宿和餐饮，包括参与会议的差旅费（如有需要）都由会议方承担，4 月 19 日晚上将由生态健康联盟的主席 Peter Daszak 博士主持欢迎晚宴。

如果您有兴趣参与此次会议，贡献您宝贵的想法和建议，请于 **2016 年 4 月 10 日**前尽快通过回复邮件(b)(6)确认参会，我们会将在接到您的报名后发给您具体的会议日程，并协助您安排各项参会事宜。

如果有任何问题，请随时通过邮箱或微信与本次会议的协调员李泓萤联系。邮箱：(b)(6)微信：(b)(6)（请注明“参会”）

我们期待您的参与，希望能与您在会上进一步交流！

此致

敬礼

(b)(6)

Peter Daszak
President
EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001
(b)(6)(direct)
+1.212.380.4465 (fax)

From: Leyva-Grado, Victor
Sent: Thu, 29 Oct 2015 17:20:30 +0000
To: NIAID DMID IDIQ; Stemmy, Erik (NIH/NIAID) [E]
Cc: Umerah, Nina; Baric, Ralph; 'Heise, Mark T'
Subject: HHSN272201000019I-HHSN27200003-Task A57 - November progress report
Attachments: A57 Task Order-Research Program Nov 2015.docx

Dear Erik,

Attached you will find the October 2015 progress report for Task A57.

Please let us know if further information is required.

Thanks a lot,

Victor

Victor H Leyva-Grado DVM, PhD
Postdoctoral Fellow
Microbiology Department
Global Health and Emerging Pathogens Institute
Icahn School of Medicine at Mount Sinai
One Gustave L Levy Place
Box 1124 Annenberg 16-15
New York, NY 10029
Phone (b)(6)
Fax 1-212-534-1684

MONTHLY REPORT

Contract HHSN272201000019I Task Order HHSN27200003 A57

Mouse Model for Evaluation of Medical Countermeasures Against Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

Period of Performance:
October 1, 2015-October 31, 2015

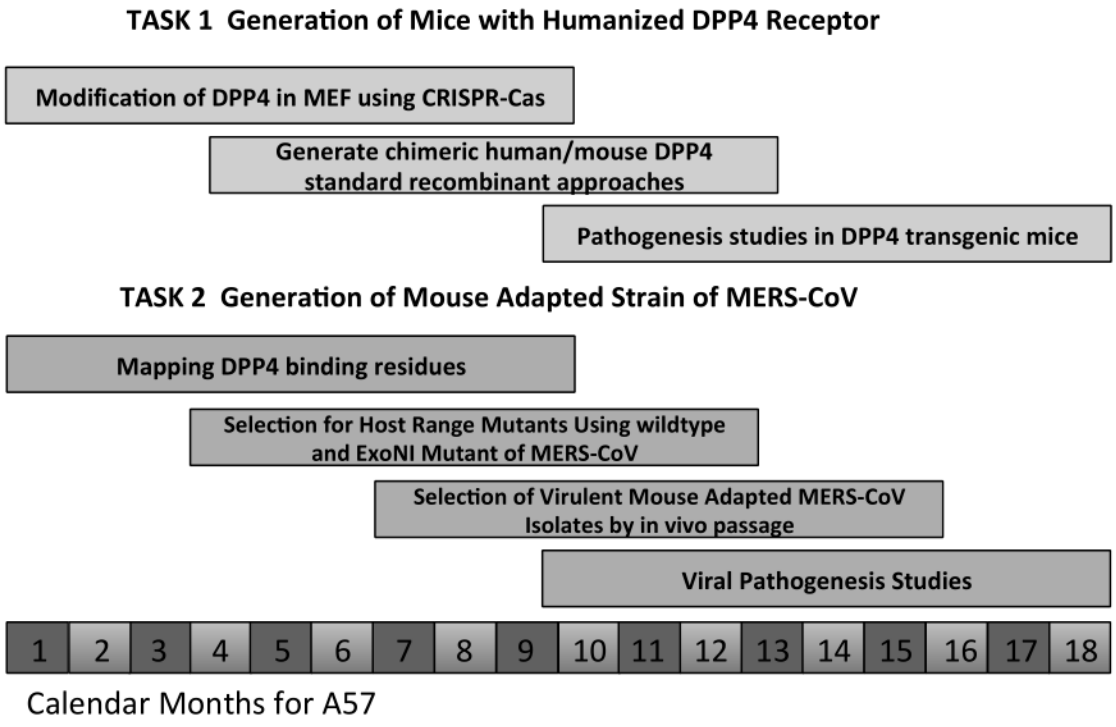
Contractor's Name and Address:
Dr. Peter Palese
Horace W. Goldsmith Professor and Chair Department of Microbiology
Professor, Department of Medicine Mount Sinai School of Medicine
1 Gustave Levy Pl.
New York, New York 10029-6574
Tel (b)(6)
Fax 212-722-3634
e-mail: (b)(6)

Date of Submission:
October 25, 2015

A. Scope

The objective of this task is to make a lethal mouse model (80% lethal by 10 days post infection) for the recently identified human coronavirus Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Our group will develop transgenic mice that express humanized dipeptidyl-peptidase receptors and select for mouse-adapted strains of the MERS-CoV.

B. Timeline



C. Progress on Model and Supporting Data

Generate hDPP4 expressing transgenic mice.

Breeding results for generation 2 (G2) progeny for each hDPP4 overexpressing transgenic mouse line is shown in **Table 1.** As anticipated G3 pups from Lines 9/21 and 24 appear to be predominantly homozygous at the DPP4 locus. G3 pups from Line 5 remain heterozygous at the DPP4 locus as we continue to have a significant number with wild type DPP4 genes. This is most likely due to random insertion or concatemerization of the insert and it can be anticipated that multiple copies were inserted and continue to segregate into G3. We are currently developing an assay sensitive enough to allow us to determine copy number, as well as the heterozygous/homozygous status of each mouse. In addition, as we establish stable lines we will develop methods that allow us to sequence the genomic sites of the insertions to better characterize our knock-in mouse models. Subsequent rounds of breeding are underway (see **Figure 1**). When this breeding is completed G2/G3 mice will also be characterized for MERS-CoV pathogenesis.

Breeding of hDPP4 lacking enzymatic activity transgenic mice. As overexpression of functional hDPP4 can be toxic to cells and has the potential to be embryonic lethal over subsequent rounds of breeding, we have generated another transgenic hDPP4 mouse line that expresses a mutant hDPP4 that lacks catalytic activity. We currently have three founder lines for these transgenic mice and breeding to wild type C57Bl/6J mice is already underway. To date we have 22 pups born from these founder lines and we anticipate additional births in the near future. They will be weaned and genotyped in time for the December report. As with the wild type hDPP4 overexpressing mice, each transgenic generation will be tested for MERS-CoV pathogenesis.

Endogenous mouse DPP4 modified to be permissive for MERS-CoV infection using CRISPR/Cas approach. *Single mutation mice:* CRISPR/Cas genome editing was used to modify endogenous mouse DPP4 in an attempt to make it amenable to infection. Currently mice are being bred for two lines of mice, one that contains only the T330R permissive mutation and a separate line of mice that have the A288L mutation. We have identified G2 mice and defined if one or both gene copies have been mutated for each strain (**Table 2**) and we are currently setting up breeding pairs to generate G3 mice that are homozygous on both chromosomes for each mutation. G3 mice will then be assessed for MERS-CoV replication and disease.

Double mutation mice: We have screened pups from the 5 founder lines with both the A288L and T330R permissive alleles present on at least one chromosome and an additional line that appears to be heterozygous. Breeding of Line 57 resulted in 6 pups (G1) that are positive for each mutation on both chromosomes. The 6 positive mice from Line 57 were set up in breeding triads to generate G2 pups, some of which are anticipated to be homozygous at both the 288 and 330 locus. From these breeding triads a total of 22 pups were recently born. Once weaned these will be

Table 1. Breeding results for G2 hDPP4 overexpressing transgenic mice (G3 pups).

Breeding Lines	Generation	Genotyping: hDPP4 Pos.
Line 5 (41) G2 ♂ x Line 5 (44) ♀	G3	2
Line 5 (60) G2 ♂ x Line 5 (65) ♀ & Line 5 (66) ♀	G3	2
Line 5 (51) G2 ♂ x Line 5 (57) ♀ & Line 5 (68) ♀	G3	11
Line 9/21 (1) G2 ♂ x Line 5 (3) ♀	G3	3
Line 9/21 (12) G2 ♂ x Line 9/21 (20) ♀ & Line 9/21 (21) ♀	G3	10
Line 24 (22) G2 ♂ x Line 24 (32) G2 ♀ & Line 24 (33) ♀	G3	14
Line 24 (24) G2 ♂ x Line 24 (34) G2 ♀	G3	6

screened and bred. We anticipate having conclusive genotyping data on these mice by the January 2015 report.

As an alternative, we are also identifying select Collaborative Cross Recombinant Inbred Lines for CRISPR/CAS genome editing; specifically, identifying lines that i) can be induced to super-ovulate, ii) have large litter sizes, ii) are genetically distinct, and iv) encode an H2B locus (less critical). We hypothesize that viral pathogenesis may be increased in a different mouse genetic background than C57Bl/6. While we have identified candidate strains of CC lines that can be used for these studies, we will hold these experiments until some positive results are seen in the current DPP4 mutant CRISPR/CAS mice. We hope to begin these studies in Dec or Jan.

Figures 1 and **2** contain updated schematics of the current DPP4 mouse breeding and infection schedule.*Note: These experiments are on hold and we are awaiting permission to continue

In vitro Selection Studies (Proposed). It is possible that hDPP4 transgenic mice or CRISPR/Cas humanized mDPP4 mice will support MERS-CoV replication, but result in limited in vivo pathogenesis. In particular, CRISPR/Cas humanized mDPP4 mice only encode the most important subsets of residues critical for MERS-CoV docking and entry. To enhance MERS-CoV binding and affinity for the chimeric receptors, we will passage virus in nonpermissive cells expressing the mDPP4 288/330 humanized receptor, selecting for second site mutations in the RBD that more effectively engage the chimeric receptor. This is based on the observation that SARS and other related bat coronaviruses evolve mutations in the RBD first, to enhance virus replication efficiency in mice. In parallel, we will select for MERS-CoV variants that more effectively use the mDPP4 chimeras encoding the single 288 or 330 humanizing mutations alone. The goal of these studies are to identify compensating mutation networks in the MERS-CoV RBD that allow for efficient chimeric receptor usage, and when introduced into the MERS-CoV molecular clone, will potentially enhance virus replication efficiency and pathogenesis in the in vivo mouse models. Finally, the availability of these mutants will speed the rate of in vivo selection for more virulent mouse adapted MERS-CoV strains for downstream studies. We note that it is likely that in vivo passage is essential for meeting the defined metrics of the animal models contract as originally stated.

Defining residues that enhance MERS-CoV permissivity using mouse DPP4. The characterization of the role of glycosylation and charge changes at the DPP4 MERS RBD interface is complete. A manuscript detailed the results of these studies is being written up and a draft will be forwarded to program as we prepare to submit it for publication.

Table 2. Breeding results for G1 hDPP4 single mutation CRISPR/Cas transgenic mice (G2 pups).

Breeding Lines	Generation	Genotyping: hDPP4 Pos.
A288L (2) G1 ♂ x A288L (14) G1 ♀	G2	2 homozygous for A288L 3 heterozygous for A288L
T330R (13) G1 ♂ x T330R (12) G1 ♀ & T330R (17) G1 ♀	G2	5 homozygous for T330R 5 heterozygous for T330R

Figure 1

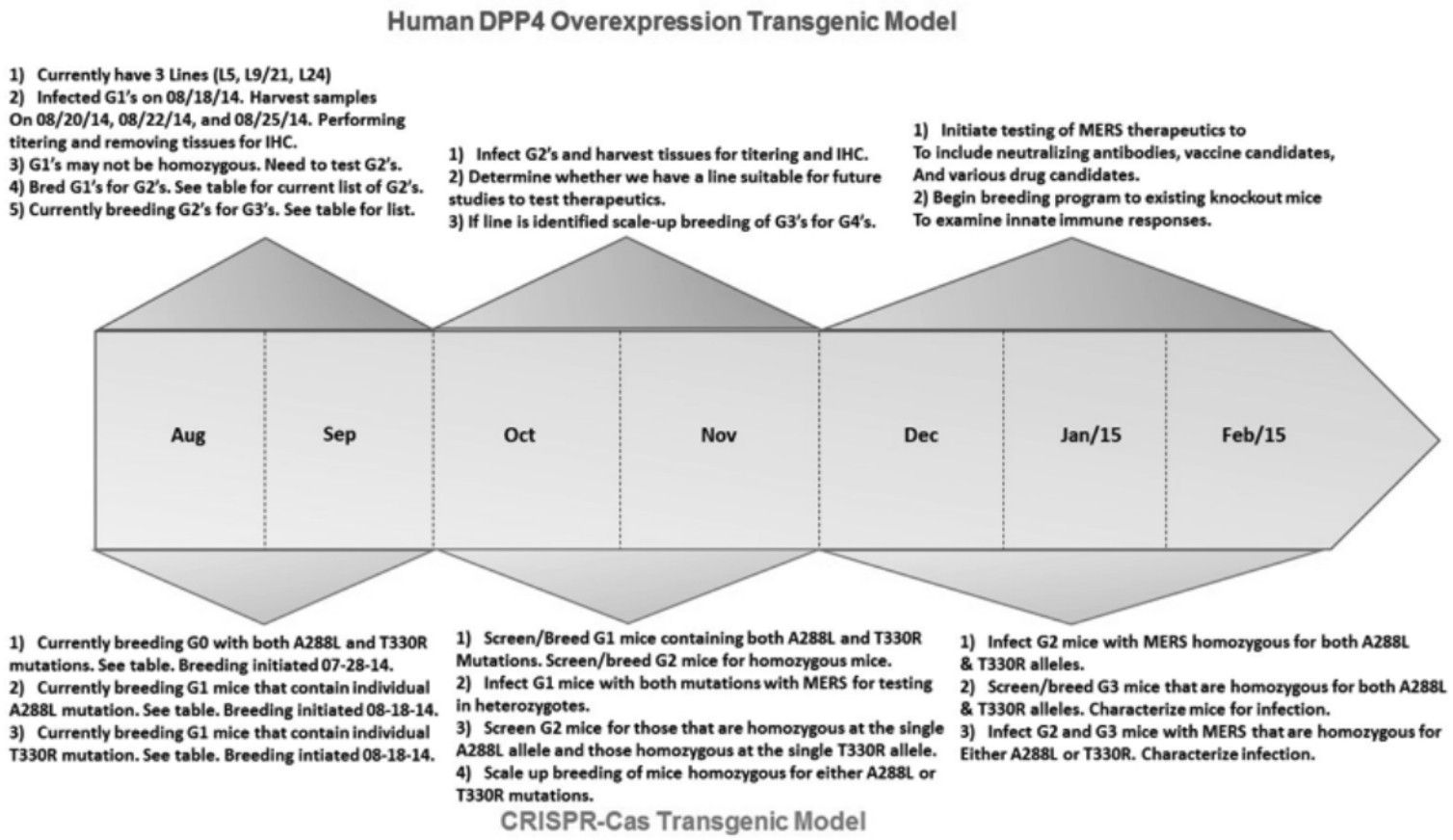
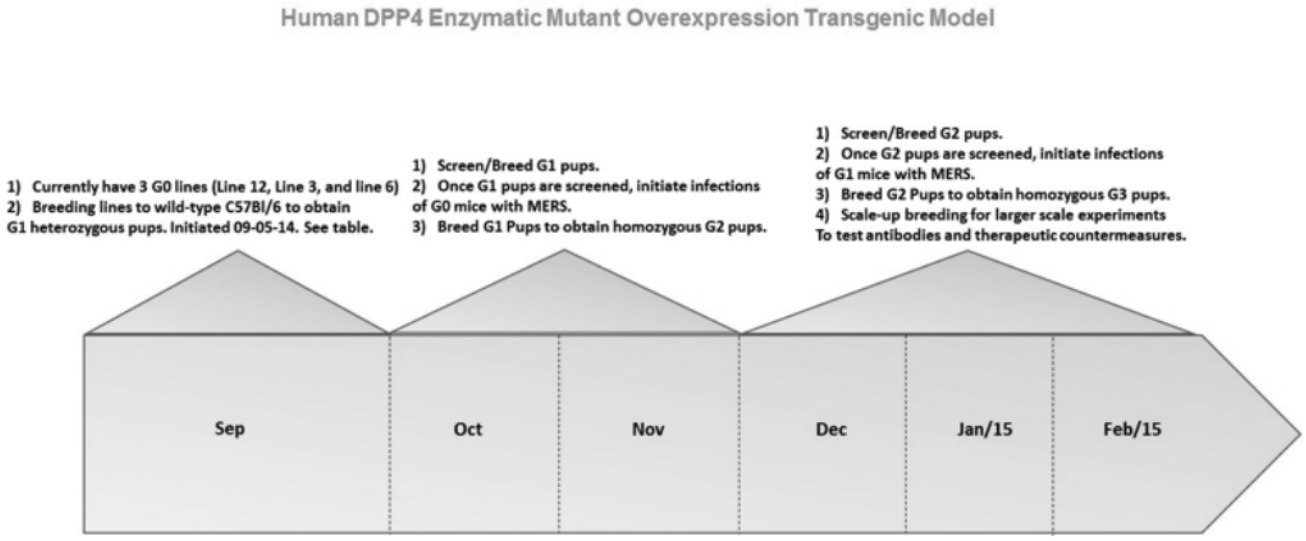


Figure 2 Timeline for human DPP4 enzymatic mutant overexpression mice



Technical/Performance Issues and Proposed Corrective Action

None

D. Expenditures Reporting

It is not possible for us to estimate what UNC Chapel Hill will bill Mt. Sinai for October 2014. We hope that this will be resolved over the next few weeks.

Please note: UNC Chapel Hill is transitioning to new purchasing software as of October 1, 2014. Our current software begins shutting down September 17, 2014. As such, we may not be able to provide billing/invoice information in the November report as there may be down time while potential issues are worked out with the new system. We appreciate your patience as the university makes this massive change, the first of its kind in 40 years!

From: Keith Wycoff
Sent: Thu, 26 Jan 2017 14:28:09 -0800
To: Cockrell, Adam
Cc: Stemmy, Erik (NIH/NIAID) [E]; Baric, Ralph
Subject: Re: timeline for testing anti-MERS therapeutic in MERS mouse model
Attachments: S2294-gal PK Summary.docx

Hi Adam,

Thanks for working this up. The timeline seems fine to me. Just to be clear, are you proposing a single dose 12 hr prior to infection, with a second dose to be added in a potential follow-up study?

The predicted size of our molecule is 222 kDa, based on amino acid sequence, which would suggest that we aim for a dose of 370 µg/mouse (typical human IgG ~150 kDa). For one dose and 5 mice I would propose sending you 7 mg of protein which should be more than enough

I am attaching the results of the small mouse PK experiment we did, using another DPP4-Fc variant that differs by only a single amino acid from S2320-Gal (by the way, the SF just indicates that the sample is sterile filtered). I'd be interested in knowing how this compares to anything you've done with human IgG.

Thanks,
Keith

On Jan 26, 2017, at 1:17 PM, Cockrell, Adam (b)(6) wrote:

Hi everyone,

I put together an outline of the study we discussed last week. I also attached a copy of our MERS mouse model manuscript.

I am currently working on the amendment for our IACUC protocol. I will be proposing the study as outlined. However, I will also add additional delivery time point post-infection in the event we decide on a follow-up study.

Currently I have this at 250ug/mouse for delivery based on efficacy that we have seen with anti-MERS antibodies delivered prophylactically, 12 hours prior to infection.

Keith, you mentioned that your group would do the calculations regarding the molar equivalent of your therapeutic compared to a typical IgG1 subtype human antibody. If the hDPP4-Fc therapeutic is larger, the molar comparison would probably indicate that we should use a higher ug amount of the hDPP4-Fc therapeutic for delivery.

Please let me know ASAP if everyone is agreeable to this timeline, dose, etc. This would require me to alter the IACUC amendment prior to submission.

Best Regards,

Adam Cockrell
Research Associate
Department of Epidemiology
University of North Carolina at Chapel Hill
Chapel Hill, NC, 27599
Lab Phone: (b)(6)
Office Phone: (b)(6)

<Cockrell AS & Baric RS et al. Nat. Micro. 2016. A mouse model for MERS-CoV-induced ARDS.published version.pdf><Timeline for initial study.pptx>

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Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

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Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

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of the Freedom of Information and Privacy Act

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Withheld pursuant to exemption

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of the Freedom of Information and Privacy Act

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Withheld pursuant to exemption

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of the Freedom of Information and Privacy Act

Page 028 of 455

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

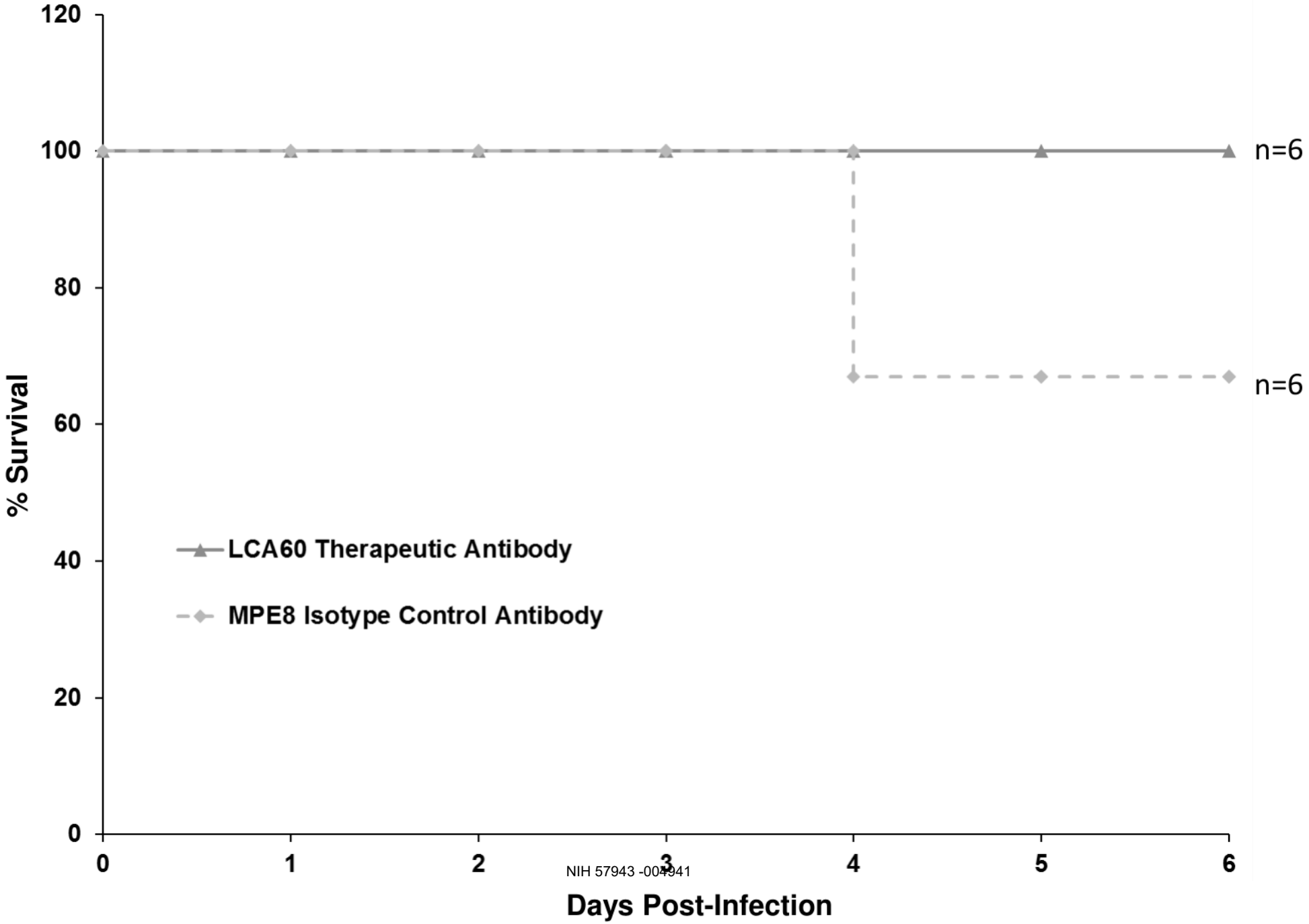
From: Cockrell, Adam
Sent: Sat, 19 Dec 2015 12:49:01 +0000
To: Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
Cc: Baric, Ralph
Subject: Summary of LCA60 data for prophylaxis study
Attachments: Summary of Data LCA60.pdf

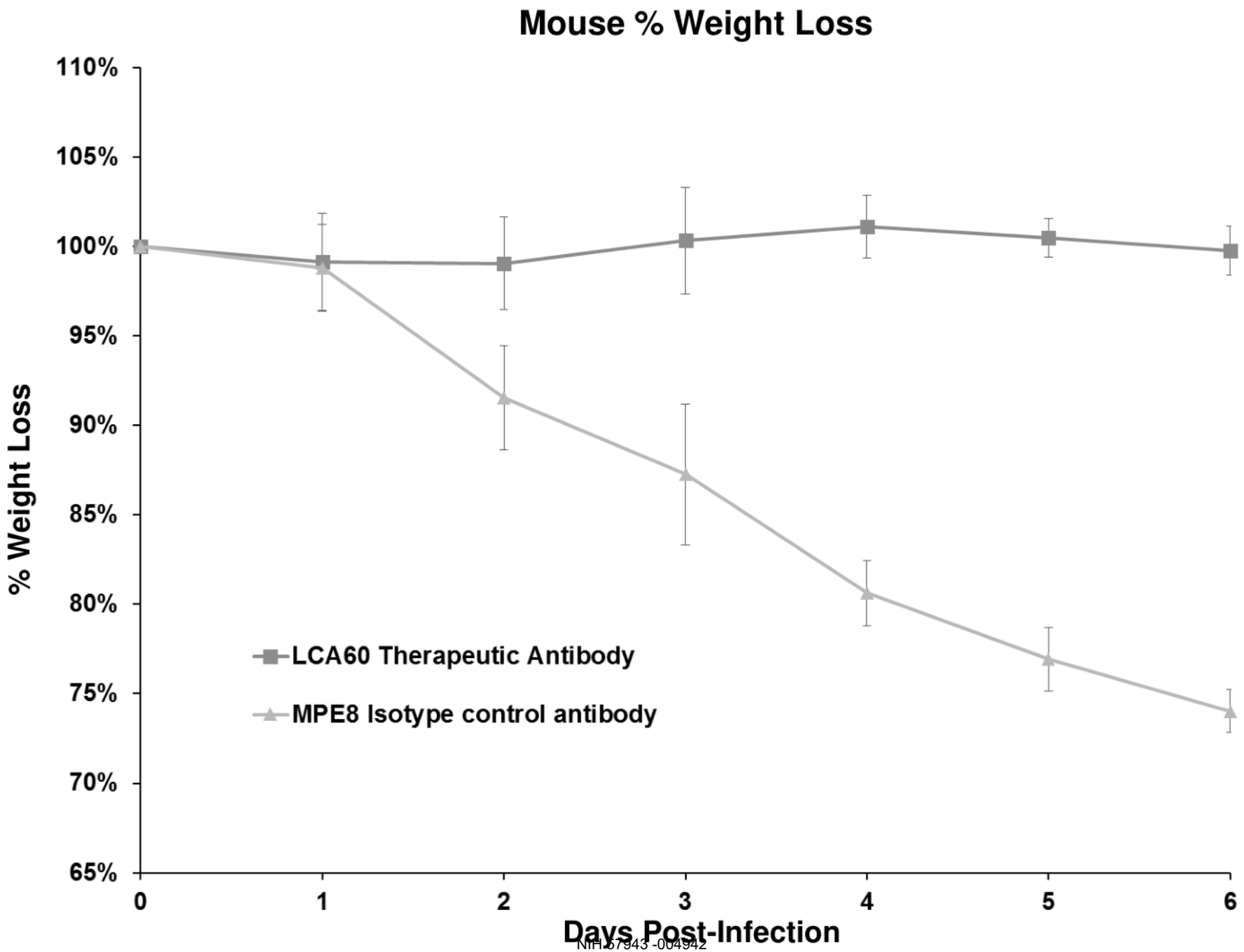
Hi everyone.

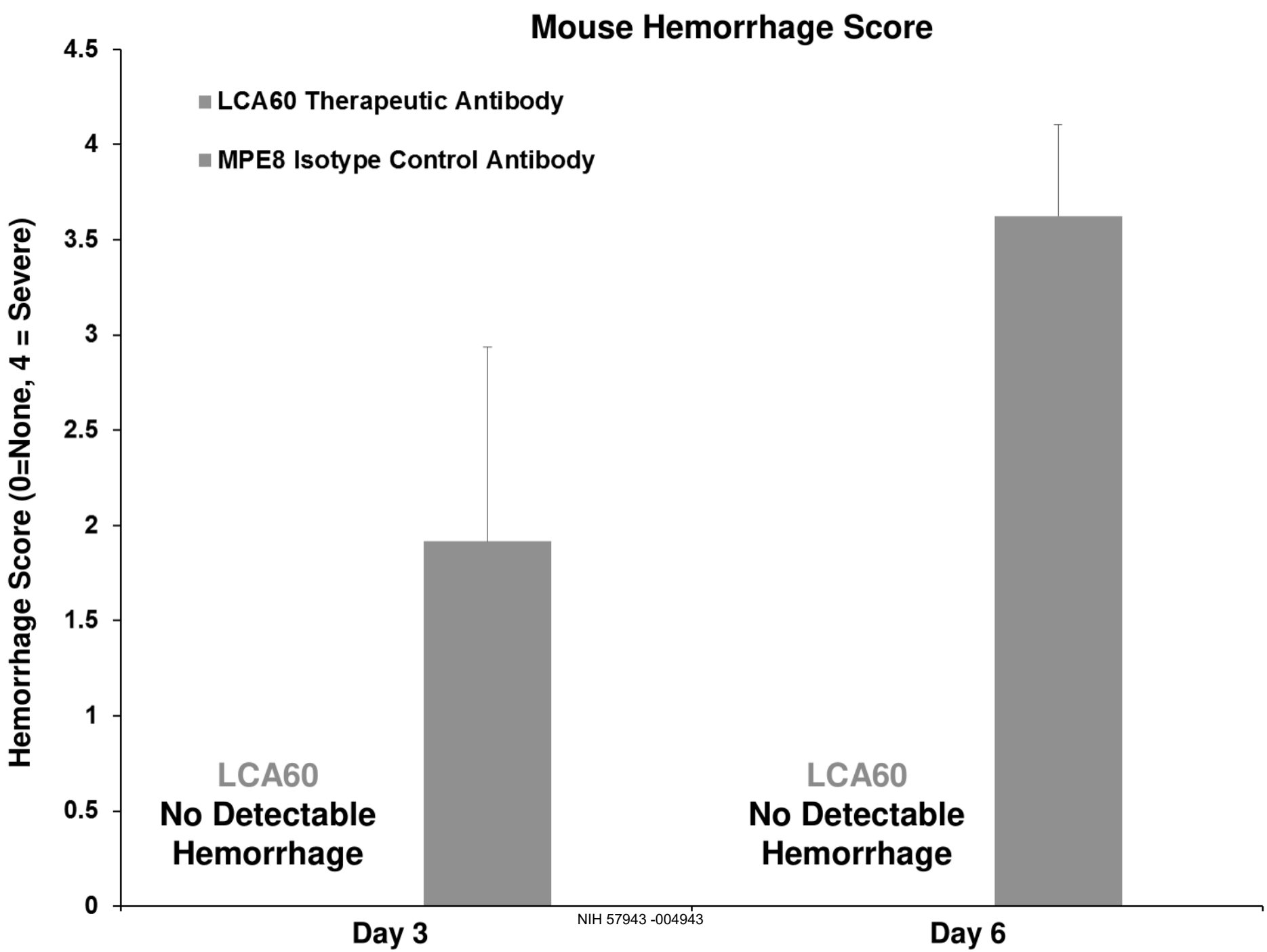
I have attached a summary of the LCA60 antibody data that includes survival, weight loss, hemorrhage score, and respiratory function for the prophylactic study. The remaining data will be collected in the new year.

Hope everyone has happy holidays.

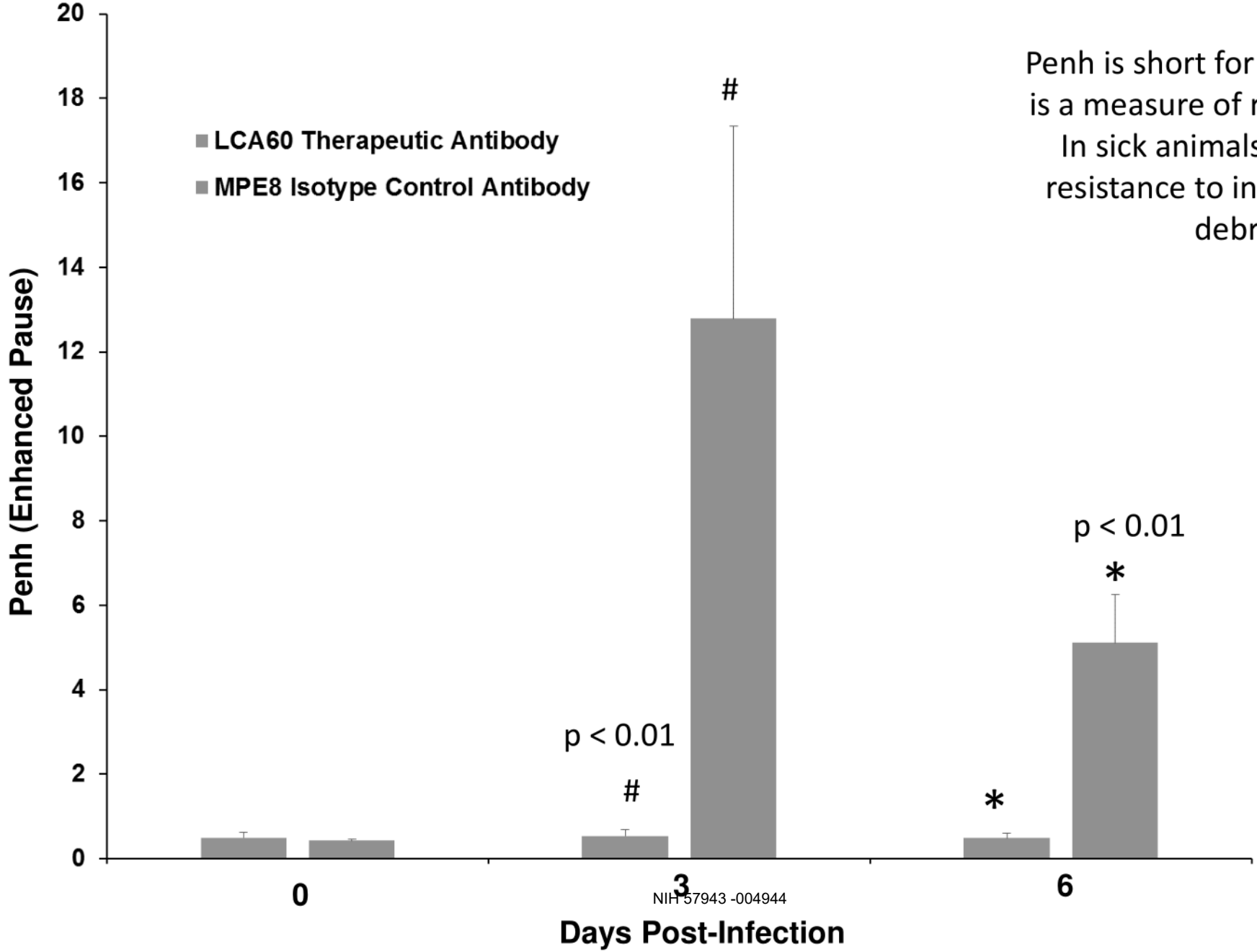
Adam Cockrell
Post-Doctoral Fellow
Department of Epidemiology
University of North Carolina at Chapel Hill
Chapel Hill, NC, 27599
Phone: (b)(6)



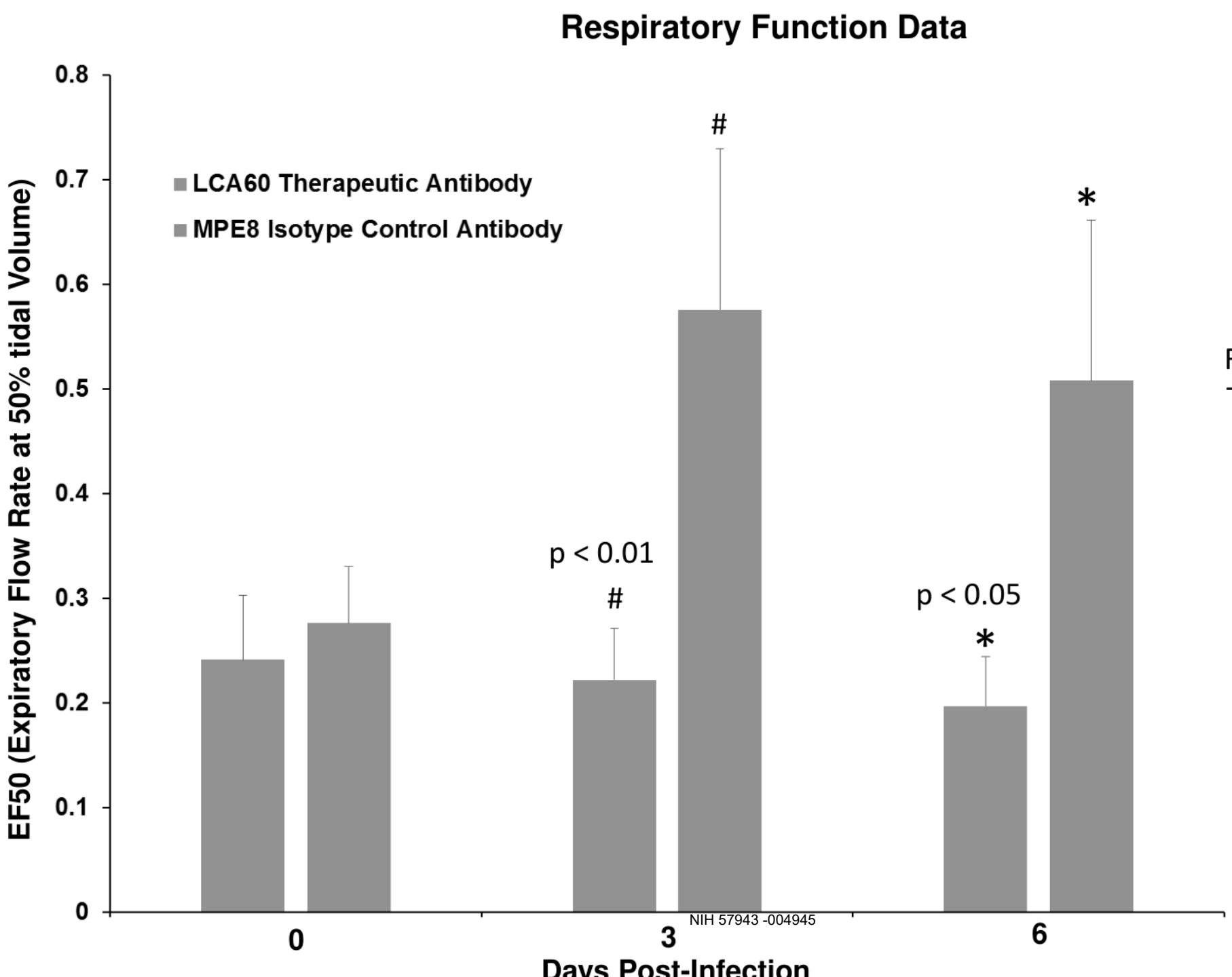

Mouse % Weight Loss
110%
105%
100%
95%
90%
85%
80%
75%
70%
65%
% Weight Loss
LCA60 Therapeutic Antibody
MPE8 Isotype control antibody
0
1
2
3
4
5
6
Days Post-Infection


Mouse Hemorrhage Score
LCA60 Therapeutic Antibody
MPE8 Isotype Control Antibody
Hemorrhage Score (0=None, 4 = Severe)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
LCA60
No Detectable
Hemorrhage
LCA60
No Detectable
Hemorrhage
Day 3
Day 6

Respiratory Function Data



Penh is short for Enhanced Pause, which is a measure of resistance in the airway. In sick animals we would anticipate resistance to increase due to possible debris in airway.



Similar to what was Previously observed with SARS infected mice, where They appear to be exhaling The breath more rapidly to 50% volume. This is an Indication that the majority Of breadth is rapidly Released with the remainder Taking a long time similar to A wheeze in humans.

From: Cockrell, Adam
Sent: Fri, 4 Nov 2016 18:17:47 +0000
To: Jeff Pouliot; Feng Wang
Cc: Stemmy, Erik (NIH/NIAID) [E]; Leyva-Grado, Victor; Umerah, Nina; Baric, Ralph; Deborah Butler; Neil Pearson; Yount, Boyd L Jr
Subject: RE: GSK A57 Study control
Attachments: Report of Initial Study with GSKXXX.pdf

Hi Jeff,

I am attaching our report of the first study for your group to review.

It appears that delivery of the vehicle and/or anesthetic enhance viral replication, which may be augmenting disease phenotypes. In addition, anesthetizing with ketamine/xylazine and intranasal delivery every 12 hours may also be contributing to significant weight loss early on, and enhanced mortality of the mice. It is important to note that the titers were increased in both the vehicle treated and drug treated, well above (>10-fold) those I have ever observed with this model. Two control animals (no drug/vehicle treatment) had lung titers similar to what we have observed previously with this model. This may be an initial indication that the drug is not reducing the viral load in the lungs.

Suggestions for a second study: I think altering the dose schedule to 24 hour increments, using ketamine/xylazine for viral administration, and using isoflurane for subsequent drug administration may result in a more effective second study. However, these are only suggestions.

We look forward to discussing the follow-up study.

Best Regards,

Adam

From: Jeff Pouliot (b)(6)
Sent: Monday, October 31, 2016 9:17 AM
To: Cockrell, Adam (b)(6) Feng Wang (b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Leyva-Grado, Victor (b)(6) (b)(6) Umerah, Nina (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6) Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study control

Hi Adam,

Any news on the viral titers? We'd like to schedule another study, but have been waiting to hear whether viral inhibition was detectable from the first run.

Nobody on this end has previously seen toxicity from intranasal dosing with a Tween formulation, so the tox we saw may be related to the infection. Do you know of any literature that suggests detergents of that sort can exacerbate MERS?

Best,

Jeff

Jeffrey Pouliot, Ph.D.
Investigator
Biology Host Defense DPU
R&D Infectious Disease

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Tel (b)(6)

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From: Cockrell, Adam (b)(6)
Sent: Wednesday, October 12, 2016 4:23 PM
To: Jeff Pouliot; Feng Wang
Cc: Stemmy, Erik (NIH/NIAID) [E]; Leyva-Grado, Victor; Umerah, Nina; Baric, Ralph S; Deborah Butler; Neil Pearson; Yount, Boyd L Jr
Subject: RE: GSK A57 Study control

EXTERNAL

Thanks Erik and Jeff.

At this time we have lost 3 vehicle treated and 1 drug treated.

Hopefully we will have the titer data by late next week, and at the latest by the following week.

Best,
Adam

From: Jeff Pouliot (b)(6)
Sent: Wednesday, October 12, 2016 4:09 PM
To: Cockrell, Adam (b)(6) Feng Wang (b)(6)

Cc: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Leyva-Grado, Victor (b)(6) (b)(6) Umerah, Nina (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6) Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study control

Adam,

How unfortunate, we had hoped the mice would respond better to the regimen. We'll defer to your experience in deciding terminate the experiment early. As you suggest, measurement of the viral load in the lungs seems the most likely way to make a conclusion at this point.

What is the distribution of the mice we lost between the control and compound groups? It will be easier to interpret the experiment if the same number of mice remain in each.

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Wednesday, October 12, 2016 3:49 PM
To: Feng Wang
Cc: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; Leyva-Grado, Victor; Umerah, Nina; Baric, Ralph S; Deborah Butler; Neil Pearson; Yount, Boyd L Jr
Subject: RE: GSK A57 Study control

EXTERNAL

Hi everyone,

Unfortunately, at this time it appears we have lost 4 of the 12 mice in the study. Most likely due to a combination of repeated anesthetic and repeated intranasal administration. I gave the fourth dose this morning, but so not think the mice will tolerate another dose. I am going to terminate the study at this time to collect the lungs for titering in an attempt to salvage some data from this experiment.

Due to the issues we are having with the dosing regimen titering may be the most telling endpoint at this time.

Please let me know ASAP if everyone is in agreement with this.

Best,

Adam

From: Feng Wang (b)(6)
Sent: Tuesday, October 11, 2016 3:28 PM
To: Cockrell, Adam (b)(6)
Cc: Jeff Pouliot (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) Leyva-Grado, Victor (b)(6) Umerah, Nina (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6) Yount, Boyd L Jr (b)(6)
Subject: Re: GSK A57 Study control

Hi Adam,

Thanks for the update! Let's see how those mice hold on.

Best wishes,
Feng

Sent from my iPhone

On Oct 11, 2016, at 11:03 AM, Cockrell, Adam (b)(6) wrote:

EXTERNAL

Thanks Feng,

Just wanted to provide a small update on the current status. After this we will wait until we have all the data for a subsequent update.

The mice have been anesthetized three times at this point. Once for intranasal administration of virus, and twice for intranasal drug/vehicle delivery. Due to the short duration between intranasal delivery times (6 hours between virus and first drug administration, and 12 hours between drug re-administration) it appears that the mice have a difficult time recovering from repeated anesthetic. Due to this fact they do not appear to be eating/drinking. In less than 24 hours the average weight loss has been 8-9% of body weight for both vehicle and drug treated. This is most likely due to lack of recovery from repeated anesthetic administration since we do not observe this in less than 24 hours after virus administration. Therefore, it may be difficult to utilize weight loss as a measure of disease outcome under this circumstance.

Mice may have tolerated 24 hour time points much better.

Best,
Adam

From: Feng Wang (b)(6)
Sent: Monday, October 10, 2016 3:59 PM
To: Cockrell, Adam (b)(6) Jeff Pouliot (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6)

'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6)
Cc: Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study control

Hi Adam,

How is the first dose going? Just a reminder, please use fresh formulation and vehicle for each dose.

Thanks,
feng

Feng Wang
Investigator
Host Defense DPU
RD Infectious Disease R&D

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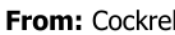

From: Cockrell, Adam (b)(6)
Sent: Thursday, October 06, 2016 12:01 PM
To: Feng Wang; Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study control

EXTERNAL

Yes 50ul/mouse intranasal. It is part of the protocol to collect weight information. I attached the agreed upon protocol/time line.

Adam

From: Feng Wang (b)(6)
Sent: Thursday, October 06, 2016 11:55 AM
To: Cockrell, Adam (b)(6) Jeff Pouliot (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6)
Cc: Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study control

Hi Adam,

Great! Let me know if you need anything else. You give 50uL intranasal dose per mouse, right? Is it possible to collect weight info?

Good luck with the study!
feng

Feng Wang
Investigator
Host Defense DPU
RD Infectious Disease R&D

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From: Cockrell, Adam (b)(6)
Sent: Thursday, October 06, 2016 11:50 AM
To: Feng Wang; Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study control

EXTERNAL

Hi Feng,

I received the drug/vehicle this morning.

Best,
Adam

From: Feng Wang (b)(6)
Sent: Wednesday, October 05, 2016 2:11 PM
To: Cockrell, Adam (b)(6) Jeff Pouliot (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6)
Cc: Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study control
Importance: High

Hi Adam,

Just an update that drugs and vehicles are to be shipped out today and they should arrive at UNC tomorrow morning. There are 7 vials of the drug solution labeled as GSKXXX and another 7 vials labeled as the blank vehicle. Since each vial has about 1.5mL solution, you would pull out one fresh vial of the drug and one fresh vial of the vehicle for each dose. If possible, please save the leftovers. Please refrigerate (i.e. 4°C) all vials upon arrival. At each dosing time, please take out vials, equilibrate them to the room temperature and mix them a little bit prior to the dosing. As we worry about the leakage and the extractable, we used HPLC (glass) vials for the formulation.
Let me know if you need additional information.

Thanks and good luck with the study!
feng

Feng Wang
Investigator
Host Defense DPU
RD Infectious Disease R&D

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Tel (b)(6)

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From: Cockrell, Adam (b)(6)
Sent: Tuesday, October 04, 2016 5:39 PM
To: Feng Wang; Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study control

EXTERNAL

Hi Feng,
The plan is to begin Monday.
Adam

Sent via the Samsung Galaxy S®6 active, an AT&T 4G LTE smartphone

-------- Original message --------

From: Feng Wang (b)(6)
Date: 10/4/2016 5:30 PM (GMT-05:00)
To: "Cockrell, Adam" (b)(6) Jeff Pouliot (b)(6) "Stemmy, Erik (NIH/NIAID) [E]" (b)(6) "'Leyva-Grado, Victor'" (b)(6) "'Umerah, Nina'" (b)(6) "Baric, Ralph S" (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6)
Cc: "Yount, Boyd L Jr" (b)(6)
Subject: RE: GSK A57 Study control

Hi Adam,

Just like to know when you are to give the first dose?

Thanks,
feng

Feng Wang
Investigator
Host Defense DPU
RD Infectious Disease R&D

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From: Cockrell, Adam (b)(6)
Sent: Tuesday, October 04, 2016 11:13 AM
To: Feng Wang; Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study control

EXTERNAL

Thanks Feng. I will hold on to it.

From: Feng Wang (b)(6)
Sent: Tuesday, October 04, 2016 11:11 AM
To: Cockrell, Adam (b)(6) Jeff Pouliot (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6)

Cc: Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study control

Hi Adam,

Would you please keep the powder and the vehicle for now? Feel free to dispose the suspensions.

Thanks,
feng

Feng Wang
Investigator
Host Defense DPU
RD Infectious Disease R&D

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Tel (b)(6)

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From: Cockrell, Adam (b)(6)
Sent: Tuesday, October 04, 2016 11:01 AM
To: Feng Wang; Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study control

EXTERNAL

Hi Feng,

I kept what remained of the previous lot of drug and vehicle. Do you mind if I discard the previous batch of drug and vehicle that you sent? At least, the vials that remain from the suspension trials.

Thanks,
Adam

From: Feng Wang (b)(6)
Sent: Monday, October 03, 2016 2:26 PM
To: Cockrell, Adam (b)(6) Jeff Pouliot (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6)

Cc: Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study control

Thanks Adam! As it stands now, it only needs refrigerated (i.e. 4°C). I will keep you updated with the shipment.

Best wishes,

Feng Wang
Investigator
Host Defense DPU
RD Infectious Disease R&D

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From: Cockrell, Adam (b)(6)
Sent: Monday, October 03, 2016 2:21 PM
To: Feng Wang; Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study control

EXTERNAL

Thanks Feng,

Just in case it was lost in the shuffle, the following is the information for delivery.

What temperature should the drug be stored at?

Adam Cockrell/Boyd Yount
University of North Carolina at Chapel Hill
Department of Epidemiology
135 Dauer Drive
Hooker Bldg./Room 3105
Chapel Hill, NC, 27599
Lab Phone: (b)(6)
Cell #: (b)(6)

Best,
Adam

From: Feng Wang (b)(6)
Sent: Monday, October 03, 2016 1:56 PM
To: Cockrell, Adam (b)(6) Jeff Pouliot (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6)
Cc: Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study control

Hi Adam,

Yes, we are on schedule to deliver the formulation to you by this Friday.

Thanks,
feng

Feng Wang
Investigator
Host Defense DPU
RD Infectious Disease R&D

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Tel

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<image001.png>

From: Cockrell, Adam (b)(6)
Sent: Monday, October 03, 2016 1:27 PM
To: Jeff Pouliot; Feng Wang; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study control

EXTERNAL

Hi Jeff,

Thanks for asking. I think for this experiment we should test for efficacy, and consider this possibility for future experiments.

Should I anticipate the drug to be delivered by this Friday?

Cheers,

Adam

From: Jeff Pouliot (b)(6)
Sent: Monday, October 03, 2016 11:29 AM
To: Cockrell, Adam (b)(6) Feng Wang (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6)
Cc: Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study control

Hi Adam,

Have you decided whether you'll be able to include our proposal to test satellite animals to ensure compound is on board during the study? If so, I can arrange for the sample shipping to GSK. If not we can reconsider while we plan the next round of experiments.

Best Regards,

Jeff

From: Jeff Pouliot
Sent: Thursday, September 08, 2016 3:48 PM
To: 'Cockrell, Adam'; Feng Wang; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study control

Hi Adam,

We were thinking of three mice to be dosed identically to those in the study. Dosing simultaneous to the infected animals won't be possible because it will be done under BSL2 conditions, but the compound dose and dosing methodology should be the same as what will be done with the infected animals.

The animals would be euthanized at T=15 minutes after dose, with blood samples and lungs to be frozen on dry ice and shipped to GSK. We can analyze them to determine amount of compound on board and can match those values to the efficacy.

Let me know if this is sufficient detail.

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Thursday, September 08, 2016 12:15 PM
To: Jeff Pouliot; Feng Wang; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study control

EXTERNAL

Hi Jeff,

When you have a chance can you please provide the exact details of what the controls might entail? Exact time point post-drug administration, exactly how to collect/prepare samples, and ship samples?

This will help provide a clearer picture for us of the extent of the work necessary for collecting/preparing these controls.

Best Regards,

Adam

From: Jeff Pouliot (b)(6)
Sent: Tuesday, September 06, 2016 10:46 AM
To: Cockrell, Adam (b)(6) Feng Wang (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6)
Cc: Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study control

Hi Adam,

It's great to hear the compound is en route. Have you had time to consider the inclusion of satellite uninfected animals in the study? We believe adding animals in parallel to test compound delivery at your site would be critical to interpretation if the efficacy is lower than we expect.

Best,

Jeff

From: Jeff Pouliot
Sent: Tuesday, August 30, 2016 12:08 PM
To: 'Cockrell, Adam'; Feng Wang; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: GSK A57 Study control

Hi Adam,

We would like to ask if a control can be added to this study. Would you be able to treat 2-3 satellite uninfected animals to test whether your dosing methodology is delivering the same amount of compound we've seen in our studies? This would entail treating uninfected mice, sacrificing them 5-15 minutes after dose and shipping blood samples and terminal lungs to GSK.

This control would provide information on compound delivery without the BSL-3 complications we discussed previously. Apologies for the late addition but this was a recent suggestion. Please let us know your thoughts.

Best Regards,

Jeff

Jeffrey Pouliot, Ph.D.
Investigator
Biology Host Defense DPU
R&D Infectious Disease

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Email (b)(6)
Tel (b)(6)

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From: Cockrell, Adam (b)(6)
Sent: Tuesday, August 30, 2016 10:41 AM
To: Feng Wang; Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study

EXTERNAL

Hi Feng,

I received the vehicle this morning. However, the address on the package had it shipped to a lab in a different building in the pharmacy department. Fortunately, they were able to find our number and let us know.

Also, I stored it at 4C, but it was shipped at ambient temperature.

I will test the formulation late next week when I return.

For shipping of the test compound please use the following address:

Boyd Yount/Adam Cockrell
UNC-CH
135 Dauer Drive
Hooker Bldg./Room 3105
Chapel Hill, NC
27599
Phone# (b)(6)

Best Regards,
Adam

From: Feng Wang (b)(6)
Sent: Tuesday, August 30, 2016 9:39 AM
To: Cockrell, Adam (b)(6) Jeff Pouliot (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6)
Cc: Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study

Hi Adam,

We shipped out study vehicle (i.e. 0.5%Tween80) yesterday and should arrive at your lab today. Please watch out and store it at 4-8°C. Due to some paper work delay, I do not think that the test compound will arrive before you leave for vacation. Is it possible that your coworker could do the formulation test in your absence? In addition, the test compound should also be stored at 4-8°C prior to use.

Thanks,
feng

Feng Wang
Investigator
Host Defense DPU
RD Infectious Disease R&D

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<image001.png>

From: Cockrell, Adam (b)(6)
Sent: Monday, August 29, 2016 9:25 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study

EXTERNAL

Hi Jeff,

Contact numbers are (b)(6) (Adam) and (b)(6) (Boyd)

Thanks,

Adam

From: Jeff Pouliot (b)(6)
Sent: Friday, August 26, 2016 4:09 PM
To: Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6) Feng Wang (b)(6)
Cc: Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study

Hi Adam

Thank you very much. Can you supply a contact phone number for shipping?

We will send the 0.5% Tween in saline with our compound. Everything should arrive by midweek.

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Friday, August 26, 2016 10:54 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang; Barb Carter

Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study

EXTERNAL

Hi Jeff,

Thanks for the update. I have addressed your questions below in red.

I will be out of town September 1st-september 7th, but Boyd Yount will be available to receive the package if I'm not here. Please advise on any special storage conditions.

Would it be possible for you ship a sample for early arrival next week, with all the components, so that I can test out the resuspension of the drug?

Also, I have attached a copy of the study as we discussed. As you suggested I eliminated the time point for drug delivery 6 hours prior to infection.

Best Regards,
Adam

From: Jeff Pouliot (b)(6)
Sent: Thursday, August 25, 2016 6:38 PM
To: Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6)
Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson
(b)(6) Feng Wang (b)(6) Barb Carter
(b)(6)
Subject: RE: GSK A57 Study

Dear Adam,

We'd like to update you on the status of the test compound shipping for the study and your formulation pre-work. We have the patent nearly completed and will be able to send the compound early next week, targeting shipping for Tuesday 8/30 with arrival by the end of the week. Please let us know if this does not agree with your planned work schedule. We also have a few shipping questions to be certain everything goes smoothly:

- Can you advise on the planned start date for the in vivo study? If you need compound on the morning of September 6 we will try to send it earlier in the week to reduce the chance of shipping delays. I have reserved time in our BSL3 facility to initiate the experiment on Monday September 12th. Therefore, we would need to have the compound by Friday September 9th.

- Will your shipping group be receiving packages next Thurs-Fri (Sep 1-2)? If I am not here when the package arrives Boyd Yount in the lab will be available to receive the package. Please advise on any special storage conditions. I have included Boyd on this email.

- Could you please confirm the shipping address we should use for the test compound? Adam Cockrell/Boyd Yount, UNC-CH, 135 Dauer Dr., Chapel Hill, NC, 27599

- Do you have 0.5% Tween-80 in saline available for the formulation or should we plan to ship some? It would be simpler if you had some on hand as it necessitates a second package, but we're happy to arrange it if you prefer. I would prefer that the GSK group provides everything relevant to the drug.

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Sunday, August 14, 2016 10:48 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Subject: RE: GSK A57 Study

EXTERNAL

Thanks Jeff,

Sounds great!

Adam

From: Jeff Pouliot (b)(6)
Sent: Saturday, August 13, 2016 5:27 PM
To: Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6) Feng Wang (b)(6)
Subject: RE: GSK A57 Study

Hi Adam,

We can send you a sample as soon as legal tells us the patent is filed. This should take roughly another week, so we should be able to get the sample to you by the end of two weeks. We will let you know if there are any unexpected delays.

Thanks for the info on dose groups. We can plan in more detail once the pilot run is complete.

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Saturday, August 13, 2016 7:43 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Subject: RE: GSK A57 Study

EXTERNAL

Thanks Jeff,

Would you guys mind sending me a sample of the drug (exactly how I will receive it for the mouse studies) in the next week, or two, so that I can validate the resuspension process in my hands?

If we see efficacy with the initial study, I believe 2-3 dose groups, with a 24 hour delivery window, would be feasible.

Thanks,
Adam

From: Jeff Pouliot (b)(6)
Sent: Thursday, August 11, 2016 3:45 PM
To: Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6) Feng Wang (b)(6)
Subject: RE: GSK A57 Study

Dear Adam,

You should be able to formulate the compound either way. It should easily go into solution in 3-5 min with a 37C water bath. Otherwise, you can vortex and leave it on a heated plate (low setting, warm) with stirring for a couple minutes.

We suggested a 24h dosing schedule for the first study, but your counterproposal of BID dosing to have the greatest chance of efficacy was a good one. A 12-hour doing schedule for the initial study is fine.

For the follow-up study we can modify dosing to qd from 6-hours post infection, presuming the initial results are robust. We can plan this in more detail once the initial test is complete. To help us think it through, though, can you let us know if it is technically feasible to run 2-3 dose groups in parallel?

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Wednesday, August 10, 2016 6:39 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Subject: RE: GSK A57 Study

EXTERNAL

Dear Jeff,

Please see responses to comments/questions below.

Regards,
Adam

From: Jeff Pouliot (b)(6)
Sent: Tuesday, August 09, 2016 5:51 PM
To: Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6) Feng Wang (b)(6)
Subject: RE: GSK A57 Study

Dear Adam,

Thanks for the note. Your research plan nicely reflects our discussion last week. We have some information below to fill in the details and a few questions for you.

- The predosing of compound is not needed as these are direct acting antivirals. In addition, only a suboptimal amount of compound would remain at the time of infection given the short T1/2 of this compound. A therapeutic model with the first dose following infection is our preferred choice. Is this acceptable? Starting with a therapeutic dose at 6 hours post-infection sounds great.

- BID dosing starting at 6 hours post infection seems the better plan. Do you know how long robust viral replication continues in an untreated test subject? Our model exhibits robust replication through day 6 post-infection with peak replication at days 2-3.
- We recommend intranasal dosing at 1 mg/kg, 50 uL volume per mouse, at a concentration of 0.5 mg/mL. This should deliver a compound concentration at Tmax of 100x EC50 to the lung. IN sounds good.
- We will plan to ship you the compound as dry powder. We're exploring stability but until we have firm data we can't guarantee that a solution prepared here would be stable long enough for the experiment. You will need to suspend by brief sonication in a dosing solution of 0.5% Tween-80 in saline. Is this acceptable? This is acceptable, however can you please define sonication? Is a water sonicator necessary for this? Or, will vortexing suffice? Does this compound readily go into solution? The 12 hour dosing schedule is quite rigorous, especially in a BSL3, therefore I am trying to get an understanding of how much additional time I will have to spend suspending the drug prior to each 12 hour administration.

We would like also to think ahead to the second round of the experiment. Presuming the outcome shows positive results, we propose a similar experiment at successive 3-fold lower drug concentrations to clarify the PK/PD relationship. If the follow up allows more than one dose group, we would dose at 0.3 mg/kg and 0.1 mg/kg (30x and 10x EC50). Does this sound reasonable to you? A dosing experiment sounds reasonable. Provided the initial study is successful, In follow-up experiments we discussed moving to a 6-7 day time course. In doing this I will have to move to delivering the drug every 24 hours. Is this reasonable to you? Would you prefer that the initial study use a 24 hour repeated dosing time course? The 24 hour time course would begin after the initial delivery of the drug at 6 hours post-infection.

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Wednesday, August 03, 2016 5:20 PM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Subject: RE: GSK A57 Study

EXTERNAL

Hi everyone. It was good to meet everyone in the gsk group.

In putting together the time line (attached to email) I had some additional thoughts.

1) There are two slides. The first is the initial time line that we discussed on the phone. The second slide takes into account the fact that the half-life of drug is really short, therefore we can adjust the drug delivery time line to bracket the initial viral delivery to be -6 hours and +6 hours if you guys would prefer. This would shorten the study on the back end by 6 hours, which should be of no consequence regarding the data we will capture.

2) This is just a thought, and not sure if this is a viable possibility given the half-life of the drug, but we could eliminate any confounding issues with repeated anesthetic administration if there was an option to deliver drug by the IP route. Thoughts?

That said I look forward to working with everyone.

Best Regards,
Adam

From: Jeff Pouliot (b)(6)
Sent: Wednesday, August 03, 2016 2:13 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6) Cockrell, Adam (b)(6) Feng Wang (b)(6)
Subject: RE: GSK A57 Study

Thank you all for the productive discussion. We look forward to working together.

I've added one person to the email list above. Please include Feng Wang on the experimental planning communications.

Best,

Jeff

Jeffrey Pouliot, Ph.D.
Investigator
Biology Host Defense DPU
R&D Infectious Disease

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(6)
Tel (b)(6)

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, August 03, 2016 1:59 PM
To: 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph; Deborah Butler; Neil Pearson; Jeff Pouliot;

'Cockrell, Adam'
Subject: GSK A57 Study

EXTERNAL

Hi Everyone,
Thanks for your time today, it was a productive call. Please feel free to email directly to work out the protocol/dosing and other study details, but be sure to CC everyone on this message.

Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email: (b)(6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.
****

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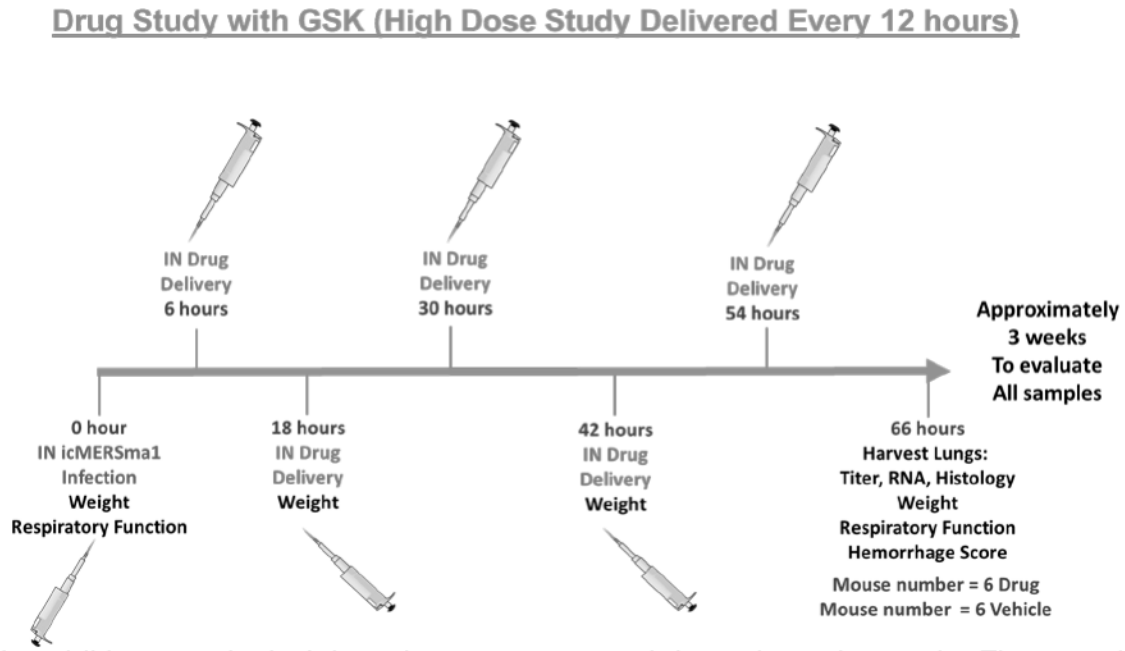
Utilizing the MERS 288-330 Mouse Model to test GSKXXX anti-MERS therapeutic. Therapeutic testing of this drug is through an agreement with Glaxo-Smith-Kline.

Please see previous reports for a description of the mouse model and viruses. 50µl of the GSKXXX anti-MERS therapeutic, or vehicle, was initially delivered intranasal at 6 hours post-infection with $5x10^6$ PFU of icMERSma1. Drug was delivered every 12 hours thereafter until 50 hours post-infection. The study was terminated early due to a clear increase in the mortality and morbidity of the mice within the first 48 hours. In previous studies mortality typically started at 4-5 days post-infection. The original study outline is depicted in figure 1. All mice were weighed daily and surviving mice were assessed for hemorrhage and viral lung titer at 50 hours post-infection.

Figure 1. GSK therapeutic study outline.



In addition, survival of the mice was assessed throughout the study. Figure 2 demonstrates that there may be a slight increase in survival with the GSKXXX treated mice (80%) compared to the vehicle treated mice (50%). In addition to the 12 mice included in the initial study, 2 mice (from another study executed concurrently) were included as no drug/vehicle treated controls. Figure 3, however, indicates that appears to be more dramatic for drug/vehicle treated animals compared to mice that did not receive drug. Thus, the GSKXXX drug did not appear to protect from weight loss since both GSKXXX and vehicle exhibited similar weight loss (figure 3). Lung hemorrhaging is scored from 0 to 4 with 4 being most severe. Figure 4 demonstrates that there was little hemorrhaging in the lungs of all surviving mice by 50 hours post-infection, and that there is no significant difference between GSKXXX and vehicle treated groups. In figure 5 lungs of surviving mice were assayed for replicating virus by plaque assay. In both the GSKXXX and vehicle treated mice there was a clear enhancement in the amount of productive MERS-CoV replicating in the lungs of these animals compared to those that were not treated with drug. The titers for GSKXXX and vehicle were higher than the range that we routinely test for viral replication in the lungs. Based on what was observed at the highest dilution by plaque assay the titers for GSKXXX and

vehicle would be, at minimum, >10^{10} PFU/ml/gram lung tissue. To date, titers this high have not been observed with this model. This would seem to indicate that the vehicle (containing 0.5% tween 80, a nonionic detergent) may be augmenting virus infection, replication and/or spread, especially since the drug and vehicle are being administered by the intranasal route, which would provide direct access to the lungs, and possibly sites of replication. Nonetheless, at this time it also cannot be ruled out that repeated ketamine/xylazine anesthetic administered at the time of GSKXXX/vehicle dosing may also influence the increase in titer, although we have not seen this phenotype in other C57BL/6 SARS infected mice. In line with the enhanced titer effect the GSKXXX therapeutic does not appear to inhibit viral replication in the lungs of this mouse model.

Figure 2. Increased survival was observed with GSKXXX (80%) compared to vehicle (50%).

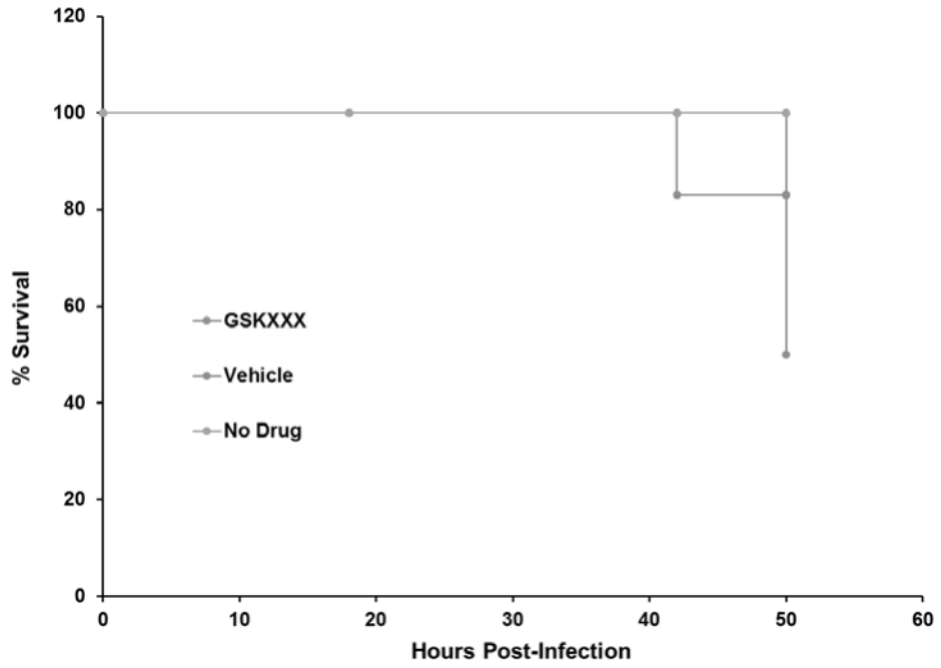


Figure 3. Mice treated with GSKXXX and vehicle exhibit similar weight loss.

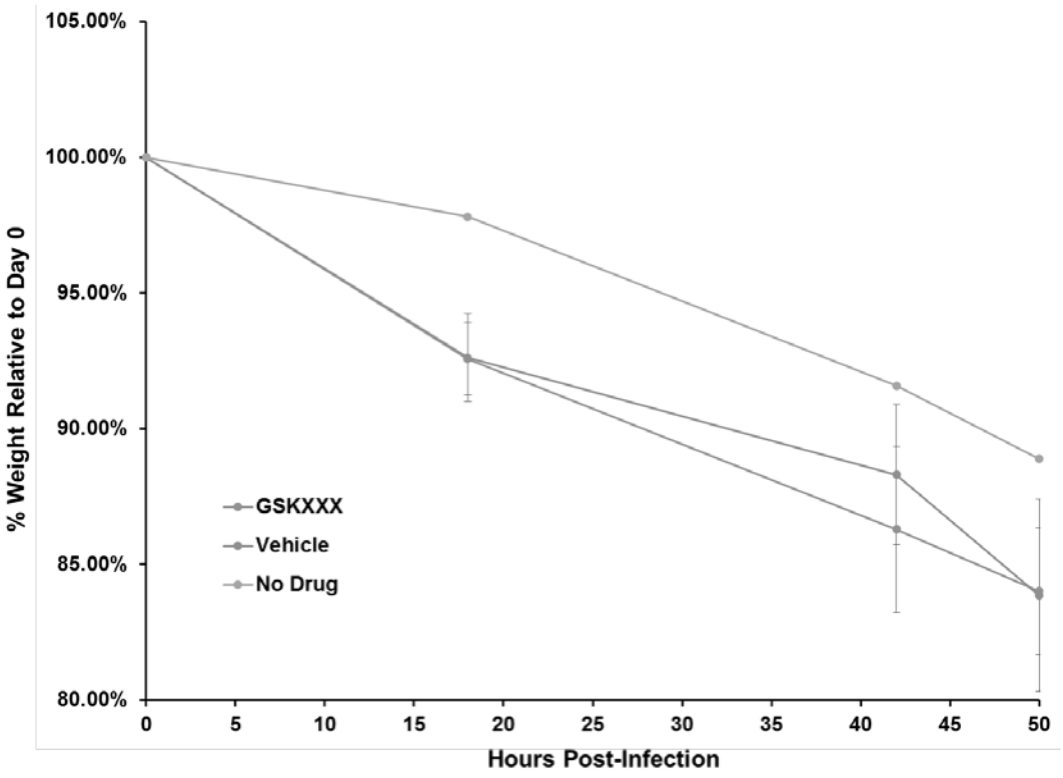


Figure 4. All mice exhibit similar levels of hemorrhaging.

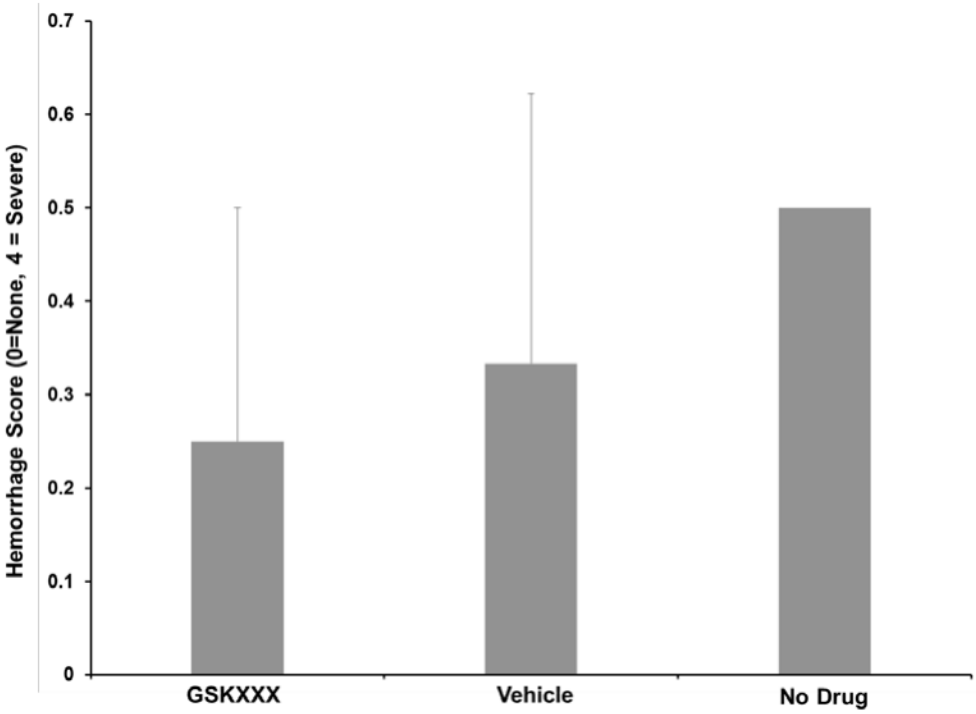
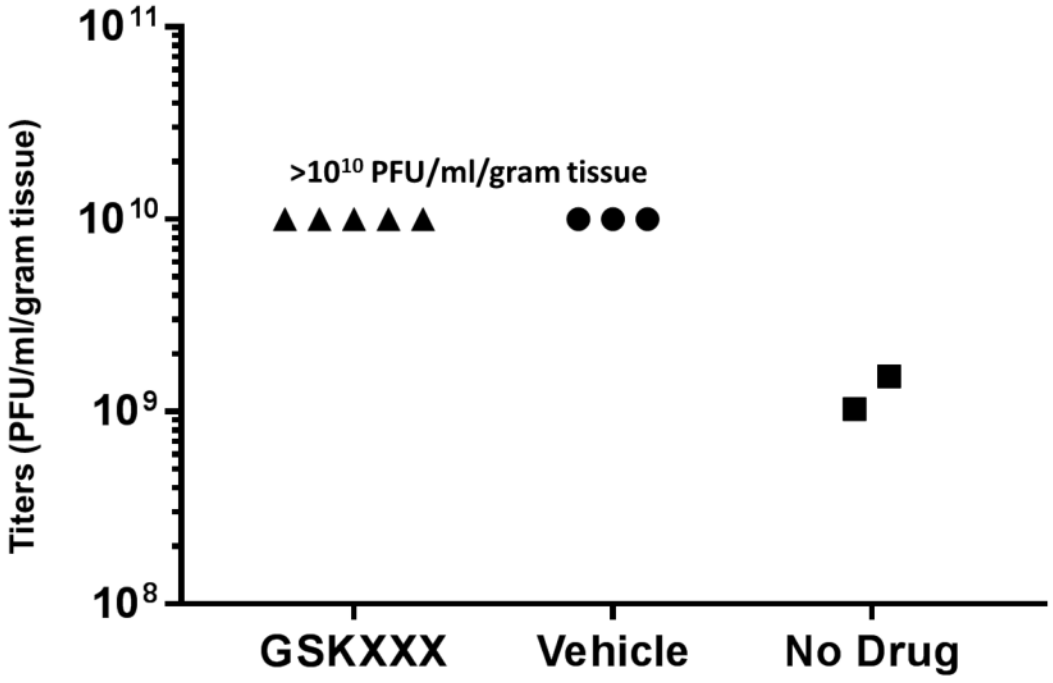


Figure 5. MERS-CoV lung titers were increased for both GSKXXX and vehicle treated mice compared to mice without drug/vehicle treatment.



From: Cockrell, Adam
Sent: Tue, 11 Apr 2017 12:28:57 +0000
To: Stemmy, Erik (NIH/NIAID) [E]; Leyva-Grado, Victor
Cc: Baric, Ralph; Sims, Amy C
Subject: Report describing planet biotech study and novel mouse-adapted MERS clones
Attachments: AMC report for April 2017.pdf

Hi Erik and Victor,

The report for the study with planet biotech is attached. In addition to information for the planet biotech study I have included novel data for new mouse-adapted MERS viruses (figures 7-10) that overcome a previous limitation of the model (i.e. severe pathology that required high dose virus at 5x10^6 PFU). We can reduce the infectious dose by 100-fold with our novel mouse-adapted MERS.

Best Regards,
Adam

Adam Cockrell
Research Associate
Department of Epidemiology
University of North Carolina at Chapel Hill
Chapel Hill, NC, 27599
Lab Phone: (b)(6)
Office Phone: (b)(6)

MONTHLY REPORT

Contract HHSN272201000019I Task Order HHSN27200003 A57

Mouse Model for Evaluation of Medical Countermeasures Against Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

Period of Performance:
March 1, 2017- March 30, 2017

Contractor's Name and Address:
Dr. Peter Palese
Horace W. Goldsmith Professor and Chair Department of Microbiology
Professor, Department of Medicine Mount Sinai School of Medicine
1 Gustave Levy Pl.
New York, New York 10029-6574
Tel (b)(6)
Fax 212-722-3634
e-mail:(b)(6)

Date of Submission:
April 11, 2017

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(b)(4)

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of the Freedom of Information and Privacy Act

From: Aleksei Chmura
Sent: Wed, 13 May 2015 20:36:11 +0000
To: Pone, Laura (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Grant Number: 5R01AI110964 - 02 PI Name: DASZAK, PETER
Attachments: Approval Letter-EcoHealth-11-14-14.pdf, ATT00001.htm
Importance: High

Dear Laura,

Apologies for any delays or confusion on my part, but I am not certain what summary statement concerns you are requesting. We provided details about protection of human subjects via our Just in Time Report in May of last year and sent our US IRB approval for our human research protocol under our award last month (attached here for reference). We expect to have an FWA for Wuhan University before the end of the month, but I will update you on our progress in the next week.

Can we have a quick chat about the summary statement concerns anytime that is good for you. Once we are clear on what is required, we will provide the requested details immediately.

Is the deleted reference with updated My NCBI report for Dr. Daszak ok as well? I have not yet had a response from the NCBI support re. removing and/or disassociating the reference.

Please call me anytime day/night at (b)(6)

Many thanks!

Aleksei Chmura
Senior Coordinator of Operations

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
(b)(6) (mobile)
(b)(6) (Skype)

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On May 13, 2015, at 11:39, Pone, Laura (NIH/NIAID) [E] (b)(6) wrote:

Hi Aleksei,

I am writing to follow up on the email below and information due 5/11.

Please submit your response to summary statement concerns right away so we can issue the award and remove the human subject restriction.

Thank you,

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
5601 Fishers Lane, Room 4E29, MSC 9824
Bethesda, MD 20892-9824
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)
please consider the environment before printing this e-mail.

From: Pone, Laura (NIH/NIAID) [E]
Sent: Friday, May 08, 2015 4:30 PM
To: (b)(6) Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
Subject: Grant Number: 5R01AI110964 - 02 PI Name: DASZAK, PETER
Importance: High

Hi Aleksei,

Please provide a response to the summary statement concern from the competing application regarding Protection of human subjects. A response by **Monday, May 11th** is appreciated.

Thank you,

Laura Pone
Grants Management Specialist

DHHS/NIH/NIAID/GMP
5601 Fishers Lane, Room 4E29, MSC 9824
Bethesda, MD 20892-9824
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

hummingbirdIRB

November 17, 2014

Peter Daszak Ph.D.
EcoHealth Alliance
460 West 34th St., 17th Floor
New York, NY 10001-2320

Protocol Title: Understanding the Risk of Bat Coronavirus Emergence

Hummingbird IRB #: 2014-23

Grant Number: 1R01AI110964-01

Sponsor: EcoHealth Alliance

Approval Period: November 14, 2014 – November 13, 2015

Dear Dr. Daszak:

At the convened board meeting of November 14, 2014, Hummingbird IRB approved the above referenced study for one year.

The following document was approved:

Protocol Date: May 27, 2014

We wish to acknowledge the approval from Wuhan University's IRB which approved the portion of the study for which there was human subject intervention. Hummingbird IRB's approval extends only to the data analysis which will take place for anonymized data transferred to Dr. Daszak.

Any changes made to the protocol must be submitted to the Hummingbird IRB. Approval from Hummingbird IRB must be secured prior to initiation of the revision(s). You will receive a reminder to renew approval of the study approximately 3 months prior to the end of the approval period.

Attached, you will find a summary of investigator commitments with which the Board requires each investigator to adhere to during the approval period.

Sincerely,

(b)(6)

Isaac M. Colbert, Ph.D.
Chairman, Hummingbird IRB

Attachment
cc: Maureen Miller, EcoHealth Alliance
Hummingbird IRB File

Investigator Commitments

All Hummingbird IRB (HIRB) approved investigators are required to fulfill these commitments.

In granting approval to the investigator for the conduct of an investigational study, Hummingbird IRB requires the investigator to understand and agree to these commitments:

1. The investigator will conduct the study(ies) in accordance with the relevant, current protocol(s) and will only make changes in a protocol when necessary to protect the safety, rights, or welfare of subjects.

2. The investigator will personally conduct or supervise the described investigation(s).

3. The investigator will delegate tasks to only trained, experienced and appropriately credentialed individuals who are familiar with the protocol and understand the tasks required to conduct the study and protect human subjects during screening and while enrolled.

4. The investigator is obligated to inform Hummingbird IRB of any financial conflicts of interest which may exist through submitting appropriate forms on an annual basis. Should a conflict arise during the course of the study, this conflict will be promptly reported to the IRB.

5. The investigator will inform any patients involved in a study involving drugs, devices or biologics, or any persons used as controls, that the drugs, devices or biologics are being used for investigational purposes and will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.

6. The investigator will report to the sponsor and Hummingbird IRB (when applicable) adverse and unanticipated problems that occur in the course of the investigation(s). If after the study has concluded, new information is made available that is relevant to ongoing health or safety, the investigator will inform subjects of these results.

7. When applicable, the investigator will read and understand the information in the investigator's brochure, device manual and other scientific background that describes the potential risks and side effects of the drug, procedure or device.

8. The investigator will ensure that all associates, colleagues, and employees assisting in the conduct of the study(ies) are informed about their obligations in meeting the commitments outlined in this document.

9. The investigator will maintain adequate and accurate records and make those records available for inspection.

10. The investigator will promptly report Hummingbird IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, the investigator will not make any changes in the research without Hummingbird IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.
11. The investigator will have in place at his or her site, a process by which the HIRB approved consent form is compared to the executed contract to ensure that consistency exists between documents in terms of procedures, study visits, payment to subjects and compensation for injury as well as other conditions effecting human subjects. The investigator and sponsor will resolve any difference and notify HIRB of any changes impacting the consent.

12. The investigator will provide referrals to any subject for whom a condition or potentially adverse information is uncovered during the study. This may include, for example, learning of suicidality or a previously unknown disease. This does not pertain to results of genetic testing unless sharing this information is part of the protocol.

From: Cockrell, Adam
Sent: Tue, 15 Aug 2017 20:59:40 +0000
To: Matthias Schnell
Cc: Baric, Ralph; Johnson, Reed (NIH/NIAID) [E]; Frieman, Matthew (b)(6) Stemmy, Erik (NIH/NIAID) [E]; Beall, Anne Elizabeth
Subject: RE: Update on Rabies Vaccine Study
Attachments: Summary of Data.pdf

Everyone,

This is the same update as last week with titers included.

Adam

From: Matthias Schnell (b)(6)
Sent: Wednesday, August 09, 2017 11:42 AM
To: Cockrell, Adam (b)(6)
Cc: Matthias Schnell (b)(6) Baric, Ralph S (b)(6)
Johnson, Reed (NIH/NIAID) [E] (b)(6) Frieman, Matthew
(b)(6) Erik [E] Stemmy
(b)(6) Beall, Anne Elizabeth (b)(6)
Subject: Re: Update on Rabies Vaccine Study

Adam:
thanks a lot for all the work. Yes looks very nice.
Thanks again
Matthias
On Aug 9, 2017, at 11:23, Cockrell, Adam (b)(6) wrote:

Hi all,

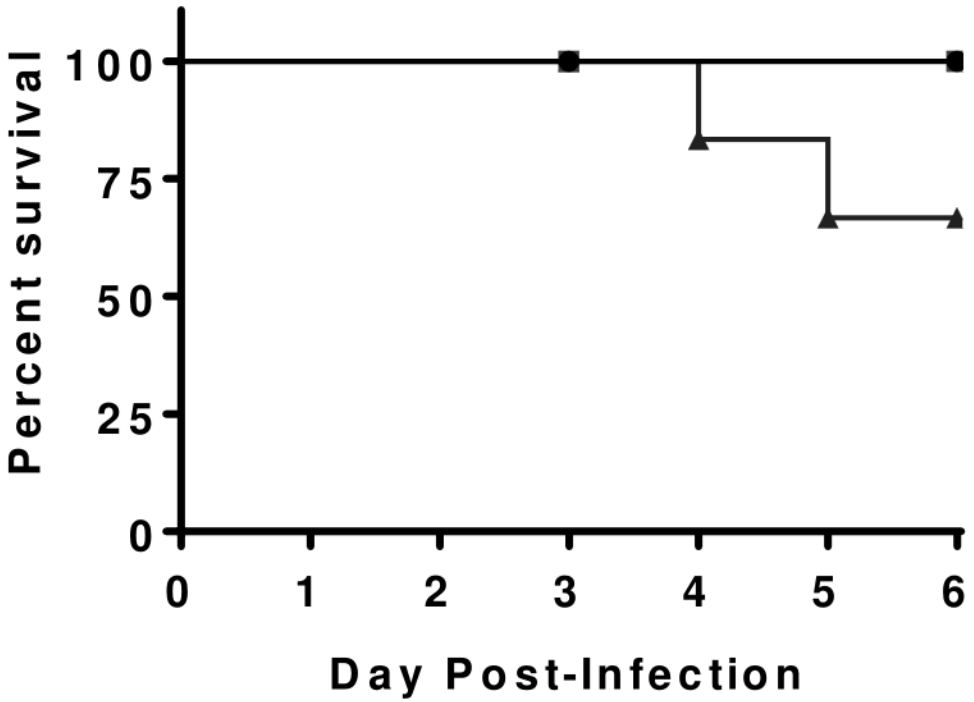
Please find an update of the data attached for the MERS Rabies vaccine in the our 288-330+/+ mouse model. I think it looks really nice!

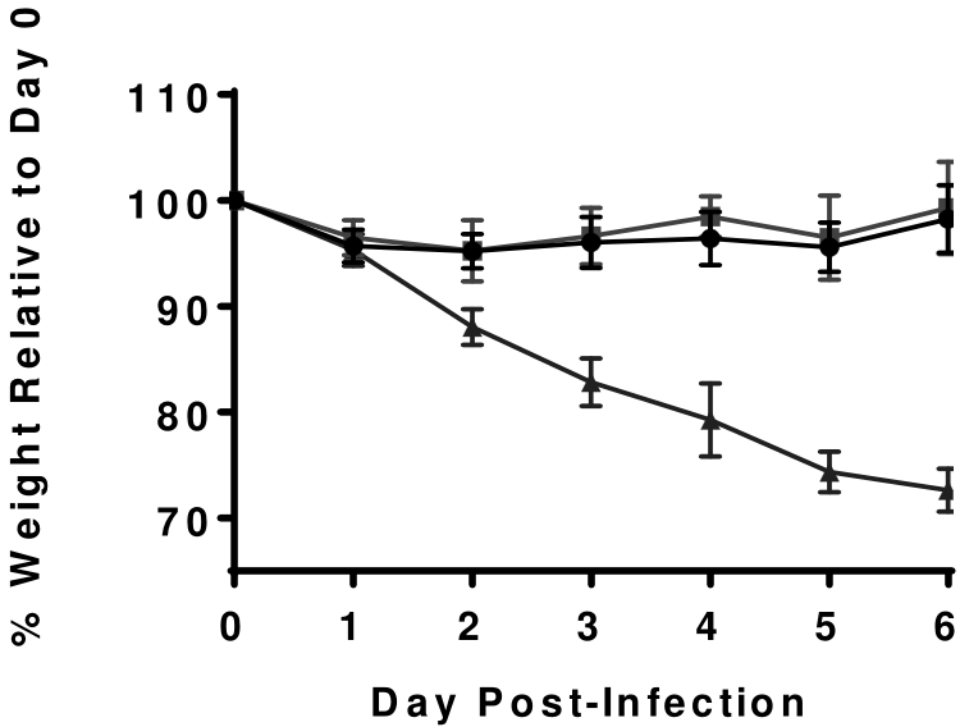
I am in the process of titering viruses (should have sometime next week), and will submit tissue for histology next week. This will probably take a few weeks for processing and analysis.

For the respiratory function data. Penh is a unit less measure that reflects airway obstruction/restriction due to debris in the airway. On day 0 and day 5 there was an issue with one of the Buxco chambers so we do not have a single data point for one mouse in the MERS-Rabies Vaccine Prime/28 cohort on day 0 and one mouse from the Rabies Ctrl. cohort on day 5.

Best,
Adam
<Summary of Data.pdf>

Both Vaccine Regimens Protect from Death and Weight Loss





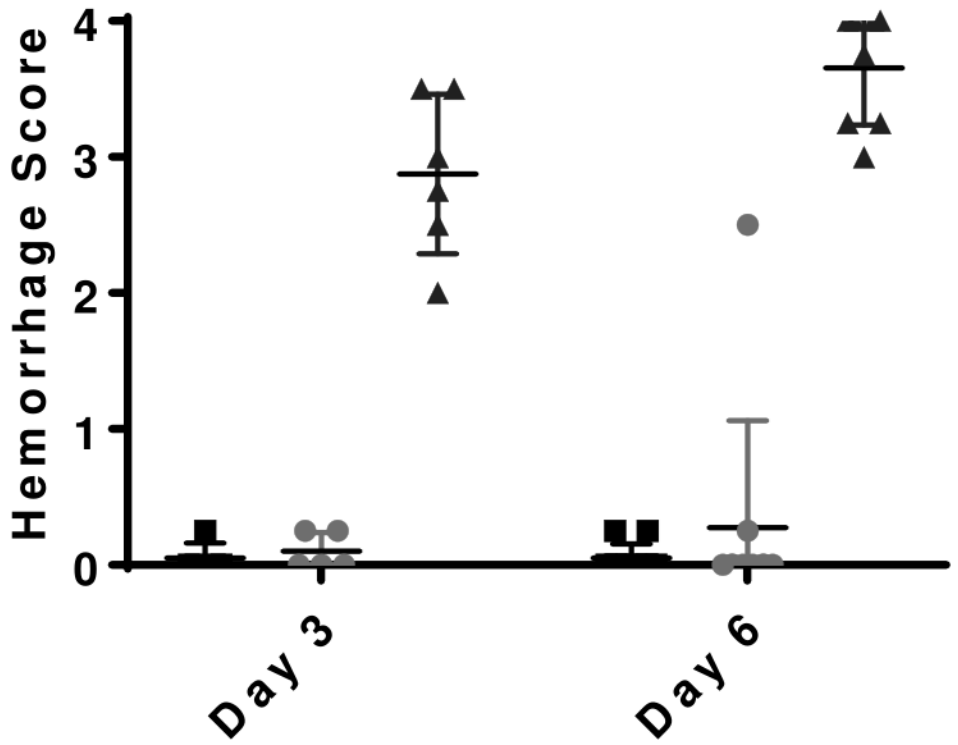
MERS-Rabies Vaccine Prime/7/28

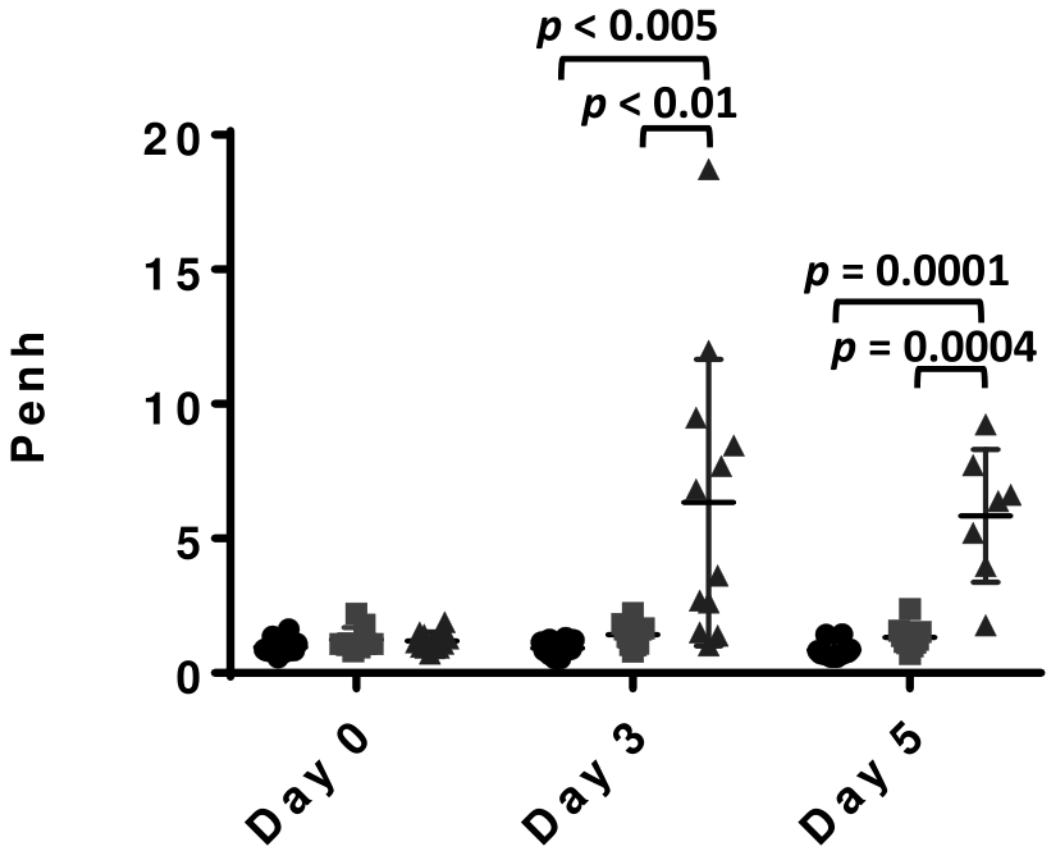
MERS-Rabies Vaccine Prime/28

Rabies Ctrl. Vaccine

Error Bars Represent 1 SD

Both Vaccine Regimens Protect from Hemorrhaging and Loss of Respiratory Function

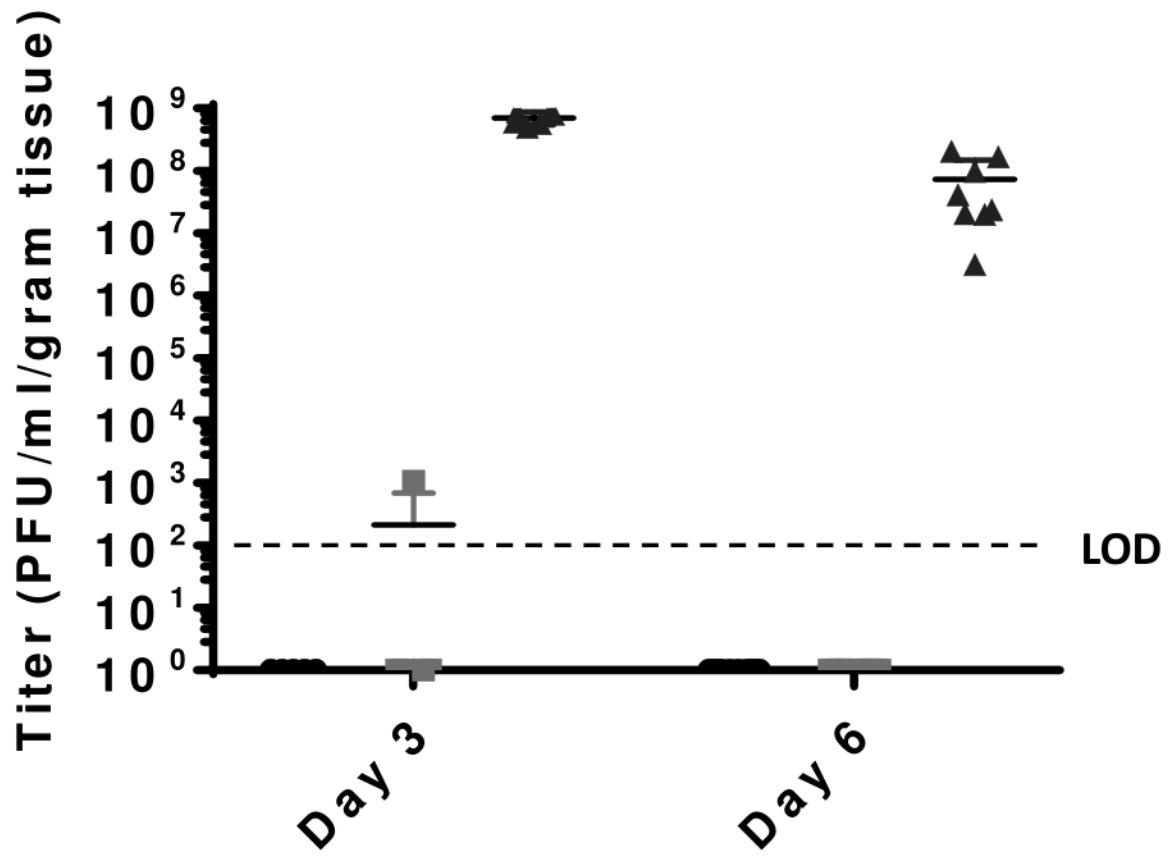




- MERS-Rabies Vaccine Prime/7/28
- MERS-Rabies Vaccine Prime/28
- Rabies Ctrl. Vaccine

Error Bars Represent 1 SD
Student T test used for comparison

Both Vaccine Regimens Prevent Viral Replication in the Lungs



Except for 1 mouse in red group
At day 3 (near limit of detection)
All vaccinated animals were free
Of virus by plaque assay.

- MERS-Rabies Vaccine Prime/7/28
- MERS-Rabies Vaccine Prime/28
- Rabies Ctrl. Vaccine

From: (b)(6)
Sent: Mon, 14 Dec 2015 11:45:08 +0100
To: Cockrell, Adam
Cc: (b)(6) Baric, Ralph; Maria Zambon; Baric, Toni C; Stemmy, Erik (NIH/NIAID) [E];(b)(6)
Subject: Re: UPDATE MERS MABS
Attachments: 151214_Cockrell UNC_letter.pdf

Dear Adam,

The antibodies were shipped today with Fedex, we expect them to arrive on December 16.

Here is the tracking nr. 775167325653

Please find attached the description of the materials sent

Best Regards

(b)(6)

On 04.12.2015 21:18, Cockrell, Adam wrote:
Dear (b)(6)

Just to be on the safe side it might be best to wait to ship the following Monday (12-14-15). That will give a week in case of unforeseen events.

Regards,

Adam

From: (b)(6)
Sent: Friday, December 04, 2015 3:14 PM
To: Cockrell, Adam (b)(6)
Cc: Baric, Ralph S (b)(6) Maria Zambon (b)(6) Baric, Toni C (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) (b)(6)
Subject: Re: UPDATE MERS MABS

Dear Adam,
Here Tuesday will be public Holiday and it will be therefore better ship on Wednesday, or alternatively organize the shipment for the following week on Monday.
What do you prefer?

Best regards,

(b)(6)

(b)(6)
Humabs Biomed SA
Via Mirasole 1
CH-6500 Bellinzona
Switzerland
Tel: (b)(6)
E-mail: (b)(6)
http://www.humabs.com

Il giorno 03 dic 2015, alle ore 23:00, Cockrell, Adam (b)(6) ha scritto:

Dear (b)(6)

That sounds good. So the shipping goes smoothly, like the last shipment, could you ship on a Monday/Tuesday, and give me a notification of shipment so I can anticipate the date of receipt?

Once I have the data compiled I will definitely share it. I am leaving for the holidays on the 23rd and will not return until January 3rd. Provided all goes well I anticipate the initial experiment to be completed the 15th. I should be able to put together the weight loss and survival data.

Yes. I plan to use a mix of females/males that are 288-330 +/+ homozygous mice. We do not currently have the numbers to include the 288-330 +/- hets.

Cheers,

Adam

From: (b)(6)
Sent: Thursday, December 03, 2015 9:23 AM
To: Cockrell, Adam (b)(6)
Cc: Baric, Ralph S (b)(6) Maria Zambon (b)(6) Baric, Toni C (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) (b)(6)
Subject: Re: UPDATE MERS MABS

Dear Adam,
thanks for the clarifications.
We will aim to ship more material next week.
Would it be possible to have interim updates about the body weight loss and survival as soon as data will be available?

Another question, will you use 288-330 +/+ homozygous mice only?

Best regards,

(b)(6)

Il giorno 02 dic 2015, alle ore 12:41, Cockrell, Adam (b)(6) ha scritto:

Dear (b)(6)

Thanks. It would be helpful to have all of the antibody on hand. The previous shipping arrangement worked well.

Regarding the timeline. I would anticipate the first results early-mid January (experiment 1). Provided our University EHS approvals have been received, and taking into account the holidays, I should initiate the second experiment by mid-January, and accumulate the necessary data to move forward on the third experiment by the end of January-beginning of February. Provided all goes well this would put us in mid-late February, possibly beginning of March to complete assays and assemble all the data.

Cheers,

Adam

From: (b)(6)
Sent: Tuesday, December 01, 2015 5:17 PM
To: Cockrell, Adam (b)(6)
Cc: Baric, Ralph S (b)(6) Maria Zambon (b)(6) Baric, Toni C (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) (b)(6)
Subject: Re: UPDATE MERS MABS

Dear Adam,
Thanks for the slides! the description of the advantages of your model is indeed very helpful. Could you clarify what could be the overall timeline for the completion of the three experiments if performed stepwise?
Since you are using 12 mice/group (6 used for the day 3 analysis and 6 for the day 6 readouts) you should need a little bit more than 20 mg. We can plan a shipment of more LCA60 and a matched IgG1 Ab (MPE8) in a week or two.

Best regards,

(b)(6)

(b)(6)

Humabs Biomed SA
Via Mirasole 1
CH-6500 Bellinzona
Switzerland

Tel: (b)(6)
E-mail: (b)(6)
http://www.humabs.com

Il giorno 01 dic 2015, alle ore 22:29, Cockrell, Adam (b)(6) ha scritto:

Hi everyone.

Here are the slides that we discussed on yesterday's phone call. The first couple describe the advantages of our model over existing mouse models and why it is the first model to recapitulate the severe respiratory disease that believe is occurring in humans.

The last 3 slides demonstrate the timeline for the study.

Please let me know if there are any comments/questions regarding the studies.

Regards,

Adam

From: Baric, Ralph S
Sent: Monday, November 30, 2015 9:06 AM
To: Maria Zambon (b)(6) Baric, Toni C (b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Cockrell, Adam (b)(6)
Subject: RE: UPDATE MERS MABS

From: Maria Zambon (b)(6)
Sent: Sunday, November 29, 2015 7:31 AM
To: (b)(6) Baric, Toni C
Cc: Stemmy, Erik (NIH/NIAID) [E]; Cockrell, Adam; Baric, Ralph S
Subject: RE: UPDATE MERS MABS

Colleagues,

Is there material in regards the mouse model set up by ralph Baric to be shared before this meeting tomorrow (as suggested in some of the earlier correspondance). I will be doing the phone call externally, so would appreciate an early view of any data, as I am not sure whether I will have good email access during the day tomorrow

thanks

Maria Zambon
Director, Reference Microbiology

Deputy Director, NIS
National Infection Service
Public Health England
(b)(6)
Tel: (b)(6)
www.gov.uk/phe Follow us on Twitter@PHE.uk
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From: (b)(6)
Sent: 10 November 2015 17:32
To: Baric, Toni C
Cc: Stemmy, Erik (NIH/NIAID) [E]; Maria Zambon; Cockrell, Adam; Ralph Baric; Robin Gopal
Subject: Re: UPDATE MERS MABS

Dear Toni,

This is fine. I can provide the call-in number.

Here it is:

UK: 0808 234 88 76
Switzerland: 0800 329 329
USA: +1 866 591 43 61 (or +1 888 50 333 35)

Participant access code: (b)(6)

Best regards,

(b)(6)

Il giorno 10 nov 2015, alle ore 17:52, Baric, Toni C (b)(6) ha scritto:

Hi Everyone,
Let's set this call for Nov 30 at 9 am EST/ 2pm UK time. Does this work? Also, does someone have a call-in number or should I set this up?
Thank you,
Toni

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Thursday, November 05, 2015 9:30 AM
To: Baric, Toni C; Maria Zambon; Cockrell, Adam; (b)(6)
Cc: Baric, Ralph S; Robin Gopal
Subject: RE: UPDATE MERS MABS

My preference would be for 11/30. I can do any time before 1pm EST.

Erik

From: Baric, Toni C (b)(6)
Sent: Thursday, November 05, 2015 9:26 AM
To: Maria Zambon (b)(6) Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Baric, Ralph (b)(6) Robin Gopal (b)(6)
Subject: RE: UPDATE MERS MABS

Hi Group,

Let's we revisit the following dates:
11/30 9-10 am EST or after 10 am EST
12/2 before 2 pm EST.

Please let me know the day and time range that works for all of you, keeping in mind that Maria will be calling in from UK.
Thanks
Toni

From: Maria Zambon (b)(6)
Sent: Wednesday, November 04, 2015 5:49 PM
To: Cockrell, Adam; Baric, Toni C; Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
Cc: Baric, Ralph S; Robin Gopal
Subject: RE: UPDATE MERS MABS

Hello,

Sorry if late to the party. I have already sent back a note saying the Monday of this week would work for me. Unfortunately I will be in Hng Kong the 17th to 20th, so would suggest we try earlier if we can

maria

Maria Zambon
Director, Reference Microbiology
Deputy Director, NIS
National Infection Service
Public Health England
(b)(6)
Tel: (b)(6)
www.gov.uk/phe Follow us on Twitter@PHE.uk
Protecting and improving the nation's health

From: Cockrell, Adam (b)(6)
Sent: 04 November 2015 20:21
To: Baric, Toni C; Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
Cc: Baric, Ralph S; Maria Zambon; Robin Gopal
Subject: RE: UPDATE MERS MABS

That sounds good for me.
Thanks,
Adam

From: Baric, Toni C
Sent: Wednesday, November 04, 2015 3:11 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Baric, Ralph S (b)(6) Maria Zambon (b)(6) Cockrell, Adam (b)(6) Robin Gopal (b)(6)
Subject: RE: UPDATE MERS MABS

11/20 sounds good. How about 10 am? If this works for everyone, please let me know. Otherwise, please suggest a time before 1 pm that suits or a different day.
Thank you,
Toni

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, November 04, 2015 3:07 PM
To: (b)(6) Baric, Toni C
Cc: Baric, Ralph S; Maria Zambon; Cockrell, Adam; Robin Gopal
Subject: RE: UPDATE MERS MABS

On 11/20 I can do any time before 1pm EST. Can we aim for that date?

Erik

From: (b)(6)
Sent: Wednesday, November 4, 2015 3:02 PM
To: Baric, Toni C (b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Baric, Ralph (b)(6) Maria Zambon (b)(6) Cockrell, Adam (b)(6) Robin Gopal (b)(6)
Subject: Re: UPDATE MERS MABS

Dear Toni,

I am available on all dates with the exception of 12/4.

Best regards,

(b)(6)

Il giorno 04 nov 2015, alle ore 20:25, Baric, Toni C (b)(6) ha scritto:

How about the following:

Friday 11/20 Ralph is open all day. Then the next day is 11/30 –after 11, Wednesday 12/2 before 2 and all day on 12/4.

Best regards,
Toni

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, November 04, 2015 2:13 PM
To: Baric, Toni C; Baric, Ralph S; Maria Zambon; Cockrell, Adam
Cc: (b)(6) Robin Gopal
Subject: RE: UPDATE MERS MABS

I am leaving for a meeting in Riyadh on 11/12, so we'll have to schedule a call after I return on 11/16.

Erik

From: Baric, Toni C (b)(6)
Sent: Wednesday, November 4, 2015 12:06 PM
To: Baric, Ralph (b)(6) Maria Zambon (b)(6) Cockrell, Adam (b)(6)
Cc: (b)(6) Robin Gopal (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Subject: RE: UPDATE MERS MABS

Hi Maria,
Ralph is available on 11/12 after 3:30 and between 11:30-3 on Friday 11/13
Best regards,
Toni

From: Baric, Ralph S
Sent: Tuesday, November 03, 2015 4:11 PM
To: Maria Zambon; Cockrell, Adam; Baric, Toni C
Cc: (b)(6) Robin Gopal; Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: UPDATE MERS MABS

Hi Maria, We have recently received 20mg of pure antibody from (b)(6) and have support by Erik Stemmy to perform your studies in the mouse model. Initially, we will evaluate protection prior to

infection. We currently don't have approval to use sharps for therapeutic intervention postinfection, but are in the process of putting in the paperwork to administer drug postinfection. I recommend a two phase study, first prior to infection to demonstrate efficacy and then drug dose at day 1 or 2 postinfection (single dose?). We likely need to set up a time to discuss the experiments. We will also share the model details at that time. Toni can assist. We also are planning on doing the protection study in early December, post infection study would likely be jan at best. Adam is a key contact person for discussion. Hope you are doing well. It's a pleasure to work with you again. Thoughts? ralph

---

From: Maria Zambon (b)(6)
Sent: Friday, October 23, 2015 3:52 PM
To: Baric, Ralph S
Cc: (b)(6) Robin Gopal
Subject: UPDATE MERS MABS

Dear Ralph,

Greetings , we have not corresponded for a while…I think another pesky virus (Ebola) has caused a bit of a diversion for all of us. (b)(6) has mentioned that you have developed a new animal model for MERS which is transgenic, and is very sensitive. This is just a brief note to explore the possibility of extending mouse model work for LCA60. We have submitted a proposal to the Medical Research Council (MRC) in the UK to take LCA60 into a Phase 1 clinical study. This proposal included the costs for Phase 1 scale up to GMP and also a Phase 1 Pk study in healthy volunteers, and is a large proposal.

The response from the MRC has been favourable, but they are requesting strengthening of the pre-clinical package in the proposal to try and give more indication of how the Mab could be used. We would appreciate your advice/collaboration in this

(1) Could we propose more work in your mouse model to extend understanding of prophylaxis duration and the window for treatment. I am thinking about extending the time points post infection at which Mab is given and also refining knowledge of the duration of protection if given before challenge. One of the questions we are asked to address is to what are the parameters under which this might be used clinically. Currently the data we hold is more of a YES/NO format, rather than a considered model approach to window of treatment opportunity.
(2) If you thought some more work was feasible, would this be possible without provision of funding from us under existing NIH contracts, or would you require additional funding, and if so, what would that be . (NB could we also slip in some work on LCA57, the non neutralising Mab that we have got ?). We would be pleased to include you as a co-applicant for MRC funding, subject to MRC rules for overseas applicants, but the full proposal application cannot be submitted before March, meaning that you might well have already done the work before we could provide any funding
(3) What is your advice about whether the animal model you have developed is suitable for use…Suggestions welcome

Grateful for a rapid response

Maria Zambon

Director, Reference Microbiology
Deputy Director, NIS
National Infection Service
Public Health England
(b)(6)
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<Slides for LCA60 Human mAb study.pdf>

--

(b)(6)

Humabs BioMed SA
Via Murate 5a
6500 Bellinzona

Ph (b)(6)
Cell (b)(6)
Fax (b)(6)

HUMABS BioMed

Adam Cockrell
UNC-CH
135 Dauer Dr.
3105 MHRC, CB#7435
Chapel Hill, NC, 27599
Phone (b)(6)
E mail (b)(6)

Bellinzona, December 14, 2015

Delivery of human monoclonal antibody

Dear Prof. Dr. Cockrell

We are sending you the following material:

Material	Concentration	Total amount	Date of preparation
MPE8	4.6 mg/ml	20 mg	2015729/JN8*
LCA60	4.7 mg/ml	20 mg	201597/S2I*

MPE8 and LCA60 antibodies were produced from CHO cell line.
The antibodies were affinity-purified using HiTrap Protein A columns (GE Healthcare, HiTrap Mab select Xtra #28-4082-61) followed by desalting against PBS using HiTrap Fast desalting columns (GE Healthcare #17-5087-01). The final product were sterilized by filtration through 0.22 µm filters and stored at +4°C.
The buffer formulation is PBS
*these are the same lots sent on September 27., 2015

Please, do not hesitate to contact me should you require additional information.

Best regards,

(b)(6)

From: Baric, Ralph S
Sent: Tue, 18 Jul 2017 16:30:47 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Baric, Toni C
Subject: RE: Grant Number: 1R01AI132178 - 01 PI Name: Baric, Ralph S
Attachments: Copy of R01 AI132178 revised budget.xlsx, R01 AI132178 JIT.pdf, R01 AI132178 revised budget.pdf

Hi Erik, thanks for your email…but this is my fault and I greatly appreciate your going the extra mile for me. I have attached the updated budget spreadsheet and the JIT information that was submitted last Friday (we weren't sure what to do so we submitted it with the Indirect costs added to the bottom line. I've also been in contact with **Kelvin Lyons,** Grants Management Specialist at NIH (Office: (b)(6) (b)(6) Email: (b)(6) who is working on this grant to explain the budget dollar discrepancy (b)(4) that now includes the indirect costs for the partner institutions (Vanderbilt, UTMB) that was accidently left out of the original revised budget that we sent you. There is no change in scope of work. He has flagged the JIT for a few days to hear the final resolution of the matter from you and the appropriate NIH decision makers. I appreciate your checking into this and if the decision is to stay within the first set of numbers, we can revise and resubmit to NIH immediately. Again, I apologize for our error and for the extra work its causing you and others at NIH. I know that the hiring freeze is layering on all kinds of additional work for program. Let me know if there is anything I or Toni can do to assist in this process.
Thanks,
Ralph

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, July 18, 2017 11:42 AM
To: Baric, Ralph S (b)(6)
Subject: Re: Grant Number: 1R01AI132178 - 01 PI Name: Baric, Ralph S

Hi Ralph,
Sorry for being slow, I'm at a meeting this week and doing triple duty! Our grant folks are still checking on this. I haven't seen your updated JIT come through yet, so can you send me the exact figure for the omitted F&A and what the updated final total costs would be?

Erik

Sent from my iPhone

On Jul 17, 2017, at 2:41 PM, Baric, Ralph S (b)(6) wrote:

Hi Erik, hope your doing well. I was wondering if you have any feedback for us regarding this issue. Really sorry to be a problem on this. Got in a bind on Friday, so to make the timeline we put in the budget with the indirect costs added (likely caused all kinds of problems). Do you have some time for a quick call? ralph

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Friday, July 14, 2017 11:57 AM
To: Baric, Ralph S (b)(6) Lyons, Kelvin (NIH/NIAID) [E] (b)(6)
Cc: Baric, Toni C (b)(6) Sheahan, Timothy Patrick (b)(6)
Subject: RE: Grant Number: 1R01AI132178 - 01 PI Name: Baric, Ralph S

Hi Ralph,
I'll have to check and will get back to you soon.

Erik

From: Baric, Ralph S (b)(6)
Sent: Friday, July 14, 2017 11:34 AM
To: Lyons, Kelvin (NIH/NIAID) [E] (b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E] Baric, Toni C (b)(6) Sheahan, Timothy Patrick (b)(6)
Subject: RE: Grant Number: 1R01AI132178 - 01 PI Name: Baric, Ralph S

Hi Kelvin and Erik, As we were putting together this budget (number below), we noticed that we had inadvertently left off the consortium F&A for the Denison and Tseng subcontracts of the revised budget, which total about (b)(4) Would it be okay for us to increase this (b)(4) by the consortium F&A costs as absorbing these costs will impact our work performance. Please excuse our error here. Thank you for your assistance in this matter. Ralph

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From: Lyons, Kelvin (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, July 11, 2017 2:51 PM
To: Burkhart, Carol J. (b)(6)
Cc: Baric, Ralph S (b)(6)
Subject: RE: Grant Number: 1R01AI132178 - 01 PI Name: Baric, Ralph S

Hello Again,

Please provide a revised budget reflecting a budget reduction to (b)(4) in addition to the original information requested. This was also addressed in some of the SRG concerns as well.

Thanks,
Kelvin Lyons
Grants Management Specialist
NIH – NIAID – DEA – GMP
5601 Fishers Lane, Room 4G26
Rockville, Maryland 20852
Office: (b)(6)

Email: (b)(6)

From: Lyons, Kelvin (NIH/NIAID) [E]
Sent: Tuesday, July 11, 2017 1:43 PM
To: (b)(6)
Cc: Baric, Ralph (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Subject: Grant Number: 1R01AI132178 - 01 PI Name: Baric, Ralph S

Hello,

The above referenced application is being considered for funding by the National Institute of Allergy and Infectious Diseases. Please note that this request is not a guarantee of funding. Official notification of funding is only made by issuance of a Notice of Award (NoA).
The following Just-In-Time information (JIT) identified is requested:

_____ Current Other Support - Provide active and pending support information for ALL individuals designated in an application as key personnel.

There is no form page for providing other support, although a sample format page is available at http://grants.nih.gov/grants/funding/2590/non-competing_othersupport.pdf

_____ IRB approval date *(NIH does not require a copy of the IRB certification/approval).* Pending or out-of-date approvals are not acceptable. **If IRB has not met, provide anticipated meeting date.**
Information regarding the Federal Wide Assurance website: http://grants.nih.gov/grants/policy/hs/faqs_aps_assurances.htm

_____ Documentation of the required education in the Protection of Human Subject Research Participants for all key personnel involved in HS research.
Information regarding this requirement can be found at the following website: http://phrp.nihtraining.com/users/login.php

_____ IACUC approval date *(NIH does not require a copy of the IACUC certification/approval).* Pending or out-of-date approvals are not acceptable**.**
If IACUC has not met, provide anticipated meeting date.
Information regarding IACUCs can be found at http://grants.nih.gov/grants/olaw/faqs.htm

_____ Other

1. Confirm your institutions Entity Identification Number (EIN) is 1566001393A1.
2. **Include a copy of your latest F&A rate agreement as well as the most recent agreement for each consortium in this application.**
3. Please provide a detailed response to the concerns listed at the end of the Summary Statement.

The requested Just In Time (JIT) information must be submitted via eRA Commons (NIH Guide Notice NOT-OD-12-101) by **07/15/2017**. If you are unable to submit the requested information through eRA Commons, please contact your Grants Management Specialist. Timely submission of the above information will enable us to expedite the issuance of an award should the application be identified for funding.

Thanks,

Kelvin Lyons
Grants Management Specialist
NIH – NIAID – DEA – GMP
5601 Fishers Lane, Room 4G26
Rockville, Maryland 20852
Office: (b)(6)
Email: (b)(6)

(b)(4); (b)(6)

UNC
GILLINGS SCHOOL OF
GLOBAL PUBLIC HEALTH

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL
DEPARTMENT OF EPIDEMIOLOGY F 919.966.2089
McGAVRAN-GREENBERG HALL
CAMPUS BOX 7435
CHAPEL HILL, NC 27599-7435

July 14, 2017

Dear Dr. Stemmy:

The purpose of this letter is to summarize the changes to our grant application submitted in response to the Partnerships for Countermeasures Against Select Pathogens (R01, RFA-AI-16-034). Our program titled "Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV" is intended to accelerate the preclinical development of GS-5734 and provide proof of concept data necessary for IND licensure and the origination of human clinical trial. Our application was received well in study section (b)(6) with a few noted weaknesses. Below, we summarize our 20% reduction in budget, and address the weaknesses noted by reviewers.

(b)(4); (b)(6)

(b)(4)

(b)(4); (b)(6)

Sincerely,

(b)(6)

Ralph S. Baric
Professor, Department of Epidemiology
University of North Carolina at Chapel Hill
(b)(6)

(b)(6)

Timothy P. Sheahan, Ph.D.
Research Assistant Professor, Department of Epidemiology
University of North Carolina at Chapel Hill
(b)(6)

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(b)(4)

(b)(4); (b)(6)

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(b)(6)

Ralph S. Baric
Professor, Department of Epidemiology
University of North Carolina at Chapel Hill
(b)(6)

(b)(6)

Timothy P. Sheahan, Ph.D.
Research Assistant Professor, Department of Epidemiology
University of North Carolina at Chapel Hill
(b)(6)

(b)(4)

PERSONNEL

Ralph Baric, Ph.D., Co-Principal Investigator (b)(4); (b)(6) Dr. Baric will lead and supervise the in vitro drug testing for this project and in collaboration with Dr. Sheahan manage the overall direction of this highly interactive proposal. He will interact closely with Gilead, Drs. Sheahan, Denison, Sims, Randell, Kocher, Tseng, and Schaefer and Ms. West and Mr. Scobey to ensure steady progress during the course of the proposal, evaluate results and propose alternative experiments. Drs. Baric and Sheahan will share the responsibility for interacting closely with all research staff, holding regular laboratory meetings, communicating research findings with the Denison and Tseng laboratories, writing progress reports and managing fiscal matters associated with the proposal. Given the extensive interaction and collaboration with Dr. Denison in the past, he will also lead efforts to coordinate and promote research efforts with the groups. Dr. Baric will communicate his findings with Gilead, Dr. Sheahan, Denison, Sims and Tseng on a regular basis via both conference calls and meetings between all laboratories working on this proposal.

Timothy Sheahan, Ph.D. Co-Principal Investigator (b)(4); (b)(6) Dr. Sheahan will lead and oversee the in vivo rodent model drug testing of the project and in collaboration with Dr. Baric manage the overall direction of this highly interactive proposal. He has extensive experience working at BSL3 containment and drug testing in rodent animal models. Dr. Sheahan will oversee Dr. Schaefer, Mr. Scobey and Mr. Dinnon to ensure daily progress on all in vivo rodent drug testing as well as assist with problem solving and experimental design. Drs. Sheahan and Baric will share the responsibility for overseeing all research staff, holding regular laboratory meetings, communicating research findings with Gilead, the Denison, Tseng, and Randell laboratories, writing progress reports and managing fiscal matters associated with the proposal. Dr. Sheahan will interact closely with and meet in regular scheduled conference calls/face to face meetings with Gilead, Drs. Baric, Sims, Denison, and Tseng to communicate all data and results in real time.

Amy Sims, Ph.D. Co-Investigator (b)(4); (b)(6) Dr. Sims has more than a decade experience working at BSL3 with primary human airway cells. She will work closely with Drs. Baric and Randell to design and execute all testing of SARS-CoV, MERS-CoV, and various mutant strains of each virus in primary culture models of the human lung. Drs. Sims and Randell will coordinate with Ms. Fulcher and Lam to ensure that the appropriate numbers of primary human lung cells and lung cell donors are available as needed for this project. (b)(3):42 U.S.C. § 262(a) She will report findings regularly to Drs. Baric and Sheahan as well as interfacing with Gilead to discuss all drug studies.

Scott Randell, Ph.D. Co-Investigator (b)(4); (b)(6) Dr. Randell will interact with Drs. Baric, Sheahan and Sims and Ms. Fulcher and Ms. Lam to ensure that specific project needs regarding primary cell cultures are met, establish standard operating procedures for production of culture substrates and media, and create new protocols as needed. Dr. Randell is expert in airway biology, and has extensive datasets evaluating genomic and metagenomic changes in these cultures following various perturbations. He will supervise Ms. M. Leslie Fulcher and Ms. Mariam Lam in the isolation, culture and distribution of primary cells (human airway epithelium nasal epithelium, type II pneumocytes, and alveolar macrophages) for this proposal, and oversee quality control and troubleshooting. Dr. Randell will consult with Drs. Baric and Sims on experimental design, methods, data analysis and publication, and ensure that all regulatory and reporting requirements are fulfilled for work with the primary cell isolates.

Alexandra Schaefer, Ph.D. Staff Scientist (b)(4); (b)(6) Dr Schaefer has extensive BSL3 experience in the Baric laboratory and is an expert at working with BSL3 pathogens in mice. She will work with Dr. Sheahan to design and execute the in vivo animal drug testing proposed in this project. She will also work

with Mr. Scobey and Mr. Dinnon to ensure there is steady progress on sample processing for viral titrations and histology.

Jacob Kocher, Ph.D. Postdoctoral Fellow (b)(4); (b)(6) Dr. Kocher has completed BSL3 training and is now working independently in the Baric containment laboratories. He will work with Dr. Sims to perform in vitro drug testing in primary cells. He will assist with the isolation and characterization of SARS-CoV and MERS-CoV strains containing resistance mutations as well as testing these mutants in the presence of drugs.

Ms. Ande West, Research Specialist (b)(4); (b)(6) . Ms. West has extensive BSL3 experience and will assist with viral titration assays and BSL3 animal husbandry. She will also support Drs. Sheahan, Sims, Schafer, and Kocher's research efforts as needed.

Ms. M. Leslie Fulcher, Research Specialist (b)(4); (b)(6) . Ms. Fulcher facilitates the day-to-day operations of the UNC Cystic Fibrosis Center Tissue Procurement and Cell Culture Core. In support of this proposal she will oversee and maintain quality control of cell isolation and culture procedures from human lung specimens and assist Drs. Baric, Sims, and Randell with the design and performance of the primary cell culture experiments.

Mr. D. Trevor Scobey, Research Technician (b)(4); (b)(6) . Mr. Scobey has extensive BSL3 experience and will assist with the generation of viral stocks, viral titration assays, daily BSL3 laboratory maintenance and BSL3 animal husbandry. He will also assist with weighing and performing whole body plethysmography measurements for infected mice. Mr. Scobey will also maintain our RAG-/- breeding colony.

Mr. Matthew Begley Research Technician (b)(4); (b)(6) Mr. Matthews will be responsible for preparing tissue culture cells for viral titration and will work closely with Drs. Sims and Kocher to anticipate the needs of the project. Mr. Begley will also be responsible for purchasing supplies and supporting Drs. Sheahan, Baric, Sims, and Kocher's research efforts as needed.

Ms. Mariam Lam, Research Specialist (b)(4); (b)(6) Ms. Lam will perform cell isolation human lungs dedicated to this project per year, following specified procedures. She will maintain inventories of frozen cells, prepare reagents and custom media, order supplies and maintain laboratory records. She is fully trained and highly experienced in the culture methods and will work closely with Drs. Baric, Sims, and Randell to provide the specific number of cultured human airway cells designated in the projects.

Mr. Kenneth Dinnon III Graduate student (b)(4); (b)(6) Mr. Dinnon is a new graduate student in the Baric/Sheahan laboratories and he will work closely with Drs. Sheahan and Kocher to perform drug testing with the resistance mutants in vivo once he had completed his BSL3 training.

Fringe Benefits: Faculty/Staff: 22.883% Social Security and Retirement; $5,659/FTE Health Insurance. Post-doctoral Research Associates: 8.99% Social Security and benefits; $4,310/FTE Health Insurance. Health Insurance for Graduate Research Assistants is $3,399. All fringe rates are prorated for effort.

Dr. Baric's compensation is above the NIH salary cap, the balance of his salary will be covered by departmental funds

EQUIPMENT

Spectra Max M3 ($35,646) Funds are requested to purchase a SpectraMax M3 plate reader/luminometer for our BSL3 laboratory for the SARS-CoV and MERS-CoV nano-luciferase assays. We are currently restricted to preforming these assays in one of our laboratories and this purchase will give us additional flexibility in performing the proposed in vitro experiments in this proposal.

Magna Lysers ($11,500) Processing mouse lung tissue for viral titration assays in the BL3 requires homogenization. The Roche Magna Lyser is the best homogenizer on the market for performing homogenization in a containment laboratory. However, constantly moving the equipment into and out of the

biosafety cabinet and daily decontamination causes key parts inside the machine to break frequently. We are requesting two of Magna Lysers to replace ones that will age and break over the course of the project.

Dual Stack CO2 Incubators ($9,989) Funds are requested to replace the dual stack incubator in one of our two BSL3 laboratories. The current incubators are more than ten years old and have issues with contamination that will be solved by the copper lined units we are requesting. Incubators are required for all in vitro virus studies and viral titration assays proposed in this grant.

Biosafety cabinet ($9,620) All work in the BSL3 laboratories must occur in biological safety cabinets. Funds are requested to add an additional biological safety cabinet to our existing facilities to allow enough space to perform the proposed experiments.

-80C Freezer ($13,942) Funds are requested to store the large number of viral primary cell, mouse and non-human primate samples to be generated over the course of this project.

Perkin Elmer Lumina Series III ($177,300) The IVIS Lumina III is an in vivo imaging instrument capable of measuring bioluminescence and fluorescence in live animals. Viruses can be engineered to express luciferase whose expression can be detected by the IVIS upon injection of luciferase substrate. Not only is this technology exquisitely sensitive, but it also allows for repeated measures in live animals eliminating the need to sacrifice multiple cohorts of mice over time and the traditional evaluation of virus replication in harvested tissues. Virus replication data as measured by IVIS is also obtained instantaneously in real time eliminating the wait time associated with traditional virus titration techniques. Thus, in vivo drug efficacy testing can be done faster with far fewer animals and greater sensitivity thus fulfilling the principles of the 3Rs (reduction, refinement, replacement) that guide humane animal research. This technology will revolutionize in vivo efficacy testing.

Abaxis Hematology Analyzer ($15,500) The Vetscan HM5c is a 5-part differential hematology analyzer displaying a comprehensive 22-parameter complete blood count (CBC). Since similar blood panels are collected in routine human clinical practice, the data obtained from the HM5c is inherently translatable. Accurate measurement of CBC should prove to be a valuable biomarker of antiviral treatment success or failure since blood cell populations in humans and mice infected with SARS and MERS-CoV are modulated during infection.

SUPPLIES

Cell culture, Serum, and media (b)(4) Funds are requested for media, serum and related cell culture supplies to maintain Vero cells (titering) in culture to measure virus growth kinetics and to characterize mutant strains containing potential resistance mutations.

BSL3 protective gear (b)(4) Personnel wear powered air purifying HEPA filtered breathing apparatuses, wear tyvek suits, tyvek aprons, hoods, booties and are double gloved when entering the BSL3 facility. These materials are expensive as the HEPA, organic chemical filters and even batteries must be replaced every ~6 months, and the tyvek suits are disposable. Moreover, the PAPR (powered air breathing apparatus) are expensive and must be replaced every ~2 years from normal wear and tear, and daily contact with EPA disinfectants. Personnel use high quantities of disinfectants like ethanol, Clorox and other EPA approved disinfectants in maintaining a safe working environment in the BSL3. Personnel spray down tyvek suits, etc. with alcohol or related disinfectants in the process of deconing and leaving the BSL3 facility. All materials that leave the BSL3 must be disinfected, packaged in disinfected, sealed containers, which are disinfected prior to removal from the BSL3 facility. In addition, funds are requested to help defray costs associated with the decontamination and maintenance of the BSL3 laboratory each year.

Plasticware (b)(4) Funds are requested to purchase tissue culture flasks, dishes, pipettes, etc. used in day to day virologic and cell culture procedures as well as in growing and titering virus growth in vitro.

Enzymes, kits and reagents (b)(4) Assembling recombinant SARS-CoV and MERS-CoV requires large amounts of highly expensive restriction enzymes (e.g., BsmB1, etc.) and large amounts of DNA ligase. In addition, funds are requested for DNA markers, high quality T7 RNA polymerase, and protein and nucleic

acid markers. As sequence confirmation is critical prior to assembly of full-length genomic cDNA, funds are also requested to sequence modified genomic fragments following introduction of resistance mutations.

Miscellaneous (b)(4) Monies are requested to purchase glassware, pipettes, etc. used in day to day virologic and cell culture procedures as well as in growing, titering and characterizing virus growth in vitro. Funds are also requested to purchase chemicals, reagents, paper products, gloves, micropipetors, autoclave supplies, plastic tips, water baths, and other small equipment items that typically have short half lives in laboratory settings.

Computer supplies ($10,000/year) Funds are requested for project specific computer and software upgrades over the course of the proposal.

RNA Seq (b)(4) RNASeq will be used to identify viral mutations that arise following passage of virus in the presence of GS-5734. Funds are requested for supplies to generate amplicon library and to prepare the library for sequencing as well as for informatics support. As such, we anticipate significant sequencing costs over the duration of this proposal.

Primary Cells ($20,000/year) Funds are requested to acquire up to 8 different primary human cell types (i.e. lung-HAE, FB, MVE, AT2; immune cell-PBMC, etc.) and testing seven different drug concentrations in triplicate. We estimate a total number of 120 wells of primary cells per year at $130 a well.

BioRad Bioplex kits (b)(4) Funds are requested to purchase BioRad Bioplex kits to analyze primary cell and mouse lung cytokine profiles. This data will contribute to understanding how the immune response contributes to the mechanism of action of GS-5734.

OTHER EXPENSES

Publishing ($2,000/year) Funds are requested to cover the publication of manuscripts.

Maintenance Contracts ($5,000/year) The Baric/Sheahan laboratory covers costs for maintenance on the Dracor Water Purifiers and Steris Autoclaves used in the BSL3 laboratories. These are sophisticated instruments, so the repairs require specialists with appropriate tools and particular replacement parts. The funds requested each year will cover a portion of these two sets of maintenance contracts.

Histology ($10,000/year) Histology slides from paraformaldehyde fixed tissues are prepared on a fee for service basis at UNC Chapel Hill. Given the large number of tissues to be analyzed each year, we are requesting funds to cover tissue/slide preparation and staining costs.

Animal Costs ($1,120 Year 1 only) Funds are requested to purchase 8 $RAG^{-/-}$ mice for breeding to generate the $Ces^{-/-}$ mice in Aim 3. All other mice will be sent to us from Jackson Laboratories courtesy of Gilead.

Animal Per Diem ($604/year) We anticipate breeding/acquiring from Jackson laboratory 534 mice for Aim 1, 768 mice for Aim 2, and 390 mice for Aim 3 for a total of 1,692 mice for five years. We estimate using approximately 338 mice a year. These mice will be housed in sets of 4 and will be maintained in UNC DLAM facilities for approximately 10 days prior to being moved to the BL3. 10 days x $0.71 per cage per day x 85 cages (to house 338 mice) =$604 a year.

Tuition (b)(4); (b)(6) In accordance with University policy, funds are requested to cover tuition costs for Mr. Dinnon's graduate studies and are prorated to his effort.

Travel

Funds are requested for the PIs and co-investigators to attend annual meetings at Gilead Sciences and one to two conferences each year. ($3,000 international and $3,000 domestic per year)

INDIRECT COST

In a DHHS agreement dated May 16, 2012, the UNC F&A rate is 52% of MTDC.

OTHER SUPPORT

BARIC, RALPH S.

ACTIVE:

U19 AI 107810 (PI: Baric) 06/21/13-05/31/18 (b)(4); (b)(6)
NIH/NIAID $1,572,931
Characterization of novel genes encoded by RNA and DNA viruses
Using highly pathogenic human respiratory and systemic viruses which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes.

U19-AI100625 (PI: Baric/Heise-MPI) 08/05/12-07/31/17 (b)(4); (b)(6)
NIH/NIAID $3,580,599
Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross
Specific Aims: In this proposal, we are utilizing the Collaborative Cross (CC), a novel panel of reproducible, recombinant inbred (RI) mouse lines to identify genes and gene interactions which regulate the induction, kinetics, and magnitude of the innate, inflammatory and adaptive arms of the immune response following virus infection. Specifically, we will develop novel modeling algorithms to predict and validate the causal relationships between natural genetic variation and host signaling networks, immune cell recruitment, and immune function.

00008956 (PI: De Silva) 07/29/15-06/30/18 (b)(4); (b)(6)
UCB/NIH $279,165
Protective immunity following dengue virus natural infections and vaccination
We will perform studies to characterize the B-cell/ antibody (responses in people who receive dengue live attenuated virus vaccines (DLAV).
Role: Co-Investigator

R01 AI 107731 (PI: De Silva) 08/05/13-07/31/17 (b)(4); (b)(6)
NIH/NIAID $621,124
Molecular Basis of Dengue Virus Neutralization by Human Antibodies
These studies proposed here are directly relevant to developing simple assays to predict the performance of the leading dengue vaccine candidates and also for developing the next generation of safe and effective dengue vaccines.
Role: Co-Investigator

R01 AI108197 (MPI: Denison/Baric) 08/01/13-07/31/17 (b)(4); (b)(6)
Vanderbilt University/NIH/NIAID $187,635
Determinants of Coronavirus Fidelity in Replication and Pathogenesis
Experiments in this aim will test the hypothesis nsp1 functions in maintaining high replication fidelity and viral RNA synthesis are coupled and that targeted engineered mutations across nsp14 alter: a) RNA fidelity outcomes; b) sensitivity nucleoside mutagens, terminators and polymerase inhibitors; c) the kinetics and magnitude of positive, negative, genomic and subgenomic RNA synthesis; and d) RNA recombination frequencies.

U19-AI106772-01 (PI: Kawaoka) 06/01/13-05/31/18 (b)(4); (b)(6)
Univ of Wisconsin/NIH $55,729
MERS-CoV Supplement for OMICs Proposal
The proposed studies will provide a more detailed look at the intracellular environment by taking “snapshots” of the lipids, metabolytes, and proteins present during viral infection time courses. These assays will allow us to determine the innate immune response occurring immediately following virus infection and to determine how the virus and cell interact over a 72 hour window.
Role: Investigator

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19 (b)(4); (b)(6)
UAB/NIH/NIAID $304,371
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Co-Investigator

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19 (b)(4); (b)(6)
Columbia/NIH/NIAID $584,891
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Project Leader, Consortium PI

246823 (PI: Baric) 01/27/15-12/30/17 (b)(4); (b)(6)
PNNL/DHS $205,569
The Generation of Predictive Models of Viral Pathogenesis
Using advances in transcriptomics, proteomics, and metabolomics, we will identify changes in the virus-host interaction expression networks associated with DENV infection of Aedes aegypti cells or human immune cells in vitro, the latter model after natural receptor-mediated or after ADE mediated entry processes.

Not assigned (PI: deSilva) 11/05/14-09/30/17 (b)(4); (b)(6)
(b)(4); (b)(6) $726,915
The dengue human infection model: Defining correlates of protection and advancing vaccine development
The goal of these studies conducted by the Baric laboratory are to use recombinant dengue viruses encoding multiple homotypic neutralizing sites from multiple strains, as well as a collection of null mutants, to characterize the homotypic immune response elicited in humans following natural infection and after challenge in GSK DENV tetravalent vaccinated individuals. This grant has been funded by (b)(4); (b)(6)
Role: Co-Investigator

R01 AI110700 (PI: Baric) 04/20/15-03/31/20 (b)(4); (b)(6)
NIH/NIAID $613,691
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

Not Assigned (PI: Baric) 01/08/16-01/07/19 (b)(4); (b)(6)
(b)(4); (b)(6) $1,243,048
In Vitro and In Vivo Characterization of Bivalent DENV Live Virus Vaccines
To provide expertise in molecular virology required for creating recombinant dengue viruses for in vitro and in vivo testing.

(b)(4); (b)(6) (PI: deSilva) 02/29/16-02/28/18 (b)(4); (b)(6)
$212,800
Pilot study to characterize human antibody response to tetravalent dengue vaccine- phase 3.
To perform experiments to produce recombinant dengue viruses using DENV1, 2, 3 and 4 infectious clones, conducting neutralization assays, culturing viruses and performing other phenotypic analyses.
Role: Co-Investigator

R01-AI125198 (PI: deSilva) 05/04/16-04/30/21 0.60 cal mos
NIH/NIAID $1,153,997

Preclinical Assays To Predict Tetravalent Dengue Vaccine Efficacy
Dengue is the most significant mosquito transmitted viral infection of humans. Vaccination is a feasible solution to prevent and control dengue. Although dengue vaccines are under development, we do not know the specific properties of antibodies induced by vaccines that are likely to protect from infection. In this project investigators from the University of North Carolina and Sanofi Pasture, a leading dengue vaccine developer, will collaborate to define properties of antibodies induced by the Sanofi vaccine that correlate with protection. The main goal of the project is to develop new assays to support the current global effort to develop dengue virus vaccines. Role: Co-Investigator

60045042 (PI: Baric) 02/01/15-01/31/18 0.12 cal mos
Ohio State Univ/USDA $44,804
Molecular attenuation mechanisms of porcine epidemic diarrhea virus in pigs
Reverse genetic strategies are used to construct a panel of live attenuated porcine epidemic diarrhea recombinant viruses for in vivo pathogenesis studies and in vitro biological characterization. We test rationale vaccine strategies to protect new born piglets against this devastating porcine epidemic virus.

(b)(4); (b)(6) (PI: Baric) 06/23/16-06/22/18 0.24 cal mos
$1,066,500
Breadth of Blockade Antibody Responses Following Norovirus Vaccination
To conduct a project as an agreement in which Dr. Ralph Baric will test (b)(4); (b)(6) provided serum samples for cross-strain blockade antibody responses.

N005402801 (PI: Li) 06/07/16-05/2831/18 0.36 cal mos
Univ Minn/NIH $120,384
Receptor recognition and cell entry of coronaviruses
To investigate how CoVs explore host receptors and host proteases for regulation of their host range, cross-species transmission, tissue tropism, and pathogenesis. Role: Subcontract PI

684K644 (PI: Sims) 06/01/16-05/31/18 0.12 cal mos
Univ of Wisconsin/NIH $200,000
Systems Virology of MERS-CoV in vivo
The major goal of this award is to define host cell gene networks and pathways that are modulated following infection in our newly developed lethal mouse model of MERS-CoV. Role: Investigator

PENDING: None

OVERLAP: None

OTHER SUPPORT

SHEAHAN, TIMOTHY

ACTIVE:

U19AI107810 (PI: Baric) 06/21/13-05/31/18 (b)(4); (b)(6)
NIH/NIAID $2,027,645
Characterization of novel genes encoded by RNA and DNA viruses
Using highly pathogenic human respiratory and systemic viruses, which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes.
Role: Investigator

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19 (b)(4); (b)(6)
UAB/NIH/NIAID $1,611,425
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Investigator

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19 (b)(4); (b)(6)
Columbia/NIH/NIAID $2,999,060
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Investigator

R01 AI110700 (PI: Baric) 04/01/15-03/31/20 (b)(4); (b)(6)
NIH $3,683,050
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.
Role: Investigator

R01 AI108197 (MPI: Denison/Baric) 08/01/13-07/31/17 (b)(4); (b)(6)
Vanderbilt University/NIH/NIAID $187,635
Determinants of Coronavirus Fidelity in Replication and Pathogenesis
Experiments in this aim will test the hypothesis nsp1 functions in maintaining high replication fidelity and viral RNA synthesis are coupled and that targeted engineered mutations across nsp14 alter: a) RNA fidelity outcomes; b) sensitivity nucleoside mutagens, terminators and polymerase inhibitors; c) the kinetics and magnitude of positive, negative, genomic and subgenomic RNA synthesis; and d) RNA recombination frequencies.
Role: Investigator

PENDING:

(b)(4); (b)(6)

(b)(4); (b)(6)

OVERLAP:

If other awards are made, Dr. Sheahan will reduce his percent effort accordingly.

Please note: Dr. Sheahan's effort on **R01 AI108197** ends July 31, 2017 and his effort can be reduced on **U19AI107810** if his pending application gets awarded.

CHAPPELL, JAMES D.

ACTIVE

(b)(4); (b)(6)	Denison	07/01/16-06/30/17 $98,020	(b)(4); (b)(6)

Identification of MERS-CoV Spike changes responsible for resistance to NHP and human Spike-specific mAbs

The goal of this project is to perform detailed scientific studies utilizing characterized pathogenic coronaviruses, passage virus in the presence of increasing amounts of mAb, identify resistant viruses, sequence the viral attachment protein (Spike), and verify that sequence mutations identified correspond to viral resistance to the antibody. Selected mutations will be applied to reconstructing molecular clones of coronaviruses.

PENDING

(b)(4); (b)(6)

OVERLAP

No overlap exists.

DENISON, MARK R.

ACTIVE

6R01AI026603-26 (2nd NCE) Denison 05/15/10-04/30/17 (b)(4); (b)(6)
NIAID $34,677
Polymerase Proteins in Coronavirus Replication

The goal of this proposal is to elucidate the mechanism of coronavirus nsp5 protease in regulating virus replication. The specific aims of the proposal are to: 1) test the functions of nsp5 precursors and membrane association on protease activity during activity; 2) define the role of structural and dimerization determinants in nsp5 protease specificity; and 3) identify intramolecular residue networks in nsp5 and determine their function in protease activity and virus replication.

5R01AI108197-05 Denison/Baric 08/01/13-07/31/17 (b)(4); (b)(6)
NIH/NIAID $450,129
Determinants of Coronavirus Fidelity in Replication and Pathogenesis

Experiments in this aim will test the hypothesis nsp1 functions in maintaining high replication fidelity and viral RNA synthesis are coupled and that targeted engineered mutations across nsp14 alter: a) RNA fidelity outcomes; b) sensitivity nucleoside mutagens, terminators and polymerase inhibitors; c) the kinetics and magnitude of positive, negative, genomic and subgenomic RNA synthesis; and d) RNA recombination frequencies.

VUMC43650(U19AI109680-04) Whitley/Denison 03/01/14-02/28/19 (b)(4); (b)(6)
UAB/NIAID/NIH $257,723
Antiviral Drug Discovery and Development Center

The goals of this project mainly consist of: 1) *in vitro* confirmation of lead compounds provided by the HTS core at SRI/UAB, 2) mechanistic studies of these compounds against SARS-CoV, 3) testing the development of resistance *in vitro*, and 4) examining the activity of these compounds against phylogenetically distant CoVs *in vitro.*

5K12HD087023-03 Webber 12/01/15-11/30/20 (b)(4); (b)(6)
NIAID/NIH $320,112
Pathogenesis, Targeted Therapeutics, and New Vaccines for Childhood Disease

The goal of this project is to establish at Vanderbilt School of Medicine a mentored training program for young pediatricians possessing both the aptitude and passion to become a new generation of basic and translational physician scientists.

(b)(4); (b)(6) Denison 07/01/16-06/30/17 (b)(4); (b)(6)
$98,020
Identification of MERS-CoV Spike changes responsible for resistance to NHP and human Spike-specific mAbs

The goal of this project is to perform detailed scientific studies utilizing characterized pathogenic coronaviruses, passage virus in the presence of increasing amounts of mAb, identify resistant viruses, sequence the viral attachment protein (Spike), and verify that sequence mutations identified correspond to viral resistance to the antibody. Selected mutations will be applied to reconstructing molecular clones of coronaviruses.

2T32AI095202-07 Denison 08/18/16-07/31/21 (b)(4); (b)(6)
NIH/NIAID $262,742
Childhood Infections Research Program (CHIRP)
The CHIRP is designed to train and facilitate the careers of investigators studying infectious diseases relevant to children. The outcomes will be both MD and PhD researchers capable of making a long-term impact on the diagnosis, prevention, and treatment of infections in children. The public health relevance is high for the direct impact on the health of children.

VUMC Denison 3/8/17-2/28/21 (b)(4); (b)(6)
DARPA $284,745
Defective Interference of Viral Infectious Diseases using Evolutionary & Computational Rationale (DIVIDE & ConqueR)
This goal of this project will be to test whether small defective genomes generated during virus infection can act to interfere with virus replication – thus resulting in "Therapeutic Interfering Particles- TIP's".

PENDING

(b)(4); (b)(6)

OVERLAP

If (b)(4); (b)(6) is fully funded effective June 1, 2016 the following effort allocations will be decreased to ensure there is no overlap for this grant. Below reduction in effort for June and July will not cause an annual reduction over (b)(4); (b)(6)

June 1st – June 30, 2017

5R01AI108197-05: Will reduce effort to (b)(4); (b)(6)

VUMC43650 (U19AI109680-04: Will reduce effort on this contract to (b)(4); (b)(6)

DARPA Grant: Will reduce effort to (b)(4); (b)(6)

5K12HD087023-03: Will reduce effort to (b)(4); (b)(6)

(b)(4); (b)(6) Will reduce effort to (b)(4); (b)(6)

July 1st – July 31st, 2017

5R01AI108197-05: Will reduce effort to (b)(4); (b)(6)

VUMC43650 (U19AI109680-04: Will reduce effort on this contract to (b)(4); (b)(6)

DARPA Grant: Will reduce effort to (b)(4); (b)(6)

RANDELL, Scott H.

ACTIVE

(b)(4); (b)(6) 7/1/07-9/30/17 (b)(4); (b)(6)

Under this agreement, the Tissue Procurement and Cell Culture Core will receive lung tissues, process the tissues for epithelial cell harvest, characterize the resulting cells, and distribute the cells for CF research by institutions as designated by (b)(4); (b)(6)

RES507797 (Gaston) 1/23/13-3/31/17* (b)(4); (b)(6)
Case Western (subc on NHLBI P01 HL101871-05)
Cellular S-Nitrosothiol Signaling in Respiratory Biology, Project 1: S-Nitrosothiol Signaling and Localization in Airway Epithelial Cells

The major goal of this project is to understand the role of S-nitrosothiols in pulmonary physiology and pathophysiology. *Overall grant runs 4/15/11-3/31/16. Project halted temporarily from 9/14/12-1/22/13 due to Dr. Gaston's relocated to Case Western. Currently in NCE.

RES507797 (Gaston) 1/23/13-3/31/17* (b)(4); (b)(6)
Case Western (subc on NHLBI P01 HL101871-05)
Cellular S-Nitrosothiol Signaling in Respiratory Biology, Core B: Cell Culture Core

The major goal of this core is to provide cultured cells and expert consultation to PPG investigators. *Overall grant runs 4/15/11-3/31/16. Project halted temporarily from 9/14/12-1/22/13 due to Dr. Gaston's relocated to Case Western. Currently in NCE.

5 P01 HL 110873-04 (Boucher) 5/15/12-4/30/17 (b)(4); (b)(6)
NIH/NHLBI
Pulmonary Surface Liquid Homeostasis, Core C: Cell Culture Core

The major goal of this core is to provide cell culture services to the projects of the PPG: tissue procurement, epithelial cell isolation and culture, genetic manipulation of cell cultures, and novel cell lines.

5 R01 HL 117843-03 (Harris/Knowles/O'Neal) 7/15/13-5/31/18 (b)(4); (b)(6)
NIH/NHLBI
Mechanistic Link between Genetic Variation and CF Lung Disease

The goals of this project are to define important mechanistic links between genetic variation and severity of CF lung disease, and offers opportunity for therapeutic insights.

5 P50 HL 120100-03 (Tarran) 9/19/13-6/30/18 (b)(4); (b)(6)
NIH/NHLBI
The Impact of Tobacco Exposure on the Lung's Innate Defense System, Core C: Tissue Culture and Smoke Exposure Core

The major goal of the overall P50 is to measure the potential adverse impact of tobacco alternatives ("little cigars" and Hookah) on the lung's innate defense system. Core C will procure, culture and characterize primary cells for use in the projects.

sub contract (Lipkin/Baric) 3/7/14-2/28/19 (b)(4); (b)(6)
Columbia Univ on NIH/NIAID 5 U19 AI 109761-02
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease

The goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.

2 P30 DK 065988-11 (Boucher) 4/23/15-3/31/20 (b)(4); (b)(6)
NIH/NIDDK
UNC Cystic Fibrosis Research and Translation Core Center, Core C: Cell Models Core

The major goal of the overall P30 is to synergize and accelerate cystic fibrosis research by creating and supporting four research cores, a pilot and feasibility program and an administrative core to coordinate the activities. Cores include 1) Preclinical Core focused on CFTR biogenesis/function, 2) Cell Models Core focused on generation/provision of relevant airway and GI epithelial cell models, 3) Mucus Biochemistry/Biophysics Core, and 4) Human Translational Studies Core. Core C will support CF research by providing normal and CF human and mouse airway epithelial cell air liquid interface cultures.

(b)(4); (b)(6) 1/1/15-12/31/17 (b)(4); (b)(6)
(b)(4); (b)(6)

The goal of the study is to determine the acute effect of (b)(4); (b)(6) on ciliary activity.

1 R01 HL 123557-01A1 (Oldenburg/Zdanski) 9/1/15-8/31/18 (b)(4); (b)(6)
NIH/NIAID
Anatomic Optical Coherence Tomography for Quantitative Bronchoscopy

We propose to address the need for quantitative, real-time, dynamic upper airway imaging by developing a technology based upon anatomic Optical Coherence Tomography (aOCT) delivered via standard bronchoscopes. The data acquired from this new technology will be manipulatable into 3D computational models of the airway upon which computational fluid dynamic (CFD) modeling will be performed.

(b)(4); (b)(6) 7/1/15-6/30/19 (b)(4); (b)(6)
Epithelial Function in Cystic Fibrosis, Core C: Tissue Procurement and Cell Culture Core

The major goals of this Core are to (1) procure tissue, (2) characterize patients, (3) culture cells and support research projects, (4) characterize atypical CF patients, and (5) develop cell lines.

(b)(4); (b)(6) (Stanton) 7/1/15-6/30/19 (b)(4); (b)(6)
Dartmouth (subcontract on (b)(4); (b)(6)
Translational Research in Cystic Fibrosis, Cell Biology and Imaging Core

The major goals of this subcontract is to provide services and cultured cells to Dr. Bruce Stanton and colleagues on the Dartmouth Research Development Program grant in support of their research on cystic fibrosis.

(b)(4); (b)(6) (Clancy) 7/1/15-6/30/19 (b)(4); (b)(6)
Cinc Child Hosp Med Ctr (subc on (b)(4); (b)(6)
(subcontract on (b)(4); (b)(6) subcontract PI: Randell)
Research Development Program

The major goal of this subcontract is to provide services to the CCHMC RDP grant in isolating primary airway epithelial cells, harvesting cells, and culturing cells.

(b)(4); (b)(6) 4/1/16-3/31/18 (b)(4); (b)(6)
Towards Somatic Airway Epithelial Stem Cell Therapy for Cystic Fibrosis

The project tests the hypothesis that endogenous airway epithelial stem and progenitor cells (so called somatic stem cells) are a viable alternative for cell therapy. Our specific Aims are to: 1) Identify the optimal somatic stem cell for CF airway epithelial cell therapy; 2) Understand requirements for airway epithelial

engraftment; and 3) Provide proof in principle for *in vivo* airway epithelial cell therapy. The fundamental early preclinical studies proposed here explore key steps towards translation of somatic airway epithelial stem cell therapy for CF.

(b)(4); (b)(6) 12/1/12-12/31/18 (b)(4); (b)(6)

Human Cystic Fibrosis Bronchial Epithelial Cell Culture Support

The major goal of this project is to provide cells specifically prepared for research projects of (b)(4); (b)(6) (b)(4); (b)(6)

Subcontract on DAVIS15XX0 (Davis, BR - UTHSC) 11/1/15-10/31/17 (b)(4); (b)(6)
Univ of TX Health Science Center, sub on (b)(4); (b)(6)
Zinc Finger Nuclease-mediated Gene Editing to Restore CFTR Function

The goals of this proposal are to optimize zinc finger nuclease-mediated gene editing to restore CFTR function, which is a vital link in the effort to create viable cell therapy for cystic fibrosis (CF).

(b)(4); (b)(6) 9/28/15-9/28/17 (b)(4); (b)(6)

Assessment of (b)(4); (b)(6) compounds
To test the effects of potential therapies for mucus obstructive diseases and provide cells for toxicology studies.

(b)(4); (b)(6) 4/15/16-04/14/17 (b)(4); (b)(6)

Airway Epithelial Cells for (b)(4); (b)(6)

The goal is to provide cells and consultation to (b)(4); (b)(6) for their in-house research.

PENDING

(b)(4); (b)(6)

(b)(4); (b)(6)

OTHER SUPPORT

SIMS, AMY

ACTIVE:

U19-AI100625 (PI: Baric) 8/05/12-07/31/17 (b)(4); (b)(6)
NIH/NIAID $4,030,980
Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross
Specific Aims: In this proposal, we are utilizing the Collaborative Cross (CC), a novel panel of reproducible, recombinant inbred (RI) mouse lines to identify genes and gene interactions, which regulate the induction, kinetics, and magnitude of the innate, inflammatory and adaptive arms of the immune response following virus infection. Specifically, we will develop novel modeling algorithms to predict and validate the causal relationships between natural genetic variation and host signaling networks, immune cell recruitment, and immune function.
Role: Investigator

U19AI107810 (PI: Baric) 06/21/13-05/31/18 (b)(4); (b)(6)
NIH/NIAID $2,027,645
Characterization of novel genes encoded by RNA and DNA viruses
Using highly pathogenic human respiratory and systemic viruses, which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes.
Role: Investigator

U19-AI106772-01 (PI: Kawaoka) 06/01/13-05/31/18 (b)(4); (b)(6)
Univ. of Wisconsin/NIH $87,000
MERS-CoV Supplement for OMICs Proposal
The proposed studies will provide a more detailed look at the intracellular environment by taking “snapshots” of the lipids, metabolytes, and proteins present during viral infection time courses. These assays will allow us to determine the innate immune response occurring immediately following virus infection and to determine how the virus and cell interact over a 72 hour window.
Role: Project PI

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19 (b)(4); (b)(6)
UAB/NIH/NIAID $1,611,425
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Investigator

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19 (b)(4); (b)(6)
Columbia/NIH/NIAID $2,999,060
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Investigator

R01 AI110700 (PI: Baric) 04/01/15-03/31/20 (b)(4); (b)(6)
NIH $3,683,050
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

Role: Investigator

INACTIVE:

Supplement to OMIC (PI: Kawaoka) 6/1/14-5/31/16
Univ. of Wisconsin/NIH/NIAID $200,000
Epigenetic Regulation of Interferon-Stimulated Genes Following MERS-CoV Infection
The overriding hypothesis of this supplemental application is that MERS-CoV and H5N1 manipulate host epigenetic programs to specifically down-regulate certain classes of ISGs, which likely antagonize virus replication efficiency in vitro. The goal is to develop systems biology datasets and unbiased modeling algorithms to deconvolute the complex pathogen-host interactions that regulate severe disease outcomes following infection and identify common host pathways/genes that can be exploited for therapeutic control.
Role: Project PI

Supplement to OMIC (PI: Kawaoka) 6/1/16-5/31/17 (b)(4); (b)(6)
Univ. of Wisconsin/NIH/NIAID $200,000
Systems Virology for MERS-CoV in vivo
The goal is to develop systems biology datasets and unbiased modeling algorithms to deconvolute the complex pathogen-host interactions that regulate severe disease outcomes following infection and identify common host pathways/genes that can be exploited for therapeutic control. These studies will build on our current data set by collecting data sets for MERS-CoV in vivo.
Role: Project PI

OVERLAP:

If other awards are made, Dr. Sims will reduce her percent effort accordingly.

Start Date: June 1, 2017-May 31, 2018 **12 Cal**

NAME	TITLE	SALARY	EFFORT	Months	SALARY	FRINGE	TOTAL
Baric, Ralph	PI	(b)(4); (b)(6)					
Sheahan, Timothy	PI						
Sims, Amy	Co-Investigator						
Randell, Scott	Co-Investigator						
Schaefer, Alexandra	Staff Scientist						
Kocher, Jacob	Postdoctoral Fellow						
West, Ande	Res. Specialist						
Fulcher, Leslie	Res. Specialist						
Scobey, Trevor	Res. Technician						
Begley, Matthew	Technician						
Lam, Mariam	Res. Specialist						
Dinnon III, Kenneth	Res. Assistant Grad						
TOTALS:			(b)(4); (b)(6)				

0

SUPPLIES		(b)(4)	
	Cell Culture, Serum and Media		
	BSL3 gear PAPR		
	Plasticware		
	Enymes, kits and reagents		
	Misc.		
	Computer Supplies	**$10,000**	
	RNA Seq	(b)(4)	
	Primary Cells	**$20,000**	
	Bioplex	(b)(4)	
Total Supplies		(b)(4)	
EQUIPMENT (Itemi ($300,000 max)			**$273,497**
SpectraMax M3		**$35,646**	
Magna lysers		**$11,500**	
Dual Stack Incubators		**$9,989**	
Biosafety Cabinet		**$9,620**	
80C Freezer		**$13,942**	
Perkin Elemer Lumina Series III		**$177,300**	
Abaxis Hematology Analyzer		**$15,500**	
		$273,497	
OTHER (Itemize by category)			
Publishing		**$2,000**	
Maint. Contracts		**$5,000**	
Tuition		(b)(4); (b)(6)	
Histology		**$10,000**	
Animal Cost		**$1,120**	
Animal per diem		**$604**	
		$0	
TOTAL OTHER:		(b)(4)	
TRAVEL			
	International	**$3,000**	

Domestic	**$3,000**	**$6,000**
TOTAL COST:		(b)(4)
CONSORTIUM DIRECT		
Vanderbilt	**$ 200,000**	
UTMB	**$ 100,000**	
		300000
SUBTOTAL DIRECT COST FOR BUDGET PERIOD		(b)(4)
CONSORTIUM F & A		
Vanderbilt	**116000**	
UTMB	**55000**	
		$171,000
TOTAL DIRECT COSTS		(b)(4)
UNC-CH F & A @ 52% MOD	(b)(4)	
TOTAL COST		

YEAR 2

6/1/18-5/31/19

NAME	TITLE	SALARY	EFFORT	Cal Months	SALARY	FRINGE	TOTAL
Baric, Ralph	PI	(b)(4); (b)(6)					
Sheahan, Timothy	PI						
Sims, Amy	Co-Investigator						
Randell, Scott	Co-Investigator						
Schaefer, Alexand	Staff Scientist						
Kocher, Jacob	Postdoctoral Fellow						
West, Ande	Res. Specialist						
Fulcher, Leslie	Res. Specialist						
Scobey, Trevor	Res. Technician						
Begley, Matthew	Technician						
Lam, Mariam	Res. Specialist						
Dinnon III, Kenne	Res. Assistant Grad						
TOTALS:			(b)(4); (b)(6)				

CONSULTANTS		**0**
SUPPLIES		
Cell Culture, Serum and Media	(b)(4)	
BSL3 gear PAPR		
Plasticware		
Enymes, kits and reagents		
Misc.		
Computer Supplies	$10,000	
RNA Seq	(b)(4)	
Primary Cells	$20,000	
Bioplex	(b)(4)	
Total Supplies		(b)(4)
EQUIPMENT (Itemize)		**$0**
OTHER (Itemize by category)		
Publishing	$2,000	
Maint. Contracts	$5,000	
Tuition	(b)(4); (b)(6)	
Histology	$10,000	
Animal Cost	$0	
Animal per diem	$604	
TOTAL OTHER:	(b)(4)	
TRAVEL		
International	$3,000	

Domestic	**$3,000**	**$6,000**
TOTAL COST:		(b)(4)
CONSORTIUM DIRECT		**300000**
SUBTOTAL DIRECT COST FOR BUDGET PERIOD		**$746,932**
CONSORTIUM F & A		
Vanderbilt	**116000**	
UTMB	**55000**	
		$171,000
TOTAL DIRECT COSTS		(b)(4)
UNC-CH F & A @ 52% MOD	(b)(4)	
TOTAL COST		

YEAR 3

6/1/19-5/31/20

NAME	TITLE	SALARY	EFFORT	Cal Months	SALARY	FRINGE	TOTAL
Baric, Ralph	PI	(b)(4); (b)(6)					
Sheahan, Timothy	PI						
Sims, Amy	Co-Investigator						
Randell, Scott	Co-Investigator						
Schaefer, Alexand	Staff Scientist						
Kocher, Jacob	Postdoctoral Fellow						
West, Ande	Res. Specialist						
Fulcher, Leslie	Res. Specialist						
Scobey, Trevor	Res. Technician						
Begley, Matthew	Technician						
Lam, Mariam	Res. Specialist						
Dinnon III, Kenne	Res. Assistant Grad						
TOTALS:			(b)(4); (b)(6)				

CONSULTANTS			**0**
SUPPLIES			
	Cell Culture, Serum and Media	(b)(4)	
	BSL3 gear PAPR		
	Plasticware		
	Enymes, kits and reagents		
	Misc.		
	Computer Supplies	**$10,000**	
	RNA Seq	(b)(4)	
	Primary Cells	**$20,000**	
	Bioplex	(b)(4)	
Total Supplies			(b)(4)
EQUIPMENT (Itemize)			**$0**
		0	
OTHER (Itemize by category)			
Publishing		**$2,000**	
Maint. Contracts		**$5,000**	
Tuition		(b)(4)	
Histology		**$10,000**	
Animal Cost		**$0**	
Animal per diem		**$604**	
TOTAL OTHER:		(b)(4)	(b)(4)
TRAVEL			
	International	**$3,000**	

Domestic	**$3,000**	**$6,000**
TOTAL COST:		(b)(4)
CONSORTIUM DIRECT		**300000**
SUBTOTAL DIRECT COST FOR BUDGET PERIOD		(b)(4)
CONSORTIUM F & A		
Vanderbilt	**116000**	
UTMB	**55000**	
	$171,000	**$171,000**
TOTAL DIRECT COSTS		(b)(4)
UNC-CH F & A @ 52% MOD	(b)(4)	
TOTAL COST		

YEAR 4

6/1/21-5/31/22

NAME	TITLE	SALARY	EFFORT	Cal Months	SALARY	FRINGE	TOTAL
Baric, Ralph	PI	(b)(4); (b)(6)					
Sheahan, Timothy	PI						
Sims, Amy	Co-Investigator						
Randell, Scott	Co-Investigator						
Schaefer, Alexand	Staff Scientist						
Kocher, Jacob	Postdoctoral Fellow						
West, Ande	Res. Specialist						
Fulcher, Leslie	Res. Specialist						
Scobey, Trevor	Res. Technician						
Begley, Matthew	Technician						
Lam, Mariam	Res. Specialist						
Dinnon III, Kenne	Res. Assistant Grad						
TOTALS:			(b)(4); (b)(6)				

CONSULTANTS			**0**
SUPPLIES			
	Cell Culture, Serum and Media	(b)(4)	
	BSL3 gear PAPR		
	Plasticware		
	Enymes, kits and reagents		
	Misc.		
	Computer Supplies	$10,000	
	RNA Seq	(b)(4)	
	Primary Cells	$20,000	
	Bioplex	(b)(4)	
Total Supplies			(b)(4)
EQUIPMENT (Itemize)			**$0**
		0	
OTHER (Itemize by category)			
Publishing		$2,000	
Maint. Contracts		$5,000	
Tuition		(b)(4)	
Histology		$10,000	
Animal Cost		$0	
Animal per diem		$604	
TOTAL OTHER:		(b)(4)	(b)(4)
TRAVEL			
	International	$3,000	

Domestic	**$3,000**	**$6,000**
TOTAL COST:		(b)(4)
CONSORTIUM DIRECT		**300000**
SUBTOTAL DIRECT COST FOR BUDGET PERIOD		(b)(4)
CONSORTIUM F & A		
Vanderbilt	**116000**	
UTMB	**55000**	
	$171,000	**$171,000**
TOTAL DIRECT COSTS		(b)(4)
UNC-CH F & A @ 52% MOD	(b)(4)	
TOTAL COST		

YEAR5

6/1/22-5/31/23

NAME	TITLE	SALARY	EFFORT	Cal Months	SALARY	FRINGE	TOTAL
Baric, Ralph	PI	(b)(4); (b)(6)					
Sheahan, Timothy	PI						
Sims, Amy	Co-Investigator						
Randell, Scott	Co-Investigator						
Schaefer, Alexand	Staff Scientist						
Kocher, Jacob	Postdoctoral Fellow						
West, Ande	Res. Specialist						
Fulcher, Leslie	Res. Specialist						
Scobey, Trevor	Res. Technician						
Begley, Matthew	Technician						
Lam, Mariam	Res. Specialist						
Dinnon III, Kenne	Res. Assistant Grad						
TOTALS:			(b)(4); (b)(6)				

CONSULTANTS		**0**
SUPPLIES		
	Cell Culture, Serum and Media	(b)(4)
	BSL3 gear PAPR	
	Plasticware	
	Enymes, kits and reagents	
	Misc.	
	Computer Supplies	**$10,000**
	RNA Seq	(b)(4)
	Primary Cells	**$20,000**
	Bioplex	(b)(4)
Total Supplies		(b)(4)
EQUIPMENT (Itemize)		**$0**
OTHER (Itemize by category)		
Publishing		**$2,000**
Maint. Contracts		**$5,000**
Tuition		(b)(4); (b)(6)
Histology		**$10,000**
Animal Cost		**$0**
Animal per diem		**$604**
Shipping		
TOTAL OTHER:		(b)(4)
TRAVEL		
	International	**$3,000**

Domestic	**$3,000**	**$6,000**
TOTAL COST:		(b)(4)
CONSORTIUM DIRECT		**300000**
SUBTOTAL DIRECT COST FOR BUDGET PERIOD		(b)(4)
CONSORTIUM F & A		
Vanderbilt	**116000**	
UTMB	**55000**	
	$171,000	**$171,000**
TOTAL DIRECT COSTS		(b)(4)
UNC-CH F & A @ 52% MOD	(b)(4)	
TOTAL COST		

From: Cockrell, Adam
Sent: Thu, 26 Jan 2017 21:17:28 +0000
To: (b)(6) Stemmy, Erik (NIH/NIAID) [E]; Baric, Ralph
Subject: timeline for testing anti-MERS therapeutic in MERS mouse model
Attachments: Cockrell AS & Baric RS et al. Nat. Micro. 2016. A mouse model for MERS-CoV-induced ARDS.published version.pdf, Timeline for initial study.pptx

Hi everyone,

I put together an outline of the study we discussed last week. I also attached a copy of our MERS mouse model manuscript.

I am currently working on the amendment for our IACUC protocol. I will be proposing the study as outlined. However, I will also add additional delivery time point post-infection in the event we decide on a follow-up study.

Currently I have this at 250ug/mouse for delivery based on efficacy that we have seen with anti-MERS antibodies delivered prophylactically, 12 hours prior to infection.

Keith, you mentioned that your group would do the calculations regarding the molar equivalent of your therapeutic compared to a typical IgG1 subtype human antibody. If the hDPP4-Fc therapeutic is larger, the molar comparison would probably indicate that we should use a higher ug amount of the hDPP4-Fc therapeutic for delivery.

Please let me know ASAP if everyone is agreeable to this timeline, dose, etc. This would require me to alter the IACUC amendment prior to submission.

Best Regards,

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ARTICLES

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A mouse model for MERS coronavirus-induced acute respiratory distress syndrome

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Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel virus that emerged in 2012, causing acute respiratory distress syndrome (ARDS), severe pneumonia-like symptoms and multi-organ failure, with a case fatality rate of ~36%. Limited clinical studies indicate that humans infected with MERS-CoV exhibit pathology consistent with the late stages of ARDS, which is reminiscent of the disease observed in patients infected with severe acute respiratory syndrome coronavirus. Models of MERS-CoV-induced severe respiratory disease have been difficult to achieve, and small-animal models traditionally used to investigate viral pathogenesis (mouse, hamster, guinea-pig and ferret) are naturally resistant to MERS-CoV. Therefore, we used CRISPR–Cas9 gene editing to modify the mouse genome to encode two amino acids (positions 288 and 330) that match the human sequence in the dipeptidyl peptidase 4 receptor, making mice susceptible to MERS-CoV infection and replication. Serial MERS-CoV passage in these engineered mice was then used to generate a mouse-adapted virus that replicated efficiently within the lungs and evoked symptoms indicative of severe ARDS, including decreased survival, extreme weight loss, decreased pulmonary function, pulmonary haemorrhage and pathological signs indicative of end-stage lung disease. Importantly, therapeutic countermeasures comprising MERS-CoV neutralizing antibody treatment or a MERS-CoV spike protein vaccine protected the engineered mice against MERS-CoV-induced ARDS.

The severity of respiratory illness caused by Middle East respiratory syndrome coronavirus (MERS-CoV), its pandemic potential through human-to-human respiratory transmission and a dearth of effective treatments necessitate the development of new MERS-CoV therapies and vaccines. Effective vaccine and therapeutic development require preclinical animal models that resemble the pathogenesis of human MERS-CoV infection. Additionally, these models should: (1) include a measure of mortality associated with severe respiratory disease; (2) not be confounded by neurological complications due to high viral loads in the brain; (3) exhibit sustained, high-level virus replication within the lungs of infected animals; (4) exhibit lung pathology associated with human acute respiratory distress syndrome (ARDS); (5) maintain innate expression of the MERS-CoV host receptor, dipeptidyl peptidase 4 (DPP4), to prevent perturbation of immunological homeostasis; (6) be genetically tractable to study host genes that regulate responses to MERS-CoV vaccines and therapeutics; and (7) exhibit reproducibility.

Conventional non-human primate (NHP) models have been established for MERS-CoV in both the rhesus macaque and common marmoset[1–4]. NHPs are instrumental for the preclinical development of therapeutics; however, these models are cost-prohibitive for initial screening of large numbers of vaccine and therapeutic candidates, challenging to work with for routine pathogenesis studies, limited in availability and typically require high virus challenge doses into multiple sites. Furthermore, two recent studies have contradicted the initial studies in NHPs, which may complicate use of the rhesus macaque[5] or the common marmoset[6] model for routine vaccine or therapeutic testing.

MERS-CoV fails to replicate in traditional small-animal models (mouse, hamster, guinea-pig and ferret) due to the inability of the receptor-binding domain in the MERS-CoV spike protein to interact with the respective DPP4 receptor[7–10]. In addition to acting as the MERS-CoV receptor, DPP4 regulates T-cell activation, cytokine function and *trans*-endothelial migration to sites of inflammation[11]. Therefore, overexpression of DPP4 may result in immune dysregulation. Effective models would therefore ideally promote functional MERS-CoV/DPP4 interactions, with minimal perturbations of innate DPP4 expression, signalling activity or tissue distribution. Classical strategies to overcome receptor incompatibilities to generate susceptible mice have relied on generalized or tissue-specific transgenic overexpression approaches to drive expression of the human receptor (hDPP4) in the mouse[12–15]. Although MERS-CoV can elicit respiratory disease in hDPP4 overexpression models, these models exhibit a fatal central nervous system (CNS) and systemic multi-organ disease[12–14], probably due to non-specific overexpression of the receptor throughout the animal, which complicates the study of MERS-CoV-induced respiratory pathogenesis in these models.

In this study, we used our knowledge of which determinants allow mouse DPP4 to act as a functional MERS-CoV receptor[7] by using CRISPR–Cas9 (clustered regularly interspaced short palindromic repeats and CRISPR-associated gene 9) genome editing technology to insert codons that match the human sequence at positions 288 and 330 in the mouse *Dpp4* gene. This strategy resulted in a mouse that is permissive for MERS-CoV infection, while maximally preserving the species-specific interaction networks critical for DPP4 immune function. Generation of mice

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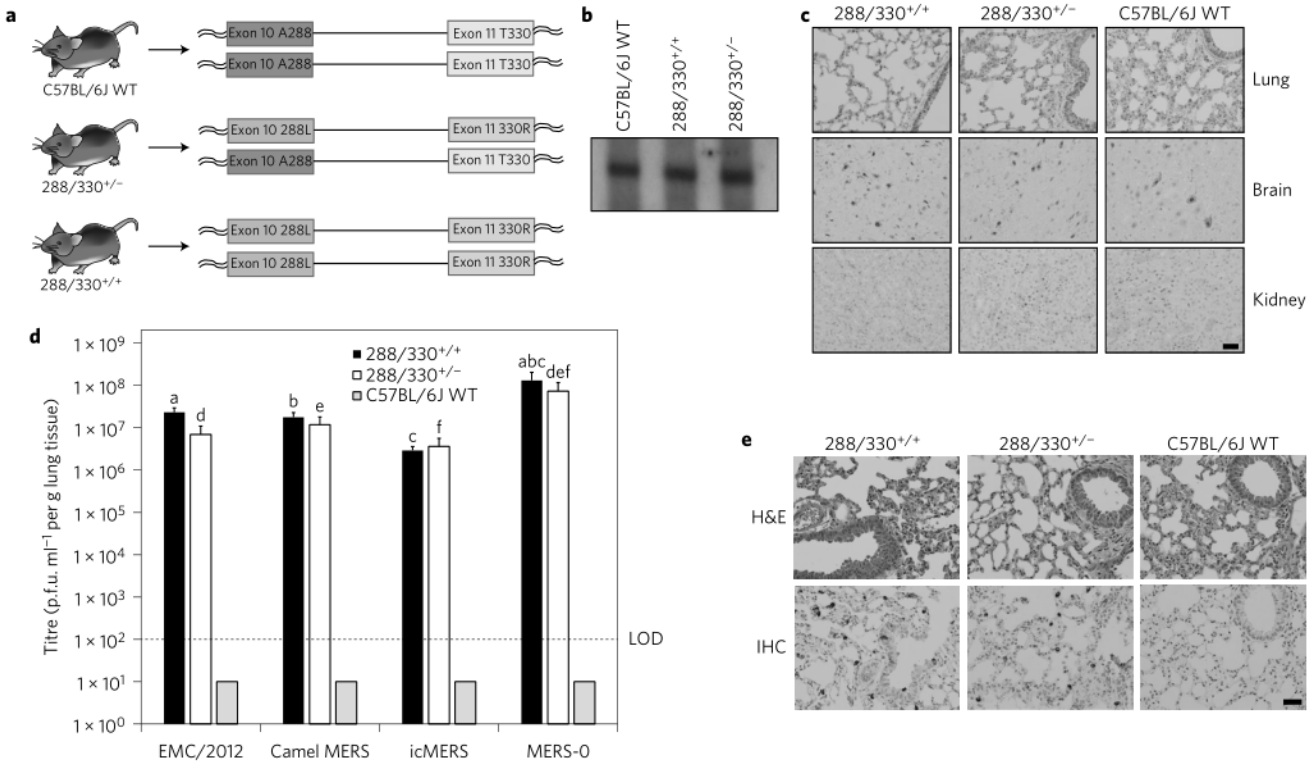

Figure 1 | A CRISPR–Cas9 genetically engineered mouse model for MERS-CoV replication. a, C57BL/6J mice were genetically engineered using CRISPR–Cas9 genomic editing to encode 288L and 330R in mDPP4 on one chromosome (heterozygous, 288/330$^{+/-}$) or on both chromosomes (homozygous, 288/330$^{+/+}$). **b**, Northern blot of mDPP4 mRNA expression. **c**, Immunohistochemistry (IHC) of mDPP4 protein in the lungs, brain and kidneys of individual C57BL/6J wild-type (WT), 288/330$^{+/-}$ and 288/330$^{+/+}$ mice. **d**, Viral titres for MERS-CoV at 3 days post-infection from C57BL/6J WT, 288/330$^{+/-}$ and 288/330$^{+/+}$ (all $n = 4$) mice infected with 5×10^5 plaque-forming units (p.f.u.) of the indicated viruses. Bar graphs show means + s.d. Student's *t*-test was used to calculate $P < 0.05$ for comparisons of MERS-0 with each virus in 288/330$^{+/+}$ mice (a–c on graph) and 288/330$^{+/-}$ mice (d–f on graph). **e**, Pathology of 288/330$^{+/+}$, 288/330$^{+/-}$ and C57BL/6J WT mice at day 3 after infection with MERS-0. Lung tissue sections were stained to examine the pathology by haematoxylin and eosin staining (H&E), or were stained by IHC to detect nucleocapsid protein from MERS-0 infection. IHC and H&E pathology images are representative of at least three samples. Scale bars (**c**,**e**), 1 mm.

carrying a chimaeric mouse DPP4 (mDPP4) molecule (A288L/T330R), combined with a mouse-adapted strain of MERS-CoV, allowed us to generate a mouse model that resembles severe MERS-CoV-induced respiratory disease without bystander neurological disease. In parallel, we demonstrated that this model system can be used for the development and testing of MERS-CoV vaccines and therapeutics.

Results

A CRISPR–Cas9-generated mouse model for MERS-CoV infection. We have demonstrated previously that the introduction of two amino acids that match the human sequence at positions 288 and 330 in the mDPP4 receptor can support MERS-CoV docking, entry and replication in cell culture[7]. These determinants are located within exons 10 and 11 of mDPP4 on chromosome 2 (Fig. 1a and Supplementary Fig. 1). Therefore, we used CRISPR–Cas9 genome editing to introduce these determinants (A288L and T330R) into the mDPP4 receptor (Fig. 1a and Supplementary Table 1). Two lines of C57BL/6J-derived mice were generated that were either homozygous (288/330$^{+/+}$) or heterozygous (288/330$^{+/-}$) for the chimaeric mDPP4 alleles (Fig. 1a). The 288/330$^{+/+}$ homozygous mice encoded the 288L and 330R changes on both chromosomes, thereby expressing only mDPP4 with both changes (Fig. 1a). The 288/330$^{+/-}$ heterozygous mice encoded the 288L and 330R changes on one chromosome and the C57BL/6J wild-type amino acids, A288 and T330, on the other chromosome, thereby expressing both mutated and wild-type mDPP4 (Fig. 1a). The innate mDPP4 expression levels and patterns in the lungs, kidneys and brains of 288/330$^{+/+}$ and 288/330$^{+/-}$ mice reflected those observed in C57BL/6J wild-type mice (Fig. 1b,c; Supplementary Fig. 2). DPP4 is central to the maintenance of glucose homeostasis in mammals[16]. Blood glucose levels were within the normal range observed in C57BL/6J wild-type mice, supporting the hypothesis that biological mDPP4 functions were not altered in the 288/330$^{+/+}$ and 288/330$^{+/-}$ mice (Supplementary Fig. 2). Moreover, basal CD4$^+$ T-cell expression of interleukin-2, tumour-necrosis factor-α, interferon-γ, CD69, CD25 and mDPP4 (CD26) from the 288/330$^{+/+}$ and 288/330$^{+/-}$ lines was comparable to the levels observed in C57BL/6J wild-type mice (Supplementary Fig. 3). Notwithstanding functional T-cell assessment, these results suggested that minimal alteration of the 288 and 330 alleles does not alter basal T-cell activation status. Overall expression levels, expression patterns, biological function and the immunological profiles of mDPP4 were comparable to those of C57BL/6J wild-type mice following site-specific modification of the 288 and 330 alleles.

The 288/330$^{+/+}$ and 288/330$^{+/-}$ mice supported efficient infection and replication of the human MERS-CoV strain HCoV-EMC/2012, the camel MERS strain Dromedary/Al-Hasa-KFU-HKU13/2013 and a recombinant virus derived from a molecular infectious clone (icMERS) in the lungs, but these virus strains could not replicate in C57BL/6J wild-type mice that retained the original murine A288 and T330 alleles (Fig. 1d). In parallel, a MERS-CoV tissue-culture-adapted variant, derived by infection of NIH/3T3 cells ectopically expressing the chimaeric mDPP4 (A288L/T330R) receptor, was found to encode a three amino acid insertion (RMR) and a

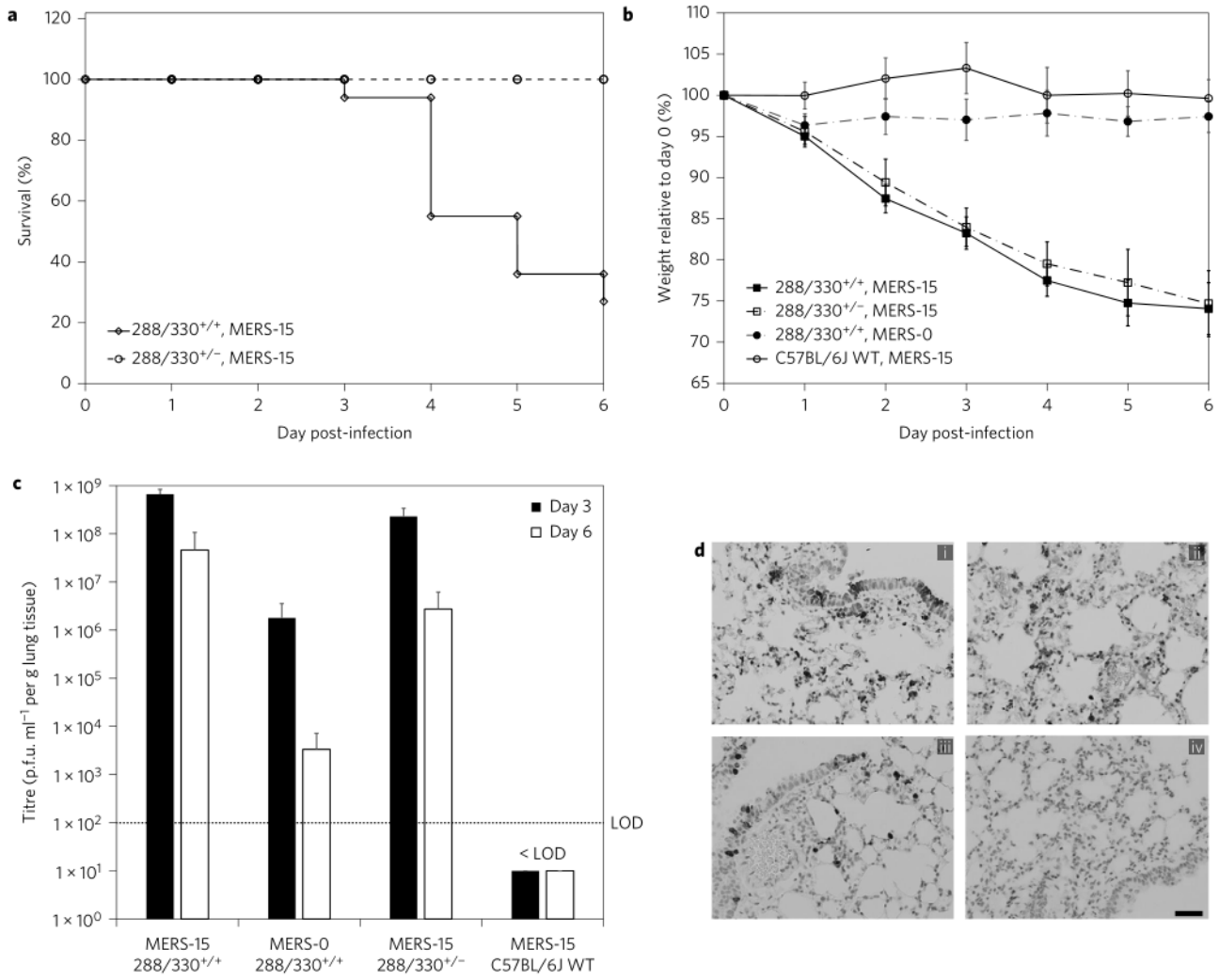

Figure 2 | Mouse-adapted MERS-CoV causes fatal disease in 288/330$^{+/+}$ mice. Mice were inoculated intranasally with 5×10^6 p.f.u. **a**, Mortality of 288/330$^{+/-}$ ($n = 10$) and 288/330$^{+/+}$ ($n = 16$) mice was monitored daily up to day 6 p.i. Data show the percentage of surviving mice. **b**, Mouse weights were measured daily up to day 6 p.i. for 288/330$^{+/+}$ mice infected with MERS-15 ($n = 16$) or MERS-0 ($n = 10$), 288/330$^{+/-}$ mice infected with MERS-15 ($n = 10$) and C57BL/6J wild-type (WT) mice infected with MERS-15 ($n = 7$). Data are daily means of the percentage weight relative to day 0 ± s.d. **c**, Viral lung titres for MERS-CoV were determined at day 3 p.i. (288/330$^{+/-}$ + MERS-15, $n = 4$; 288/330$^{+/+}$ + MERS-15, $n = 5$; 288/330$^{+/+}$ + MERS-0, $n = 5$; C57BL/6J WT + MERS-15, $n = 4$) and day 6 p.i. (288/330$^{+/-}$ + MERS-15, $n = 4$; 288/330$^{+/+}$ + MERS-15, $n = 4$; 288/330$^{+/+}$ + MERS-0, $n = 5$; C57BL/6J WT + MERS-15, $n = 3$). The limit of detection (LOD) is indicated. Bars are means + s.d. **d**, Immunohistochemistry of lung sections for anti-MERS nucleocapsid at 3 days p.i. Results show 288/330$^{+/+}$ mice + MERS-15 (i), 288/330$^{+/-}$ mice + MERS-15 (ii), 288/330$^{+/+}$ mice + MERS-0 (iii) and C57BL/6J WT mice + MERS-15 (iv). IHC images are representative of at least three samples. Scale bar (**d**), 1 mm.

single amino acid change (S885L) in the S2 region of the spike gene. This recombinantly derived virus, MERS-0, encoding the RMR and S885L S2 mutations, demonstrated significantly enhanced replication both in cell culture (Supplementary Fig. 4) and in the lungs of 288/330$^{+/+}$ and 288/330$^{+/-}$ mice (Fig. 1d; $P<0.05$). Despite replicating to significantly higher virus titres *in vivo* than the other isolates, MERS-0 exhibited no evidence of severe clinical disease symptoms (Supplementary Fig. 4). Lung histology demonstrated that nucleocapsid antigen from MERS-0 (Fig. 1e), and from the other strains (not shown), was readily detected in the lungs of infected mice by immunohistochemistry, but infected lungs exhibited only moderate signs of respiratory pathology and inflammation. These results demonstrated that we had developed a MERS-CoV model that could support high levels of virus replication up to day 3 post-infection (p.i.), but that further *in vivo* adaptation was required to achieve the respiratory symptoms characteristic of MERS-CoV infection in humans.

Mouse adaptation of MERS-CoV induces severe ARDS-like disease. The recombinantly derived MERS-0 virus was mouse adapted by serial passage for 15 rounds through the lungs in 288/330$^{+/-}$ mice at 3-day intervals, resulting in the MERS-15 strain. Infection of 288/330$^{+/+}$ mice via the intranasal route with MERS-15 resulted in ~70% mortality (genuine mortality, rather than mice meeting the typical 20% weight loss cut-off associated with humane euthanasia criteria), while 100% of infected 288/330$^{+/-}$ mice survived (Fig. 2a). However, both lines exhibited 20–25% weight loss by day 6 p.i., in contrast to MERS-0-infected 288/330$^{+/+}$ mice or MERS-15-infected C57BL/6J wild-type mice, which exhibited no weight loss (Fig. 2b). Significantly higher levels of MERS-15 replication were detectable in the lungs of both 288/330$^{+/+}$ and 288/330$^{+/-}$ mice at days 3 and 6 p.i., while MERS-0 was mostly cleared from the lungs by day 6 p.i. (Fig. 2c,d). Similar to MER-0, MERS-15 did not replicate in C57BL/6 wild-type mice. Importantly, the observed decreases in survival and weight loss

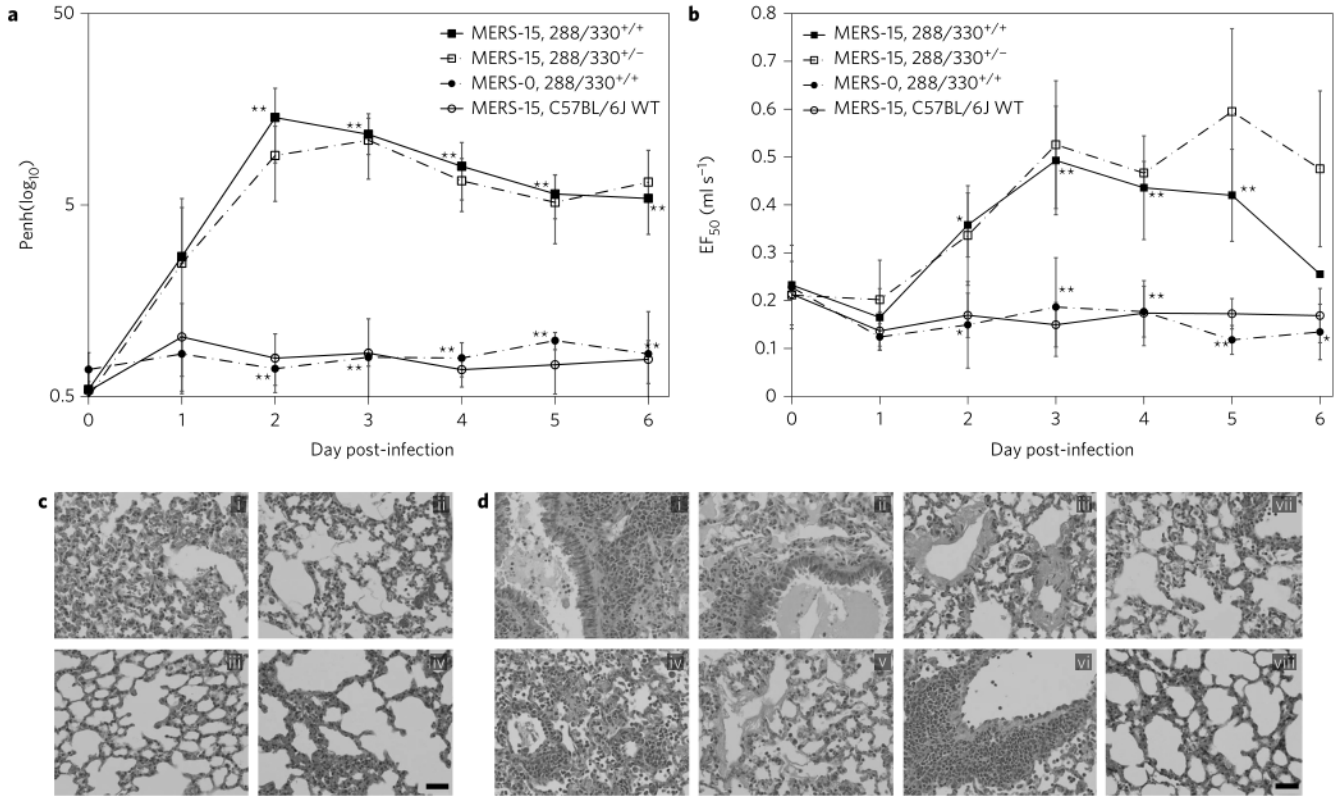

Figure 3 | Lung function in MERS-15-infected mice. a,**b**, Respiratory function was monitored in live mice up to day 6 p.i. using whole-body plethysmography to measure Penh (**a**) and EF_{50} (**b**) in 288/330$^{+/+}$ mice infected with MERS-15 ($n = 9$) or MERS-0 ($n = 3$), 288/330$^{+/-}$ mice infected with MERS-15 ($n = 4$) and C57BL/6J wild-type (WT) mice infected with MERS-15 ($n = 3$). Data are daily means ± s.d. Student's *t*-test was used to compare 288/330$^{+/+}$ mice infected with MERS-15 and MERS-0 (*$P < 0.05$; **$P < 0.01$). **c**, Pathology of lungs from infected mice at day 3 p.i. demonstrating severe inflammation for 288/330$^{+/+}$ (i) and 288/330$^{+/-}$ (ii) mice infected with MERS-15, and moderate inflammation for 288/330$^{+/+}$ mice infected with MERS-0 (iii) and C57BL/6J WT mice infected with MERS-15 (iv). **d**, Pathology at day 6 p.i. infected in mice. In 288/330$^{+/+}$ mice infected with MERS-15, there was severe inflammation and oedema in the large airways (i) and alveoli (ii), and hyaline membrane formation (iii). The 288/330$^{+/-}$ mice infected with MERS-15 exhibited severe inflammation throughout the parenchyma (iv), hyaline membrane formation (v) and perivascular cuffing (vi). The 288/330$^{+/+}$ mice infected with MERS-0 (vii) and C57BL/6J WT mice infected with MERS-15 (viii) exhibited mild-to-moderate inflammation. Samples were stained with haematoxylin and eosin and are representative of at least three samples. Scale bars (**c**,**d**), 1 mm.

induced by MERS-15 were not confounded by neurological complications from brain infection, as plaque assays for replication-competent virus and PCR with reverse transcription (RT-PCR) at days 3 and 6 p.i. were negative (Supplementary Fig. 5). Moreover, quantitative RT-PCR on the same samples demonstrated an increase of $>10^6$ detectable viral transcripts in infected lungs compared with similarly infected C57BL/6J mice, with no detectable viral transcripts in the brains of these mice (Supplementary Fig. 5c).

Although mortality and weight loss provide important measures of MERS-CoV-induced disease, these parameters do not directly assess the impact of virus replication on respiratory function. Therefore, to directly assess the impact of MERS-15 infection on respiratory function in 288/330$^{+/+}$ and 288/330$^{+/-}$ mice, we measured respiratory function using unrestrained plethysmography, as demonstrated previously for respiratory pathogenesis in mouse models of severe acute respiratory syndrome (SARS) and influenza[17]. MERS-15 elicited severe lung disease as quantified by enhanced pause (Penh), a unitless measure that reflects airway obstruction/restriction due to debris in the airway, and by midtidal expiratory flow (EF_{50}), which represents the flow rate at which 50% of the tidal volume has been expelled in a single breath[17]. MERS-15 infection led to significant increases in both Penh and EF_{50} in 288/330$^{+/+}$ and 288/330$^{+/-}$ mice up to day 6 p.i. compared with 288/330$^{+/+}$ mice infected with MERS-0 and C57BL/6J wild-type mice infected with MERS-15 (Fig. 3a,b), demonstrating that MERS-15 elicited severe respiratory distress in mice carrying the chimaeric DPP4 receptor. This was further supported by our observation of severe haemorrhage in the lungs of both 288/330$^{+/+}$ and 288/330$^{+/-}$ mice infected with MERS-15 at days 3 and 6 (Supplementary Fig. 6), inflammatory infiltrates by day 3 (Fig. 3c) and respiratory pathology associated with severe acute respiratory distress, including hyaline membrane formation, intra-alveolar oedema, perivascular cuffing and severe inflammation, at day 6 p.i. (Fig. 3d). Quantitative comparison of the lung pathology in 288/330$^{+/+}$ mice infected with MERS-15 and MERS-0 demonstrated that MERS-15 induced significant levels of pathology commensurate with ARDS by day 6 p.i. (Supplementary Fig. 7). Although we did not conduct an exhaustive assessment of all extrapulmonary tissues in the 288/330$^{+/+}$ mice, we could not detect virus replication in the brain, even at doses of 5×10^6 plaque-forming units (p.f.u.) up to day 6 when the humane euthanasia end points were reached, and the pathology observed in our model was consistent with the severe respiratory pathology associated with fatal ARDS in the only published case study of a human MERS-CoV infection[18].

Identification of MERS-CoV-adapted mutations associated with severe respiratory disease. We anticipated that the MERS-CoV genome would acquire mutations due to immunological pressure and/or enhanced virus fitness during mouse adaptation. Two viral

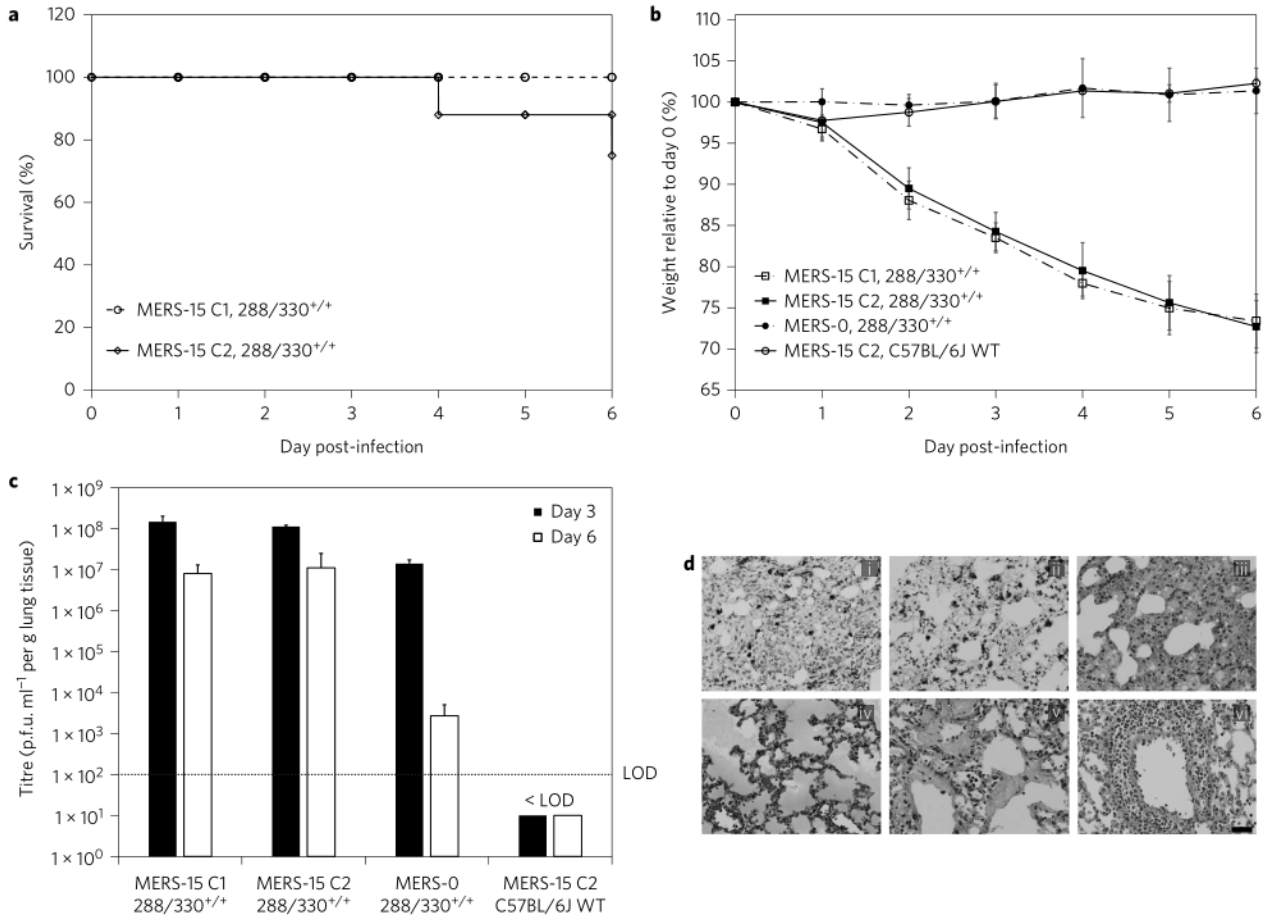

Figure 4 | Clonal isolates of mouse-adapted MERS-CoV exhibit severe respiratory disease. Mice were inoculated intranasally with 5×10^6 p.f.u. **a**, The mortality of 288/330$^{+/+}$ mice infected with MERS-15 C1 ($n = 7$) or MERS-15 C2 ($n = 11$) was monitored daily up to day 6 p.i. Data reflect the percentage of surviving mice. **b**, Mouse weights were monitored daily for 288/330$^{+/+}$ mice infected with MERS-15 C1 ($n = 7$), MERS-15 C2 ($n = 11$) or MERS-0 ($n = 6$), and C57BL/6J wild-type (WT) mice infected with MERS-15 C2 ($n = 6$). Data are daily means ± s.d. **c**, Viral lung titres were determined at day 3 p.i. (288/330$^{+/+}$ + MERS-15 C1, $n = 4$; 288/330$^{+/+}$ + MERS-15 C2, $n = 3$; 288/330$^{+/+}$ + MERS-0, $n = 3$; C57BL/6J WT + MERS-15 C2, $n = 3$) and day 6 p.i. (288/330$^{+/+}$ + MERS-15 C1, $n = 4$; 288/330$^{+/+}$ + MERS-15 C2, $n = 6$; 288/330$^{+/+}$ + MERS-0, $n = 3$; C57BL/6J WT + MERS-15 C2, $n = 3$). The limit of detection (LOD) is indicated. Bars are means + s.d. **d**, IHC of lung sections at 3 days p.i. from 288/330$^{+/+}$ mice infected with MERS-15 C1 (i) or MERS-15 C2 (ii) and stained for nucleopcapsid. The pathology of the lungs from 288/330$^{+/+}$ mice infected with MERS-15 C2 was assessed by haematoxylin and eosin staining (H&E) at day 6 p.i. and demonstrated severe inflammation (iii), oedema (iv), hyaline membrane formation (v) and perivascular cuffing (vi). All H&E images are representative of at least three samples. Scale bar (**d**), 1 mm.

clones, MERS-15 clone 1 (MERS-15 C1) and MERS-15 clone 2 (MERS-15 C2), were isolated by plaque purification from the MERS-15 heterogeneous virus population. MERS-15 C2 infection resulted in increased mortality of the mice (Fig. 4a) and significantly increased haemorrhage up to day 6 p.i. compared with MERS-15 C1 (Supplementary Fig. 8), whereas both clonal isolates caused 25–30% weight loss (Fig. 4b) and high levels of virus replication in the mice up to day 6 p.i., when humane euthanasia end points were reached (Fig. 4c). The lung pathology elicited by MERS-15 C2 resembled that obtained with the primary MERS-15 virus (Fig. 4d), demonstrating similar pathologies including oedema, hyaline membrane formation and perivascular cuffing. Sequencing of the entire MERS-15 C2 genome revealed a set of unique missense mutations, acquired during *in vivo* passaging, in the genes encoding the non-structural proteins, *nsP2*, *nsP6* and *nsP8*, and a large deletion in *orf4b* that may be responsible for the enhanced disease observed with MERS-15 (Supplementary Fig. 9). Sequencing of MERS-15 C1 revealed some differences that may influence the capacity of the virus to elicit the increased mortality observed with MERS-15 C2, namely mutations in nsP2 and an expanded deletion that extended from *orf4b* into *orf5* (Supplementary Fig. 9). Moreover, 5′ rapid amplification of cDNA ends revealed that the nucleotide at position 2 of the 5′ untranslated region was deleted in both clones (Supplementary Fig. 9). Generation of an infectious clone harbouring all of the MERS-15 C2 mutations (icMERSma1) demonstrated that the disease could be reproduced with an infectious dose of 5×10^6 p.f.u. (Supplementary Fig. 10). Decreasing the dose by 10-fold to 5×10^5 p.f.u. resulted in weight loss that paralleled that observed with 5×10^6 p.f.u.; however, the 5×10^5 p.f.u. dose exhibited a slight decrease in mortality up to day 7 p.i. (Supplementary Fig. 10). Although additional studies will be needed, comparative genomic analysis of the two clones, C1 and C2, indicated that nsP2 and Orf5 may have significant roles in determining disease outcome. Therefore, the MERS-15 C2 derived virus, combined with the 288/330$^{+/+}$ mouse line, represent useful tools for studying MERS-CoV pathogenesis or assessing therapeutic countermeasures against MERS-CoV-induced ARDS.

Human monoclonal antibody 3B11 protects from MERS-CoV-elicited severe respiratory disease. Human monoclonal antibodies provide a robust strategy for the treatment of newly emerged viruses

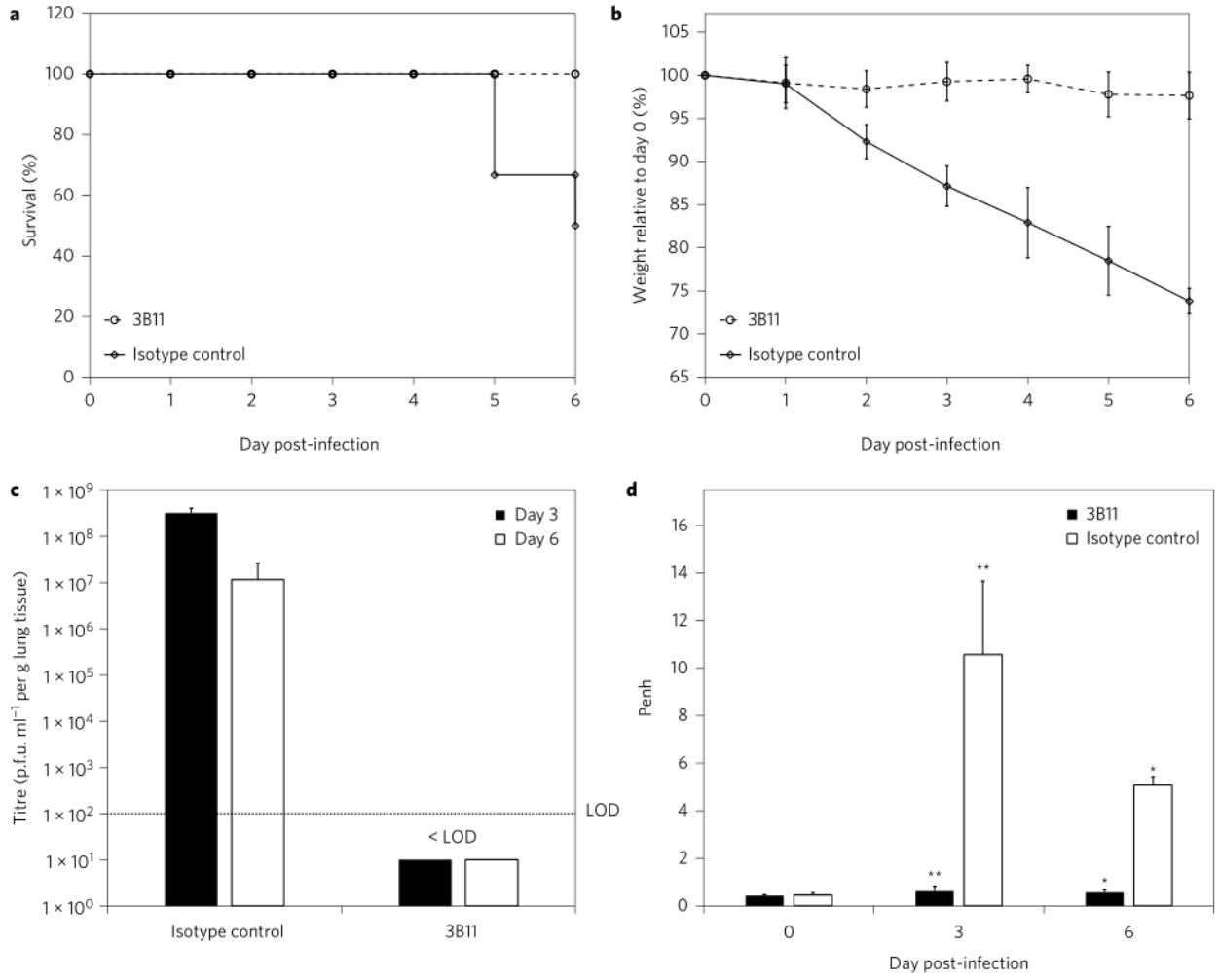

Figure 5 | Human monoclonal antibody 3B11 protects mice from severe respiratory disease. The 288/330$^{+/+}$ mice were intraperitoneally administered 250 μg of 3B11 human monoclonal antibody or isotype control antibody 12 h before challenge with 5×10^6 p.f.u. MERS-15 C2. **a**, **b**, Mortality (**a**) and mouse weight (**b**) were monitored daily for mice receiving 3B11 ($n = 12$) or an isotype control ($n = 12$) up to day 6 p.i. Data show the percentage of surviving mice (**a**) or daily mean weight ± s.d. (**b**). **c**, Viral lung titres were determined at day 3 p.i. ($n = 6$ for both 3B11- and isotype control-treated mice) and day 6 p.i. ($n = 6$ for 3B11-treated mice; $n = 3$ for isotype control-treated mice). The limit of detection (LOD) is indicated. Bars are means + s.d. **d**, Lung function was assessed by Penh at 0, 3 and 6 days p.i. for mice receiving 3B11 ($n = 6$ at days 0, 3 and 6) or isotype control ($n = 6$ at days 0 and 3; $n = 3$ at day 6). Data are means + s.d. Student's *t*-test was used to compare mice receiving 3B11 with those receiving the isotype control at day 3 and day 6 p.i. (*$P < 0.05$; **$P < 0.01$).

in humans. 3B11 is a human monoclonal antibody that targets the receptor-binding domain of the MERS-CoV spike protein[19], and is effective in NHPs[5]. As the MERS-15 C2 adapted virus acquired no receptor-binding domain mutations (Supplementary Fig. 9), we reasoned that 3B11 should protect 288/330$^{+/+}$ mice from MER-15 C2 challenge. Pretreating mice for 12 h with 3B11 provided 100% protection against MERS-15 C2 challenge (Fig. 5a,b). Moreover, 3B11 treatment reduced viral loads in the lungs of infected mice to undetectable levels (Fig. 5c and Supplementary Fig. 11) and protected from loss of respiratory function (Fig. 5d) (Supplementary Fig. 11), pulmonary haemorrhage (Supplementary Fig. 11) and severe pathological changes (Supplementary Fig. 12). In contrast, pretreatment with isotype control antibody provided no protective effect. Therefore, these data convincingly demonstrated that our preclinical mouse model of severe respiratory disease and mortality can serve as a platform for assessing MERS-CoV therapeutics.

Spike protein vaccines derived from Venezuelan equine encephalitis virus replicon particles protect from lethal infection. To examine vaccine efficacy in the 288/330$^{+/+}$ MERS-15 C2 model, mice were vaccinated with Venezuelan equine encephalitis replicon particles (VRPs) expressing MERS-CoV spike protein (spike–VRP) or mock vaccinated with VRPs expressing green fluorescent protein (GFP–VRP), boosted at 4 weeks postprime and challenged with MERS-15 C2 at 4 weeks postboost. All mice receiving spike–VRP survived and exhibited no weight loss following challenge compared with GFP–VRP mock-vaccinated animals (Fig. 6a,b). Spike–VRP vaccination significantly reduced MERS-15 C2 replication in the lungs of infected mice as shown by both plaque titre (Fig. 6c) and viral antigen staining (Supplementary Fig. 14), while also protecting against severe respiratory disease, as assessed by measurement of Penh (Fig. 6d) and EF_{50} (Supplementary Fig. 13), lung haemorrhage (Supplementary Fig. 13) and pathological indications of severe acute respiratory disease (Supplementary Fig. 14). Neutralization of MERS-15 C2 with prechallenge serum from spike–VRP-vaccinated mice validated the presence of high-titre neutralizing antibodies in the serum of vaccinated 288/330$^{+/+}$ mice (Supplementary Fig. 13). Our data demonstrated that the spike–VRP vaccine provoked an adaptive immune response capable of protecting mice from a lethal challenge with MERS-CoV, thereby extending the utility of our preclinical mouse model of severe respiratory disease to include vaccine evaluation.

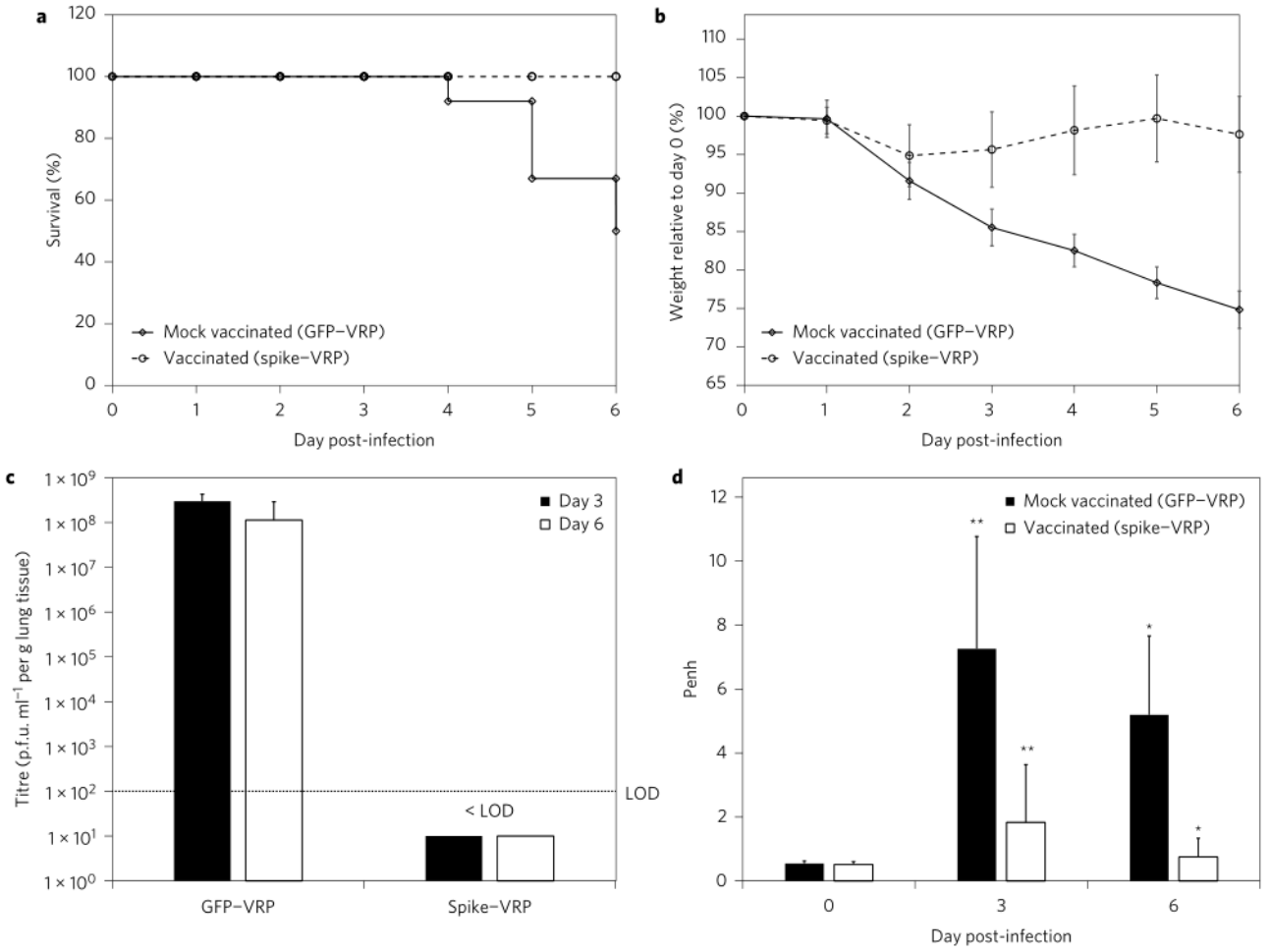

Figure 6 | Vaccination of 288/330$^{+/+}$ mice with a VRP delivering MERS-CoV spike protein protects mice from challenge with MERS-CoV. The vaccination protocol is described in Methods. **a**,**b**, After a 5×10^6 p.f.u. challenge, mortality (**a**) and mouse weight (**b**) were monitored daily for mice receiving GFP–VRP ($n = 19$) or spike–VRP ($n = 19$) vaccine up to day 6 p.i. Data reflect the percentage survival (**a**) or daily mean weight ± s.d. (**b**). **c**, Viral lung titres were determined on day 3 p.i. ($n = 7$ for spike–VRP and GFP–VRP) and day 6 p.i. ($n = 12$ for spike–VRP; $n = 6$ for GFP–VRP). The limit of detection (LOD) is indicated. Bars are means + s.d. **d**, Lung function was assessed by Penh at 0, 3 and 6 days p.i. for mice receiving GFP–VRP ($n = 12$ at days 0 and 3 p.i.; $n = 6$ at day 6 p.i.) or spike–VRP ($n = 12$ at all days p.i.). Data represent means + s.d. Student's *t*-test was used to compare mice receiving GFP–VRP with those receiving spike–VRP at days 3 and 6 p.i. (*$P < 0.05$; **$P < 0.01$).

Discussion

The MERS-CoV preclinical mouse model described here demonstrates for the first time that the CRISPR–Cas9 system can be used to genetically edit a non-permissive host receptor to generate a susceptible model for an emerging infectious pathogen. The 288/330$^{+/+}$ MERS-CoV mouse model resembles the severe, and often fatal, respiratory distress syndrome observed in humans, and can be prevented through treatment with the 3B11 neutralizing monoclonal antibody[19] or a VRP-based vaccine directed against the MERS-CoV spike protein[15,20]. Coupled with an inability to elicit escape mutants, 3B11 has also been tested in NHPs[5], making it an excellent candidate for downstream human studies. However, existing NHP models rely on quantitative RT-PCR, rather than measures of infectious virus, to quantify viral loads, and these models do not reproducibly result in the severe respiratory disease or mortality observed in human MERS patients[1–4]. Therefore, the results from our model complement the NHP 3B11 studies by directly demonstrating that 3B11 reduces levels of infectious MERS-CoV in the lungs, while preventing severe virus-induced respiratory pathology, loss of respiratory function and mortality.

In fatal cases of human MERS-CoV infections, individuals exhibit severe respiratory distress requiring mechanical ventilation, and the only published human autopsy from a MERS-CoV fatality reported histopathology that included diffuse alveolar damage with denuding of bronchiolar epithelium, hyaline membrane formation, type II pneumocyte hyperplasia and oedema[18]. Furthermore, MERS-CoV antigen staining localized the virus to pneumocytes and syncytial cells[18]. Infection of type I and II pneumocytes can lead to cell death, as observed in autopsies of patients who have died from severe respiratory infections with influenza virus and SARS-CoV[21,22]. Moreover, pneumocyte cell death has been proposed to cause decreased respiratory function, as measured by whole-body plethysmography in a mouse model of influenza[23]. Commensurate with these previous studies, our MERS-CoV mouse model demonstrated widespread infection of pneumocytes and pathology consistent with diffuse alveolar damage and severe respiratory disease. This was corroborated by decreased pulmonary function in the MERS-CoV model, as measured by plethysmography, which may be associated with widespread infection and possibly the death of pneumocytes and airway epithelial cells. Therefore, this model system provides the field with the opportunity to investigate the mechanisms that lead to MERS-CoV-induced pathology and severe respiratory disease, in the absence of any CNS complications.

Other MERS-CoV mouse models have used the more traditional method of expressing the full-length human DPP4 receptor to facilitate MERS-CoV infection[12–15,24]. These models exhibited infection/

replication in the lungs following intranasal administration at low viral doses (10^2–10^5 p.f.u.), which in some cases resulted in pathology indicative of pneumonia-like disease[12–14]. One limitation of the 288/330$^{+/+}$ MERS-CoV model described here was the use of high viral loads to achieve severe, and often fatal, respiratory disease. Further adaptation may allow the use of lower infectious doses that would produce a model of mild disease with subsequent recovery at later time points, as has been described with SARS-CoV[25]. In this context, it is interesting that the deletion of MERS-CoV ORF4b, a phosphodiesterase and antagonist of RNase L activity in human cells, appeared less critical for eliciting severe disease in rodents[26], suggesting possible species-specific modes of action *in vivo*. Deletions in some SARS-CoV interferon antagonist genes and accessory open reading frames have also yielded subtle changes in overall virulence *in vivo*[27,28]. However, it is important to point out that a balance must be achieved between the virulence of mouse-adapted virus, which enhances the model's capacity to replicate human disease phenotypes, and the number of mutations required to significantly reduce the dose lethal to 50% of animals tested.

Conventional models using constitutive overexpression of the hDPP4 MERS-CoV receptor have demonstrated widespread infection of extrapulmonary tissues including brain, kidney, liver, spleen and heart[12–14], and two of these studies indicated that the mice exhibited multi-organ failure[13,14]. Furthermore, high viral loads were detected in the brains of mice in the transgenic hDPP4 overexpression models[12–14]. Importantly, Li *et al.*[13] concluded that "mortality correlated with brain infection, suggesting that infection of this organ was most important for the high mortality observed in K18-hDPP4 mice". While these lethal models have value for vaccine and immunotherapeutic testing[29], small-molecule inhibitors that are effective in the lung may be limited in their efficacy due to an inability to cross the blood–brain barrier[30]. Similar neurological complications have been observed in model systems for SARS that used overexpression, or tissue-specific constitutive promoters, to express human angiotensin 1-converting enzyme 2 (ACE2) in mice[31]. While additional human pathology studies are needed to determine the extent of extrapulmonary sites of MERS-CoV replication and their impact on MERS-CoV disease, it is clear that respiratory replication and pathology is an important aspect of human MERS-CoV disease. Therefore, an important attribute of the 288/330$^{+/+}$ model is that the lack of detectable virus replication in the CNS means this model can be used to study MERS-CoV-induced pulmonary disease without the confounding effects of death due to CNS infection.

Recently, a debate has emerged around the safety of performing gain-of-function (GOF) studies with highly pathogenic viruses (such as MERS-CoV, SARS-CoV, influenza virus H5N1) (http://www.gryphonscientific.com/gain-of-function/). As demonstrated here, GOF studies were absolutely necessary to develop a mouse model that reflects the ARDS pathology observed previously in humans infected with respiratory pathogens. Importantly, the GOF studies performed here yielded MERS-CoV strains that reflect the complexity of clinical isolates identified recently in humans, where deletions were identified in ORF3 and ORF4A[32]. Moreover, these GOF studies have allowed us to identify mutations in MERS-CoV proteins that may influence how MERS-CoV interacts with, and possibly circumvents, host immune responses. Nevertheless, future studies to evaluate host factors that contribute to MERS-CoV disease will be constrained in this model, as well as in hDPP4 expression models, by the fact that the mice must be backcrossed to mouse lines harbouring modified endogenous genes, such as knock-out mice. This limitation may be overcome through additional GOF studies that facilitate MERS-CoV adaptation to the innate mDPP4 receptor molecule. The continued threats from novel emerging pathogens, such as Zika virus, will demand the rapid development of physiologically relevant animal models to evaluate therapeutic countermeasures, thereby necessitating virus adaptation to host immunity to achieve effective models. It is critical that the GOF regulatory structure does not impede the development of robust animal models of human disease, which are essential for protecting the public health.

Methods

Viruses, cells and plaque assays. All virus stocks were prepared on Vero CCL81 cells (ATCC). CCL81 cells were maintained routinely in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% fetal bovine serum (FBS; Sigma) and 1× antibiotic/antimycotic (Gibco). All viruses were harvested in Opti-MEM medium (Gibco) supplemented with 3% FBS, 1× antibiotic/antimycotic, 1× non-essential amino acids (Gibco) and 1× 1 mM sodium pyruvate (Gibco). The wild-type HCoV-EMC/2012 strain of MERS-CoV was used at passage 10 (originally provided by Bart Haagmans (Erasmus Medical Center Rotterdam, The Netherlands) at passage 8), icMERS was generated previously by Scobey *et al.*[33], MERS camel strain Dromedary/Al-Hasa-KFU-HKU13/2013 was used at passage 5 and was provided at passage 4 by Malik Peiris (University of Hong Kong, China) and MERS-0 was generated in the laboratory of R.S.B. as described below. Virus titres were determined by plaque assays on Vero CCL81 cells[33]. All viruses were maintained under Biosafety Level (BSL) 3 conditions with redundant fans and personnel powered air-purifying respirators, scrubs, Tyvek suits, Tyvek aprons and double layers of gloves.

An icMERS virus tagged with tomato red fluorescent protein[33] was passaged for ten rounds on NIH/3T3 cells (ATCC) that were generated to stably overexpress the mDPP4 receptor containing A288L and T330R. The mDPP4 A288L/T330R expression cassette used to generate the cell line has been described previously[7]. Sequencing of the passaged virus identified an insertion of three amino acids (RMR) after amino acid 884 in the spike protein and an S885L change. These changes were subcloned back into the MERS-CoV infectious clone by overlap-PCR of the F fragment with the following primers: 5′-GGTTTCCAGAAGTGTGAGCAATTA CTGCGCG-3′, 5′-GCAGGCCTCTGCAGTCGACGGGCCCGGGATCCAA TGCC-3′, 5′-CCTGTTTCTATATCTACTGGCAGTCG TAGAATGCGGCTTGCA CGTAGTGCTATTGAGGATTTGC-3′ and 5′-GCAAATCCTCA ATAGCACTA CGTGCAAGCCGCATTCTACGACTGCCAGTAGATATAGAAACAGG-3′. The F fragment is one part of a seven-plasmid system (A, B, C, D1, D2, E and F) that permits partitioning of the entire genome to generate infectious MERS-CoV, as described previously[33]. The PCR product encoding the insertion was subcloned back into the F fragment using the restriction enzymes *Msc*I and *Bam*HI and validated by sequencing. Recombinant MERS-0 virus was used for the *in vivo* passage experiments, which were GOF studies reviewed and approved by the National Institutes of Health (NIH). Vero CCL81 and NIH/3T3 cells were originally received from the ATCC, which indicates that cell lines are authentic and confirms that cell lines are mycoplasma free. None of the working cell line stocks was authenticated or tested for mycoplasma recently, although the original seed stocks used to create the working stocks are free from contamination. Additionally, both cell lines have been authenticated by morphological and cytopathological evaluation. Furthermore, Vero CCL81 cells were confirmed for DPP4 overexpression by the capacity to be infected and to replicate MERS-CoV.

Generation of mice with mDPP4 modified at positions 288 (exon 10) and 330 (exon 11). The alleles encoding amino acids 288 and 330 are shown in Supplementary Fig. 1. Genomic engineering of these alleles with the CRISPR–Cas9 genome editing system was performed at the University of North Carolina at Chapel Hill (UNC-CH) Animal Models Core Facility. The messenger RNA (mRNA) encoding Cas9 endonuclease and guide RNAs (gRNAs) were based on the system established by Mali *et al.*[34] and prepared as described below. The gRNAs (Supplementary Table 1) were generated by an *in vitro* transcription reaction using a T7 High Yield RNA Synthesis kit (NEB), where 1 μg *Dra*I-linearized template DNA was used in a 20 μl reaction following the kit guidelines for short RNA transcripts. The reaction was incubated at 37 °C overnight, followed by DNase I (RNase-free) digestion for 15 min at 37 °C. The gRNAs were then purified using an RNeasy column (Qiagen) following the guidelines for short RNA purification. Capped and polyadenylated Cas9 mRNA was prepared by an *in vitro* transcription reaction using an mMESSAGE mMACHINE T7 ULTRA kit (Life Technologies). Capped mRNA was generated with 1 μg hCas9-T7 linearized plasmid DNA in a 20 μl reaction containing 1× NTP/ARCA Solution, 1× T7 reaction buffer and 2 μl T7 enzyme. The reaction was incubated at 37 °C for 1 h, followed by addition of 1 μl TURBO DNase and digestion at 37 °C for 15 min. To add a poly(A) tail to the capped mRNA, the 20 μl reaction mix from step 1 was mixed with nuclease-free water (36 μl), 5× *E*-PAP buffer (20 μl), 25 mM $MnCl_2$ (10 μl), ATP solution (10 μl) and *E*-PAP (4 μl) to a final reaction volume of 100 μl. The reaction was incubated at 37 °C for 30–45 min. The capped and polyadenylated RNA was purified by lithium chloride precipitation and resuspended in microinjection buffer (5 mM Tris/HCl buffer, 0.1 mM EDTA, pH 7.5).

Fertilized zygotes were collected from C57BL/6J females that had been superovulated and mated to C57BL/6J males. Pronuclear microinjection was performed with 50 ng μl^{-1} Cas9 mRNA, 50 ng μl^{-1} gRNA Dpp4-g59B, 25 ng μl^{-1} gRNA Dpp4-g88B and 50 ng μl^{-1} each of Dpp4-A288L-B and Dpp4-T330R-B

donor oligonucleotides (Supplementary Table 1). Injected embryos were implanted into pseudopregnant recipients, and the resulting pups were screened for alleles encoding changes (Supplementary Fig. 1) at positions 288 and 330. Mutations at the 288 position were detected by amplifying biopsy DNA samples with the following primers: Dpp4-E10ScF1: 5′-GATTCTGAGCAAGCAAACACGC-3′, and Dpp4-E10ScR1: 5′-CCACAAGGTATCCCACAGAGACG-3′. The 752 bp PCR product was sequenced with primer Dpp4-E10-SqR1: 5′-CAAGAACCACACCAATGGAAAGTC-3′. Mutations at the 330 position were detected by amplifying biopsy DNA samples with the following primers: Dpp4-E11ScF1: 5′-AAGTGCTGGGATTATAGGTGGTCAC-3′, and Dpp4-E11ScR1: 5′-GTGTTTACATTCTAAGTTGGGTTTCTGC-3′. The 767 bp PCR product was sequenced with primer Dpp4-E11-SqF1: 5′- GCATGTTATCCACTGTGCCATCTC-3′. Five of 66 live animals produced showed evidence of both the 288 and 330 modifications. Founders with both expected modifications were backcrossed to C57BL/6J mice to identify animals with the 288 and 330 modifications *in cis*. F_1 animals with both the 288 and 330 modifications were then intercrossed to produce homozygous breeder pairs for colony enrichment and downstream studies.

Mouse infections. Genetically engineered mice with a modified mDPP4 receptor were housed and bred in accordance with guidelines established by the Department of Laboratory Animal Medicine at UNC-CH. As the 288/330$^{+/+}$ and 288/330$^{+/-}$ mice are novel mouse lines developed in the laboratory of R.S.B., experiments utilized available male and female mice that ranged from 12 to 20 weeks of age. Based on availability at the time of each experiment, experimental and control animals were age- and sex-matched. No blinding was used in any animal experiments, and animals were not randomized. Sample sizes were determined from preliminary data that would yield statistically significant differences. Mouse studies were executed under animal BSL3 conditions as described previously[35]. Before viral infection, mice were anesthetized by administering 50 µl ketamine/xylazine mixture intraperitoneally and then infected intranasally with 50 µl virus solution containing 5×10^6 p.f.u. Incomplete infections due to bubbling of inoculum from the nasal cavity, the inability to inhale the entire dose or inoculum going into the mouth were noted, and these mice were considered failures and were excluded, as described previously[35]. Following sedation and infection, mice were monitored daily for weight loss and survival, as well as for signs that the animals were moribund (including laboured breathing, lack of movement and lack of grooming). Mice that reached 20% weight loss were placed under exception and monitored at least twice daily. Mice that approached 30% weight loss were euthanized immediately. Mice deemed moribund were euthanized at the discretion of the researcher. Mice were euthanized with an isoflurane overdose followed by a secondary thoracotomy, at various time points, to collect lung tissues. In the absence of a thoracotomy, cervical dislocation was used as a secondary euthanasia method. All are approved methods of the Institutional Animal Care and Use Committee (IACUC) at the UNC-CH.

Ethics statement. Mouse studies were carried out in accordance with the recommendations for the care and use of animals by the Office of Laboratory Animal Welfare at NIH. IACUC at UNC-CH approved the animal studies performed here (protocol, IACUC 13-272), using a weight loss cut-off point of ~30% for humane euthanasia. Synthetically reconstructed MERS-CoVs were approved by the UNC-CH Institutional Biosafety Committee, which also considered GOF research concerns before execution of these experiments.

Analysis of serum glucose levels. All blood glucose measurements were taken following a 6 h fast. Blood glucose was measured by tail clip sampling using an AlphaTRAK 2 glucometer (Abbott Laboratories)[36]. This system is designed for use in laboratory mice, with a normal range of 111–205 mg dl^{-1} (6.1–11.38 mmol l^{-1}) blood glucose and a detection limit of 20–750 mg dl^{-1} (1.1–41.62 mmol l^{-1}) blood glucose.

Adaptation of MERS-0 in humanized mice. The recombinant virus MERS-0 was passed through the lungs of 288/330$^{+/-}$ mice every 3 days for 15 passages to obtain a MERS-CoV that was adapted to cause respiratory disease in mice (MERS-15). At each passage, the lungs were homogenized and 50 µl lung homogenate was used for intranasal infection of a naïve 288/330$^{+/-}$ mouse. The MERS-15 mouse-adapted virus was assessed in mice by survival, weight loss, lung titre, haemorrhaging, respiratory function and histopathology indicative of diffuse alveolar damage and ARDS. Clonal isolates of the MERS-15 virus were obtained by plaque purification and amplification in Vero CCL81 cells, and were sequenced to determine the mutations acquired during mouse adaptation of the virus. All sequencing was performed at the UNC-CH Genome Analysis Facility. MERS-15 C2 reproduced the severe respiratory disease observed with MERS-15; therefore, all subsequent experiments were performed with the plaque-purified MERS-15 C2.

Human monoclonal antibody 3B11 protection study. The 288/330$^{+/+}$ mice were prophylactically administered 250 µg of human monoclonal antibody 3B11 or isotype control antibody F10 by intraperitoneal injection[19] 12 h before infection with MERS-15 C2. Mice were monitored daily for weight and survival, and killed at days 3 and 6 p.i. to collect the lungs for histology, for viral titre assessment by plaque assay and for evaluation of lung haemorrhaging. Respiratory function was measured at day 0 to establish a baseline and again at days 3 and 6 p.i. The isolation and production of the 3B11 and F10 antibodies has been described previously[19].

Spike–VRP vaccine study and virus neutralization assay. The MERS-CoV gene encoding the spike protein and GFP gene were packaged into VRPs generated with helper constructs from the V3526 attenuated strain of Venezuelan equine encephalitis virus, under BSL2 conditions, as described previously[20]. Mice were administered a primary vaccination of 10 µl by footpad injection of spike–VRP (1×10^5 p.f.u.) or control GFP–VRP (1×10^5 p.f.u.). After 28 days, the mice were boosted with the same dose of their respective VRP strain. At 28 days postboost, all vaccinated mice were challenged with 5×10^6 p.f.u. MERS-15 C2. Mice were monitored daily for weight and survival, and killed at days 3 and 6 p.i. to collect lungs for histology, assessment of viral titre by plaque assay and evaluation of lung haemorrhaging. Respiratory function was measured at day 0 to establish a baseline and then again at days 3 and 6 p.i.

The presence of MERS-15 C2-specific serum antibodies was assessed by a plaque reduction neutralization titre assay. Prechallenge serum samples were collected at 25 days postboost with the spike–VRP vaccine or the GFP–VRP control. Virus neutralization assays were performed as described previously[35]. The percentage of plaque reduction was calculated as: 1 − (number of plaques with spike–VRP or GFP–VRP serum/number of plaques with serum from naïve 288/330$^{+/+}$ mice) × 100.

Respiratory function. Respiratory function was measured for individual mice, at the indicated time points, as demonstrated previously for SARS-CoV and influenza A virus[17]. Briefly, individual mice were acclimated for 30 min in individual plethysmography chambers (Buxco Systems) and each breath was then quantified over a 5 min period. Mice were routinely randomized into different chambers to avoid measurement biases that could result between chambers. Data for Penh and EF_{50} were analysed as described previously by our group[17].

Histology. Lung, brain and kidney samples were placed in 10% phosphate-buffered formalin for >7 days at 4 °C for fixation. Fixed tissue samples were then removed from the BSL3, placed into cassettes for embedding in paraffin and submitted to the Lineberger Comprehensive Cancer Center Animal Histopathology Core for processing, sectioning and staining. Tissue sections (5 µm) were stained with haematoxylin and eosin, and MERS-CoV nucleocapsid antigen was detected using mouse anti-MERS nucleocapsid serum at a 1:250 dilution. The nucleocapsid antiserum was generated in the laboratory of R.S.B. using MERS-CoV nucleocapsid–VRP particles in BALB/c mice as described previously[20]. Antigen was visualized using 3,3′-diaminobenzidine staining. An Olympus DP71 camera attached to an Olympus BX41 microscope was used to capture images with ×10 and ×40 objectives. Histopathology was scored, blinded to infection and animal status, for airway disease, vascular disease, parenchymal pneumonia, diffuse alveolar damage, eosinophils and immunohistochemistry on a scale of 0–3 (0, none; 1, mild; 2, moderate; 3, severe).

Flow cytometry analysis. Whole peripheral blood from each mouse strain was collected in EDTA and peripheral blood mononuclear cells were purified using density-gradient centrifugation over Ficoll-Paque PLUS (GE Healthcare Life Sciences). Mouse lung tissues were first dissociated mechanically using scalpel blades and then digested further using type A collagenase (Worthington Biochemical) in medium prepared with DNase I (1 mg ml^{-1}) for 1 h in a shaking incubator at 37 °C. Digested lung suspensions were filtered, centrifuged and treated with ACK lysis buffer (Gibco). Single-cell suspensions from blood and lung tissue were resuspended at 1×10^7 cells ml^{-1} in RPMI 1640 medium supplemented with 10% heat-inactivated FBS and antibiotic/antimycotic cocktail (all from Gibco), and were used immediately for flow cytometry. Single-cell suspensions were plated in 200 µl per well on 96-well, round-bottomed plates and stained for flow cytometry using an Intracellular Fixation and Permeabilization Buffer Set (eBioscience) according to the manufacturer's protocol and using antibodies specific for CD3 (clone 145-2C11; BD Biosciences), CD4 (clone GK1.5; BD Biosciences), CD8 (clone 53-6.7; eBioscience), CD25 (clone PC61; BD Biosciences), CD26/DPP4 (clone H194-112; Biolegend), CD69 (clone H1.2F3; eBioscience), interferon-γ (clone XMG1.2; BD Biosciences), tumour-necrosis factor-α (clone MP6-XT22; eBioscience) and interleukin-2 (clone JES6-5H4; eBioscience). Cells were also treated with a fixable LIVE/DEAD discriminator (Invitrogen). Stained cells were fixed for 20 min in 1% paraformaldehyde, resuspended in PBS and stored at 4 °C before acquisition within 24 h. Cell populations were first gated to exclude (1) debris, (2) doublet events and (3) dead cells before phenotypic and functional gating. All samples were stained in parallel with controls of fluorescence – 1. A minimum of 100,000 live, singlet events were acquired per sample, and data were analysed using FlowJo software (TreeStar).

Northern blot analysis and RT-PCR. Northern blot analysis was performed for detection of full-length mDPP4 in the lungs of 288/330$^{+/+}$, 288/330$^{+/-}$ and C57BL/6J wild-type mice. Poly(A) RNA was isolated to eliminate ribosomal RNA (rRNA; Qiagen). Equivalent amounts of RNA were resolved on 0.8% agarose gels and transferred to nitrocellulose membrane. A biotinylated probe (5′-biotin-gATg (biotin-dT)gCTggTgAgCTgTgCTgCTAgCgATCCCgTggTCTTCATCC-3′) was used to detect mDPP4.

To quantify viral and targeted host mRNAs, MERS-CoV, mDPP4 and mouse glyceraldehyde 3-phosphate dehydrogenase (GAPDH) RNAs were measured in brain and lung tissue from MERS-CoV-infected mice. Briefly, tissues were removed and placed into RNALater (Ambion) solution and stored at −80 °C until analysis by RT-PCR. Lung tissue was homogenized in TRIzol reagent (Invitrogen) and isolated according to the manufacturer's instructions. Standard RT-PCR was performed with the following primer pairs for each of the indicated RNAs: MERS-CoV subgenomic leader sequence: 5′-CTATCTCACTTCCCCTCgTTCTC-3′ and 5′- GAATCATTG TTAGGGTTCCG-3′; mouse GAPDH: 5′-CAACgACCCCTTCATTgACC-3′ and 5′-GCAGGGATGATGTTCTGGGC-3′; and mDPP4: 5′-TAACGACACAGGAGTG CCGC-3′ and 5′-TCTGCTTTTTGACTACAGGG-3′. Equivalent volumes of PCR product were resolved on gels for a non-quantitative answer (presence or absence) of viral subgenomic transcripts in the brain and lung.

Quantitative RT-PCR was carried out on a Roche LightCycler 480 II, with accompanying software, to analyse MERS-CoV viral RNA and mDPP4 mRNAs, and 18S rRNA as an endogenous control. All RNAs were reverse transcribed under standard conditions in a 20 µl reaction volume using SuperScript III reverse transcriptase (Invitrogen). MERS-CoV viral RNA was assessed using the following primers at 900 nM in a 20 µl reaction in a SYBR Green assay with 2× SsoAdvanced Universal SYBR Green Supermix (Biorad). The forward primer anneals at the MERS-CoV leader sequence in the 5′ untranslated region (5′-GAATAGCTTGGCTATCTCAC-3′) and the reverse primer anneals in the N gene (5′-TTGTTATCGGCAAAGGAAAC-3′). PCR conditions were 45 cycles of 95 °C for 10 s, 59 °C for 10 s and 72 °C for 15 s. Standard Taqman conditions were used to analyse expression of mDPP4 and 18S rRNAs. The following primers were used for mDPP4: 5′-CCCCAAGACAGTGTGGATTC-3′ and 5′-GAGGATGAGCTGAGAGAGTCTATATTT-3′; probe no. 51 was used from the Roche Universal ProbeLibrary. PCR conditions were 45 cycles of 95 °C for 15 s and 60 °C for 50 s. A 20× commercially available primer/probe set was utilized to quantitate the 18S rRNA (Life Technologies).

Statistical analysis. All quantitative data are presented as means ± 1 s.d. Significance between specific data sets is described in the respective figure legends and was determined by Student's *t*-test using a one-tailed or two-tailed distribution in Microsoft Excel software.

Biosafety and biosecurity. *In vivo* adaptation of the MERS-0 virus in mice had been executed before publication of the US Government's statement on funding pause on certain types of GOF research[37]. When notice to cease all *in vivo* passage experiments was received, all studies to adapt the MERS-0 virus *in vivo* were immediately halted. Following our formal written request for continuation, and on receiving an exemption from the pause and approval to continue after a National Institute of Allergy and Infectious Diseases/NIH review, the *in vivo* adaptation of MERS-0 was then continued by serial passage in mice. All studies using MERS-CoV were executed in BSL3 facilities at UNC-CH, under conditions described previously[35].

The following biosafety and biosecurity paragraph is as described by Menachery *et al.*[35] All work for these studies was performed with approved standard operating procedures and safety conditions for MERS-CoV (not a select agent), SARS-CoV (select agent) and derivatives therein. Our institutional CoV BSL3 facilities have been designed to conform to the safety requirements that are recommended by the Biosafety in Microbiological and Biomedical Laboratories, the US Department of Health and Human Services, the Public Health Service, the Centers for Disease Control and the NIH. Laboratory safety plans were submitted to, and the facility has been approved for use by, the UNC Department of Environmental Health and Safety (EHS) and the CDC. Electronic card access is required for entry into the facility. All workers have been trained by EHS to safely use powered air-purifying respirators, and appropriate work habits in a BSL3 facility and active medical surveillance plans are in place. Our BSL3 facilities contain redundant fans, emergency power to fans, and biological safety cabinets and freezers, and our facilities can accommodate SealSafe mouse racks. Materials classified as BSL3 agents consist of MERS-CoV, SARS-CoV, bat CoV precursor strains and mutants derived from these pathogens. Within the BSL3 facilities, experimentation with infectious virus is performed in a certified Class II Biosafety Cabinet. All members of staff wear scrubs, Tyvek suits and aprons, powered air-purifying respirators and shoe covers, and their hands are double-gloved. BSL3 users are subject to a medical surveillance plan monitored by the University Employee Occupational Health Clinic (UEOHC), which includes a yearly physical, annual influenza vaccination and mandatory reporting of any symptoms associated with coronvirus infection during periods when working in the BSL3. All BSL3 users are trained in exposure management and reporting protocols, are prepared to self-quarantine and have been trained for safe delivery to a local infectious disease management department in an emergency situation. All potential exposure events are reported and investigated by EHS and UEOHC, with reports filed to both the CDC and the NIH.

Data availability. The data that support the findings of this study are available from the corresponding author on request.

Received 27 April 2016; accepted 14 October 2016; published 28 November 2016

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Acknowledgements

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Author contributions

A.S.C. conceived/designed, coordinated and executed the experiments, analysed the data and wrote the manuscript. B.L.Y. developed and recovered infectious clone viruses. T.S. completed mouse experiments. K.J. designed and completed immunological experiments. M.D. helped establish and maintain the mouse colony and perform molecular analyses. A.B. helped complete the mouse experiments. X.-C.T. and W.A.M. provided critical monoclonal antibody reagents. M.T.H. and R.S.B. conceived/designed the experiments and wrote the manuscript.

Additional information

Supplementary information is available for this paper.

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Correspondence and requests for materials should be addressed to A.S.C., M.T.H. and R.S.B.

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Competing interests

W.A.M. has a financial interest in AbViro. The other authors declare no competing financial interests.

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From: Cockrell, Adam
Sent: Fri, 11 Mar 2016 17:24:56 +0000
To: Stu Greenberg; Stemmy, Erik (NIH/NIAID) [E]
Cc: Baric, Ralph; 'Leyva-Grado, Victor'; (b)(6) (b)(6)
Subject: RE: GoToMeeting Invitation - A57 Call with Ostrigen
Attachments: Timeline for OstriGen Prophylactic Study.pdf

Hi everyone,

It was good meeting everyone this morning. I have attached a PDF with timeline for the study.

Once we have the antibodies in hand we will have a better idea of when we can initiate the study.

Regards,
Adam

From: Stu Greenberg (b)(6)
Sent: Friday, March 11, 2016 11:42 AM
To: 'Stemmy, Erik (NIH/NIAID) [E]' (b)(6)
Cc: Baric, Ralph S (b)(6) Cockrell, Adam (b)(6) 'Leyva-Grado, Victor' (b)(6)
Subject: RE: GoToMeeting Invitation - A57 Call with Ostrigen

Hi Erik,

I think my Japanese colleagues were able to hear most of what went on.

Here are their email addresses:

Dr. Yasuhiro Tsukamoto - (b)(6)

Koichi Kimura – (b)(6)

Makoto Goda - (b)(6)

Regards,
Stu Greenberg

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Friday, March 11, 2016 10:22 AM
To: 'Stu Greenberg' (b)(6) Baric, Ralph (b)(6) Cockrell, Adam (b)(6) 'Leyva-Grado, Victor' (b)(6)
Subject: RE: GoToMeeting Invitation - A57 Call with Ostrigen

Hi Everyone,

Thanks for all your patience with the audio difficulties. Stu, I don't believe I have email contacts from your Japanese colleagues. Could you please send them along so UNC can circulate the protocol directly?

Thanks!
Erik

-----Original Appointment-----
From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Wednesday, March 9, 2016 6:40 PM
To: Stemmy, Erik (NIH/NIAID) [E]; 'Stu Greenberg'; Baric, Ralph; Cockrell, Adam; 'Baric, Toni C'; 'Leyva-Grado, Victor'; Umerah, Nina
Subject: GoToMeeting Invitation - A57 Call with Ostrigen
When: Friday, March 11, 2016 9:30 AM-10:30 AM (UTC-05:00) Eastern Time (US & Canada).
Where:

Hi Everyone,
Please see below for dial in information the call to discuss the Ostrigen study. You should use the link to join, and then choose either VoIP through your computer or dial in using the toll free numbers for the audio. Let me know if you have trouble connecting. A brief agenda of the call is below as well.

Stu, please forward this information to your colleagues.

Thanks!
Erik

1. Overview of the therapeutic (Ostrigen)
2. Discuss details/requirements of the study (all)
3. Discuss scheduling (UNC)

1. Please join my meeting.
https://global.gotomeeting.com/join/(b)(6)

2. Use your microphone and speakers (VoIP) - a headset is recommended. Or, call in using your telephone.

United States (toll-free): 1 877 309 2070
Japan (toll-free): 0 120 242 200

Access Code: (b)(6)
Audio PIN: Shown after joining the meeting

Meeting ID: (b)(6)

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From: Cockrell, Adam
Sent: Tue, 1 Dec 2015 21:29:24 +0000
To: Baric, Ralph; Maria Zambon; (b)(6) Baric, Toni C
Cc: Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: UPDATE MERS MABS
Attachments: Slides for LCA60 Human mAb study.pdf

Hi everyone.

Here are the slides that we discussed on yesterday's phone call. The first couple describe the advantages of our model over existing mouse models and why it is the first model to recapitulate the severe respiratory disease that believe is occurring in humans.

The last 3 slides demonstrate the timeline for the study.

Please let me know if there are any comments/questions regarding the studies.

Regards,

Adam

From: Baric, Ralph S
Sent: Monday, November 30, 2015 9:06 AM
To: Maria Zambon (b)(6) Baric, Toni C
(b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Cockrell, Adam (b)(6)
Subject: RE: UPDATE MERS MABS

From: Maria Zambon (b)(6)
Sent: Sunday, November 29, 2015 7:31 AM
To: (b)(6) Baric, Toni C
Cc: Stemmy, Erik (NIH/NIAID) [E]; Cockrell, Adam; Baric, Ralph S
Subject: RE: UPDATE MERS MABS

Colleagues,

Is there material in regards the mouse model set up by ralph Baric to be shared before this meeting tomorrow (as suggested in some of the earlier correspondance). I will be doing the phone call externally, so would appreciate an early view of any data, as I am not sure whether I will have good email access during the day tomorrow

thanks

Maria Zambon
Director, Reference Microbiology
Deputy Director, NIS
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From: (b)(6)
Sent: 10 November 2015 17:32
To: Baric, Toni C
Cc: Stemmy, Erik (NIH/NIAID) [E]; Maria Zambon; Cockrell, Adam; Ralph Baric; Robin Gopal
Subject: Re: UPDATE MERS MABS

Dear Toni,

This is fine. I can provide the call-in number.

Here it is:

UK: 0808 234 88 76
Switzerland: 0800 329 329
USA: +1 866 591 43 61 (or +1 888 50 333 35)

Participant access code: (b)(6)

Best regards,

(b)(6)

Il giorno 10 nov 2015, alle ore 17:52, Baric, Toni C (b)(6) ha scritto:

Hi Everyone,
Let's set this call for Nov 30 at 9 am EST/ 2pm UK time. Does this work? Also, does someone have a call-in number or should I set this up?
Thank you,
Toni

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Thursday, November 05, 2015 9:30 AM

To: Baric, Toni C; Maria Zambon; Cockrell, Adam; (b)(6)
Cc: Baric, Ralph S; Robin Gopal
Subject: RE: UPDATE MERS MABS

My preference would be for 11/30. I can do any time before 1pm EST.

Erik

From: Baric, Toni C (b)(6)
Sent: Thursday, November 05, 2015 9:26 AM
To: Maria Zambon (b)(6) Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Baric, Ralph (b)(6) Robin Gopal (b)(6)
Subject: RE: UPDATE MERS MABS

Hi Group,

Let's we revisit the following dates:
11/30 9-10 am EST or after 10 am EST
12/2 before 2 pm EST.

Please let me know the day and time range that works for all of you, keeping in mind that Maria will be calling in from UK.
Thanks
Toni

From: Maria Zambon (b)(6)
Sent: Wednesday, November 04, 2015 5:49 PM
To: Cockrell, Adam; Baric, Toni C; Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
Cc: Baric, Ralph S; Robin Gopal
Subject: RE: UPDATE MERS MABS

Hello,

Sorry if late to the party. I have already sent back a note saying the Monday of this week would work for me. Unfortunately I will be in Hng Kong the 17th to 20th, so would suggest we try earlier if we can

maria

Maria Zambon
Director, Reference Microbiology
Deputy Director, NIS
National Infection Service
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From: Cockrell, Adam (b)(6)
Sent: 04 November 2015 20:21
To: Baric, Toni C; Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
Cc: Baric, Ralph S; Maria Zambon; Robin Gopal
Subject: RE: UPDATE MERS MABS

That sounds good for me.
Thanks,
Adam

From: Baric, Toni C
Sent: Wednesday, November 04, 2015 3:11 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Baric, Ralph S (b)(6) Maria Zambon (b)(6) Cockrell, Adam (b)(6) Robin Gopal (b)(6)
Subject: RE: UPDATE MERS MABS

11/20 sounds good. How about 10 am? If this works for everyone, please let me know. Otherwise, please suggest a time before 1 pm that suits or a different day.
Thank you,
Toni

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, November 04, 2015 3:07 PM
To: (b)(6) Baric, Toni C
Cc: Baric, Ralph S; Maria Zambon; Cockrell, Adam; Robin Gopal
Subject: RE: UPDATE MERS MABS

On 11/20 I can do any time before 1pm EST. Can we aim for that date?

Erik

From: (b)(6)
Sent: Wednesday, November 4, 2015 3:02 PM
To: Baric, Toni C (b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Baric, Ralph (b)(6) Maria Zambon (b)(6) Cockrell, Adam (b)(6) Robin Gopal (b)(6)
Subject: Re: UPDATE MERS MABS

Dear Toni,

I am available on all dates with the exception of 12/4.

Best regards,

(b)(6)

Il giorno 04 nov 2015, alle ore 20:25, Baric, Toni C (b)(6) ha scritto:

How about the following:

Friday 11/20 Ralph is open all day. Then the next day is 11/30 –after 11, Wednesday 12/2 before 2 and all day on 12/4.

Best regards,
Toni

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, November 04, 2015 2:13 PM
To: Baric, Toni C; Baric, Ralph S; Maria Zambon; Cockrell, Adam
Cc: (b)(6) Robin Gopal
Subject: RE: UPDATE MERS MABS

I am leaving for a meeting in Riyadh on 11/12, so we'll have to schedule a call after I return on 11/16.

Erik

From: Baric, Toni C (b)(6)
Sent: Wednesday, November 4, 2015 12:06 PM
To: Baric, Ralph (b)(6) Maria Zambon (b)(6) Cockrell, Adam (b)(6)
Cc: (b)(6) Robin Gopal (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Subject: RE: UPDATE MERS MABS

Hi Maria,
Ralph is available on 11/12 after 3:30 and between 11:30-3 on Friday 11/13
Best regards,
Toni

From: Baric, Ralph S
Sent: Tuesday, November 03, 2015 4:11 PM
To: Maria Zambon; Cockrell, Adam; Baric, Toni C
Cc: (b)(6) Robin Gopal; Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: UPDATE MERS MABS

Hi Maria, We have recently received 20mg of pure antibody from (b)(6) and have support by Erik Stemmy to perform your studies in the mouse model. Initially, we will evaluate protection prior to infection. We currently don't have approval to use sharps for therapeutic intervention postinfection, but are in the process of putting in the paperwork to administer drug postinfection. I recommend a two phase study, first prior to infection to demonstrate efficacy and then drug dose at day 1 or 2 postinfection (single dose?). We likely need to set up a time to discuss the experiments. We will also share the model details at that time. Toni can assist. We also are planning on doing the protection study in early December, post infection study would likely be jan at best. Adam is a key contact person for discussion. Hope you are doing well. It's a pleasure to work with you again. Thoughts? ralph

---

From: Maria Zambon (b)(6)
Sent: Friday, October 23, 2015 3:52 PM
To: Baric, Ralph S
Cc: (b)(6) Robin Gopal
Subject: UPDATE MERS MABS

Dear Ralph,

Greetings , we have not corresponded for a while…I think another pesky virus (Ebola) has caused a bit of a diversion for all of us. (b)(6) has mentioned that you have developed a new animal model for MERS which is transgenic, and is very sensitive. This is just a brief note to explore the possibility of extending mouse model work for LCA60. We have submitted a proposal to the Medical Research Council (MRC) in the UK to take LCA60 into a Phase 1 clinical study. This proposal included the costs for Phase 1 scale up to GMP and also a Phase 1 Pk study in healthy volunteers, and is a large proposal.

The response from the MRC has been favourable, but they are requesting strengthening of the pre-clinical package in the proposal to try and give more indication of how the Mab could be used. We would appreciate your advice/collaboration in this

(1) Could we propose more work in your mouse model to extend understanding of prophylaxis duration and the window for treatment. I am thinking about extending the time points post infection at which Mab is given and also refining knowledge of the duration of protection if given before challenge. One of the questions we are asked to address is to what are the parameters under which this might be used clinically. Currently the data we hold is more of a YES/NO format, rather than a considered model approach to window of treatment opportunity.
(2) If you thought some more work was feasible, would this be possible without provision of funding from us under existing NIH contracts, or would you require additional funding, and if so, what would that be . (NB could we also slip in some work on LCA57, the non neutralising Mab that we have got ?). We would be pleased to include you as a co-applicant for MRC funding, subject to MRC rules for overseas applicants, but the full proposal application cannot be submitted before March, meaning that you might well have already done the work before we could provide any funding
(3) What is your advice about whether the animal model you have developed is suitable for use…Suggestions welcome

Grateful for a rapid response

Maria Zambon
Director, Reference Microbiology
Deputy Director, NIS
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From: Cockrell, Adam
Sent: Mon, 20 Feb 2017 15:12:00 +0000
To: Keith Wycoff
Cc: Stemmy, Erik (NIH/NIAID) [E]; Leyva-Grado, Victor; Baric, Ralph
Subject: RE: Study with Planet Biotech.
Attachments: Timeline for initial study.pdf

Hi Keith,

You are correct. We are definitely going with 400ug/mouse. I just did not change it on the outline. Corrected outline is attached.

Thanks,
Adam

From: Keith Wycoff (b)(6)
Sent: Monday, February 20, 2017 10:09 AM
To: Cockrell, Adam (b)(6)
Cc: Erik [E] Stemmy (b)(6) Leyva-Grado, Victor (b)(6)
Baric, Ralph S (b)(6)
Subject: Re: Study with Planet Biotech.

Hi Adam,

I just want to make sure I understand the doses, and thus how much drug we need to supply. Our original discussion contemplated administering an amount, on a molar basis, equivalent to antibodies you have tested before. I had understood that 250 µg of antibody had been administered, and due to the greater molar mass of our protein the equivalent amount of DPP4-Fc would be 380 µg, which you suggested rounding up to 400 µg. Did you intend to divide the 400 µg into two doses (of 200 µg each) or administer two doses of 400 µg each? In any case, the numbers differ from the two 250 µg doses on the study design you sent. Please confirm how much protein you want to administer at -12 and +12 hours. Also, please confirm that you wanted the concentration to be 2 mg/ml (if that is the case).

Thanks,
Keith

On Feb 20, 2017, at 6:40 AM, Cockrell, Adam (b)(6) wrote:

Hi everyone,

We have approval to begin the study with Planet Biotech. I would like to schedule this to begin on Friday March 10. I have included the study time line in this email just as a reminder. Also, I bumped the mouse

numbers for the Day 6 time point to 20 (10 for each of the therapeutic and control). Want to make sure that we have enough mice by day 6.

Keith: The address to send S2320-Gal-SF, and control, to is as follows:

Attn: Adam Cockrell
University of North Carolina at Chapel Hill
Department of Epidemiology/#4635
135 Dauer Dr.
Room 3105 MHRC
Chapel Hill, NC
27599

Phone number is below.

Adam Cockrell
Research Associate
Department of Epidemiology
University of North Carolina at Chapel Hill
Chapel Hill, NC, 27599
Lab Phone: (b)(6)
Office Phone: (b)(6)

<Timeline for initial study.pdf>

Page 167 of 455

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

From: Hobbs, Ron Lee
Sent: Wed, 22 Mar 2017 18:37:51 +0000
To: Normil, Carine (NIH/NIAID) [C]; Stemmy, Erik (NIH/NIAID) [E]
Cc: Baric, Ralph; Caldwell, Chandra
Subject: FW: Publication Compliance for Grant Number: 5R01AI110700 - 03 PI Name: Baric, Ralph S
Attachments: Baric Response_R01AI110700-03.pdf

Carine,

Attached, please find the requested publication compliance for Grant Number: 5R01AI110700 – 03. At any time, please feel free to inform us if additional documentation is required. Thanks in advance.

Ronald L. Hobbs Sr.
Contracts and Grants Specialist
The University of North Carolina at Chapel Hill
Office of Sponsored Research
104 Airport Drive, Suite 2200
CB#1350
Chapel Hill, NC 27599-1350
(b)(6)

From: "Normil, Carine (NIH/NIAID) [C]" (b)(6)
Date: March 17, 2017 at 5:14:41 PM EDT
To: (b)(6)
Cc: "Stemmy, Erik (NIH/NIAID) [E]" (b)(6) "Baric, Ralph" (b)(6)
Subject: Publication Compliance for Grant Number: 5R01AI110700 - 03 PI Name: Baric, Ralph S

Dear Authorized Organization Representative,

NIAID has found publication compliance issues on the RPPR for this award because three publications listed in Section B., were not reported in Section C. Public Access MyNCBI report.

The publications below are funded from this award and must to be reported for compliance with NIH public access. The publications below will need to be submitted via email in pdf copy from MyNCBI.

- Structure, Function, and Evolution of Coronavirus Spike Proteins. Li F. Annu Rev Virol. 2016 Sep 29;3(1):237-261.PMID: 27578435
- MERS-CoV spike protein: a key target for antivirals. Du L, Yang Y, Zhou Y, Lu L, Li F, Jiang S. Expert Opin Ther Targets. 2017 Feb;21(2):131-143. doi: 10.1080/14728222.2017.1271415. PMID: 27936982
- Recombinant Receptor-Binding Domains of Multiple Middle East Respiratory Syndrome Coronaviruses (MERS-CoVs) Induce Cross-Neutralizing Antibodies against Divergent Human and Camel MERS-CoVs and Antibody Escape Mutants.Tai W, Wang Y, Fett CA, Zhao G, Li F, Perlman S, Jiang S, Zhou Y, Du L.J Virol. 2016 Dec 16;91(1). pii: e01651-16. PMID: 27795425

Additionally, please provide an updated other support document for Fr. Fang Li which includes the level of effort for each active support.

Submission deadline for the above documents is **March, 22, 2017**.

Thank you,
Carine

Carine Normil

Grants Management Specialist (Contractor)

Grants Management Program, DEA, NIAID, NIH, HHS
5601 fishers Lane, Rm 4G46, Bethesda , Maryland 20892

Phone: (b)(6)

Fax: (301)-493-0597
Email: (b)(6)

NIH National Institute of Allergy and Infectious Diseases

UNC
GILLINGS SCHOOL OF
GLOBAL PUBLIC HEALTH

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

DEPARTMENT OF EPIDEMIOLOGY F 919.966.2089
McGAVRAN-GREENBERG HALL
CAMPUS BOX 7435
CHAPEL HILL, NC 27599-7435

March 22, 2017

Carine Normil
Grants Management Specialist (Contractor)
Grants Management Program, DEA, NIAID, NIH, HHS
5601 fishers Lane, Rm 4G46,
Bethesda , Maryland 20892

RE: Publication Compliance for Grant Number: 5R01AI110700-03, PI Name: Baric, Ralph S

Dear Ms. Normil,

Per the email notice you sent on March 17, the updated other support document for Dr. Fang Li including his level of effort is attached. Also, I have attached the PDF file from MyNCBI in regards to the following three publications:

- Structure, Function, and Evolution of Coronavirus Spike Proteins. Li F. Annu Rev Virol. 2016 Sep 29;3(1):237-261.PMID: 27578435 – Undergoing NIHMS submission
- MERS-CoV spike protein: a key target for antivirals. Du L, Yang Y, Zhou Y, Lu L, Li F, Jiang S. Expert Opin Ther Targets. 2017 Feb;21(2):131-143. doi: 10.1080/14728222.2017.1271415. PMID: 27936982 – Undergoing NIHMS submission
- Recombinant Receptor-Binding Domains of Multiple Middle East Respiratory Syndrome Coronaviruses (MERS-CoVs) Induce Cross-Neutralizing Antibodies against Divergent Human and Camel MERS-CoVs and Antibody Escape Mutants.Tai W, Wang Y, Fett CA, Zhao G, Li F, Perlman S, Jiang S, Zhou Y, Du L.J Virol. 2016 Dec 16;91(1). pii: e01651-16. PMID: 27795425 – Publication is compliant per the attached documentation

If you have any other additional questions, please let me know.

Sincerely,

(b)(6)

Ralph Baric, PhD
Professor of Epidemiology
Principal Investigator of Grant No: R01AI110700-03

Other Support

LI, FANG

Active

National Institutes of Health 04/01/15 – 03/31/20
R01AI110700 Annual Direct Costs $245,000 4.8 cal months
Role: Co-Principal Investigator (contact Co-PI: Ralph Baric, University of North Carolina)
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
Goal: This research investigates genetic pathways regulating MERS coronavirus cross species transmission and receptor homolog usage, establishes robust animal models of human disease, and discovers critical reagents for therapeutic and vaccine testing.

National Institutes of Health 06/07/16 – 05/31/21
R01AI089728 Annual Direct Costs $217,800 3.0 cal months
Role: Principal Investigator
Receptor recognition and cell entry of coronaviruses
Goal: This research investigates how coronaviruses recognize their receptors and how they interact with receptors from different hosts. It explores novel principles governing viral evolution, virus-receptor interactions, viral host ranges and cross-species infections, and may lead to new approaches in the prevention and treatment of coronavirus infections in humans and other animals.

AHC Faculty Research Development Grant, 09/01/16 – 08/31/18
University of Minnesota Annual Direct Costs $30,000 0.12 cal month
Role: Co Principal Investigator (contact PI: Robert Geraghty, University of Minnesota)
Development of biological and structural approaches to Zika virus drug discovery
Goal: This research develops and implements the tools necessary to identify small molecule inhibitors of Zika virus, and also elucidates the structure/function of the viral RNA-dependent RNA polymerase.

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Non-compliant	Du L, Yang Y, Zhou Y, Lu L, Li F, Jiang S. MERS-CoV spike protein: a key target for antivirals. Expert Opin Ther Targets. 2017 Feb;21(2):131-143. doi: 10.1080/14728222.2017.1271415. PubMed PMID: 27936982.
Complete	Tai W, Wang Y, Fett CA, Zhao G, Li F, Perlman S, Jiang S, Zhou Y, Du L. Recombinant Receptor-Binding Domains of Multiple Middle East Respiratory Syndrome Coronaviruses (MERS-CoVs) Induce Cross-Neutralizing Antibodies against Divergent Human and Camel MERS-CoVs and Antibody Escape Mutants. J Virol. 2016 Dec 16;91(1). pii: e01651-16. PubMed PMID: 27795425; PubMed Central PMCID: PMC5165220.
Non-compliant	Li F. Structure, Function, and Evolution of Coronavirus Spike Proteins. Annu Rev Virol. 2016 Sep 29;3(1):237-261. PubMed PMID: 27578435.

Manuscript Summary

Status	Undergoing NIHMS submission review and file preparation
Manuscript Title	Structure, Function, and Evolution of Coronavirus Spike Proteins.
Journal Title	Annual review of virology
NIHMSID	861907
PDF Receipt	PDF Receipt [2017-03-21 15:42:56, 5,416.4 KB]
Release Delay	Set to release to PubMed Central **immediately** after publication in the journal.
Reviewer	Fang Li

Manuscript Summary

Status	Undergoing NIHMS submission review and file preparation
Manuscript Title	MERS-CoV spike protein: a key target for antivirals.
Journal Title	Expert opinion on therapeutic targets
NIHMSID	861913
PDF Receipt	PDF Receipt [2017-03-21 15:49:30, 1,399.9 KB]
Release Delay	Set to release to PubMed Central **immediately** after publication in the journal.
Reviewer	Fang Li

From: Peter Daszak
Sent: Wed, 14 Jun 2017 16:29:32 +0000
To: Park, Eun-Chung (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]
Cc: Kevin Olival, PhD; Anthony Ramos; Coleman, Amanda (NIH/NIAID) [C]
Subject: RE: Potential media interest from a Nature paper in press based on our work funded by two NIAID R01s
Attachments: Olival_etal_Nature2016-01-00163D_5May2017_FINAL-mergedPDF.pdf
Importance: High

No problem.

Hi Amanda. I've attached the pdf of the final version as accepted – not yet in Nature typesetting. We're just waiting on the corrected proofs from Nature and we'll send these on as soon as we get them.

As you know this is embargoed, but unfortunately right now we don't know the official publication date. We think it might be released online next Wednesday June 21st, but will confirm as soon as we hear back from Nature.

By the way – if you want a quote from me or Kevin, or have any questions – no problem – we're around this week and would be happy to help!

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. (b)(6)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Park, Eun-Chung (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, June 14, 2017 10:36 AM
To: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Cc: Kevin Olival, PhD; Anthony Ramos; Coleman, Amanda (NIH/NIAID) [C]

Subject: RE: Potential media interest from a Nature paper in press based on our work funded by two NIAID R01s

Peter,
Our communication office asks if you can provide the manuscript. I copy Amanda Coleman here, and if you can send to all of us, that will be helpful. Thank you.

Sincerely,
Eunchung

Eun-Chung Park, PhD
Program Officer,
NIAID, NIH
PH:(b)(6)
(b)(6)

From: Peter Daszak (b)(6)
Sent: Tuesday, June 13, 2017 10:08 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Park, Eun-Chung (NIH/NIAID) [E] (b)(6)
Cc: Kevin Olival, PhD (b)(6) Anthony Ramos (b)(6)
Subject: Potential media interest from a Nature paper in press based on our work funded by two NIAID R01s
Importance: High

Hi Erik and Eun-Chung

Good News! I want to give you advance notice about a paper Kevin Olival and I have in press with *Nature* that might generate some publicity. It's called "Host and Viral Traits Predict Zoonotic Spillover from Mammals". We acknowledge the current R01 (R01AI110964) on SARS-like CoVs in China that you're Program Officer for, Erik, as well as the R01 on predicting spillover from bat-origin viruses (R01AI079231) that you were Program Officer for a few years ago Eun-Chung – the work for this paper began under that R01, and it's taken a few years of database building and analysis to get to this stage!

I've inserted the abstract below, as accepted by Nature so you can see the content, as well as a draft Press Release we're working on. I don't know what the current standard is for publicity from NIAID-funded work, but I wanted to let you know in advance, in case you'd like to put a story up about this on your website, or talk to the media about it prior to the embargo.

The timing is tight. As always, we don't know exactly when Nature will release it, but we expect it will be online next week, maybe as early as **Wednesday 21st June.** We've already had pre-proofs and have corrected these so we're getting our ducks in a row for that date so that we don't miss any publicity. We'll let you know as soon as we hear the final decision.

Host and viral traits predict zoonotic spillover from mammals

Kevin J. Olival1, Parviez R. Hosseini1, Carlos Zambrana-Torrelio1, Noam Ross1, Tiffany L. Bogich1 & Peter Daszak1

The majority of human emerging infectious diseases are zoonotic, with viruses that originate in wild mammals of particular concern (for example, HIV, Ebola and SARS)1–3. Understanding patterns of viral diversity in wildlife and determinants of successful crossspecies transmission, or spillover, are therefore key goals for pandemic surveillance programs4. However, few analytical tools exist to identify which host species are likely to harbour the next human virus, or which viruses can cross species boundaries5–7. Here we conduct a comprehensive analysis of mammalian host–virus relationships and show that both the total number of viruses that infect a given species and the proportion likely to be zoonotic are predictable. After controlling for research effort, the proportion of zoonotic viruses per species is predicted by phylogenetic relatedness to humans, host taxonomy and human population within a species range—which may reflect human–wildlife contact. We demonstrate that bats harbour a significantly higher proportion of zoonotic viruses than all other mammalian orders. We also identify the taxa and geographic regions with the largest estimated number of 'missing viruses' and 'missing zoonoses' and therefore of highest value for future surveillance. We then show that phylogenetic host breadth and other viral traits are significant predictors of zoonotic potential, providing a novel framework to assess if a newly discovered mammalian virus could infect people.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. (b)(6)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

Host and viral traits predict zoonotic spillover from mammals

Kevin J. Olival[1], Parviez R. Hosseini[1], Carlos Zambrana-Torrelio[1], Noam Ross[1], Tiffany L. Bogich[1], and Peter Daszak[1]

[1] EcoHealth Alliance, 460 W. 34th Street, New York, NY, 10001, USA.

The majority of human emerging infectious diseases (EIDs) are zoonotic, with viruses originating in wild mammals of particular concern (e.g. HIV, Ebola, SARS)[1-3]. Understanding patterns of viral diversity in wildlife and determinants of successful cross-species transmission, or spillover, are therefore key goals for pandemic surveillance programs[4]. However, few analytical tools exist to identify which host species likely harbor the next human virus, or which viruses can cross species boundaries[5-7]. Here we conduct the most comprehensive analysis yet of mammalian host-virus relationships and show that both the total number of viruses that infect a given species, and the proportion likely to be zoonotic are predictable. After controlling for research effort, the proportion of zoonotic viruses per species is predicted by phylogenetic relatedness to humans, host taxonomy, and human population within a species range – which may reflect human-wildlife contact. We demonstrate for the first time that bats harbor a significantly higher proportion of zoonotic viruses than all other mammalian orders. We identify the taxa and geographic regions with the largest estimated number of 'missing viruses' and 'missing zoonoses' and therefore of highest value for future surveillance. We then

show that phylogenetic host breadth and other viral traits are significant predictors of zoonotic potential, providing a novel framework to assess if a newly discovered mammalian virus could infect people.

Viral zoonoses are a serious threat to public health and global security, and have caused the majority of recent pandemics in people[4], yet our understanding of the factors driving viral diversity in mammals, viral host range, and cross-species transmission to humans remain nascent. Recent studies have described broad patterns of pathogen host range[1,3] and various host or microbial factors that facilitate cross-species transmission[5,7,8], or have focused on factors promoting pathogen and parasite sharing within specific mammalian taxonomic groups including primates[9-11], bats[12-14], and rodents[12,15]— but to date there has been no comprehensive, species-level analysis of viral sharing between humans and all mammals. Here we create then analyze a database of 2,805 mammal-virus associations, including 754 mammal species (14% of global mammal diversity) from 15 orders and 586 unique viral species (every recognized virus found in mammals[16]) from 28 viral families (Methods). We use these data to test hypotheses on the determinants of viral richness and viral sharing with humans. We fit three inter-related models to elucidate specific components of the process of zoonotic spillover (Extended Data Fig. 1). First, we identify factors that influence total viral richness (i.e. 'baseline' number of unique viral species found in a given host, including those which may have the potential to infect humans). Second, we identify and rank the ecological, phylogenetic, and life-history traits that make some species more likely hosts of zoonoses than others. Third, recognizing that not all mammalian viruses will have the biological

46 capacity to infect humans, we identify and rank viral traits that increase their likelihood
47 of being zoonotic.

48 In examining the raw data, we found that observed viral richness within mammals
49 varies at a host order and viral family level, and is highest for Bunya-, Flavi-, and
50 Arenaviruses in rodents; Flavi-, Bunya-, and Rhabdoviruses in bats; and Herpesviruses in
51 non-human primates (Extended Data Fig. 2). Of 586 mammalian viruses in our dataset,
52 263 (44.8%) have been detected in humans, 75 of which are exclusively human and 188
53 (71.5% of human viruses) zoonotic – defined operationally here as viruses detected at
54 least once in humans and at least once in another mammal species (Methods). The
55 proportion of zoonotic viruses is higher for RNA (159/382, 41.6%) than DNA (29/205,
56 14.1%) viruses. The observed number of viruses per wild host species was comparable
57 when averaged across orders, but bats, primates, and rodents had a higher proportion of
58 observed zoonotic viruses compared to other groups of mammals (Fig. 1). Species in
59 other orders (e.g. Cingulata, Pilosa, Didelphimorphia, Eulipotyphla) also shared a
60 majority of their observed viruses with humans, but data were limited in these less
61 diverse and poorly-studied orders. Several domestic ungulate species (orders
62 Cetartiodactyla and Perissodactyla) are outliers for their number of observed viruses, but
63 these species have a relatively low proportion of zoonotic viruses (Fig. 1; Supplementary
64 Discussion).

65 Previous analyses show that zoonotic disease emergence events and human
66 pathogen species richness are spatially correlated with mammal and bird diversity[2,17].
67 However, these studies weight all species equally. In reality, the risk of zoonotic viral
68 transmission, or spillover, likely varies among host species due to differences in

underlying viral richness, opportunity for contact with humans, propensity to exhibit clinical signs that exacerbate viral shedding[18], other ecological, behavioral and life-history differences[5,12,15], and phylogenetic distance from humans[10]. We hypothesize that the number of viruses a given mammal species shares with humans decreases with phylogenetic distance from humans and increases with opportunity for human contact. We used generalized additive models (GAMs) to identify and rank host-specific predictors (ecological, life history, taxonomic, and phylogenetic traits, and a control for research effort) of the number of total and zoonotic viruses in mammals (Methods; Supplementary Table 1).

The best-fit model for total viral richness per wild mammal species explained 49.2% of the total deviance, and included per species measures of disease-related research effort, phylogenetically-corrected body mass, geographic range, mammal sympatry, and taxonomy (order) (Fig. 2a-e). Not surprisingly, research effort had the strongest effect on the total number of viruses per host, explaining 31.9% of the total deviance for this model (Extended Data Table 1). The remaining 17.3% can be explained by biological factors, a value greater than or comparable to studies examining much narrower groups of mammal hosts[10,12,15] (Supplementary Discussion). Mammal sympatry was the second most important predictor of total viral richness (Fig. 2d). Our model selection consistently identified mammal sympatry calculated at a ≥20% area overlap over other thresholds explored (Methods), providing insight into the minimum geographic overlap needed to facilitate viral sharing between hosts. Host geographic range was also significantly associated with increasing total viral richness, although the strength of this effect was low (Fig. 2c). Several mammalian orders, Chiroptera (bats),

92 Rodentia (rodents), Primates, Cetartiodactyla (even-toed ungulates), and Perissodactyla
93 (odd-toed ungulates) listed here in order of relative deviance explained, had a
94 significantly greater viral richness than predicted (Fig. 2e). This finding highlights these
95 taxa as important targets for global viral discovery in wildlife[4], and suggests that traits
96 not captured in our analysis (e.g. immunological function, social structure, and other life-
97 history variables) may underlie their capacity to harbor a greater number of viral species.
98 Our models to predict total viral richness were comparable when excluding virus-host
99 associations detected by serology, i.e. using the 'stringent data', and were robust when
100 validated with random cross-validation tests (Extended Data Table 1; Supplementary
101 Table 2). However, we identified several regions that showed significant bias when cross
102 validated by excluding mammals from zoogeographic areas, suggesting that there are
103 location-specific factors that remain unexplained in our models (Methods; Supplementary
104 Table 3).
105 Our best model to predict the number of zoonotic viruses per wild mammal
106 species explained 82% of the deviance, and included phylogenetic distance from humans,
107 a ratio of urban to rural human population across a species range, host order, whether or
108 not a species is hunted, research effort, and total viral richness (Extended Data Table 1).
109 A large fraction of the deviance explained is driven by the observed total viral richness
110 per host – supporting the biological assumption that the number of viruses that infect
111 humans scales positively with the size of the potential 'zoonotic pool'[19] in each reservoir
112 host. Removing this contribution by including observed total viral richness per host as an
113 offset, the model explains 33% of the total deviance in the *proportion* of viruses that are
114 zoonotic (Methods), with 30% of total deviance explained by biological factors (Fig. 2f-

115 i). Several mammalian orders had both a significant positive (bats) and negative (two
116 ungulate orders) effect on the proportion of zoonotic viruses (Fig. 2i). A number of
117 previous studies have proposed that bats are special among mammals in being reservoir
118 hosts of a large number of recently emerging high profile zoonoses (e.g. SARS, Ebola
119 virus, MERS)[12,13,20]. Our study, for the first time, tests this hypothesis in the context of all
120 known mammalian viruses and hosts. While other mammalian orders have relatively high
121 proportions of observed zoonoses and others have been poorly studied (Fig. 1a), our
122 model results show that bats are host to a significantly higher proportion of zoonoses than
123 other mammalian orders after controlling for reporting effort and the other predictor
124 variables.

125 We found that the proportion of zoonotic viruses per species increases with host
126 phylogenetic proximity to humans, and that this relationship is significant even when we
127 removed 'reverse zoonoses' primarily associated with transmission from humans to
128 primates (Methods). This is the first time this relationship has been demonstrated using
129 data for all mammals and specifically as a determinant of zoonotic spillover, and is
130 supported by previous taxon-specific studies that have examined host relatedness and
131 parasite/pathogen sharing in primates[9,10], bats[14], and plants[21]. The proportion of zoonotic
132 viruses shows some upward drift for mammals that are very phylogenetically distant from
133 humans (Fig. 2g) that may represent an artifact of preferentially screening marsupials for
134 human viruses. While primate species largely drive the phylogenetic effect, our best-fit
135 model excluded the effect of the order Primates as a discrete variable (Fig. 2i) –
136 suggesting that continuous variation in phylogenetic distance across primate species is
137 more important, and is significant even when all mammals are included. This finding

highlights the need to uncover the mechanism by which phylogeny affects spillover risk, e.g. evolutionarily related species sharing host cell receptors and viral binding affinities[22,23] and specific viral mutations that may expand host range in related mammal species[24].

We tested several measures to estimate human-wildlife contact at a global scale for the 721 wild mammals in our dataset, but only the ratio of urban to rural human population (all data model), the change in human population density, and the change in urban to rural population ratio from 1970-2005 across a species range (stringent data model) were included (Extended Data Table 1). The response curve for urban to rural population suggests that increasing urbanization raises the risk of zoonotic spillover (Fig. 2h), as does increasing human population density and the change in urban to rural population ratio over time. A single global metric of human-wildlife ecological contact did not emerge across models. However, the alternate inclusion of these related variables points to the importance of human-animal contact in defining per species spillover risk globally, and the need for controlled field experiments and human behavioral risk studies to uncover the mechanisms underlying this risk. Overall, the strength of the effect of phylogenetic distance was stronger than our proxies for animal-human contact in predicting proportion of zoonoses (30-44% stronger explanatory factor), but both remained significant after controlling for research effort (Extended Data Table 1).

The predominance of zoonoses of wildlife origin in emerging diseases has led to a series of programs to sample wildlife, discover novel viruses, and assess their zoonotic potential[4,23,25-27]. To inform their scale and scope we calculate the expected number of as-yet undiscovered viruses and zoonoses per host species using our best-fit GAMs and a

scenario of increased research effort (Methods, Supplementary Table 4). We then project these 'missing viruses' and 'missing zoonoses' geographically (Fig. 3, Extended Data Figs. 3-8) to identify regions of the world where targeted, future surveillance to find new viruses and zoonoses will be most effective. In the process of translating our non-spatial, species-level predictions to geographic space, we identified several regions where our model predictions of the number of total and zoonotic viruses were systematically biased (hatched regions – Fig. 3 and Extended Data Figs. 3-8; Methods). Local factors contributing to this bias may include geographic variation in the detection probability of human and/or wildlife viruses, indicating areas where additional research and capacity strengthening for viral detection are most needed. Our model predictions were not systematically biased or clustered across host phylogeny (Extended Data Fig. 9).

Geographic hotspots of 'missing zoonoses' vary by host taxonomic order, with foci for carnivores and even-toed ungulates in Eastern and Southern Africa, bats in South and Central America and parts of Asia, primates in specific tropical regions in Central America, Africa, and Southeast Asia; and rodents in pockets of North and South America and Central Africa. Areas where 'missing zoonoses' predictions were systematically biased varied by taxonomic order, but included large parts of Africa for the all mammal dataset (Fig. 3a, Extended Data Figs. 3-8f). In contrast, the distribution of bias in predicting the 'missing viruses' for all mammals was limited to patches of northeastern Asia, Greenland, Peninsular Malaysia, and scattered grid cells in Western Asia and Patagonia (Extended Data Fig. 3c). We also identify geographic regions with large numbers of mammal species currently lacking any information regarding their viral diversity (Extended Data Figs. 3i-8i). In combination, these maps can be used for cost

184 effective allocation of future resources for viral discovery programs, such as the recently
185 proposed "Global Virome Project"[27].

186 Finally, a significant challenge to preventing future disease emergence is
187 estimating the zoonotic potential of a newly-discovered viral species or strain based on
188 viral traits[4-6,28]. The best model for determining whether or not a known virus (n=586
189 mammalian viruses) has been observed as zoonotic explained 27.2% of total deviance
190 and included maximum phylogenetic host breadth (PHB – a virus-specific trait that
191 measures the phylogenetic range of known hosts, excluding humans), research effort,
192 whether or not a virus replicates in the cytoplasm, is vector-borne, or is enveloped, and
193 average genome length (Fig. 4). Using the 'stringent' dataset to define whether a virus is
194 zoonotic resulted in a reduced model that excluded enveloped status and genome length
195 (Extended Data Table 1). Our findings confirm a positive relationship between zoonotic
196 potential and ability to replicate in the cytoplasm[7], and that viruses with arthropod vectors
197 may be able to infect a wider range of mammalian hosts[5]. Our phylogenetically-explicit
198 measure of host breadth, PHB, can be used at various hierarchical taxonomic levels to
199 quantify and rank viruses from specialist to generalist, and was the strongest predictor of
200 zoonotic potential (12.4% of total deviance explained). This highlights the value of field
201 programs to identify the natural host range of newly discovered pathogens in order to
202 develop early proxies for their zoonotic potential[4]. Significant variation in PHB across
203 viral families is suggestive of intrinsic differences in a virus' ability to infect diverse
204 hosts, and this relates to the proportion of observed zoonoses in each family (Fig. 4a).

205 We acknowledge several important caveats. First, our estimates of 'missing
206 viruses' and 'missing zoonoses' per species are based on the current maximum observed

research effort from the literature, and these estimates should be viewed as relative, not absolute. The true size of the undiscovered mammalian virome will likely increase with new genetic tools for unbiased viral discovery and in depth studies that repeatedly sample wildlife populations over time[25]. Second, our ecological and biological predictor variables only explain a portion of the total variation in viral richness per host and zoonotic potential based on viral traits, although this is greater than that reported in comparable order-specific studies[10,12]. Third, while we control for research effort we cannot account for viruses or host associations that have completely evaded human detection to date, nor those identified but not published. Additional resources to support better data-sharing and on-the-ground viral surveillance in the species and regions we identify would help validate predictive models to identify zoonotic viral hotspots, and streamline costly efforts to develop measures to prevent their future emergence.

The analyses reported herein have broad potential to assist in expediting viral discovery programs for public health. Our host-specific analyses and estimates of 'missing zoonoses' allow us to identify which species and regions should be preferentially targeted to characterize the global mammalian virome. Our viral trait framework then allows prioritization of newly-discovered wildlife viruses for detailed characterization (e.g. by sequencing receptor binding domains, and conducting *in vitro* and *in vivo* infection experiments[23]) to assess their potential to threaten human health.

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Author contributions

K.J.O., T.L.B., and P.D. designed the study and supervised the collection of data. N.R., P.R.H. and K.J.O. designed the statistical approach, wrote the code, and generated figures. K.J.O. performed phylogenetic analyses. C.Z-T. performed spatial analyses. All authors were involved in writing the manuscript.

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Figure Legends

Figure 1. Observed viral richness in mammals. Boxplots of **a,** Proportion of zoonotic viruses, and **b**, total viral richness per species, aggregated by order. Data points represent wild (light grey, n=721) and domestic (dark red, n=32) mammal species; lines represent median, boxes interquartile range. Animal silhouettes from PhyloPic. Data based on 2805 host-virus associations.

Figure 2. Host traits that predict total viral richness (top row) and proportion of zoonotic viruses (bottom row) per wild mammal species. Partial effect plots show the relative effect of each variable included in the best-fit Generalized Additive Model, given the effect of the other variables. Shaded circles represent partial residuals; shaded areas, 95% confidence intervals around mean partial effect. **a-e,** Best model for total viral richness includes: **a,** number of disease-related citations per host species (research effort, log); **b,** Phylogenetic Eigenvector Regression (PVR) of body mass (log); **c,** geographic

range area of each species (log km^2); **d,** number of sympatric mammal species overlapping with at least 20% area of target species range; and **e,** mammalian orders. **f-i,** Best model for proportion of zoonoses includes: **f,** research effort, log; **g,** phylogenetic distance from humans (cytochrome b tree constrained to the topology of the mammal supertree[29]); **h,** ratio of urban to rural human population within species range; and **i,** three mammalian orders. Bats are the only order with a significantly larger proportion of zoonotic viruses than would be predicted by the other variables in the all-data model. Three additional mammalian orders, and whether or not a species is hunted, improved the overall predictive power of the best zoonotic virus model but were non-significant, and are not shown (see Extended Data Table 1).

Figure 3. Global distribution of the predicted number of 'missing zoonoses' by order. Warmer colors highlight areas predicted to be of greatest value for discovering novel zoonotic viruses. **a,** All wild mammals (n=584 spp. included in the best-fit model). **b,** Carnivores (Order Carnivora, n=55). **c,** Even-toed ungulates (Order Cetartiodactyla, n=70). **d,** bats (Order Chiroptera, n=157). **e,** Primates (Order Primates, n=73). **f,** Rodents (Order Rodentia, n=183). Hatched regions represent areas where model predictions deviate systematically for the assemblage of species in that grid cell (approximately 18 km x 18 km, see Methods). Animal silhouettes from PhyloPic.

Figure 4. Traits that predict zoonotic potential of a virus. a, Boxplot of maximum phylogenetic host breadth per virus (PHB, see methods) for each of 586 mammalian viruses, aggregated by 28 viral families. Individual points represent viral species, color-

366 coded by zoonotic status. Boxplots colored and sorted by the proportion of zoonoses in
367 each viral family. **b-d,** Partial effect plots for the best-fit Generalized Additive Model to
368 predict a virus' zoonotic potential. **b,** Maximum PHB. Viruses that infect more
369 phylogenetically diverse hosts are more likely to be zoonotic. **c,** Research effort (log,
370 number of PubMed citations per viral species). **d,** Whether or not a virus replicates in the
371 cytoplasm or is vector-borne. Viral genome length and whether or not a virus is
372 enveloped improved the overall predictive power but were non-significant, and are not
373 shown (see Extended Data Table 1).
374
375 **Methods**
376 **Database**
377 To construct the mammal–virus association database we initially extracted all viruses
378 listed as occurring in any mammal from the International Committee on Taxonomy of
379 Viruses database (ICTVdb), and further individually went through each virus listed in the
380 ICTV 8th edition master list and searched the literature for mammalian hosts. All viral
381 species names were synonymized to ICTV 8th edition, which was the global authority on
382 viral taxonomy at the start of our data collection in 2010[16]. From 2010-15 the authors and
383 a team of research assistants and interns at EcoHealth Alliance compiled mammal species
384 associations for each of 586 unique viruses published in the literature between 1940-2015
385 initially by using the virus name and synonyms as the search keywords in the major
386 online reference databases (Web of Science, PubMed, and Google scholar) in addition to
387 searching in books, reviews, and literature cited in sources we had already obtained. To
388 narrow the search for hosts for well-researched viruses, we additionally included the

terms “host(s)”, “reservoir”, “wildlife”, “animals”, “surveillance”, and other relevant terms to find publications related to host range. Associations were cross-checked for completeness with the Global Mammal Parasite Database for primate, carnivore and ungulate viruses, version as of Nov 2006 (GMPD, www.mammalparasites.org)[30] and other published reviews specific to bats and rodents[12,31,32]. We excluded all records without species-level host information, and those where we could not track down the primary references. Records of mammal-virus associations from experimental infection studies, zoological parks or captive breeding facilities, or cell culture discoveries were excluded. Host species were defined as domestic vs. wild following the list of domestic animal species from the Food and Agriculture Organization (FAO)[33], and we removed the black rat (*Rattus rattus*) and domestic mouse (*Mus musculus*) from the domesticated list as these two species make up their own “peri-domestic” category. Host species were categorized as either occurring in human modified habitats or being hunted by humans – both estimates for human contact – according to the IUCN Red List species descriptions[34].

To control for the fact that some detection methods are more reliable than others in identifying the pathogen of interest, we recorded the detection method used for each host-virus association and scored these as 0, 1, or 2 according to the reliability of detection method used. Viral isolation and PCR detection with sequence confirmation were scored as a 2 (= stringent data); and serological methods were scored as a 0 or 1, with viral or serum neutralization tests (=1), and enzyme-linked immuno assays (ELISA), antigen detection assays, and other serological assays scored as (=0). ‘Stringent data’ were

412 analyzed separately to remove potential uncertainty due to cross-reactivity with related
413 viruses. We exhaustively searched the literature to identify a 'stringent' detection for
414 each mammal-virus pair, and only included the serological finding for that pair if no
415 molecular or viral isolation studies were available. We partitioned data and conducted
416 separate analyses for the entire dataset (0 +1 + 2 detection quality) and the 'stringent'
417 data (score of 2) to reduce the noise from potential serological cross-reactivity. Full list of
418 host-virus associations, detection methods, and associated references are provided in our
419 data and code repository at http://doi.org/10.5281/zenodo.569079.
420
421 Our operational definition of a zoonotic virus includes any virus that was detected in
422 humans and at least one other mammalian host in at least one primary publication, and
423 does not imply directionality. Our complete dataset of mammalian viral associations
424 demonstrates evidence of past or current viral infection which we believe is a reasonable
425 proxy for measuring spillover, and our 'stringent' dataset specifically is more robust to
426 exclude species that may have been exposed to a given virus versus those that show some
427 evidence for replication within the host species. Our bi-directional definition of spillover
428 follows a proposal by the WHO that defines a zoonosis as "any disease or infection that is
429 naturally transmissible from vertebrate animals to humans and vice-versa"
430 (http://www.who.int/zoonoses/en/) and excludes any human pathogens that recently
431 evolved from nonhuman pathogens (e.g. HIV in primates), as per Woolhouse and
432 Gowtage-Sequeria 2005[1].
433

434 In order to address influence of transmission from humans to wildlife in our models, we
435 also ran our GAM model fitting and selection procedure (see below) on a subset of data
436 that excluded any probable 'reverse zoonotic' viruses. We first searched our entire
437 dataset and removed any clear instances of transmission from humans to primates, e.g.
438 including records from zoological parks and wildlife rehabilitation centers (as previously
439 noted). We then additionally removed several human viruses most commonly transmitted
440 from humans back to non-human primates to create a subset of data without the most
441 common 'reverse zoonotic' viruses (Adeno-associated virus-2; Human adenovirus D;
442 Human herpesvirus 4; Human metapneumovirus; Human respiratory syncytial virus;
443 Measles virus; Mumps virus)[35,36]. We present these additional analyses excluding
444 'reverse zoonoses' and associated code at http://doi.org/10.5281/zenodo.569079.
445
446 Total viral richness was calculated as the number of unique ICTV-recognized viruses
447 found in a given host species, and zoonotic viral richness was defined as the number of
448 unique ICTV-recognized viruses in a given host species that were also detected in
449 humans in our database.
450
451 To assess research bias for both host and virus, we searched ISI Web of Knowledge,
452 including Web of Science and Zoological Record, and PubMed for the number of
453 research publications for a given host or pathogen. We recorded two values for the
454 number of research papers for a host. The first was a simple search by scientific binomial
455 in Zoological Abstracts where we recorded the number of papers published between
456 1940-2013 for each host species. We also recorded the number of disease-related

457 publications for each species using the scientific binomial AND topic keyword: disease*
458 OR virus* OR pathogen* OR parasit*. The * operator was used in our search criteria to
459 capture all words that begin with each term, e.g. “parasit” (= “parasite”, “parasites”, and
460 “parasitic”). These search criteria broadly included papers that examined disease or
461 diseases, virus or viruses, pathogen or pathogens, parasite parasites, or parasitology, for
462 each species. Only one measure of per host research effort was included at a time in
463 model selection. As these metrics are highly correlated and the number of disease related
464 citations per host outperformed the total number of publications per host in all but one
465 model (all-data zoonoses), we decided to use disease-related publications as our per-
466 species research effort measure for all models to improve interoperability. We also
467 recorded the number of publications for each of 586 virus species using a keyword search
468 by virus name in PubMed and Web of Science. Only one measure of per virus research
469 effort was included at a time in model selection.
470
471 We used a phylogenetically-corrected measure of body mass (see details below under
472 ‘Phylogenetic Signal’) as our main life history predictor variable, because it was the only
473 one for which a nearly complete data set existed for the species in our dataset. We used
474 the body mass recorded in the PanTHERIA database[37] for 709 species. For 3 species, we
475 used the second choice option, body mass recorded in the AnAge database[38]. For 11
476 species, we used the third choice option of the extrapolated body mass recorded in
477 PanTHERIA, which is based on body length or forearm length, depending on species. For
478 36 species, we used the average body mass for members of the genus that had a recorded
479 body mass. We explored other life-history variables related to longevity[39], reproductive

success, and basal-metabolic rate but these were ultimately excluded due to the high number of missing records.

Phylogenetic Signal.

We address the issue of non-independence of host species traits due to shared ancestry[40] in our analyses by first quantifying the phylogenetic signal for each variable in our model using Blomberg's K[41]. Blomberg's K measures phylogenetic signal in a given trait by quantifying trait variance relative to an expectation under a Brownian motion null model of evolution using a phylogenetic tree with varying branch lengths. Blomberg's K-values are scaled from 0 to infinity, with a value of 0 equal to no phylogenetic signal and values greater than 1 equal to strong phylogenetic signal for closely related species that share more similar trait values. While there is no clearly defined K-value cut-off in which to apply phylogenetic comparative methods, non-significant value of <1, or more conservatively <0.5, are typical for traits that are phylogenetically independent. The only host variables we examined with significant K-values >0.5 were host body mass, and our direct measure of phylogenetic distance to humans. While there are several tools available to control for phylogeny in multivariate analyses, e.g. using phylogenetic generalized least square models (e.g. PGLS)[42], there is currently no modeling approach to control for phylogeny using GAMs. More importantly, a wholesale effort to control for phylogeny across all variables in our analysis was not appropriate here, as we are explicitly testing the relative importance of phylogenetic distance to humans vs. other host traits including measures of human-wildlife contact to predict the proportion of zoonotic viruses for a given host species. This left body mass as the only variable in our

503 models, excluding our direct measures of phylogenetic distance, with a significant
504 Blomberg K value that was greater than one. We controlled for the significant effect of
505 shared evolutionary history using a phylogenetic eigenvector regression (PVR)[43,44] on
506 body mass. The PVR approach allowed us to remove phylogenetic signal for any
507 phylogenetically non-independent variables and then include the corrected values back in
508 our GAMs, while retaining predictor variables like phylogenetic distance to humans as
509 unmodified. We calculated PVR for body mass using the R package 'PVR' and our
510 custom-build maximum likelihood host phylogeny using cytochrome B sequences
511 constrained to the order-level topology of the mammalian supertree[29,45]. Our new variable
512 for body mass that controls for phylogenetic signal (*PVRcytb_resid)* removed most of the
513 phylogenetic signal, with K= 3.5 unadjusted, and K<0.5 after PVR correction. Our new
514 metric of body mass scales in the same way, with larger values equal to species with
515 larger body mass. PVR body mass was included in our GAM model selection for the total
516 viral richness and zoonotic virus models.
517
518 **Host Phylogenetic Analysis and Phylogenetic Host Breadth**
519 We used two different mammal phylogenetic trees in our analyses and used a model
520 selection framework to determine which best explained our observed association with
521 zoonotic viral richness. First the mammal supertree was pruned in R (package *ape*,
522 function 'drop.tips') to include only synonymous species for the 753 species in our
523 database[29,46]. We synonymized all host species names between the mammal supertree
524 and the host associations in our database using the IUCN Red List[34]. If the species was
525 listed as 'cattle' it was assumed to be *Bos taurus*, all other records were excluded if there

was ambiguity as to the scientific name for the host species. Second, a maximum likelihood cytB tree was generated using the constraint of a multifurcating tree with taxa constrained to their respective orders and the order-level topology matching that of the mammal supertree[6], as per this Newick tree file: (MONOTREMATA,((DIDELPHIMORPHIA,(DIPROTODONTIA,PERAMELEMORPHIA)),(PROBOSCIDEA,((PILOSA,CINGULATA),((((RODENTIA,LAGOMORPHA),(PRIMATES,SCANDENTIA)),((((CETARTIODACTYLA,PERISSODACTYLA),CARNIVORA),CHIROPTERA),EULIPOTYPHLA))))))). This generated a higher resolution species-level mammal tree using cytb data, with more reliable positioning of the higher-level taxonomic relationships than was obtained in exploratory phylogenetic analyses using cytb data alone. Genbank accession numbers and cytb sequence lengths for each species provided in in our data and code repository. Cytb gene fragments ranged from 143 to 1140bp, with >1000bp available for 558/665 (84%) of the taxa. Data derived from the cytochrome b tree constrained to the topology of the mammal supertree was selected as the best option in all best-fit GAMs.

Sequences were aligned using MUSCLE with default setting in Geneious R6, and checked visually for errors[47]. The best maximum likelihood tree with and without the constraint tree were generated using RAxML-HPC2 on XSEDE via the CIPRES Science Gateway server v3.1[48] using a GTR model with parsimony seed, 1000 bootstrap replicates, and the following, specific parameters (raxmlHPC-HYBRID -s infile -n result -x 12345 -g constraint.tre -N 1000 -c 25 -p 12345 -f a -m GTRCAT).

549 Matrices of pairwise patristic distances between all species, including *Homo sapiens*,
550 were calculated from the two phylogenies using the 'cophenetic' function in the R
551 package *ape*[46]. Phylogenetic trees (Newick format for pruned Supertree and cytb tree)
552 and matrices of phylogenetic distance from humans are provided in the data and code
553 repository.
554
555 We calculated mean, median, max, min, IQR, and standard deviation (represented as
556 generic function F in equation 1) of phylogenetic host breadth (PHB) from all known
557 mammalian hosts for each virus using the pairwise patristic distances $\left(d_{i,j}\right)$ for each
558 mammal-mammal association for all hosts of a given virus excluding humans, where i
559 indexes each mammal in the database, as does j , and J represents the total mammals
560 in the database. We aggregated these PHB values using mean, median, or maximum
561 values at a viral species, genus and viral family level to generate higher-level taxonomic
562 variables of host breadth per viral group. Our measure is similar to those developed by
563 previous studies to understand parasite host specificity[49-51], but here we create a
564 generalizable variable to measure viral host breadth that can be aggregated at different
565 viral taxonomic levels.
566
567 (1) $PHB_i = \mathrm{F}^{\mathrm{J}}_{j=0}\, \mathrm{d}_{i,j}$
568
569 To make Extended Data Figure 9, taxon names and terminal branches of cytb tree
570 constrained to supertree were color-coded using residual from the best-fit zoonotic virus
571 GAM (predicted-observed zoonotic viral richness) for wildlife species, and plotted using

572 the plot.phylo function in the R package *ape*[46]. Symbols (circles) at terminal taxa
573 additionally added to better visualize residual value colors were added using
574 willeerd.nodelabels function (dx.doi.org/10.5281/zenodo.10855). All marine mammals,
575 domestic animals, and other taxa with missing data were coded as grey for missing data.
576
577 Viral richness heatmap (Extended Data Figure 2) was generated using the R package
578 *pheatmap*, and the 'complete' hierarchical clustering algorithm to sort cells across rows
579 and columns by similar values of viral richness. All boxplots, histograms and all other
580 figures generated in R v.3.3.0[52]. R code for primary figure generation is provided in the
581 code repository.
582
583 **GAM Fitting and Selection**
584 We fit a set of generalized additive models (GAMs) that included all of our selected
585 potential variables explaining the number of total viruses or number of zoonoses in hosts,
586 as well as whether viruses were zoonotic (for conceptual framework and summary of
587 each GAM see Extended Data Figure 1; for full variable list and data sources see
588 Supplementary Table 1). Our use of GAMs, an incorporation of smooth spline predictor
589 functions into the generalized linear model (GLM) framework, allowed us to examine the
590 functional form of our predictor variables (e.g. Fig 2 and 4). Categorical and binary
591 variables (e.g. host order, IUCN status of hunted or not, and certain viral traits) were fit
592 as random effects of each variable level. We used automated term selection by double
593 penalty smoothing[53] to eliminate variables from the models. This method removes
594 variables with little to no predictive power and has been shown to be comparable or

595 superior to comparing alternate models with and without variables. We did use the model
596 comparison method in for the case of domestic animals, where the sample size was not
597 sufficient for fitting all variables. In this case dropping variables by double penalty
598 smoothing still allowed pruning the model list to eliminate redundant models. Where
599 there were competing variables measuring the same mechanistic effect, we fit alternate
600 GAMs using only one of each of these variables (as specified in below and in the
601 Extended Data Figure 1). These included phylogenetic variables, citation counts from
602 alternate databases, and different measures of human population/host overlap. For
603 example, to capture host phylogeny we used phylogenetic distance based on either the
604 mammal supertree[20] or a purpose-built CytB constrained by the topology of the mammal
605 supertree, but never both in the same model. For human population variables, we looked
606 at either variables measuring overlap of species range with human-occupied areas, or
607 human population in those areas, as area- and population-based measures were highly co-
608 linear. For citation variables, we looked at either all citations or the number of disease-
609 related citations for each host species, not both, and similarly citations in either PubMed
610 or Web of Knowledge. We used a binomial GAM to analyze the 586 mammalian viruses
611 in our database and identify viral traits that may serve as predictors of zoonotic potential.
612 Collinearity was not a major issue among variables included in the same model.
613
614 We inspected models within 2 AIC units of the model with the lowest AIC, and present
615 the outputs of the best-fit and all other top models (<2 ΔAIC) in our data and code
616 repository. In general, variable effects retained the same functional form and effect size
617 across models within 2 ΔAIC – differences were limited to the adding or dropping of

very weak, insignificant effects, or switching between highly correlated competing variables such as citation counts from different databases.

For our model of number of zoonoses per host, we used the total number of observed viruses per host as an offset, effectively fitting a model of expected number of zoonoses per host virus. We found this variable had a coefficient near to one when it was used as a linear predictor, indicating its appropriateness as an offset.

We repeated the model selection process for all models using the more 'stringent' set of data that used only virus identified in mammal hosts using viral isolation, PCR, or other methods of nucleic acid sequence confirmation, i.e. that excluded all associations detected via serology.

All models were fit using the MGCV package for R (version 1.8-12.). We used the model with the lowest AIC to predict the number of expected zoonotic viruses for each host species, using all the data from our database that had complete observations for the best model. Our top models consistently outperform the alternatives by wide margins, as measured by AIC. We used standard methods in the R package MGCV to calculate deviance explained, which is defined as (D_null – D_model)/D_null. In this formula, D_null is the deviance (-2*likelihood) of an intercept-only, (or, in the case of the zoonoses model, offset-only), model, while D_model is the deviance of our best-fit model.

641 Analyses were limited to terrestrial mammal species as defined by the IUCN Red List
642 (marine mammals were excluded) and we ran separate analyses for wild and domestic
643 animals. As domestic animals made up a much smaller data set (n=32 species) with a
644 unique set of explanatory variables that differed from the wild species analyses, these
645 models were fit separately. Domestic species results are also discussed separately (see
646 Supplementary Discussion) as they are tangential to the primary findings.
647
648 **Model cross-validation**
649 We used k-fold cross-validation to evaluate goodness of fit for all models. The data was
650 divided into 10 folds, selected randomly. For each fold, the model was re-fit based on the
651 other 9 folds, and goodness of fit was assessed by conducting a nonparametric
652 permutation test of comparing the predicted values vs. the real values for the k^{th} fold,
653 where a non-significant result indicates that predictions are unbiased. Poisson models
654 goodness-of-fit may be compared via a parametric Chi-squared permutation test on
655 deviance values, but this test is inappropriate in the case of models with low mean values,
656 as is our case for some of our GAMs[54]. The k-fold cross-validation confirmed the
657 robustness of our model predictions for wild mammals, code and outputs from these tests
658 for each best-fit GAM are provided in Supplementary Table 2.
659
660 In addition to randomly-selected k-fold cross-validation, we evaluated the robustness of
661 our models via a non-random geographic cross-validation, code and summary document
662 provided in our code and data repository. In order to meaningfully organize species in our
663 dataset by geographic areas, we used the 34 zoogeographic regions for terrestrial

mammals recently redefined by Holt et al.[55]. Using QGIS[56], a mammal-specific zoogeographical shapefile provided by Holt's group at the University of Copenhagen (http://macroecology.ku.dk/resources/wallace) was intersected (using QGIS Vector > Geoprocessing Tools > Intersect) with a shapefile of IUCN's host ranges for all mammals in our database. Areas of these intersections were then calculated using an equal-area projection (Mollweide), and each host was assigned to only the region that contained the greatest proportion of its range. We systematically removed all observations (species) from each given zoogeographical region, re-fit the model using all observations from outside the region, then performed a non-parametric permutation test comparing the predicted values to the observed values for that region. Non-significant results indicate that model predictions are unbiased. Significant results for a given zoogeographic region suggest that there are location-specific biases that remain unexplained. This systematic zoogeographic cross-validation supported the overall robustness of our model predictions for several models, i.e. all-data zoonoses, all-data total viral richness, and stringent-data total viral richness models. For these models, even though a majority of zoogeographic regions were unbiased, we still identified several zoogeographic regions that showed significant bias. Our zoogeographic cross-validation was equivocal for the stringent-data zoonoses model, with 8 regions that showed evidence of bias and 7 regions which showed no evidence of bias (Supplementary Table 3).

The presence of biased regions in our zoogeographic cross-validation suggested the possibility that there is a systematic bias associated with geography not captured by the predictor variables in our models. To further investigate this, we added zoogeographical

687 region as a categorical random effect to each of our best-fit models. For three of our best-
688 fit GAMs (all-data total viruses, stringent-data total viruses, and stringent-data zoonoses)
689 the addition of zoogeographical region as a categorical random effect decreased the
690 model AIC and increased the total deviance explained by 3-5%. The all-data zoonoses
691 model, which was used to create the series of maps in the main manuscript, does not
692 improve with the inclusion of zoogeographical region. However, the improved predictive
693 power of models using region-specific terms is offset by the increase in degrees of
694 freedom (i.e. if we included 31 zoogeographic regions as separate terms) and, more
695 importantly, a decreased interpretability of our models – especially when compared to the
696 geographical variables we used, such as host area or species range overlap with human
697 modified habitat. We opted not to include these random effects in our final GAMs in
698 favor of keeping only variables interpretable in the context of our host trait-specific
699 framework. Instead, we indicate areas of geographic bias directly on our spatially mapped
700 outputs. (See "Calculating and visualizing 'missing viruses' and 'missing zoonoses'",
701 below.) Summaries of these models, along with changes in relative deviance explained
702 for the other explanatory variables when zoogeographic region is added as a random
703 effect, are provided in our code and data repository.
704
705 **Spatial variables**
706 For all the wildlife hosts we used the geographic range information obtained from the
707 IUCN spatial database version 2015.2. Wildlife host species shapefiles needed to
708 replicate analysis are now hosted on our Amazon S3 storage
709 (https://s3.amazonaws.com/hp3-shapefiles/Mammals_Terrestrial.zip)[34]. IUCN depict

710 species' range distributions as polygons based on the extent of occurrence (EOO), which
711 is defined as the area contained within a minimum convex hull around species'
712 observations or records. This convex hull or polygon is further improved by including
713 areas known to be suitable or by removing unsuitable or unoccupied areas based on
714 expert knowledge. To accurately calculate the area in km^2 of each host species we
715 projected the polygons to an equal area projection (Mollweide).
716
717 We calculated various thresholds of mammal sympatry based on percentage of range
718 overlap for each wild species in our database using IUCN shape files for all mammals
719 globally. We define mammal sympatry as the number of mammalian species that overlap
720 with the target species' geographic range. We calculated mammal sympatry for each wild
721 species in our database at six different thresholds based on the percentage area overlap
722 with the target species geographic range, i.e. the number of other wild mammal species
723 with any (>0%), ≥20%, ≥40%, ≥50%, ≥80%, or 100% range overlap. The six different
724 thresholds for mammal sympatry were included as competing terms in our model
725 selection for the total viral richness models.
726
727 We derived and tested several global measures to estimate the level of human contact
728 with each wild species in our database. To estimate the area of host geographic range
729 covered by crops, pastures, rural and urban areas – as measures of global human contact
730 with a given wildlife species – each species polygon was intersected (overlapped) with
731 spatial data representing those land cover types. Additionally, we calculated the total
732 number of people within each host geographic range using data from HYDE database[57],

and also separately totaled the number of people in rural and urban populations. We obtained data on the distribution of cropland, pastures, rural and urban areas (i.e., grazing land) also from the HYDE database[57] for the years 1970, 1980, 1990, 2000 and 2005 with a spatial resolution of 5' x 5', equivalent to $10km^2$ by $10km^2$. These datasets were created by combining information from satellite imagery and sub-national crop and pasture statistics[57]. In our GAMs, we used several transformations of these variables as competing proxies for human-wildlife contact: the log-transformed area of host range that overlapped each type of human-modified land cover, log-transformed human population in the host range, log-transformed human population density in the host range, and the log-ratio of urban and rural human populations in the host range. For each of these, we also included as a variable the change in value from 1970 to 2005. Human-wildlife contact variables that significantly covaried were excluded (set as competing terms) during the model selection process. The ratio of urban to rural human population was used to disentangle variables of human-wildlife contact that significantly covaried. For example, the total area of a species range that overlapped with urban and rural areas was highly correlated with the total geographic area variables we examined (e.g. total area, and area in crop, pasture, rural, and urban). The ratio of urban to rural population allowed us to separate these signals and best represent this proxy of per species human-wildlife contact. All spatial analyses were performed in R (3.3.2)[52], using the following R libraries: raster[58], rgdal[59], and sp[59].

Calculating and visualizing 'missing viruses' and 'missing zoonoses'

755 We used each respective best-fit, all data GAM from the total viral richness and
756 proportion zoonoses models to calculate the estimated number of viruses that would be
757 observed if the research effort variable for each species was equal to that of the most-
758 studied wild species in our database (*Vulpes vulpes* with 4,433 total publications and
759 1,477 disease-related publications). We used the prediction of the total virus richness
760 GAM as the offset for the zoonoses GAM. We then calculated the 'missing viruses' and
761 'missing zoonoses' by subtracting the observed number of viruses and zoonoses from the
762 predictions based on maximum research for each wild mammalian species.
763
764 We used geographic range maps from the IUCN spatial database (2015.2) to visualize the
765 spatial distribution of observed host-virus associations, observed host-zoonoses
766 association, these associations as predicted under maximum research, and the maximum
767 predicted – observed viruses, or the 'missing viruses' and 'missing zoonoses' (e.g. Fig 3;
768 Extended Data Figures 3-8; Supplementary Table 4). We also generated maps comparing
769 species richness of all species in the IUCN database against those with viral associations
770 in our database. For each species, the distribution range was converted to a grid system
771 with cells 1/6 of a geographic degree (approximately 18 km x 18 km at the Equator line).
772 Each grid cell was assigned a value of one to indicate presence. We repeated this process
773 and assigned the observed and predicted-under-max-effort number of zoonotic viruses to
774 their correspondent grid cells. Viral and host species richness maps, and both the
775 'missing viruses' and 'missing zoonoses' maps were calculated by overlying individual
776 grids. Each richness map represents the sum of all values for a given grid cell. We
777 repeated the process for all the host species in our database and created viral and species

778 richness maps for the following orders: Carnivora, Cetartiodactyla, Chiroptera, Primates
779 and Rodentia. These taxa were selected because they represent 681/736 (92.5%) of wild
780 mammal species in our database.
781
782 In the process of translating our non-spatial, species-level predictions to geographic space
783 (i.e. layered raster maps), we identified several geographic areas where our model
784 predictions of the number of total and zoonotic viruses were systematically biased, i.e. p-
785 value <0.05 (Supplementary Table 3). In order to visualize the geographic biases of our
786 non-spatial model predictions in our maps (see above regarding zoogeographic cross-
787 validation), we demarcate regions with significant bias with hatching. Hatched regions
788 represent areas where model predictions of total or zoonotic viral richness deviate
789 systematically for the collection of species in that grid cell. For each grid cell we
790 calculated whether the bias exceeded that expected from a random sampling of hosts.
791 This was accomplished by summing the residuals from 100,000 random draws of species
792 in our dataset that was equal to the number of species present in that grid cell, then
793 identifying grid cells where the observed bias was outside the middle 95% of the
794 randomly drawn distribution. We calculated this for all mammals, and separately for each
795 order across all grid cells. Areas with observed bias (outside of 95% of the randomly
796 drawn distribution) are shown with hatched regions on each 'missing virus' and 'missing
797 zoonoses' map.
798
799 **Animal images used in figures**

Animal silhouettes added to Figs 1 and 3 and Extended Data Figs 1 and 2 to visually represent each mammalian order were downloaded from PhyloPic (www.phylopic.org). Chiroptera, Cingulata, Diprotodontia, Lagomorpha, Peramelemorphia, and Primates were available for use under the Public Domain Dedication license. Carnivora and Rodentia (by Rebecca Groom), Didelphimorphia, Pilosa, and Probscidea (by Sarah Werning), Eulipotyphyla (by Claus Rebler), Certartiodactyla and Perissodactyla (by Jan A. Venter, Herbert H. T. Prins, David A. Balfour & Rob Slotow and vectorized by T. Michael Keesey) are provided under the creative commons license (https://creativecommons.org/licenses/by/3.0/). We created the silhouette for Scandentia, and waive all rights.

Data availability statement

All datasets (host traits, viral traits, full list of host-virus associations and associated references, phylogenetic trees, and phylogenetic distance matrices) along with the R code and R package dependencies needed to fully replicate and evaluate these analyses are provided at http://doi.org/10.5281/zenodo.569079. The top-level README.txt file in the directory details the file structure and metadata provided.

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Extended Data Legends

Extended Data Table 1. Summary of best-fit generalized additive models (GAMs) for total and zoonotic viral richness per wild mammal species, and probability of a virus being zoonotic. Models were selected separately using the entire dataset and a 'stringent' dataset that excluded host-virus associations detected by serology. Variables are sorted by relative percent deviance explained with in each model.

Extended Data Figure 1. Conceptual model of zoonotic spillover, viral richness, and summary of models. a, Conceptual model of zoonotic spillover showing primary risk

909 factors examined, color-coded according to generalized additive models used. **b,**
910 Conceptual model of observed, predicted, and actual viral richness in mammals. **c,**
911 Generalized Additive Models (GAMs) used in our study to address specific components
912 of **a** and **b**, color-coded by model. Variables listed with "or" under each GAM covaried
913 and were provided as competing terms in model selection, and those in bold were
914 included in the best-fit model using all host-virus associations. Significant variables from
915 each best-fit GAM are noted with an *. Zoonotic viral spillover firstly depends on the
916 underlying total viral richness in mammal populations and the ecological, taxonomic, and
917 life-history traits that govern this diversity (GAM 1). Secondly, host and virus specific
918 factors may facilitate viral spillover. We examine the relative importance of host
919 phylogenetic distance to humans, ecological opportunity for contact, or other species-
920 specific life-history and taxonomic traits (GAM 2), and identify viral traits associated
921 with a higher likelihood of an observed virus being zoonotic (GAM 3). We estimate the
922 total and zoonotic viral richness per host species using GAMs 1 and 2, and calculate the
923 'missing viruses' and 'missing zoonoses' under a scenario of increased research effort (**b,**
924 Methods**)**. Due to imperfect surveillance in both humans and wildlife and biases in viral
925 detection, there may be uncertainty in the exact proportion of viruses that are zoonotic (**b**,
926 light grey), and also between the actual, or true, viral richness (dotted lines) and the
927 predicted maximum viral richness per host (dashed line).
928
929 **Extended Data Figure 2. Heatmap of observed total viral richness by mammalian**
930 **order and viral family.** Data set includes 754 mammalian species and 586 unique ICTV

recognized viral species. Heatmap aggregated by rows and columns to group taxa with similar levels of observed viral richness.

Extended Data Figure 3. Global distribution of viral and host species richness for all wild mammals. a, Observed total viral richness (for n=576 host spp.); **b,** predicted total viral richness given maximum research effort; **c,** 'missing viruses' or predicted – observed total viral richness; **d,** observed zoonotic viral richness (n=584); **e,** predicted zoonotic viral richness given maximum research effort; **f,** 'missing zoonoses' or predicted – observed zoonotic viral richness (same as included in Fig 3a); **g,** global mammal species richness (n=5290); **h,** mammal richness for species in database (n=753); **i,** mammal species with no described viruses in literature. Warmer colors (larger values) in panels **c** and **f** highlight areas predicted to be of greatest value for discovering novel viruses or novel viral zoonoses, respectively, in mammals. Red/pink colors in panel **i** highlight areas with poor viral surveillance in mammal species to date. Hatched regions represent areas where model predictions deviate systematically for the collection of species in that grid cell (see Methods).

Extended Data Figure 4. Global distribution of viral and host species richness for wild carnivores (Order Carnivora). a, Observed total viral richness (for n=55 host spp.); **b,** predicted total viral richness given maximum research effort; **c,** 'missing viruses' or predicted – observed total viral richness; **d,** observed zoonotic viral richness (n=55); **e,** predicted zoonotic viral richness given maximum research effort; **f,** 'missing zoonoses' or predicted – observed zoonotic viral richness (same as included in Fig 3b); **g,**

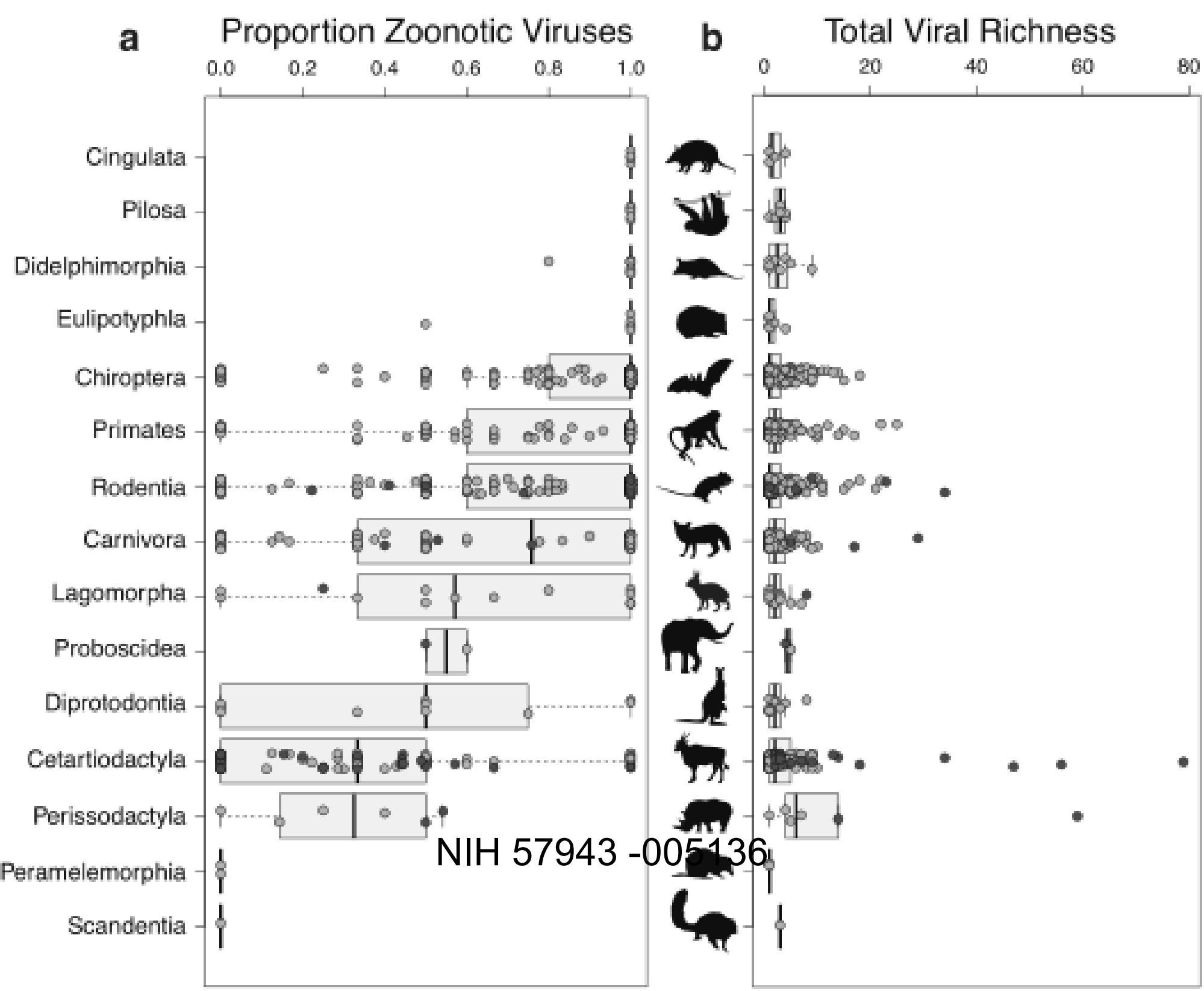
global host species richness for Carnivora (n=276); **h,** host species richness for Carnivora in database (n=79); **i,** Carnivora species with no described viruses in literature. Warmer colors (larger values) in panels **c** and **f** highlight areas predicted to be of greatest value for discovering novel viruses or novel viral zoonoses, respectively, in carnivores. Red/pink colors in panel **i** highlight areas with poor viral surveillance in carnivore species to date. Hatched regions represent areas where model predictions deviate systematically for the collection of species in that grid cell (see Methods).

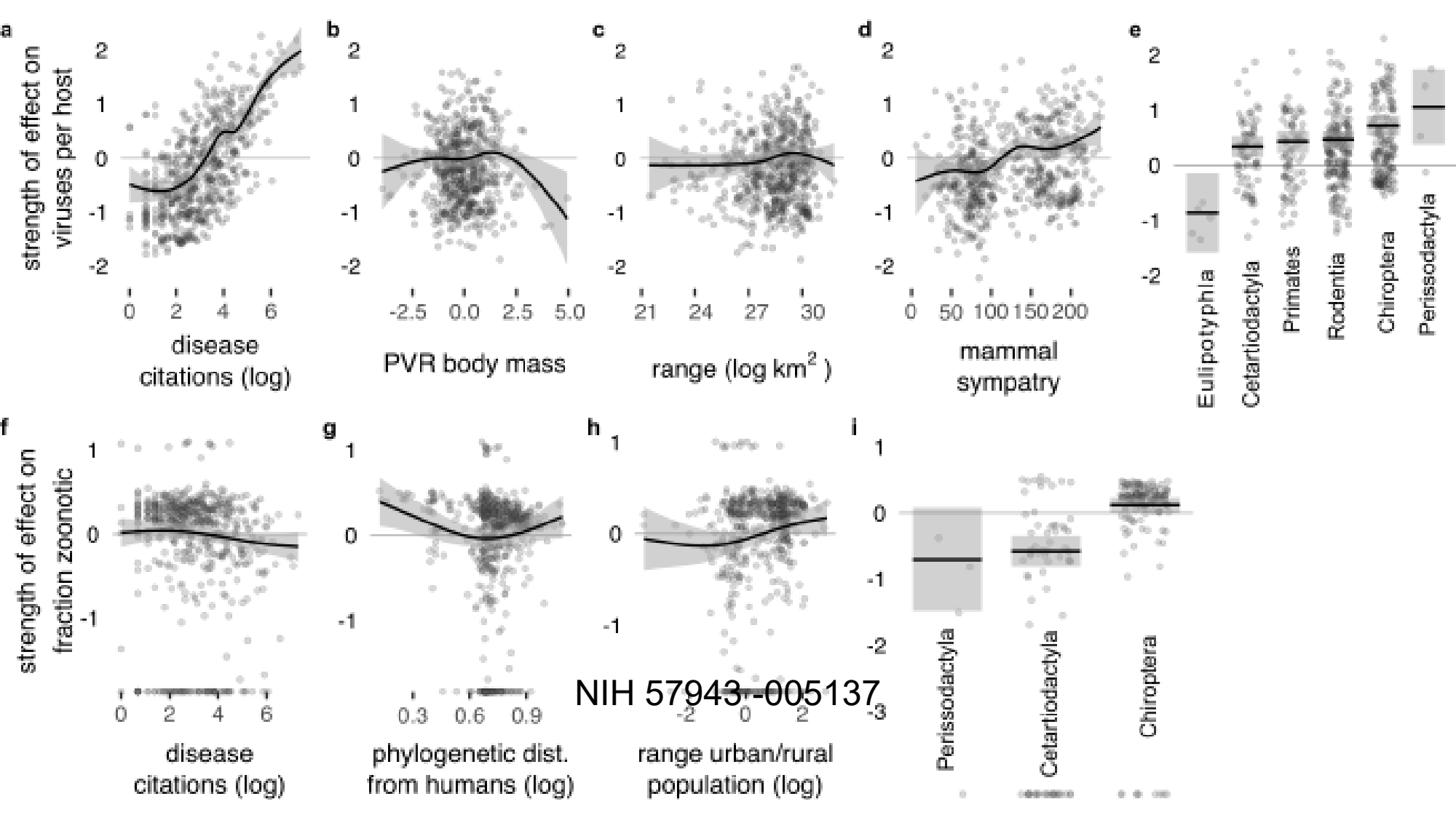
Extended Data Figure 5. Global distribution of viral and host species richness for wild even-toed ungulates (Order Cetartiodactyla). a, Observed total viral richness (for n=70 host spp.); **b,** predicted total viral richness given maximum research effort; **c,** 'missing viruses' or predicted – observed total viral richness; **d,** observed zoonotic viral richness (n=70); **e,** predicted zoonotic viral richness given maximum research effort; **f,** 'missing zoonoses' or predicted – observed zoonotic viral richness (same as included in Fig 3c); **g,** global host species richness for Cetartiodactyla (n=229); **h,** host species richness for Cetartiodactyla in database (n=105); **i,** Cetartiodactyla species with no described viruses in literature. Warmer colors (larger values) in panels **c** and **f** highlight areas predicted to be of greatest value for discovering novel viruses or novel viral zoonoses, respectively, in even-toed ungulates. Red/pink colors in panel **i** highlight areas with poor viral surveillance in even-toed ungulates species to date. Hatched regions represent areas where model predictions deviate systematically for the collection of species in that grid cell (see Methods).

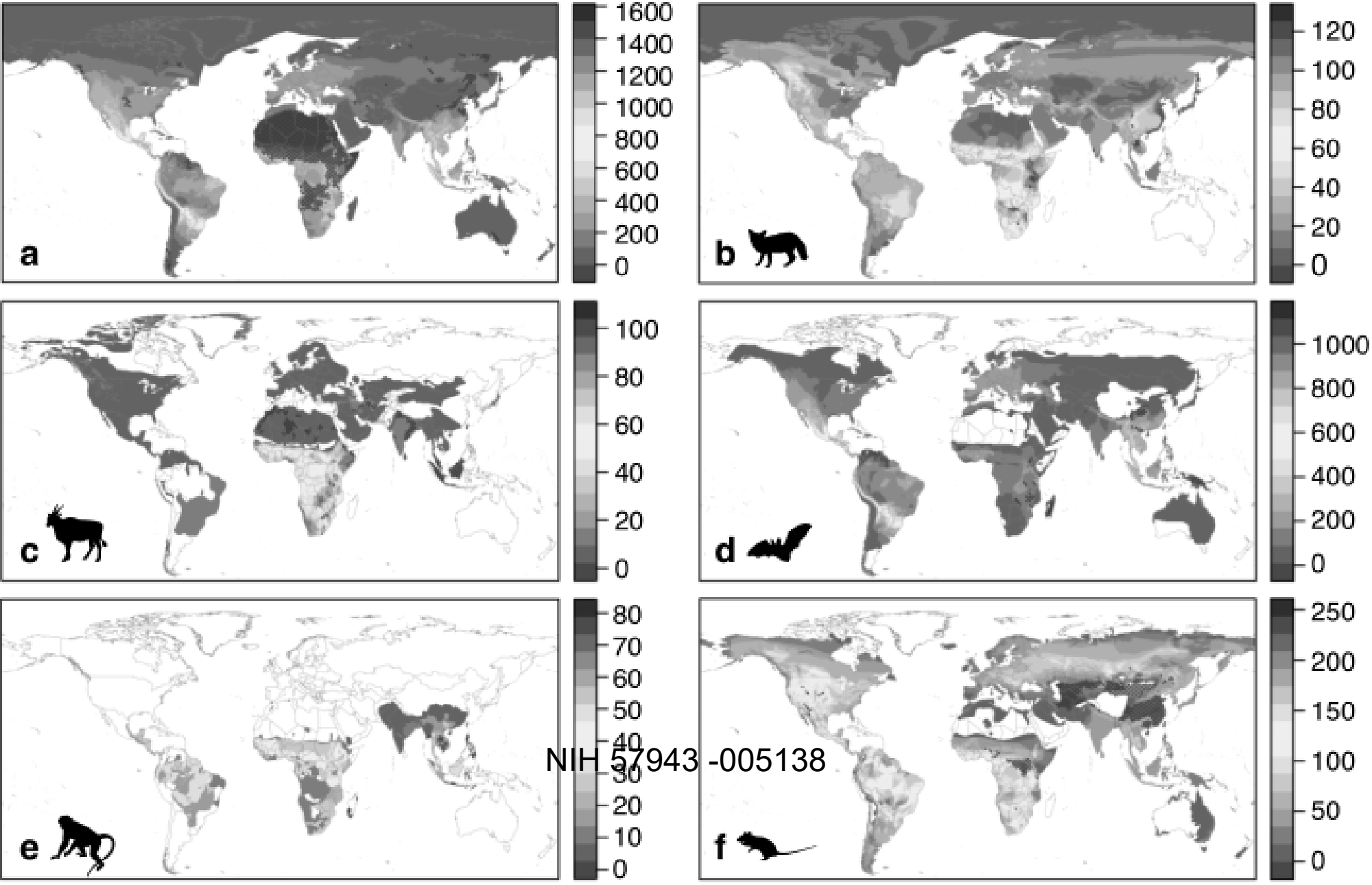
977 **Extended Data Figure 6. Global distribution of viral and host species richness for**
978 **bats (Order Chiroptera). a,** Observed total viral richness (for n=156 host spp.); **b,**
979 predicted total viral richness given maximum research effort; **c,** 'missing viruses' or
980 predicted – observed total viral richness; **d,** observed zoonotic viral richness (n=157); **e,**
981 predicted zoonotic viral richness given maximum research effort; **f,** 'missing zoonoses' or
982 predicted – observed zoonotic viral richness (same as included in Fig 3d); **g)** global host
983 species richness for Chiroptera (n=1117); **h,** host species richness for Chiroptera in
984 database (n=192); **i,** Chiroptera species with no described viruses in literature. Warmer
985 colors (larger values) in panels **c** and **f** highlight areas predicted to be of greatest value for
986 discovering novel viruses or novel viral zoonoses, respectively, in bats. Red/pink colors
987 in panel **i** highlight areas with poor viral surveillance in bat species to date. Hatched
988 regions represent areas where model predictions deviate systematically for the collection
989 of species in that grid cell (see Methods).
990
991 **Extended Data Figure 7. Global distribution of viral and host species richness for**
992 **primates (Order Primates). a,** Observed total viral richness (for n=71 host spp.); **b,**
993 predicted total viral richness given maximum research effort; **c,** 'missing viruses' or
994 predicted – observed total viral richness; **d,** observed zoonotic viral richness (n=73); **e,**
995 predicted zoonotic viral richness given maximum research effort; **f,** 'missing zoonoses' or
996 predicted – observed zoonotic viral richness (same as included in Fig 3e); **g,** global host
997 species richness for Primates (n=400); **h,** host species richness for Primates in database
998 (n=98); **i,** Primates species with no described viruses in literature. Warmer colors (larger
999 values) in panels **c** and **f** highlight areas predicted to be of greatest value for discovering

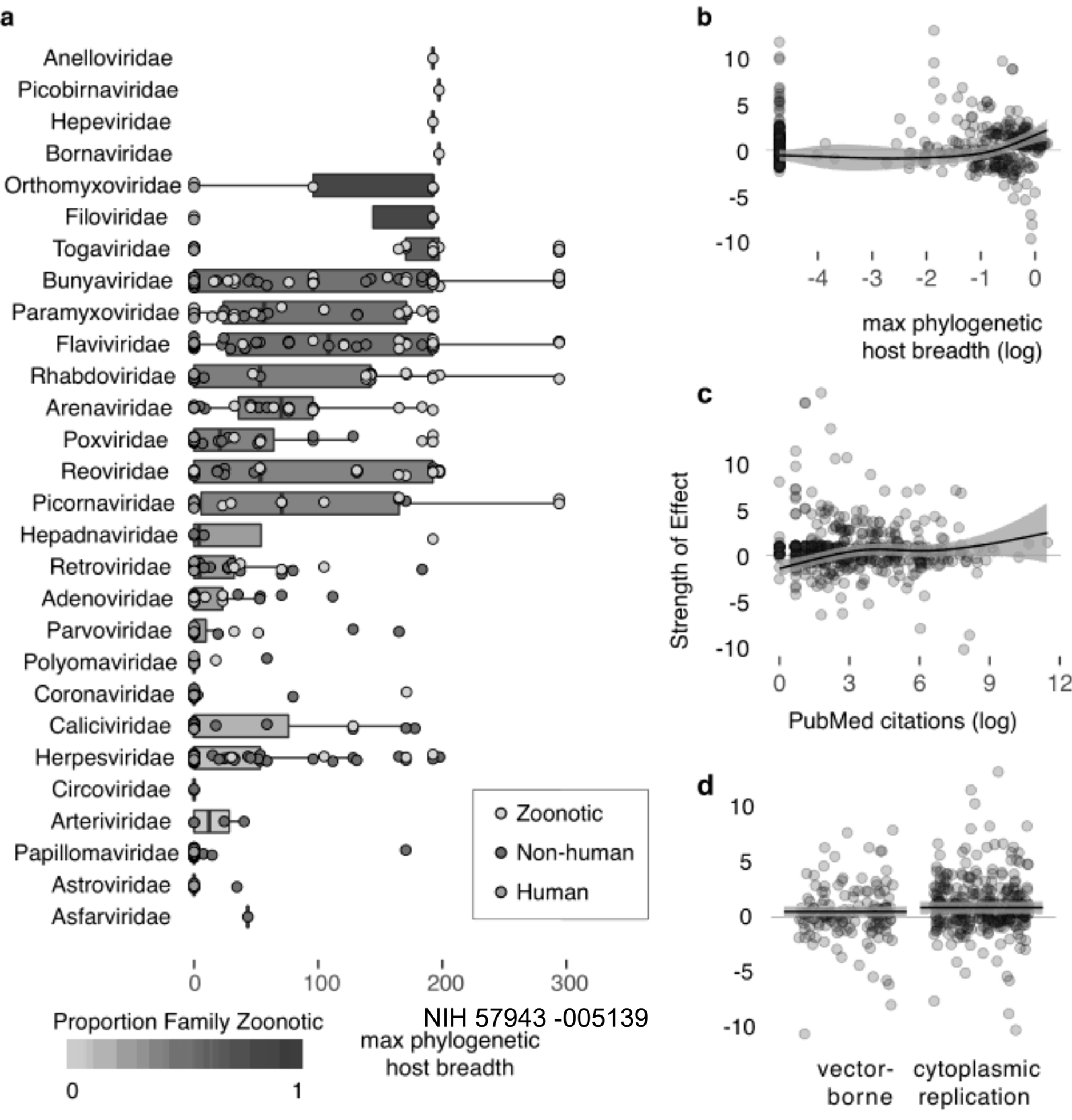
1000 novel viruses or novel viral zoonoses, respectively, in primates. Red/pink colors in panel **i**
1001 highlight areas with poor viral surveillance in primate species to date. Hatched regions
1002 represent areas where model predictions deviate systematically for the collection of
1003 species in that grid cell (see Methods).
1004
1005 **Extended Data Figure 8. Global distribution of viral and host species richness for**
1006 **rodents (Order Rodentia). a,** Observed total viral richness (for n = 178 host spp.); **b,**
1007 predicted total viral richness given maximum research effort; **c,** 'missing viruses' or
1008 predicted – observed total viral richness; **d,** observed zoonotic viral richness (n=183); **e,**
1009 predicted zoonotic viral richness given maximum research effort; **f,** 'missing zoonoses' or
1010 predicted – observed zoonotic viral richness (same as included in Fig 3f); **g,** global host
1011 species richness for Rodentia (n=2206); **h,** host species richness for Rodentia in database
1012 (n=221); **i,** Rodentia species with no described viruses in literature. Warmer colors (larger
1013 values) in panels **c** and **f** highlight areas predicted to be of greatest value for discovering
1014 novel viruses or novel viral zoonoses, respectively, in wild rodents. Red/pink colors in
1015 panel **i** highlight areas with poor viral surveillance in rodent species to date. Hatched
1016 regions represent areas where model predictions deviate systematically for the collection
1017 of species in that grid cell (see Methods).
1018
1019 **Extended Data Figure 9. Order-level phylogenies showing residuals from zoonoses**
1020 **model.** Subtrees from cytochrome b maximum likelihood phylogeny for 558 mammal
1021 species (constrained to order-level topology of mammal supertree) for **a,** bats; **b,**
1022 carnivores; **c,** even-toed ungulates; **d,** rodents; and **e,** primates. Species included have at

least 1 described virus association and available genetic data. Wildlife species names and terminal branches are color-coded by the residuals (predicted-observed) from the best-fit generalized additive model (GAM) to predict the number of zoonotic viruses using all data. Species with residual values between -1 and 1 (black) are accurately predicted within one virus. Warm colors represent species with positive residuals (orange >1 to 3; red >3). Cool colors represent species with negative residuals (green <-1 to -3; blue <-3). Marine mammals, domestic animals, and species with missing data and not included in the best-fit models are shown in grey.









From: Park, Eun-Chung (NIH/NIAID) [E]
Sent: Wed, 14 Jun 2017 17:19:11 -0400
To: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Cc: Kevin Olival, PhD; Anthony Ramos; Coleman, Amanda (NIH/NIAID) [C]
Subject: RE: Potential media interest from a Nature paper in press based on our work funded by two NIAID R01s
Attachments: Nature HP3 Press release 2017 EHA Draft 2 ed.docx

Peter,
I am attaching the document containing comments from our communication office.

Sincerely,
Eunchung

Eun-Chung Park, PhD
Program Officer,
NIAID, NIH
PH: (b)(6)
(b)(6)

From: Peter Daszak (b)(6)
Sent: Wednesday, June 14, 2017 12:30 PM
To: Park, Eun-Chung (NIH/NIAID) [E] (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Kevin Olival, PhD (b)(6) Anthony Ramos (b)(6) Coleman, Amanda (NIH/NIAID) [C] (b)(6)
Subject: RE: Potential media interest from a Nature paper in press based on our work funded by two NIAID R01s
Importance: High

No problem.

Hi Amanda. I've attached the pdf of the final version as accepted – not yet in Nature typesetting. We're just waiting on the corrected proofs from Nature and we'll send these on as soon as we get them.

As you know this is embargoed, but unfortunately right now we don't know the official publication date. We think it might be released online next Wednesday June 21st, but will confirm as soon as we hear back from Nature.

By the way – if you want a quote from me or Kevin, or have any questions – no problem – we're around this week and would be happy to help!

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. (b)(6)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Park, Eun-Chung (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, June 14, 2017 10:36 AM
To: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Cc: Kevin Olival, PhD; Anthony Ramos; Coleman, Amanda (NIH/NIAID) [C]
Subject: RE: Potential media interest from a Nature paper in press based on our work funded by two NIAID R01s

Peter,
Our communication office asks if you can provide the manuscript. I copy Amanda Coleman here, and if you can send to all of us, that will be helpful. Thank you.

Sincerely,
Eunchung

Eun-Chung Park, PhD
Program Officer,
NIAID, NIH
PH: (b)(6)
(b)(6)

From: Peter Daszak (b)(6)
Sent: Tuesday, June 13, 2017 10:08 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Park, Eun-Chung (NIH/NIAID) [E] (b)(6)
Cc: Kevin Olival, PhD (b)(6) Anthony Ramos (b)(6)
Subject: Potential media interest from a Nature paper in press based on our work funded by two NIAID R01s
Importance: High

Hi Erik and Eun-Chung

Good News! I want to give you advance notice about a paper Kevin Olival and I have in press with *Nature* that might generate some publicity. It's called "Host and Viral Traits Predict Zoonotic Spillover from Mammals". We acknowledge the current R01 (R01AI110964) on SARS-like CoVs in China that you're Program Officer for, Erik, as well as the R01 on predicting spillover from bat-origin viruses (R01AI079231) that you were Program Officer for a few years ago Eun-Chung – the work for this paper began under that R01, and it's taken a few years of database building and analysis to get to this stage!

I've inserted the abstract below, as accepted by Nature so you can see the content, as well as a draft Press Release we're working on. I don't know what the current standard is for publicity from NIAID-funded work, but I wanted to let you know in advance, in case you'd like to put a story up about this on your website, or talk to the media about it prior to the embargo.

The timing is tight. As always, we don't know exactly when Nature will release it, but we expect it will be online next week, maybe as early as **Wednesday 21st June.** We've already had pre-proofs and have corrected these so we're getting our ducks in a row for that date so that we don't miss any publicity. We'll let you know as soon as we hear the final decision.

Host and viral traits predict zoonotic spillover from mammals

Kevin J. Olival1, Parviez R. Hosseini1, Carlos Zambrana-Torrelio1, Noam Ross1, Tiffany L. Bogich1 & Peter Daszak1

The majority of human emerging infectious diseases are zoonotic, with viruses that originate in wild mammals of particular concern (for example, HIV, Ebola and SARS)1–3. Understanding patterns of viral diversity in wildlife and determinants of successful crossspecies transmission, or spillover, are therefore key goals for pandemic surveillance programs4. However, few analytical tools exist to identify which host species are likely to harbour the next human virus, or which viruses can cross species boundaries5–7. Here we conduct a comprehensive analysis of mammalian host–virus relationships and show that both the total number of viruses that infect a given species and the proportion likely to be zoonotic are predictable. After controlling for research effort, the proportion of zoonotic viruses per species is predicted by phylogenetic relatedness to humans, host taxonomy and human population within a species range—which may reflect human–wildlife contact. We demonstrate that bats harbour a significantly higher proportion of zoonotic viruses than all other mammalian orders. We also identify the taxa and geographic regions with the largest estimated number of 'missing viruses' and 'missing zoonoses' and therefore of highest value for future surveillance. We then show that phylogenetic host breadth and other viral traits are significant predictors of zoonotic potential, providing a novel framework to assess if a newly

discovered mammalian virus could infect people.

Cheers,

Peter

Peter Daszak
President

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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

Contact: Anthony M. Ramos
1.212.380.4469
ramos@ecohealthalliance.org

FOR IMMEDIATE RELEASE – DRAFT FOR APPROVAL

ECOHEALTH ALLIANCE MAPS GLOBAL DISTRIBUTION OF 'MISSING' VIRUSES ACROSS WILDLIFE SPECIES

Scientists Identify Highest Risk Mammal Species and Locations for Emerging Viruses

NEW YORK – June X, 2017 – EcoHealth Alliance, a global nonprofit organization working at the intersection of environmental, animal and public health, announced a paper published online in the journal, *Nature*, highlighting the first comprehensive analysis of all viruses known to infect mammals. The study shows that bats carry a significantly higher proportion of viruses able to infect people than any other group of mammals; and it identifies the species and geographic regions on the planet with the highest estimated proportion of yet-to-be discovered, or 'missing', viruses likely to infect people. This work provides a new way to predict where and how we should work to identify and pre-empt the next potential viral pandemic before it emerges.

The study team built a comprehensive database of all known viruses infecting over 700 mammal species (including people). They used mathematical models to identify the host species characteristics associated with having a larger number of viruses capable of infecting people (zoonotic viruses). They show that zoonotic potential is predicted by a host species evolutionary relatedness to humans, the degree of human-wildlife contact, and other factors including the taxonomic order it belongs to. They used this analysis to demonstrate for the first time that, after correcting for uneven research effort and other variables, bats harbor the highest proportion of zoonotic viruses of any mammal group. "In 2005, our team showed that SARS originates in bats. Ever since that finding, scientists have wondered whether bats are 'special' reservoirs for viruses. We now show definitively that bats carry a higher estimated proportion of yet-to-be-identified viruses of potential risk to people than any other mammal group," says EcoHealth Alliance's President and senior author on the study, Dr. Peter Daszak. The paper points out that viral discovery research on wild bats could help prevent pandemics. Bats are important to ecosystem health, through pollinating tropical fruits, removing crop pests like moths and disease vectors like mosquitoes, and providing other critical ecosystem services globally. "While the data show bats carry potentially important viruses, it's important to remember that the only way

Commented [NIAID1]: Hard to understand this concept of missing viruses.

Commented [ED2]: This seems like a massive database—might be impressive to include the approximate number of viruses studied!

Commented [NIAID3]: Unclear what this is.

Commented [NIAID4]: Not sure about using "definitive" given this is all based on predictive theory.

these viruses can emerge in people is if we make contact with bats, alter their environment, hunt them or otherwise disturb their ecology" -- BCI or us.

The study uses the analyses to produce detailed maps showing where on the planet we are most likely to find as-yet-undiscovered viruses that could emerge in people, or 'missing zoonoses.'. These maps differ among mammal groups. For example, hotspots of 'missing zoonoses' for bats are in South and Central America and parts of Asia, for primates in tropical Central America, Africa, and southeast Asia. "The holy grail in pandemic prevention is to understand where the next zoonotic virus is likely to emerge and from what species. Our study provides the first ever predictive map of where these undiscovered zoonoses can be found across the world. This information will be critical to guide future surveillance to identify and stop the next pandemic before it has chance to emerge," says Dr. Kevin Olival, lead author on the study. Finally, the paper provides a new way to estimate how likely a newly-discovered virus from wildlife could be to infect people. It shows that measuring the evolutionary breadth of its host species can predict its potential to infect people. This approach is already being used as part of a multi-country project to identify new viruses in wildlife and help prevent their emergence – the USAID PREDICT program (http://www.ecohealthalliance.org/program/predict).

Commented [ED5]: Does it count as a "new way" if USAID is already using it? Would it be better to recast this as confirming that USAID's techniques work?

This work was funded by grants from the National Institute of Allergy and Infectious Diseases (NIH-NIAID, https://www.niaid.nih.gov/) and from the USAID Emerging Pandemic Threats program (www.usaid.gov//what-we-do/global-health/pandemic-influenza-and-other-emerging-threats).

Commented [NIAID6]: Please include specific grant numbers.

About EcoHealth Alliance

Building on over 45 years of groundbreaking science, EcoHealth Alliance is a global, nonprofit organization dedicated to pandemic prevention and ecosystem health. Approximately 60 percent of emerging infectious diseases like Ebola, HIV, Zika, SARS, and MERS originated in animals before spilling over to human populations. Using environmental and health data covering the past 60 years, EcoHealth Alliance scientists created the first-ever, global disease hotspots map that identified at-risk regions to determine where field programs can help predict and prevent the next pandemic crisis. That work is the foundation of EcoHealth Alliance's rigorous, science-based approach working in more than 30 countries worldwide.

For more information, please visit www.ecohealthalliance.org

From: Peter Daszak
Sent: Sun, 1 Oct 2017 17:21:51 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Morens, David (NIH/NIAID) [E]; David Morens (b)(6) Kurilla, Michael (NIH/NCATS) [E]; (b)(6) Stemmy, Erik (NIH/NIAID) [E]; Alison Andre; Aleksei Chmura
Subject: Confidential - A new bat-origin coronavirus emerging in pigs in China discovered under our NIAID R01
Attachments: Nature 2017-05-06890-main text with figures and tables.pdf
Importance: High

Dear Dr Fauci and NIAID colleagues,

It was a pleasure to meet you again today. I've attached an unpublished paper, currently in the second round of review with *Nature* that describes a novel bat-origin Coronavirus (SADS-CoV: Swine Acute Diarrheal Syndrome coronavirus) that recently spilled over into pig farms in Southern China, leading to the death of over 25,000 piglets in 5+ farms in Guandong Province.

The virus originates in the same group of bats as SARS-CoV, and emerged in the same place. It's not known to be zoonotic (we've tested 35+ pig farm workers with an antibody assay and none are positive. The pig farm owners tell us the virus is now under control, thanks to culling and separation of infected herds. It's not yet known if this virus has appeared elsewhere, but we are looking. We're also doing assays to find out if it can infect human cells in the lab – so far no evidence of this.

I hope this paper is of interest. You should know that this work was supported by a NIAID R01 that Erik Stemmy is the Program Officer for, and that I'm PI on, with Zhengli Shi as co-PI.

If you want any other information at all, please don't hesitate to email or call and I'd be happy to come over to NIAID to brief you further. I'll also let you know if/when it will be published so that we can try to foster some publicity as appropriate.

Cheers,

Peter

Peter Daszak
President

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www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

Title: Fatal Swine Disease Outbreak Caused by a Novel HKU2-related Coronavirus of Bat Origin

Authors: Peng Zhou[1]*, Hang Fan[2]*, Tian Lan[3]*, Xing-Lou Yang[1], Wei-Feng Shi[4], Wei Zhang[1], Yan Zhu[1], Ya-Wei Zhang[2], Qing-Mei Xie[3], Shailendra Mani[5], Xiao-Shuang Zheng[1], Bei Li[1], Jin-Man Li[2], Hua Guo[1], Guang-Qian Pei[2], Xiao-Ping An[2], Jun-Wei Chen[3], Ling Zhou[3], Kai-jie Mai[3], Zi-Xian Wu[3], Di Li[3], Danielle E. Anderson[5], Li-Biao Zhang[6], Shi-Yue Li[7], Zhi-Qiang Mi[2], Tong-Tong He[2], Feng Cong[8], Peng-Ju Guo[8], Ren Huang[8], Yun Luo[1], Xiang-Ling Liu[1], Jing Chen[1], Yong Huang[2], Qiang Sun[2], Xiang-Li-Lan Zhang[2], Yuan-Yuan Wang[2], Shao-Zhen Xing[2], Yan-Shan Chen[3], Yuan Sun[3], Juan LI[4], Peter Daszak[9]†, Lin-Fa Wang[5]†, Zheng-Li Shi[1]†, Yi-Gang Tong[2]†, Jing-Yun Ma[3]†

Affiliations:

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[3]College of Animal Science, South China Agricultural University & Key Laboratory of Animal Health Aquaculture and Environmental Control, Guangdong, Guangzhou 510642, China

[4]Shandong Universities Key Laboratory of Etiology and Epidemiology of Emerging Infectious Diseases, Taishan Medical College, Taian 271000, China

[5]Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore 169857, Singapore

[6]Guangdong Key Laboratory of Animal Conservation and Resource Utilization, Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Guangdong Institute of Applied Biological Resources, Guangzhou 510260, China

[7]School of Public Health, Wuhan University, 430072, China

[8]Guangdong Key Laboratory of Laboratory Animals, Guangdong Laboratory Animals Monitoring Institute, Guangzhou 510640, China

[9]EcoHealth Alliance, New York, USA

*These authors contributed equally to this work

†To whom correspondence should be addressed: daszak@ecohealthalliance.org; linfa.wang@duke-nus.edu.sg; tong.yigang@gmail.com; majy2400@scau.edu.cn; zlshi@wh.iov.cn

Spillover of bat-origin coronaviruses is implicated in the origin of two high-impact emerging zoonoses, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome coronavirus. Here, we report virological, epidemiological, evolutionary and experimental infection evidence that a novel HKU2-related bat coronavirus, Swine Acute Diarrhea Syndrome coronavirus (SADS-CoV), is the cause of an ongoing outbreak of lethal diarrheal disease in pigs in China. The outbreak began in Guangdong Province in the vicinity of the origin of the SARS pandemic in 2002, and has resulted in the death of 24,693 piglets in four farms to date. We identified SADS related-CoVs with 96-98% sequence identity to SADS-CoV in 11.9% (71/596) of anal swabs collected from bats in Guangdong Province during 2013-16, predominantly in *Rhinolophus* spp. horseshoe bats that are known reservoirs of SARS-related CoVs. The geographic, temporal, ecological and etiological similarities in the emergence of SADS and SARS highlight the urgent need to identify coronavirus diversity in bats to mitigate future outbreaks that threaten livestock, public health and economic growth.

The emergence of severe acute respiratory syndrome (SARS) in southern China in 2002, which was caused by a previously unknown coronavirus (SARS-CoV)[1-5] and led to more than 8,000 human infections and 774 deaths [http://www.who.int/csr/sars/en/], heralded two new frontiers in emerging infectious diseases. Firstly, it demonstrated that coronaviruses are capable of causing fatal

diseases in humans. Secondly, the identification of bats as the reservoir for SARS-related coronaviruses, and likely origin of SARS-CoV[6-8] firmly established bats as an important source of highly lethal zoonotic viruses that include Hendra, Nipah, Ebola and Marburg viruses[9].

Here we report a series of fatal swine disease outbreaks in Guangdong Province, China, approximately 100 km from the location of the purported index case of SARS. Most strikingly, we found that the causative agent for this swine acute diarrhea syndrome (SADS) is a novel HKU2-related coronavirus which is 98.48% identical in genome sequence to a bat coronavirus we detected in 2016 from a bat cave in the vicinity of the index pig farm. This new virus (SADS-CoV) originated from the same genus of horseshoe bats (*Rhinolophus*) as SARS-CoV.

From 28 October 2016, fatal swine disease outbreaks were observed in a pig farm in Qingyuan, Guangdong Province, China, very close to the location of the first known index case of SARS in Foshan (**Extended Data Figure 1**). Porcine epidemic diarrhea virus (PEDV, a coronavirus) had caused prior outbreaks at this farm, and was detected in the intestine of deceased piglets at the start of the outbreak. However, PEDV could no longer be detected in deceased piglets after 12th January 2017, despite accelerating mortality (**Fig. 1A**) and extensive testing for other common swine viruses yielded negative results (**Extended Data Table 1**). These findings suggested an outbreak of a novel disease. Clinical signs are similar to those caused by other known swine enteric coronaviruses [10,11] and include severe and acute diarrhea, and rapid weight loss, leading to death, which we conclude is due to nutritional exhaustion

(denoted by rapid weight loss) in addition to dehydration in newborn piglets less than five days of age. Vomiting, a hallmark for PEDV, PDCoV and TGEV infections in seronegative piglets, was not observed in SADS piglets, either in the farms or in experimentally-infected pigs (below). Further detailed work on the clinical course and pathology of SADS may identify more distinct clinical markers that allow it to be distinguished more readily from other pig intestinal infections. Infected piglets died 2-6 days following disease onset, while infected sows suffered only mild diarrhea and most recovered in two days. The disease caused no signs of febrile illness in piglets or sows. The death toll reached as high as 90% for piglets five days or younger, while for piglets older than eight days the death rate dropped to 5%. We randomly sampled 12 sick piglets (>5 day old) and 5 sick sows, all were SADS-CoV positive (negative for healthy piglets/sows). For deceased piglets, intestines were tested by either PCR or IHC. All piglets tested were SADS-CoV positive (**Fig. 3**). Subsequently, SADS-related outbreaks were found in three additional pig farms within 20-150 km of the index farm (**Extended Data Figure 1**) and, as of 2nd May 2017, had resulted in the death of 24,693 piglets from four farms (**Fig. 1A**). In Farm A alone, 64% (4659/7268) of all piglets born in February died. The outbreak appears now to be under control. Measures taken included separating sick sows and piglets from the rest of the herd and using qPCR was the main diagnostic tool for SADS-CoV (see primer sequence in **Extended Data Table S3**).

A small intestinal sample from diseased piglets was subjected to metagenomics analysis by next generation sequencing (NGS) for identification of

potential etiologic agent. Of the 15,256,565 total reads obtained, 4,225 matched sequences of bat CoV HKU2, first detected in Chinese horseshoe bats in Hong Kong and Guangdong Province, China[12]. By *de novo* assembly and targeted PCR we obtained a 27,173-bp CoV genome that shared 95% sequence identity to HKU2 (Genbank accession number NC009988.1). Thirty-three genomes of SADS-CoV were subsequently obtained (8 from farm A; 5 from B; 11 from C and 9 from D), and these are 99.9% identical to each other (**Extended Data Table 2**).

Using quantitative PCR based on the nucleocapsid gene (**Extended Data Table 3**), we detected SADS-CoV in acutely sick piglets and sows, but not in recovered or healthy pigs on the four farms, nor in nearby farms that showed no evidence of SADS. The virus replicated to higher titers in piglets than in sows (**Fig. 1B**). SADS-CoV displayed tissue tropism for intestine (**Fig. 1C**), as observed for other swine enteric coronaviruses[13]. Retrospective PCR analysis revealed that SADS-CoV was present on Farm A during the PEDV epidemic, where the first strongly positive SADS-CoV sample was detected on 6 December 2016. From mid-January onwards, SADS-CoV was the dominant viral agent detected in diseased animals (**Extended Data Figure 2**). Although PEDV was also detected occasionally during the outbreaks in Farms B, C and D, SADS-CoV was the dominant virus (**Extended Data Figure 2 & Table 1**). It is possible that the presence of PEDV early in the SADS-CoV outbreak may have somehow facilitated or enhanced spillover and amplification of SADS. However the fact that the vast majority of piglet mortality occurred after PEDV infection had become undetectable suggests that SADS-CoV

125 itself causes a lethal infection in pigs that was responsible for these large scale
126 outbreaks and that PEDV does not directly contribute to its severity in individual pigs.
127 We rapidly developed an antibody assay based on the S1 domain of the spike
128 (S) protein using the Luciferase Immunoprecipitation System (LIPS)[14]. As SADS is
129 acute with rapid onset in piglets, serological investigation was conducted only in
130 sows. Among 46 recovered sows tested, 12 were seropositive for SADS-CoV within
131 three weeks of infection (**Fig. 1D**). To investigate possible zoonotic transmission,
132 serum samples from 35 farm workers who had close contact with sick pigs were
133 subjected to the same LIPS test and none was positive for SADS-CoV.
134 While the overall genome identity of SADS-CoV and HKU2 is 95%, the S
135 gene sequence identity is only 86%, suggesting that previously reported HKU2 is not
136 the direct progenitor of SADS-CoV but that they may have originated from a common
137 ancestor. To test the hypothesis, we developed a qPCR assay based on the
138 SADS-CoV RNA dependent RNA polymerase (RdRp) gene (**Extended Data Table**
139 **3**) and screened 596 bat anal swabs collected from 2013-2016 from seven different
140 locations in Guangdong Province (**Extended Data Figure 1**). A total of 71 samples
141 (11.9%) tested positive (**Extended Data Table 4**), the majority (94.3%) was from
142 *Rhinolophus* spp. bats which are also the natural reservoir hosts of SARS-related
143 coronaviruses[6-8, 15-18]. Complete genome sequences were determined by NGS from
144 four samples selected based on their highest sequence identity to SADS-CoV. These
145 four genomes are very similar in size (27.2 kb) to SADS-CoV (**Fig. 2A**) and we
146 tentatively nominate them SADS related coronaviruses (SADSr-CoV). Overall

147 sequence identity between SADSr-CoV and SADS-CoV ranges from 96-98%. More
148 importantly, S proteins of two SADSr-CoVs (162149 and 141388) shared more than
149 98% identity to SADS-CoV, compared to 86% for HKU2. The major differences
150 among SADSr-CoVs lie in the predicted coding regions of the S and 3'-terminal
151 ORF7a and ORF7b genes (**Fig. 2A**). Additional 19 S1 genes of bat-derived strains
152 were sequenced.

153 The phylogeny of S1 and full-length genome revealed a high genetic diversity
154 of alpha coronaviruses among bats and strong coevolutonary relationships to their
155 hosts(**Fig. 2B and Extended Data Figure 3**), with SADS-CoVs closely related to
156 SADSr-CoVs from *Rhinolophus affinis* rather than *Rhinolophus sinicus* where HKU2
157 was found (**Fig. 2B**). Both phylogenetic and haplotype network analyses
158 demonstrated that viruses from the four farms likely originated from their reservoir
159 hosts independently (**Extended Data Figure 4**), and a few viruses might have
160 undergone further genetic recombination (**Extended Data Figure 5**). However,
161 molecular clock analysis of the 33 SADS-CoV genome sequences failed to establish
162 the positive association between sequence divergence and sampling date. Therefore,
163 we speculate that either the virus was introduced into pigs from bats multiple times, or
164 the virus was introduced into pigs once but subsequent genetic recombination
165 disturbed the molecular clock.

166 Known coronavirus host cell receptors include angiotensin-converting enzyme
167 2 (ACE2) for SARS-related CoV, aminopeptidase N (APN) for PEDV, and dipeptidyl
168 peptidase 4 (DPP4) for MERS-CoV[19-21]. To investigate the receptor usage of

169 SADS-CoV, we used SADS-CoV positive samples or HIV pseudoviruses carrying the
170 SADS-CoV S protein to infected HeLa cells which over-expressed each of the three
171 molecules. While the positive control worked for SL-CoV and MERS-CoV
172 pseudovirus with successful infection or entry, we found no evidence of infection or
173 entry for SADS-CoV, suggesting that none of them is a functional receptor of
174 SADS-CoV (**Extended Data Table 5**).

175 Swine enteric coronaviruses including PEDV, transmission gastroenteritis
176 virus (TGEV) and porcine diarrhea coronavirus (PDCoV) are known to cause severe
177 watery diarrhea and dehydration accompanied by histopathological lesions in the
178 infected pigs. Clinically PEDV, TGEV, and PDCoV are indistinguishable[22]. In
179 contrast, piglets infected with SADS-CoV mainly die of nutritional exhaustion rather
180 than severe dehydration. Efforts to isolate virus from intestinal tissues of infected
181 piglets and from bat samples with low PCR Ct values have been unsuccessful to date.
182 However, we successfully conducted animal challenge experiments using NGS to
183 identify and confirm causality relationship. Tissue samples positive for SADS-CoV
184 and negative for PEDV or any other known swine diarrhea virus by both NGS and
185 PCR were fed to 3-day old specific pathogen free (SPF) piglets. Most of the piglets
186 (5/7) inoculated with SADS-CoV positive tissues exhibited severe diarrhea one day
187 after challenge, while control animals remained healthy. On day 2 post infection, all
188 animals in the infected group suffered severe diarrhea or death (**Extended Data**
189 **Table 6 & Figure 6**). Animals were euthanized on day 3 for further analysis.
190 Histopathological examinations showed similar lesions in the challenged piglets to

those in naturally infected piglets (**Fig. 3A and 3B**). Using antibodies specific for the NP (prepared in house) and epithelial cell markers, specific staining was detected mainly in small intestine epithelial cells (**Fig. 3C and 3D**). Finally, qPCR and NGS were used to verify that all diseased piglets were SADS-CoV positive and negative for other known swine diarrhea viruses; and that all control piglets were negative for SADS-CoV.

The current study highlights the value of proactive viral discovery in wildlife, and targeted surveillance in response to an emerging infectious disease event, as well as the disproportionate importance of bats as reservoirs of viruses that threaten veterinary and public health[23]. It also demonstrates that by using modern technological platforms such as NGS, LIPS serology and phylogenic analysis, key experiments that traditionally rely on isolation of live virus can be performed rapidly prior to virus isolation.

METHODS

Sample collection

Bats were captured and sampled in their natural habitat in Guangdong Province (**Extended Data Figure 1**) as described previously[6]. Fecal swab samples were collected in viral transport medium (VTM) composed of Hank's balanced salt solution at pH7.4 containing BSA (1%), amphotericin (15 μg/ml), penicillin G (100 units/ml), and streptomycin (50 μg/ml). Stool samples from sick pigs were collected in VTM. When appropriate and feasible, intestinal samples were also taken from

deceased animals. Samples were aliquoted and stored at -80 ^{0}C until use. Blood samples were collected from recovered sows and farm workers who had close contact with sick pigs. Serum was separated by centrifugation at 3,000 *g* for 15 min within 24 h of collection and preserved at 4 ^{0}C. Human serum collection was approved by the Medical Ethics Committee of the Wuhan School of Public Health, Wuhan University and Hummingbird IRB.

Virus isolation

The following cells were used for virus isolation in this study: VeroE6 (cultured in DMEM +10% FBS); *Rhinolophus sinicus* primary or immortalized cells generated by our laboratory (all cultured in DMEM/F12 +15% FBS): kidney primary RsKi9409, lung primary RsLu4323, lung immortalized RsLuT, brain immortalized RsBrT and heart immortalized RsHeT; and swine cell lines: two intestinal IPEC (RPMI1640+10%FBS) and SIEC (DMEM+10%FBS), three kidney PK15, LLC-PK1 (DMEM+10% FBS for all) and IBRS (MEM+10%FBS), and one testes ST (DMEM+10%FBS).

Cultured cell monolayers were maintained in their respective medium. PCR-positive pig fecal or supernatant from homogenized pig intestine (in 200 µl VTM) were filtered and diluted 1:10 with serum-free medium before being added to cells. After incubation at 37 ^{0}C for 1 h, the inoculum was removed and replaced with fresh culture medium containing 2% FCS. The cells were incubated at 37 ^{0}C and observed daily for cytopathic effect (CPE). Four blind passages (three-day interval between every passage) were performed for each sample. After each passage, both the

culture supernatant and cell pellet were examined for presence of virus by RT-PCR using the SADS-CoV primers listed in **Extended Data Table 3**. Penicillin (100 units/ml) and streptomycin (15 μg/ml) were included in all tissue culture media.

RNA extraction, S1 gene amplification and qPCR

Whenever commercial kits were used, manufacturer's instructions were followed without modification. RNA was extracted from 200 μl of swab samples (bat), feces or homogenized intestine (pig) with the High Pure Viral RNA Kit (Roche). RNA was eluted in 50 μl of elution buffer and used as the template for RT-PCR. Reverse transcription was performed using the SuperScript III kit (Invitrogen).

To amplify S1 genes from bat samples, nested PCR was performed with primers designed based on HKU2 (Genbank accession number NC009988.1)[12] (**Extended Data Table 3**). The 25 μl first-round PCR mixture contained 2.5 μl 10X PCR reaction buffer, 5 pmol of each primer, 50 mM MgCl2, 0.5 mM dNTP, 0.1 μl Platinum Taq Enzyme (Invitrogen) and 1 μl cDNA. The 50 μl second-round PCR mixture was identical to the first-round PCR mixture except for primers. Amplification of both rounds was performed as follows: 94 °C for 5 min followed by 60 cycles consisting of 94 °C for 30 s, 50 °C for 40 s, 72 °C for 2.5 min, and a final extension of 72 °C for 10 min. PCR products were gel purified and sequenced.

For qPCR analysis, primers based on SADS-CoV RdRp and NP genes were used (**Extended Data Table 3**). RNA extracted from above was reverse-transcribed using PrimeScript RT Master Mix (Takara). The 10-μl qPCR reaction mix contained

5 μl 2× SYBR premix Ex Taq II (Takara), 0.4 μM of each primer and 1 μl cDNA. Amplification was performed as follows: 95 °C for 30 s followed by 40 cycles consisting of 95 °C for 5 s, 60 °C for 30 s, and a melting curve step.

Luciferase Immunoprecipitation System (LIPS) assay

The SADS-CoV S1 gene was codon optimized for eukaryotic expression, synthesized (GenScript) and cloned in frame with the Renilla luciferase gene (Rluc) and a FLAG tag in the pREN2 vector[14]. pREN2-S1 plasmids were transfected into Cos-1 cells using Lipofectamine (Invitrogen). At 48 h post-transfection, cells were harvested, lysed and a luciferease assay was performed to determine Rluc expression for both the empty vector (pREN2) and the pREN2-S1 construct. For testing of unknown pig or human serum samples, 1 μl of serum was incubated with 10 million units of Rluc alone (vector) and Rluc-S1, respectively, together with 3.5 μl of a 30% protein A/G ultralink bead suspension (Thermo Scientific). After extensive washing to remove unbounded luciferase-tagged antigen, captured luciferase amount was determined using the commercial luciferase substrate kit (Promega). The ratio of Rluc-S1/Rluc (Vector) was used to determine the specific S1 reactivity of pig and human sera. Commercial FLAG antibody (Life Technologies) was used as the positive control, and various pig sera (from uninfected animals in China or Singapore; or pigs infected with PEDV, TGEV or Nipah virus) were used as a negative control.

Protein expression and antibody production

The NP gene from SADSr-CoV 3755 (GenBank accession number MF094702), which shared a 98% aa sequence identity to the SADS-CoV NP gene,

was inserted into pET-28a+ (Novagen) for prokaryotic expression. Transformed *E. coli* were grown at 37 °C for 12-18 h in media containing 1 mM IPTG. Bacteria were collected by centrifugation and resuspended in 30 ml of 5 mM imidazole and lysed by sonication. The lysate, from which NP protein expression was confirmed with an anti-HIS-tag antibody, was applied to the Ni2+ resin (Thermo Scientific). The purified NP protein, at a concentration of 400 μg/ml, was used to immunize rabbits for antibody production following published methods[24]. After immunization and two boosts, rabbits were euthanized and sera were collected. Rabbit anti-NP sera were diluted 1:10,000 for subsequent Western blots.

Amplification, cloning and expression of the human and swine genes

Construction of expression clones for human ACE2 in pcDNA3.1 has been described previously[8, 25]. Human DPP4 was amplified from human cell lines. Human APN gene was commercially synthesized. Swine APN and ACE2 genes were amplified from piglet intestine. Full-length gene fragments were amplified using specific primers (provided upon request). The human APN, DPP4 and ACE2 genes were cloned into pCDNA3.1 fused with HIS tag. The pig APN and ACE2 genes were cloned into pCAGGS fused with S tag. Purified plasmids were transfected to HeLa cells. After 24 h, HeLa cells expressing human or swine genes were confirmed by immunofluorescence assay (IFA). Human APN, ACE2 and DPP4 expression was detected using mouse anti-HIS tag monoclonal antibody or rabbit anti-human APN polyclonal antibody (produced in house) followed by cyanin 3-labeled goat anti-mouse/rabbit IgG (Proteintech Group). Swine APN and ACE2 expression was

detected using mouse anti-S tag monoclonal antibody followed by cyanin 3-labeled goat anti-mouse IgG from proteintech (Proteintech Group).

Pseudovirus preparation

The codon-humanized S protein genes of SADS-CoV and MERS-CoV cloned into pcDNA3.1(+) and pHIV-Luc (pNL4.3.Luc.R$^-$E$^-$Luc) were used for pseudovirus construction as described previously[8, 25]. Briefly, 15 μg of each pHIV-Luc (pNL4.3.Luc.R$^-$E$^-$Luc) and the S protein expressing plasmids (or empty vector control) were co-transfected into 4 x 10^6 293T cells using Lipofectamine 3000 (Invitrogen). After 4 h, the medium was replaced with fresh medium. Supernatants were harvested at 48 h post transfection and clarified by centrifugation at 3,000g, then passed through a 0.45μm filter (Millipore). The filtered supernatants were stored at -80°C in aliquots until use. To evaluate the incorporation of S proteins into the core of HIV virions, pseudoviruses in the supernatant (20 ml) were concentrated by ultracentrifugation through a 20% sucrose cushion (5ml) at 80,000g for 90 min using a SW41 rotor (Beckman). Pelleted pseudoviruses were dissolved in 50μl phosphate-buffered saline (PBS) and examined by electron microscopy (EM).

Pseudovirus infection

HeLa cells transiently expressing APN, ACE2 or DPP4 were prepared by a Lipofectamine 2000 system (Invitrogen). Pseudoviruses prepared above were added to each 96-well plate seeded with HeLa cells at 24 hpi of APN, ACE2 or DPP4 expression plasmids. The unabsorbed viruses were removed and replaced with fresh medium at 3 hpi. The infection was monitored by measuring the luciferase activity

conferred by the reporter gene carried by the pseudovirus, using the Luciferase Assay System (Promega) as follows: cells were lysed at 48 hpi, and 20 µl of the lysates was taken for determining luciferase activity by the addition of 50 µl of luciferase substrate.

SADS-CoV positive samples infection and IFA.

HeLa cells transiently expressing APN, ACE2 or DPP4 were prepared by a lipofectamine 2000 system (Invitrogen) in 96-well plate, with mock-transfected cells as controls. SADS-CoV RNA positive samples were used to infect Hela cells at 24 hpi. The inoculum was removed after 1h absorption and washed twice with PBS and supplemented with medium. PEDV, SARS-related-CoV WIV16[18] and MERS-CoV HIV-pseudovirus were used as positive control for swine APN, human/swine ACE2 and human DPP4, respectively. At 24 hpi, cells were washed with PBS and fixed with 4% formaldehyde in PBS (pH 7.4) for 20 min at room temperature. SL-CoV WIV16 replication was detected using rabbit antibody against the SL-CoV Rp3 nucleocapsid protein followed by cyanin 3-conjugated goat anti-rabbit IgG[18]. PEDV and SADS-CoV replication was detected using rabbit antibody against the HKU2 NP followed by cyanin 3-conjugated goat anti-rabbit IgG. Nucleus was stained with 4',6'-diamidino-2-phenylindole (DAPI). Staining patterns were examined using the FV1200 confocal microscopy (Olympus). The successful infection of MERS CoV HIV-pseudovirus was indicated by luciferase on 48 hpi.

High throughput sequencing, pathogen screening and genome assembly

Tissue from the small intestine of deceased pigs was homogenized and filtered through 0.45 μm filters before nucleic acid was extracted and ribosomal RNA were depleted using NEBNext rRNA Depletion Kit (New England Biolabs). Metagenomics analysis of both RNA and DNA viruses was performed. For the RNA virus screening, the sequencing library was constructed using Ion Total RNA-Seq Kit v2 (ThermoFisher). For the DNA virus screening, NEBNext Fast DNA Fragmentation & Library Prep Set for Ion Torrent (New England Biolabs) was used for library preparation. Both libraries were sequenced on an Ion S5 sequencer (Thermal Fisher). An analysis pipeline was applied on the sequencing data which perform the following analysis steps: 1) raw data quality filtering, 2) host genomic sequence filtering, 3) blastn against virus nucleotide database using BLAST, 4) blastx against virus protein database using DIAMOND; 5) contig assembling and blastx against virus protein database. For whole viral genome sequencing, amplicon primers (**Extended Data Table 3**) were designed using the ThermoFisher online tool with HKU2 and SADS-CoV-farm A genome as reference, and the sequencing libraries were constructed using NEBNext Ultra II DNA Library Prep Kit for Illumina and sequenced on an MiSeq sequencer. PCR and Sanger sequencing was performed to fill gaps in the genome. TGenome sequences were assembled using CLC Genomic Workbench (ver 9.0). 5'-RACE was performed to determine the 5'-end of the genomes. Genomes were annotated using Clone Manager Professional Suite 8 (Sci-Ed Software).

Phylogenetic analysis

SADS-CoV genome sequences and other representative coronavirus sequences (obtained from GenBank) were aligned using MAFFT (ver 7.221). Phylogenetic analyses with full-length genome, S gene and RdRp were performed using MrBayes v3.2. Twenty million to fifty million steps were run, with GTR+G+I model (General Time Reversible model of nucleotide substitution with a proportion of invariant sites and γ-distributed rates among sites). The first 10% were removed as burn-in. The association between phylogenies and phenotypes (e.g. host species and farms) was assessed by BaTS, with the trees obtained in the previous step used as input. For SADS-CoVs, a median-joining network analysis was performed using PopART v1.7, with $\varepsilon = 0$. Phylogentic analysis of the 33 full-length SADS-CoV genome sequences was also performed using RAxML, with GTRGAMMA as the nucleotide substitution model and 1,000 bootstrap replicates. The maximum likelihood tree was used to test the molecular clock by TempEst v1.5. Potential genetic recombination events in our datasets were detected using RDP.

Animal infection study

Experiments were carried out strictly in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The use of animals in this study was approved by the South China Agricultural University Committee of Animal Experiments (approval ID: 201004152).

Animal challenge experiments were performed using Chinese Bamaxiang SPF piglets (see details in **Extended Data Table 6**). Experiments were performed in

388 animal BSL3 facility in Guangdong Key Laboratory of Laboratory Animals. Healthy,
389 swine diarrhea virus free, piglets (3-day old) were orally fed with homogenized
390 intestinal samples from SADS-CoV infected piglets. Inocula were confirmed as
391 SADS-CoV positive, but negative for all other known swine diarrhea viruses. Control
392 group of piglets were fed with homogenized intestine from healthy piglets. Animals
393 were observed daily for signs of disease, such as diarrhea and weight loss. Fecal
394 swabs were collected daily from all animals and screened for all known swine
395 diarrhea viruses. At experimental endpoints, piglets were humanely euthanized and
396 necropsies performed. Ileal, jejunal and duodenal tissues were taken from selected
397 animals and store in at -80 ^{0}C for further analysis.

398 **Hematoxylin and eosin (H&E) and immunohistochemistry (IHC) analysis**

399 Frozen (-80 ^{0}C) small intestinal tissues including duodenum, jejunum, and
400 ileum taken from the above experimentally infected pigs were pre-frozen at -20 ^{0}C for
401 10 min. Tissues were then embedded in optimal cutting temperature compound and
402 cut into 8-μm sections using the Cryotome FSE machine (Thermo Scientific).
403 Mounted microscope slides were fixed with paraformaldehyde and stained with H&E
404 for histopathological examination.

405 For IHC analysis, the rabbit antibody raised against the NP protein was used
406 for specific staining of SADS-CoV antigen. Slides were blocked by incubating with
407 10% goat serum (Beyotime) at 37 ^{0}C for 30 min, followed by overnight incubation at
408 4 ^{0}C with the rabbit anti-3755 N protein serum (1:1000) and mouse anti-Cytokeratin
409 8+18+19 mAb (Abcam) diluted at 1:100 in PBST buffer containing 5% goat serum.

After washing, slides were then incubated for 50 min at room temperature with FITC conjugated goat-anti-rabbit IgG and Cy3 conjugated goat-anti-mouse IgG (Proteintech) diluted at 1:100 in PBST buffer containing 5% goat serum. Slides were stained with DAPI (Beyotime) and observed under fluorescence microscope (Nikon).

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AUTHOR CONTRIBUTIONS: L.F.W, Z.L.S, P.Z, T.Y.G, M.J.Y conceived the study. P.Z, W.Z, Y.Z, M.S, X.S.Z, B.L, X.L.Y, H.G, D.S, Y.L, X.L.L, J.C performed qPCR, serology, histology and virus culturing. H.F, Y.W.Z, J.M.L, G.Q.P, X.P.A, Z.Q.M, T.T.H, Y.H, Q.S, Y.Y.W, S.Z.X, X.L.L.Z, W.F.S, J.L performed genome sequencing and annotations. T.L, Q.M.X, J.W.C, L.Z, K.J.M, Z.X.W, L.B.Z, S.Y.L, Y.S,C, D.L, Y.S, F.C, P.J.G, R.H prepared the samples and animal challenges. Z.L.S., P.D., L.B.Z, S.Y.L coordinated collection of bat samples. P.Z, L.F.W, Z.L.S, P.D prepared the draft.

SEQUENCES INFORMATION

Full-length genomic sequences or S sequences of SADS-CoV and SARSr-CoV have been deposited in GenBank under accession numbers MF094681-MF094688 & MF769416-MF769444 and MF094697-MF094701 & MF769406-MF769415, respectively. NGS raw data for SADS-CoV sequencing can be found in GenBank under the accession number PRJNA400643.

The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to ZLS. (zlshi@wh.iov.cn).

FIGURE LEGENDS

Figure 1. Detection of SADS-CoV infection in pigs in Guangdong, China.

(A) Chronology of outbreaks and the mortality rate on the four different farms. Daily number of pig deaths was recorded from 26 October 2016 to 2 May 2017. The outbreak is ongoing as of the current date. (B) Detection of SADS-CoV by qPCR in different groups of pigs. (C) Tissue distribution of SADS-CoV in diseased pigs. LA- Lymphonodi abdominals; LM- Lymphoglandulae mesentericae. (D) Detection of SADS-CoV antibodies using S1-specific LIPS assay. Infected sows were bled during the initial three weeks of the outbreak, then >1 month after the beginning of the outbreak. Healthy pig sera were set as control.

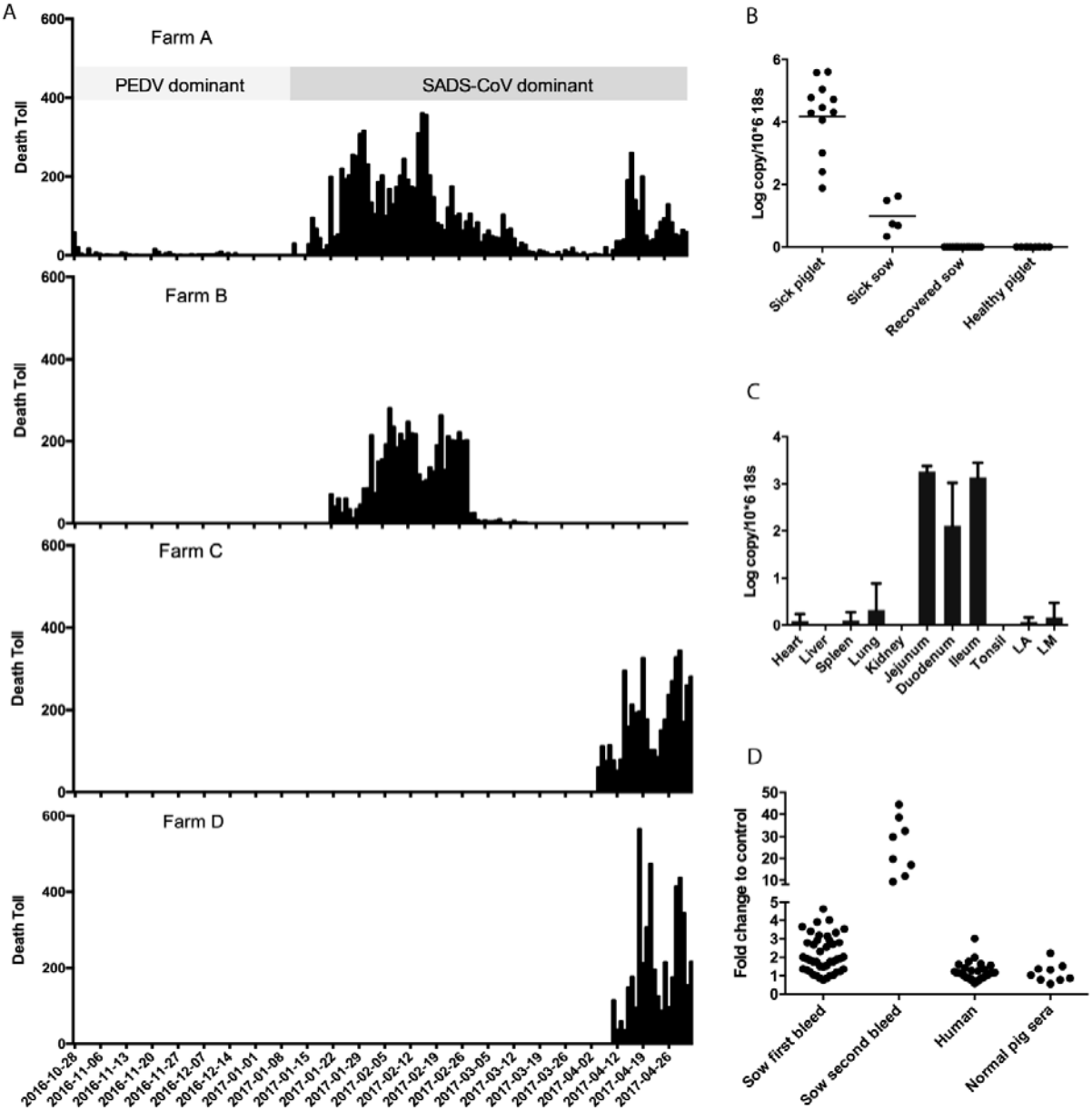


Figure 2. Genome and phylogenetic analysis of SADS-CoV and SADSr-CoV.

(A) Genome organization and comparison. Colored boxes represent genes or UTRs: Green- nonstructural polyproteins ORF1a and 1b; Yellow- structural proteins S, E, M and N; Blue- nonstructural accessory proteins NS3a, NS7a and Ns7b; Orange- UTRs. The level of sequence identity of SADSr-CoV to SADS-CoV was illustrated by patterns; Open box- highly similar; Dots- moderately similar; Dashed line- least similar. (B) Phylogenetic analysis of 57 spike gene sequences using MrBayes. Different colors represent different host species.

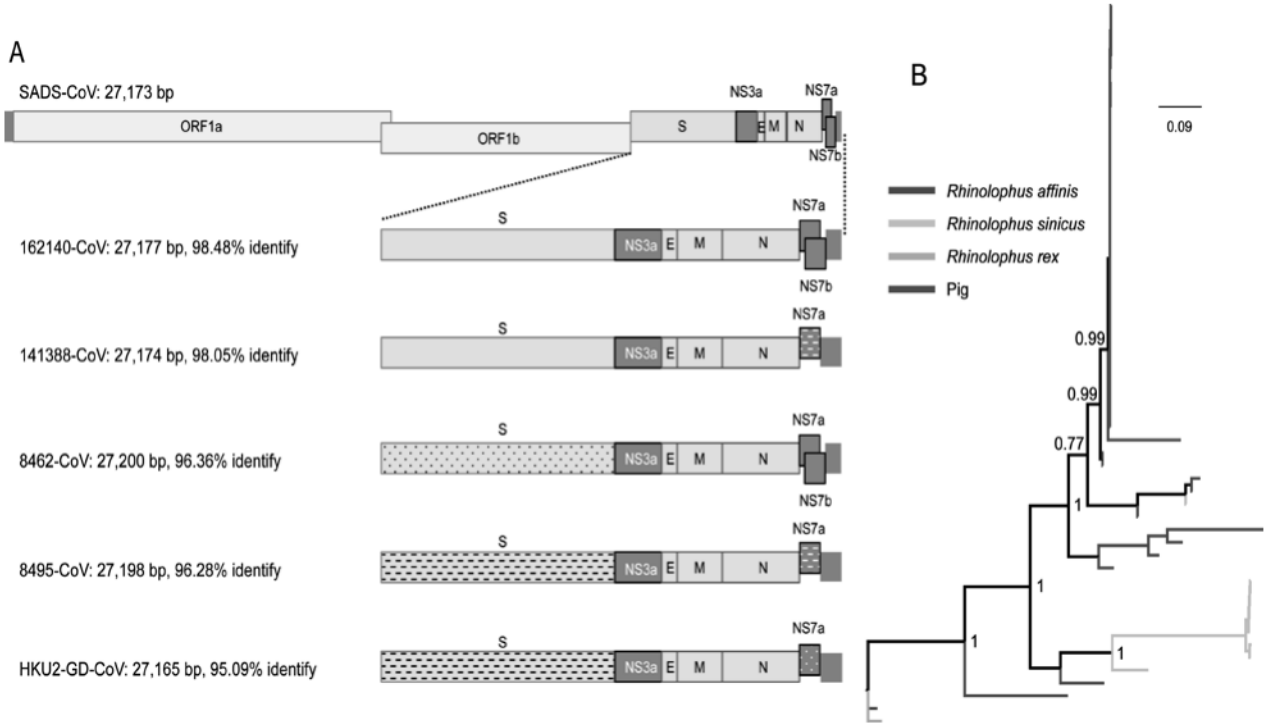
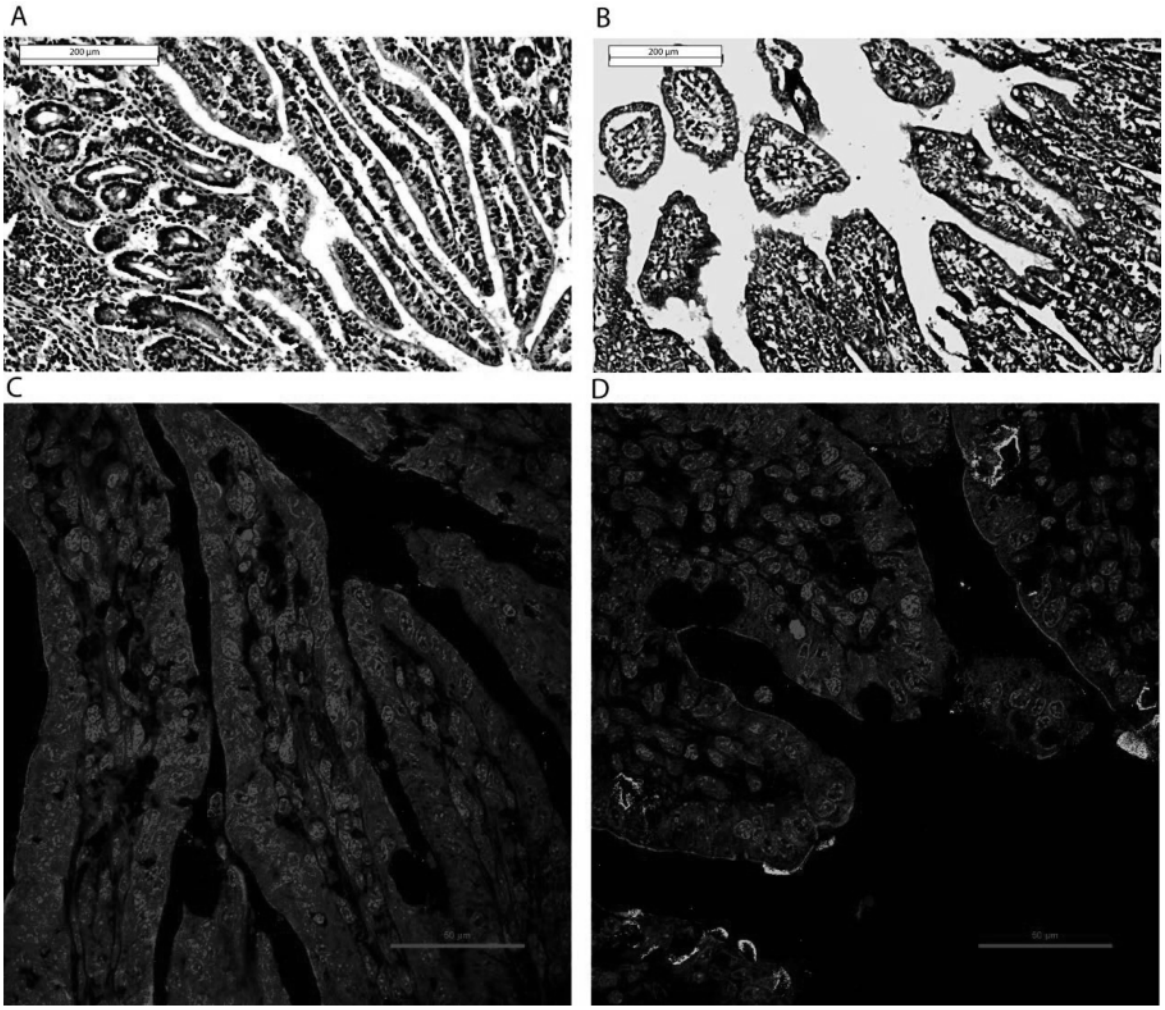


Figure 3 Immunohistopathology of SARS-CoV infected tissues.

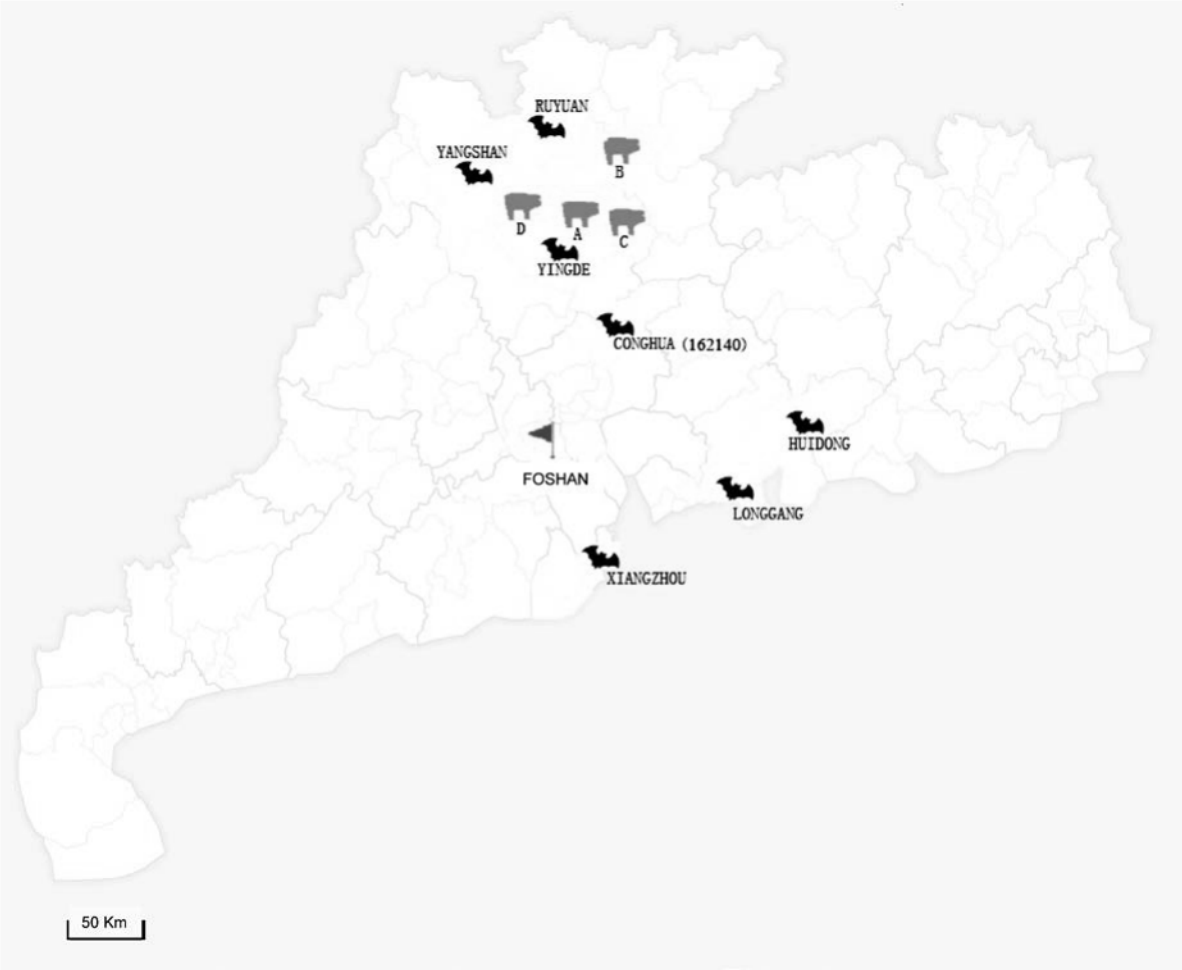
(A) and (B), Hematoxylin and eosin staining of jejunum without (A) and with (B) infection. (C) and (D), Immunohistochemistry staining of jejunum without (C) and with (D) infection using rabbit serum raised against the recombinant SADSr-CoV NP protein (green), DAPI against the nucleus (blue) and mouse anti-cytokeratin 8,18 and19 mAb against epithelial cells (red).



EXTENDED DATA LEGENDS

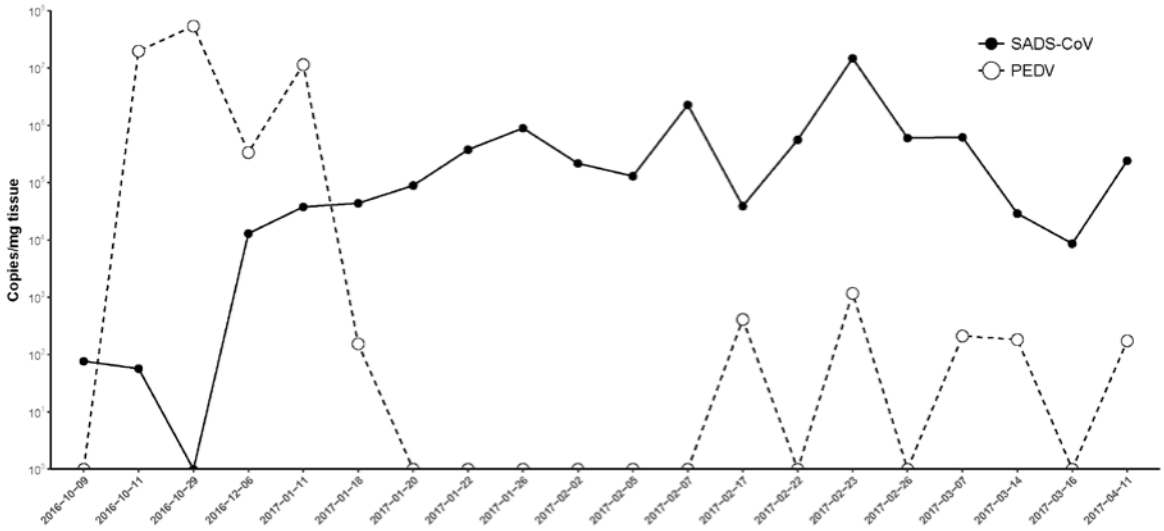
Extended Data Figure 1. Map of Guangdong Province, China.

SADS-affected farms are labeled A to D with blue swine symbols following the temporal sequence of the outbreaks. Bat sampling sites are identified by black bat symbols. The bat SADSr-CoV most closely related to SADS-CoV (sample 162140) originated Conghua. The red flag marks Foshan city, site of the index case of SARS..

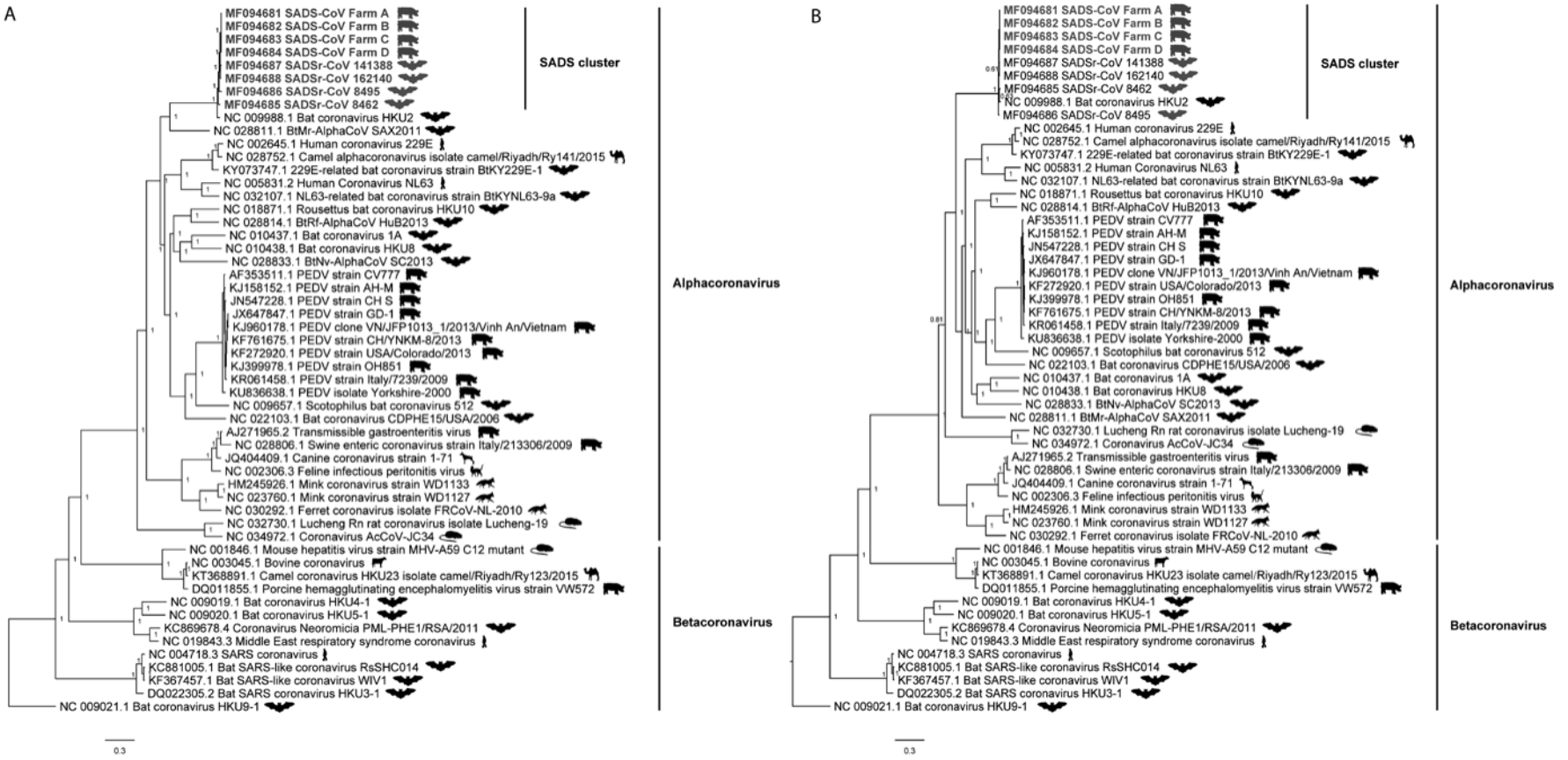


Extended Data Figure 2. Co-circulation of PEDV and SADS-CoV during the initial outbreak on Farm A.

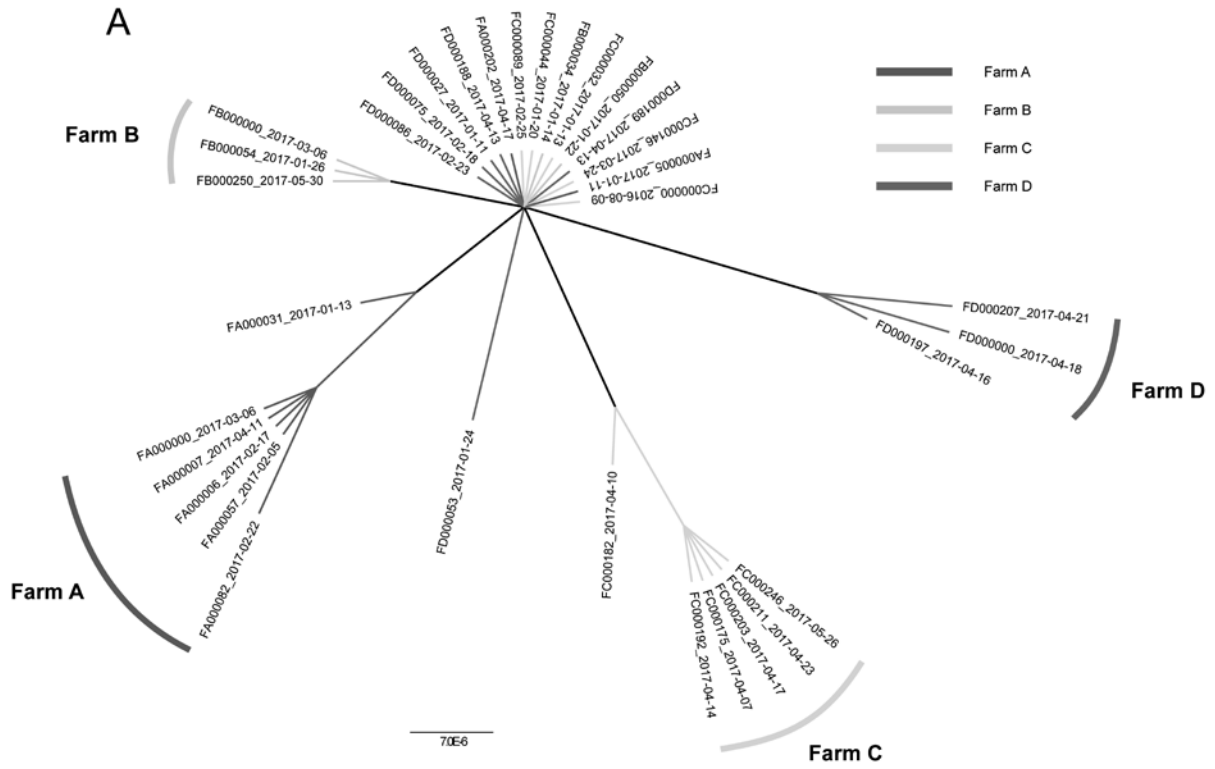
Pooled intestinal samples were collected at dates given on the x-axis from deceased piglets and analyzed by qPCR. The intensity infection for each piglet is shown as a copy number per milligram of intestine (y-axis).



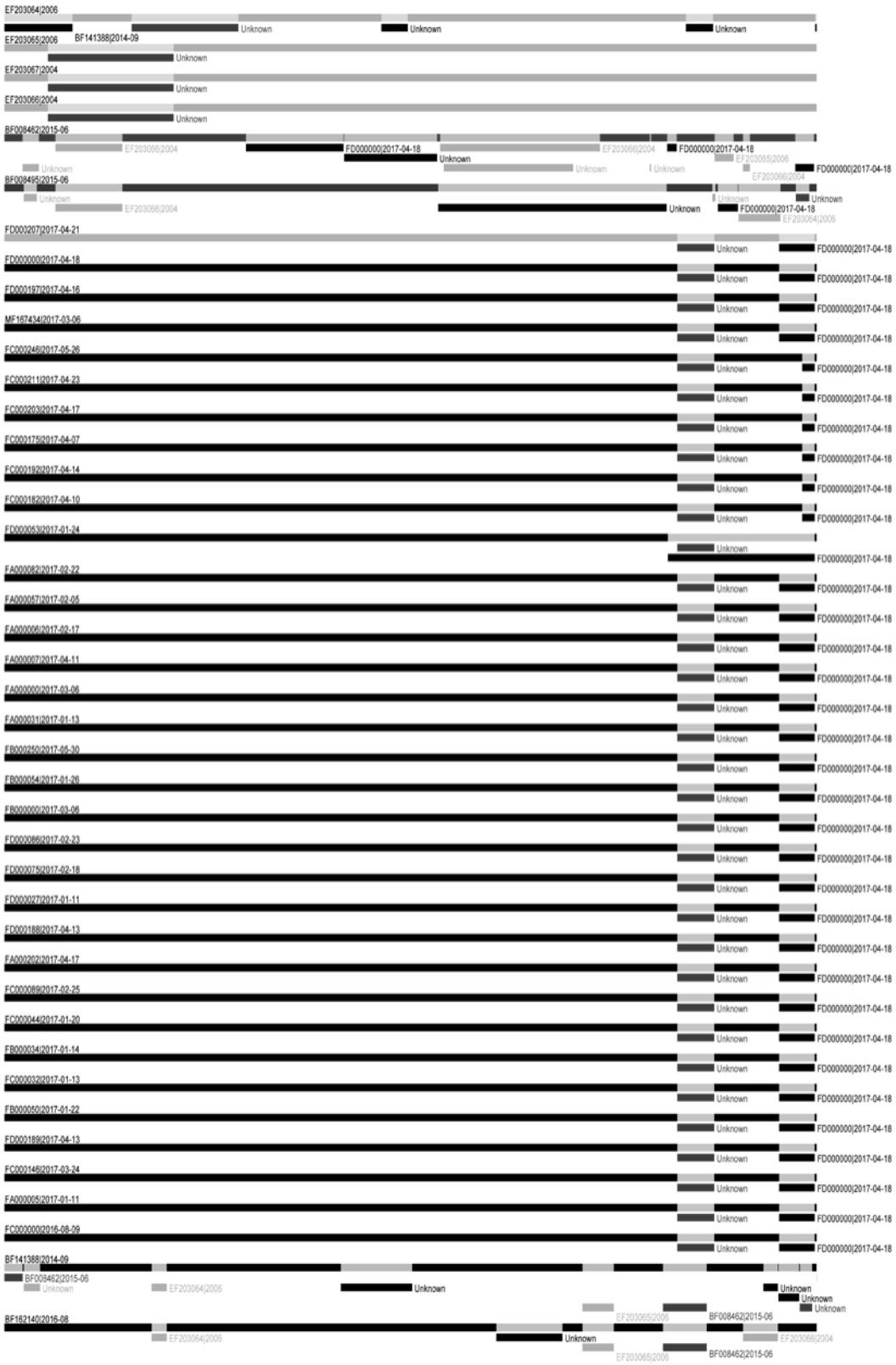
Extended Data Figure 3. **Bayesian phylogenetic tree of the full-length genome (A) or ORF1ab sequences (B) of SADS-CoV and related coronaviruses.** Tree was constructed using MrBayes v3.2 with the average standard deviation of split frequencies under 0.01. The host of each sequence is represented pictorially. Newly sequenced SADS-CoVs are highlighted in red while bat SADSr-CoVs are highlighted in blue.



Extended Data Figure 4. Phylogenetic and haplotype network analyses of the 33 SADS-CoV strains from the four farms. Panel A shows the phylogenetic tree estimated using MrBayes. The GTR+GAMMA model was applied and 20 million steps were run, with the first 10% of which were removed as burnin. Viruses from different farms were labeled with different colors. Panel B demonstrated the the median-joining haplotype network constructed using ProART v1.7. In this analysis $\varepsilon = 0$ was applied. In this network, size of the circles represents the number of samples. The larger the circle is, the larger number of samples it includes. Different colors indicate samples from different farms.

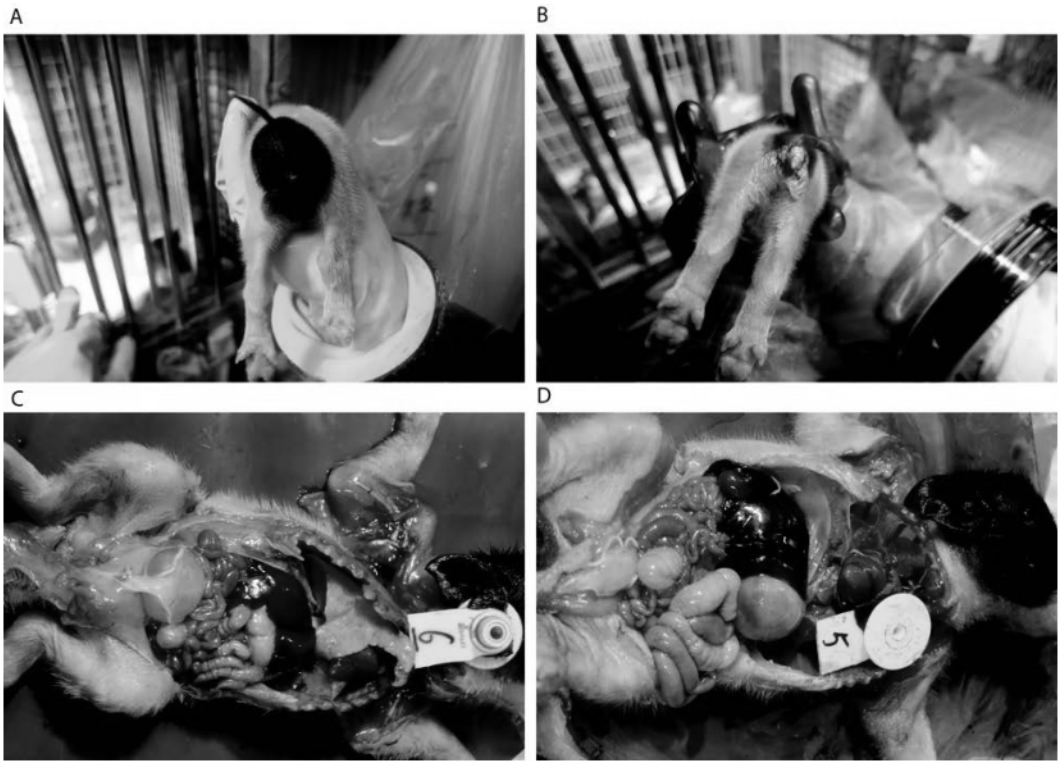


Extended Data Figure 5. Recombination of SADS-CoV and related CoVs. The potential genetic recombination events were detected using RDP.



Extended Data Figure 6. SADS-CoV experimentally infected and healthy SPF piglets.

(A) Mock infected piglet on day 2. (B) Piglet on day 2 post SADS-CoV infection. (C) Intestine from mock-infected piglet at necropsy. (D) Intestine from infected piglet at necropsy..



Extended Data Table 1. List of all known swine viruses tested by PCR at the beginning of the of SADS outbreak investigation on the four farms *.

	PEDV	PDCoV	TGEV	RV	PBV	PSV	SVA	SIV	NADC30	PRV	FMDV	CSFV	PCV2	PCV3	APPV	PPV	Norovirus
Farm A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	-
Farm B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	-
Farm C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND
Farm D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND

* Dash indicates negative PCR result. ND, not done. Virus abbreviations: PEDV- Porcine Epidemic Diarrhea Virus; PDCoV- Porcine Delta Coronavirus; TGEV-Porcine Transmissible Gastroenteritis Virus; RV- Porcine Rotavirus; PBV- Porcine Picobirnavirus; PSV- Porcine Sapelo Virus; SVA- Porcine Senecavirus A; SIV- Swine Influenza Virus; NADC30- Porcine Reproductive and Respiratory Syndrome Virus, strain NADC30; PRV- Porcine Pseudorabies Virus; FMDV- Foot and Mouth Disease Virus; CSFV- Classical Swine Fever Virus; PCV2- Porcine Circovirus 2; PCV3- Porcine Circovirus 3; APPV- Atypical Porcine Pestivirus; PPV- Porcine Parvovirus.

Extended Data Table 2. List of nucleotide and amino acid (aa) residue variants among the 33 SADS-CoV genomes obtained from the four different farms. Of note, there is one sample from a fifth farm where no disease outbreak

No.	Farm	Date	2116	2236	2955	3285	10860	15395	18410	20219	21139	21580	21622	21985	22180	26669
27	DB (farm D)	2017/1/11	c	g	t	g	t	c	t	t	t	t	c	g	c	c
53	DB (farm D)	2017/1/24	c	g	t	g	t	c	t	t	t	t	c	c	t	c
75	DB (farm D)	2017/2/18	c	g	t	g	t	c	t	t	t	t	c	g	c	c
86	DB (farm D)	2017/2/23	c	g	t	g	t	c	t	t	t	t	c	g	c	c
188	DB (farm D)	2017/4/13	c	g	t	g	t	c	t	t	t	t	c	g	c	c
189	DB (farm D)	2017/4/13	c	g	t	g	t	c	t	t	t	t	c	g	c	c
197	DB (farm D)	2017/4/16	c	g	t	t	t	t	t	t	t	t	a	g	c	c
D	DB (farm D)	2017/4/18	c	t	t	t	t	t	t	t	t	t	a	g	c	c
207	DB (farm D)	2017/4/21	t	g	t	t	t	t	t	t	t	t	a	g	c	c
DCD5	DCD (farm A)	2017/1/11	c	g	t	g	t	c	t	t	t	t	c	g	c	c
31	DCD (farm A)	2017/1/13	c	g	t	g	t	c	t	c	t	t	c	g	c	c
57	DCD (farm A)	2017/2/5	c	g	t	g	t	c	c	c	t	t	c	g	c	c
DCD6	DCD (farm A)	2017/2/17	c	g	t	g	t	c	c	c	t	t	c	g	c	c
82	DCD (farm A)	2017/2/22	c	g	t	g	c	c	c	c	t	t	c	g	c	c
A	DCD (farm A)	2017/3/6	c	g	t	g	t	c	c	c	t	t	c	g	c	c
DCD7	DCD (farm A)	2017/4/11	c	g	t	g	t	c	c	c	t	t	c	g	c	c
202	DCD (farm A)	2017/4/17	c	g	t	g	t	c	t	t	t	t	c	g	c	c
B	QJ (farm B)	2017/3/6	c	g	c	g	t	c	t	t	t	t	c	g	c	c
C	SC (farm B)	2016/8/9	c	g	t	g	t	c	t	t	t	t	c	g	c	c
34	SC (farm B)	2017/1/14	c	g	t	g	t	c	t	t	t	t	c	g	c	c
50	SC (farm B)	2017/1/22	c	g	t	g	t	c	t	t	t	t	c	g	c	c
54	SC (farm B)	2017/1/26	c	g	c	g	t	c	t	t	t	t	c	g	c	c

250	SC (farm B)	2017/5/30	c	g	c	g	t	c	t	t	t	t	c	g	c	c
32	WT (farm C)	2017/1/13	c	g	t	g	t	c	t	t	t	t	c	g	c	c
44	WT (farm C)	2017/1/20	c	g	t	g	t	c	t	t	t	t	c	g	c	c
89	WT (farm C)	2017/2/25	c	g	t	g	t	c	t	t	t	t	c	g	c	c
146	WT (farm C)	2017/3/24	c	g	t	g	t	c	t	t	t	t	c	g	c	c
175	WT (farm C)	2017/4/7	c	g	t	g	t	c	t	t	c	c	c	g	c	t
182	WT (farm C)	2017/4/10	c	g	t	g	t	c	t	t	c	t	c	g	c	t
192	WT (farm C)	2017/4/14	c	g	t	g	t	c	t	t	c	c	c	g	c	t
203	WT (farm C)	2017/4/17	c	g	t	g	t	c	t	t	c	c	c	g	c	t
211	WT (farm C)	2017/4/23	c	g	t	g	t	c	t	t	c	c	c	g	c	t
246	WT (farm C)	2017/5/26	c	g	t	g	t	c	t	t	c	c	c	g	c	t

600

Extended Data Table 3. List of PCR primers used in this study. The numbering system of SADS-CoV from Farm A was used here.

Gene	Primer name and location	Primer sequence	Purpose
RdRp gene	SADS-RdRp-F (19512-19531)	GTTGATTGTAAGGCTTGGCG	qPCR
	SADS-RdRp-R (19590-19608)	AACCACACTTCCACTCAGC	
N gene	SADS-N-F (25810-25830)	CTAAAACTAGCCCCACAGGTC	qPCR
	SADS-N-R (25938-25957)	TGATTGCGAGAACGAGACTG	
S gene	HKU2-S1-1F (20066-20085)	GGCGCTATGGCTGTTAAGAT	Amplification
	HKU2-S1-1R (22317-22336)	CACGAATGTCAGCCTCAACT	
S gene	HKU2-S1-2F (20157-20176)	CCAGTGTCAACACGTCATCT	Amplification
	HKU2-S1-2R (22218-22238)	ACGCTGAACTTAGGCATTGTA	

Primers for amplification of full-length genome sequence are provided upon request.

605 **Extended Data Table 4. List of SADSr-CoVs detected in bats in Guangdong,**
606 **China.**

Sampling			PCR analysis	
Time (Month-Year)	Location	Bat Species	Fecal swabs sampled	PCR Positive
Jun 13	Yingde	*Rhinolophus sinicus*	1	1
		Pipistrellus abramus	8	0
		Myotis ricketti	2	0
Jul 13	Yangshan	*Pipistrellus abramus*	1	0
		Hipposideros pratti	36	1
Jul 13; May 14; Jun 15; Aug 16	Ruyuan	*Rhinolophus sinicus*	27	6
		Rhinolophus affinis	11	2
		Rhinolophus macrotis	3	0
		Rhinolophus pusillus	41	6
		Rhinolophus rex	9	7
		Hipposideros pratti	7	0
Sep 14; Jun 15; Aug 16	Conghua	*Rhinolophus sinicus*	70	2
		Rhinolophus affinis	34	7
		Rhinolophus pusillus	11	2
		Hipposideros pomona	10	0
		Myotis ricketti	1	0
Jun 13; Nov 13; Aug 14; Jun 15	Huidong	*Rhinolophus sinicus*	37	2
		Rhinolophus affinis	59	29
		Rhinolophus macrotis	15	2
		Rhinolophus pusillus	1	0
		Hipposideros pomona	2	0
		Myotis ricketti	84	1
Apr 14; Jun 15	Longgang	*Rhinolophus sinicus*	55	1
		Pipistrellus abramus	5	1
Sep 14	Xiangzhou	*Rhinolophus pusillus*	28	0
		Hipposideros pomona	38	1
		Total	596	71 (11.9%)

607 See Fig. S1 for sampling sites in relation to SARS and SADS outbreak locations

Extended Data Table 5. Multiple human CoV receptors as well as swine APN cannot be utilized as entry receptor for SADS-CoV.

	HuAPN★	HuACE2★	HuDPP4★	SwAPN★	SwACE2★
SADS-CoV*	-	-	-	-	-
SARS-related-CoV	NA	+	NA	NA	+
MERS-CoV#	NA	NA	+	NA	NA
Expression$	+ (APN Ab)	+ (HIS-tag Ab)	+ (DPP4 Ab)	+ (S-tag Ab)	+ (S-tag Ab)

★Gene accession numbers for the genes used in this study: human APN, M22324.1; human ACE2, NM_021804; human DPP4, NM_001935.3; swine ACE2, XM_021079374.1

* For SADS-CoV infection, both positive samples and HIV-pseudovirus were used. Viral positive samples were from SADS infected pig anal swabs: SusAS-7 (4.0×10^5 copy/μl), SusAS-20 (4.3×10^5 copy/μl), SusAS-22 (2.4×10^5 copy/μl).

For MERS-CoV infection, HIV-pseudovirus were used.

$ Expression of APN, DPP4 and ACE2 was confirmed by antibodies against the targeting proteins or fused tags.

Extended Data Table 6. Experimental outline of SADS-CoV infection of piglets.

Experiments were performed with 3-day old SPF piglets. Infection was performed as described in the Material and Methods. According to the sign of diseases, we term the severity of diarrhea into four different categories: no diarrhea; mild diarrhea (diluted feces); severe diarrhea (watery diarrhea, normally with dehydration); deadly diarrhea (vomiting and watery diarrhea, end up with death quickly). Only severe or deadly (shown as severe diarrhea) are counted in the following table.

Infection route	SADS-CoV titer (copy/μl)	First day				Second day				
		Severe diarrhea	Weight loss	SADS-CoV positive	PEDV/PDCoV/RV positive	Severe diarrhea	Weight loss	SADS-CoV positive	PEDV/PDCoV/RV positive	Died
Oral+milk	1.155×10*6	5 (no death)	4	7	0	4 (another three died)	3 (in 4)	7	0	3
Oral+milk	0	0 (no death)	1	0	0	1 (another one died)	1 (in 4)	0	0	1

From: Baric, Ralph
Sent: Tue, 4 Oct 2016 20:14:07 +0000
To: Mathur, Punam (NIH/NIAID) [E]; Graham, Rachel
Cc: Yao, Alison (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: latest paper drafts
Attachments: Zikv Reverse Genetics Figures-Combined.pdf, 160923 Clone Manuscript Draft 8.docx, Cockrell et al 2016 Nature Microbiology.pdf

Hi Punam, Alison and Erik, As promised, still some minor tweeking on the zikv clone paper. Thanks, ralph

---

From: Mathur, Punam (NIH/NIAID) [E] (b)(6)
Sent: Thursday, September 08, 2016 3:41 PM
To: Baric, Ralph S; Graham, Rachel; Baric, Toni C
Cc: Yao, Alison (NIH/NIAID) [E]
Subject: Semi-annual report due date

Hi Ralph and Rachel,

As discussed on our call, this is to confirm that the ORFEOME semi-annual report is due October 15th, 2016.

Thank you,
Punam

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Supplementary Information

A Mouse Model for MERS Coronavirus Induced Acute Respiratory Distress Syndrome

Adam S. Cockrell, Boyd L. Yount, Trevor Scobey, Kara Jensen, Madeline Douglas, Anne Beall, Xian-Chun Tang, Wayne A. Marasco, Mark T. Heise, Ralph S. Baric

Address Correspondence: R.S.B. (b)(6)

Supplementary Table 1

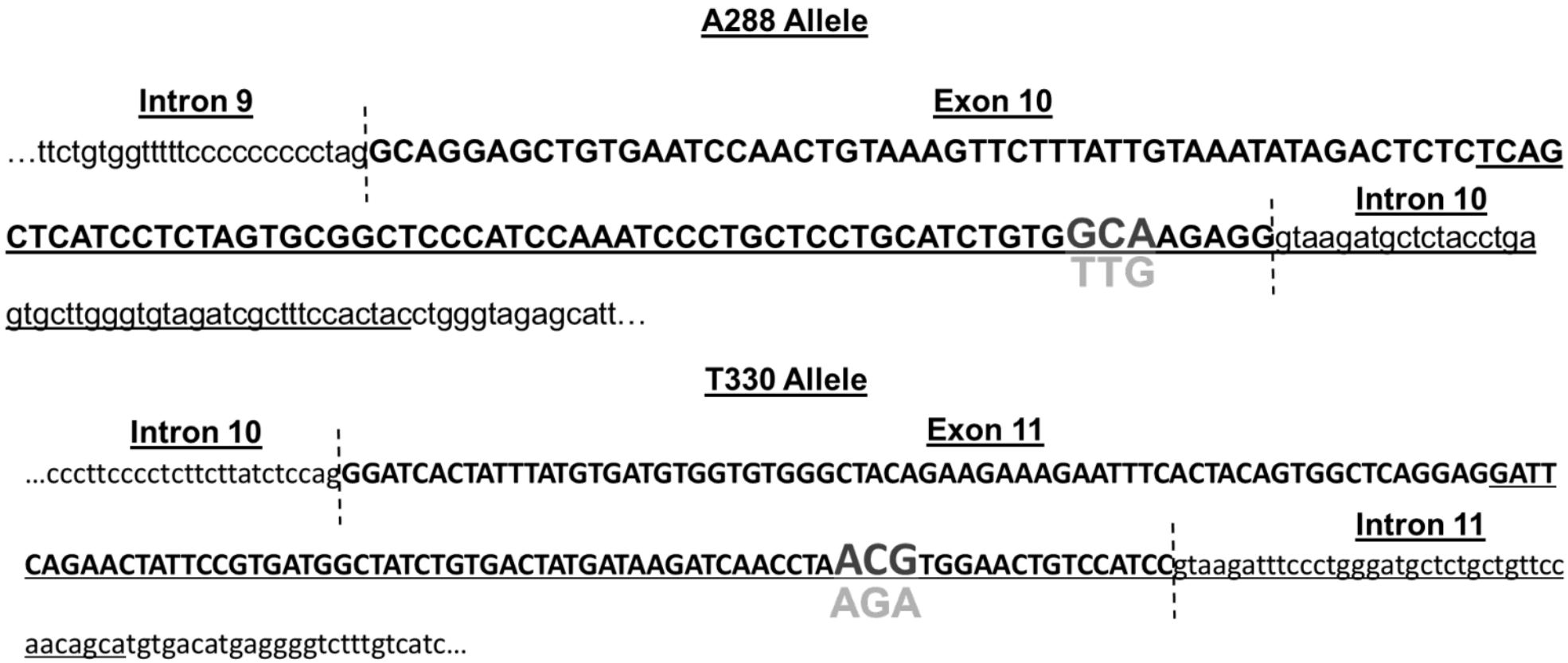
Supplementary Figures 1-14

Supplemental Table 1

Guide RNAs and oligo sequences to humanize the alleles encoding amino acids 288 and 330 in mouse DPP4

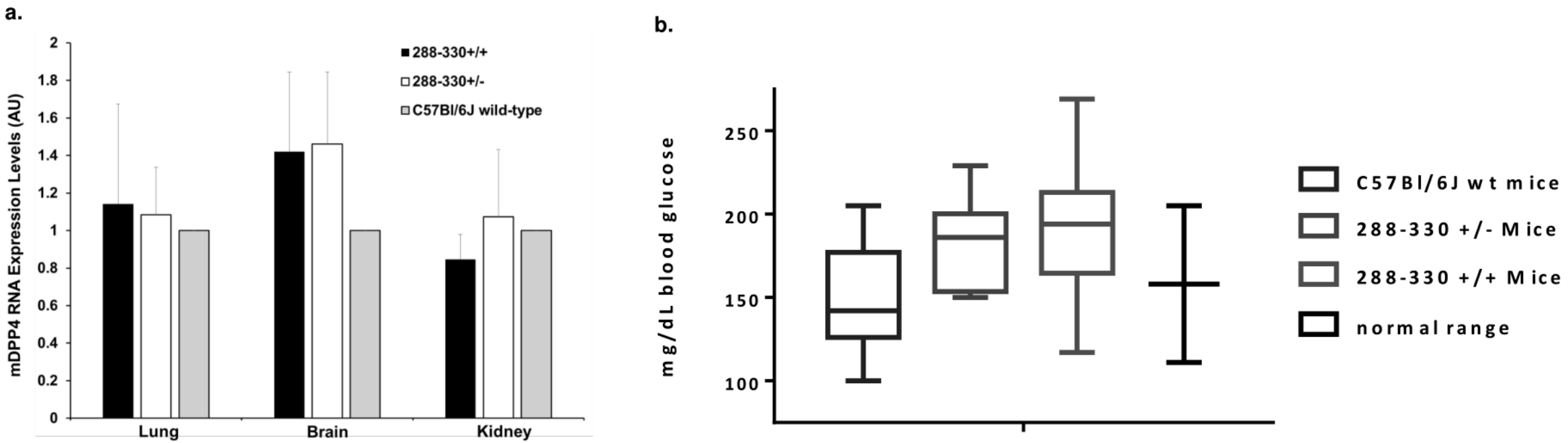
Edited Amino Acid	Cas 9 Guide Sequence	Oligo for Homology Directed Repair
A288L	5'-GCTCCTGCATCTGTGGCAAG-3'	5'-TCAGCTCATCCTCTAGTGCGGCTCCCATCCAAATCCCTGCTCCTGCATCTGTGTTGAGAGGGTAAGATGCTCTACCTGAGTGCTTGGGTGTAGATCGCTTTCCACTAC-3'
T330R	5'-ATGATAAGATCAACCTAACG-3'	5'-GATTCAGAACTATTCCGTGATGGCTATCTGTGACTATGATAAGATCAACCTAAGATGGAACTGTCCATCCGTAAGATTTCCCTGGGATGCTCTGCTGTTCCAACAGCA-3'

Supplemental Figure 1



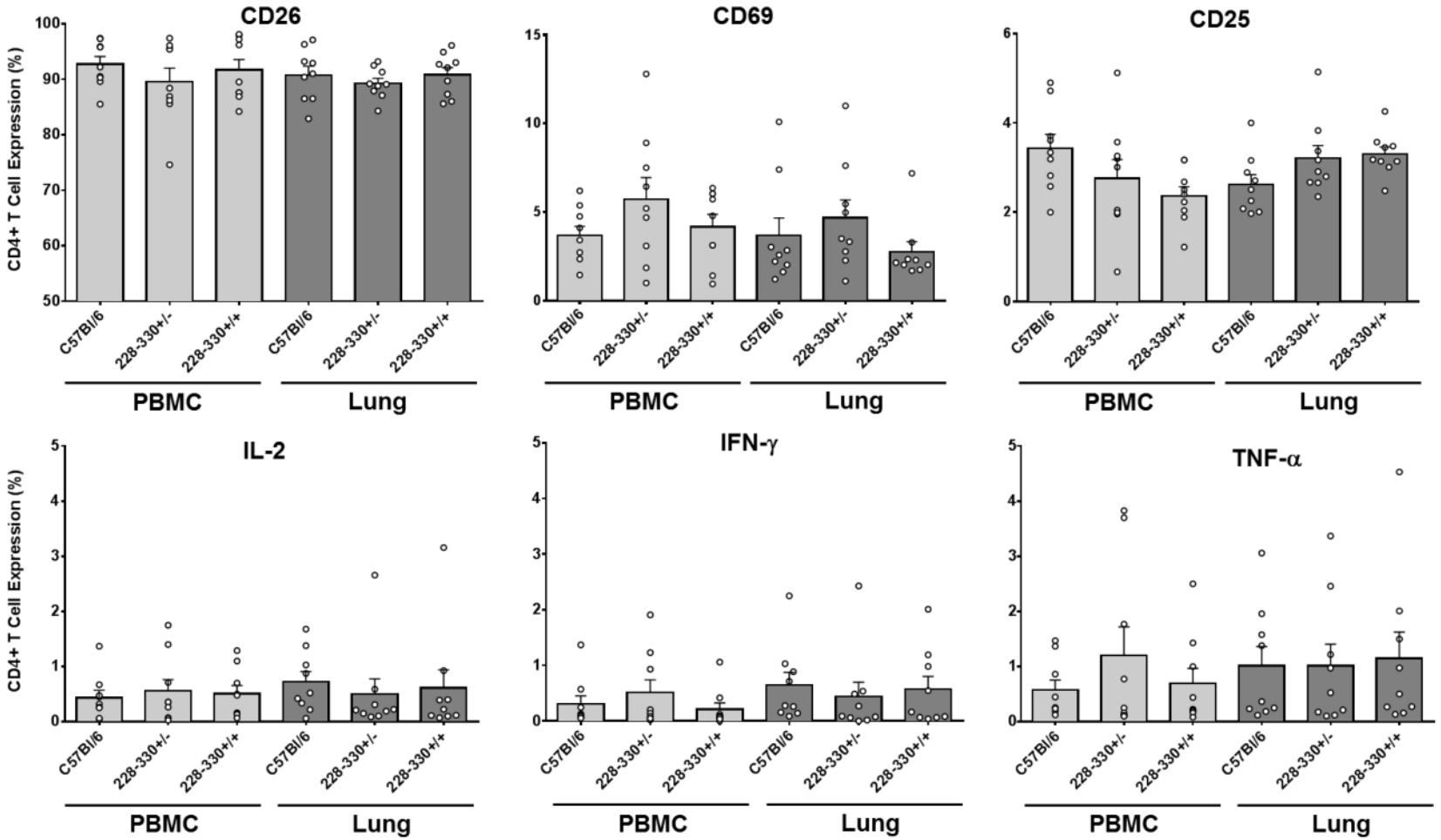
Supplemental Figure 1. Mouse DPP4 288 and 330 alleles modified by CRISPR/Cas9. Mouse alleles encoding A288 and T330 (red) are shown on exon 10 and 11 of mDPP4, respectively. Underlined regions indicate sequence encoded in oligo's for homology-directed repair with CRISPR/Cas9 to introduce the modified nucleotides (green). Vertical hashes indicate intron/exon borders.

Supplemental Figure 2



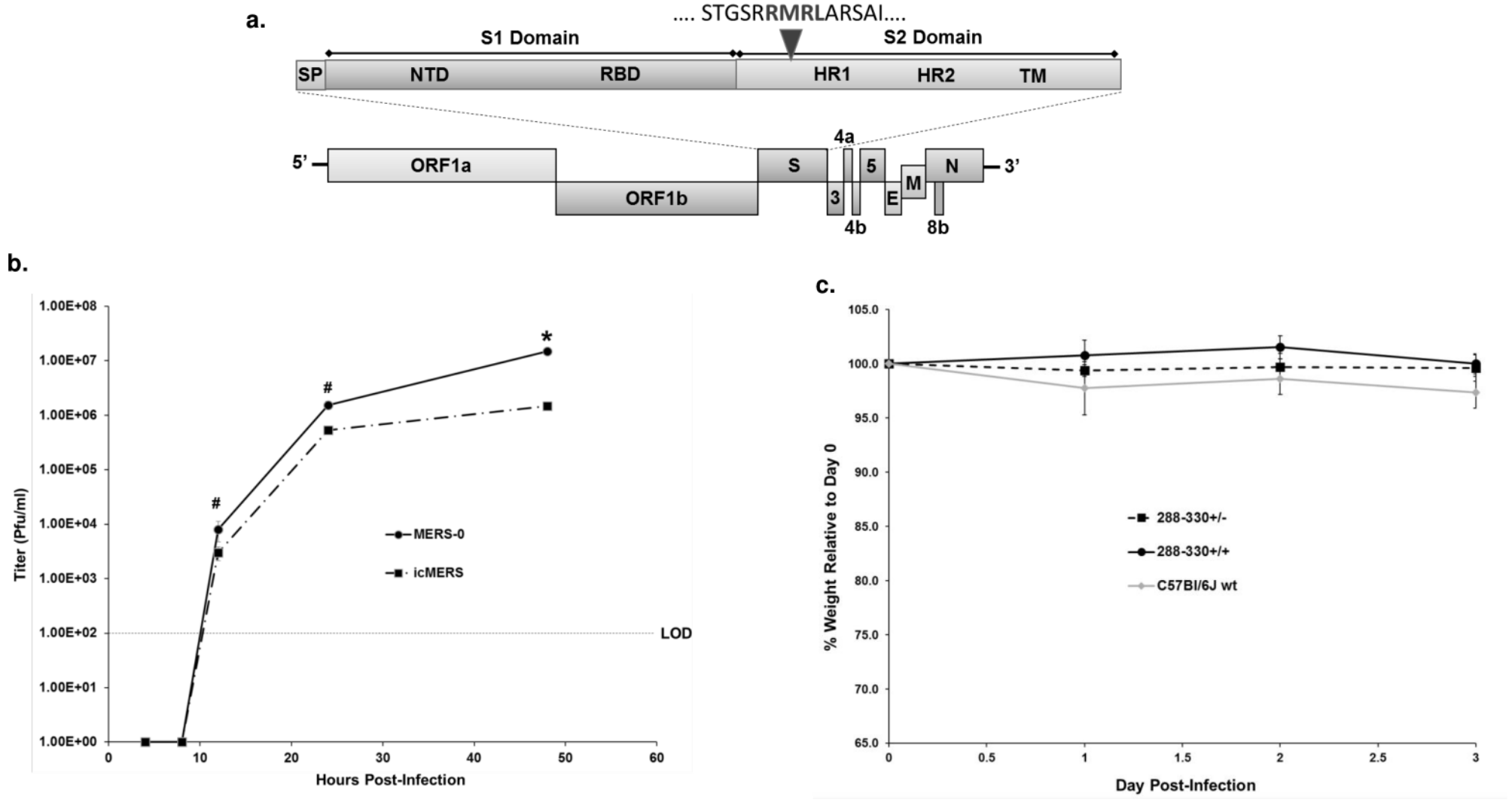
Supplemental Figure 2. Bio-distribution and functional characterization of modified mouse DPP4. (a) RT-qPCR was used to evaluate the mRNA levels of mDPP4 in the lungs, brain, and kidneys of 288-330$^{+/+}$ (n = 5) and 288-330$^{+/-}$ (n = 5) compared with C57Bl/6J wild-type mice (n = 5). Values are expressed as arbitrary units that were normalized to 18S ribosomal RNA and C57Bl/6J wild-type mouse samples using the equation $2^{-\Delta\Delta Cp}$. Error bars are +SD. (b) Blood glucose levels were measure from whole blood samples of 288-330$^{+/+}$ (n = 16), 288-330$^{+/-}$ (n = 14), and C57Bl/6J wild-type mice (n = 15). Values are compared to the anticipated normal range for mice. Error bars are +/- SD.

Supplemental Figure 3



Supplemental Figure 3. Modification of mDPP4 does not alter activation profiles of CD4+ T cells. Lymphocytes from peripheral blood mononuclear cells (PBMCs) and lungs of C57Bl/6J wild-type (n = 9), 288-330$^{+/-}$ (n = 9), and 288-330$^{+/+}$ (n = 9) were analyzed by flow cytometry. The percent of CD4+ T cells were assessed for expression of mouse DPP4 (CD26), CD69, CD25, IL-2, IFNg, and TNF-a. Error bars are +SD.

Supplemental Figure 4



Supplemental Figure 4. MERS-0 replicates to high titer *in vitro*, but causes no disease. (a) Cartoon of MERS-CoV with an expanded view of the spike protein showing the amino acid changes in the S2 domain (red). (b) Growth curves on Vero81 cells of MERS-0 ($n = 3$) compared to the MERS-CoV infectious clone (icMERS) ($n = 3$). Student *t*-test was used to compare titers at indicated time points (# = $p < 0.05$, and * = $p < 0.01$). (c) MERS-0 does not cause weight loss in 288-330$^{+/-}$ ($n = 4$) or 288-330$^{+/+}$ ($n = 4$) mice. Error bars are +/- SD.

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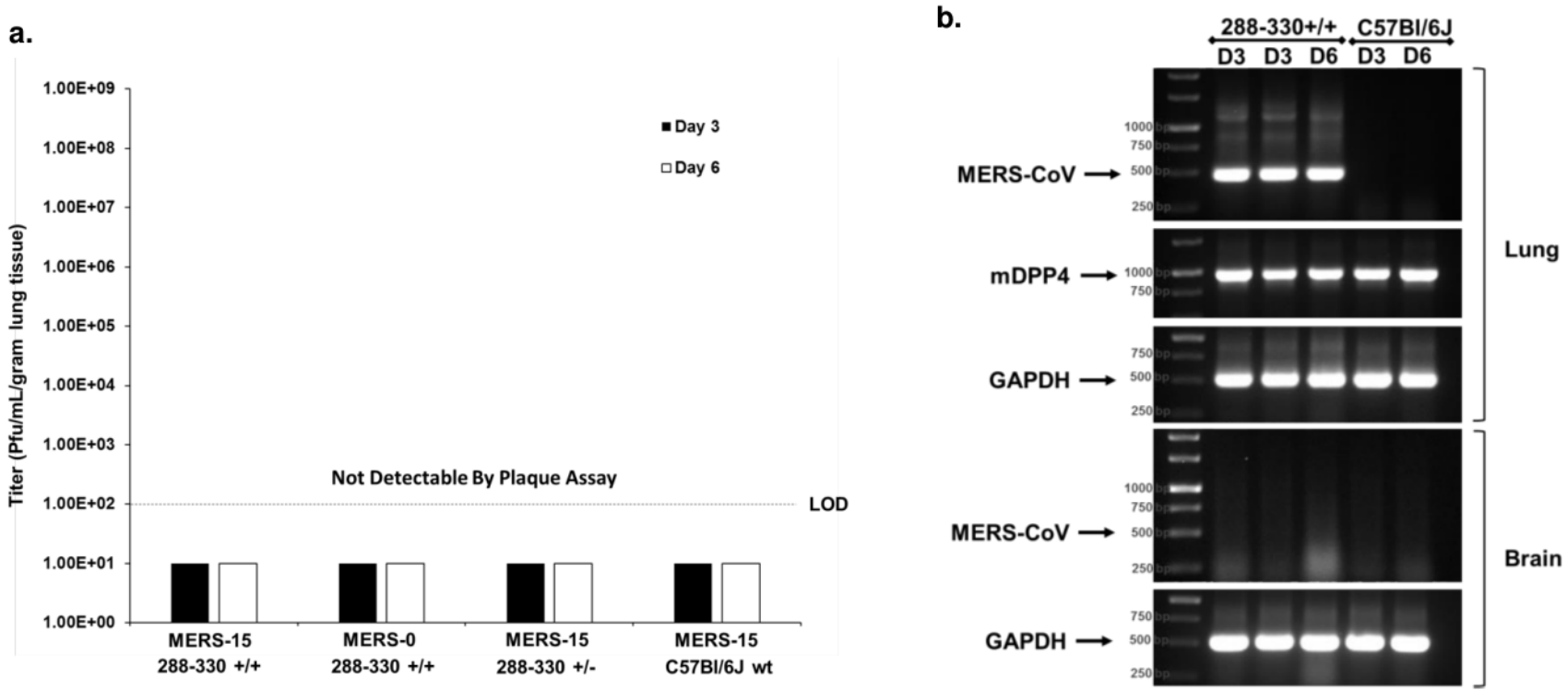
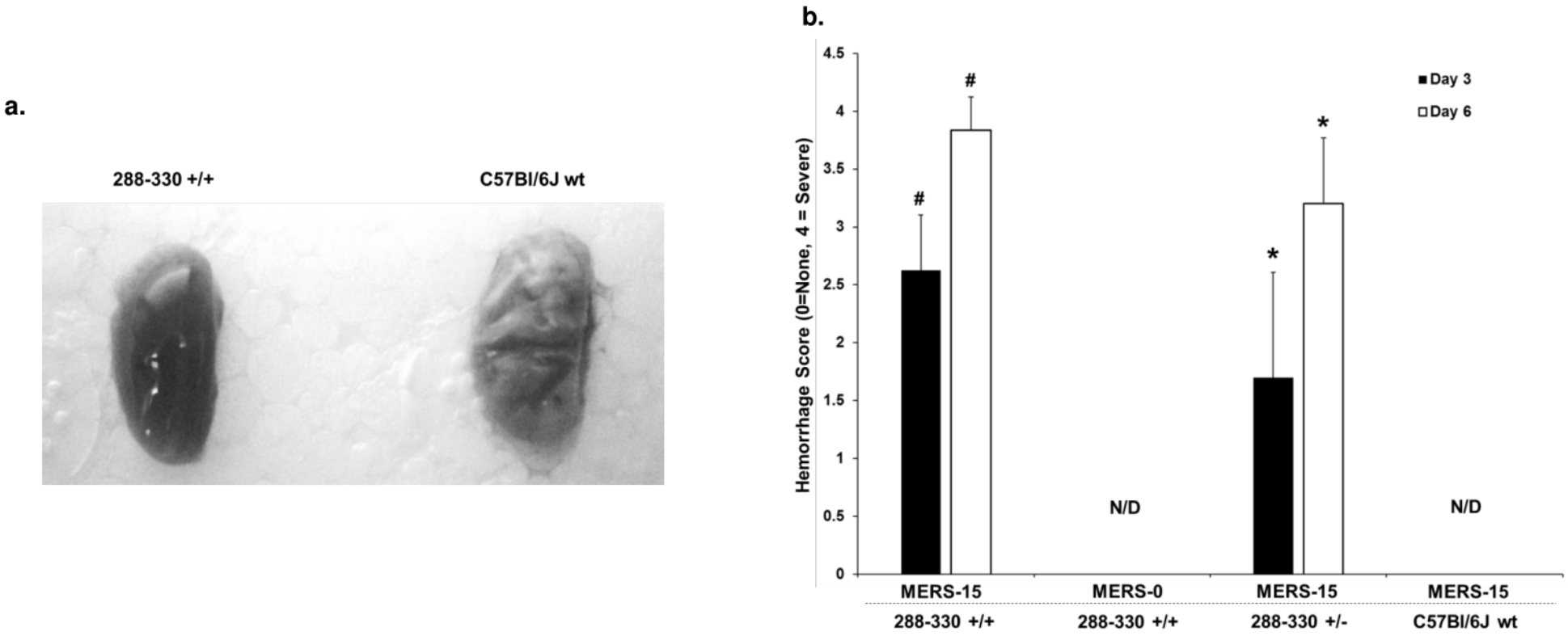
Supplemental Figure 5



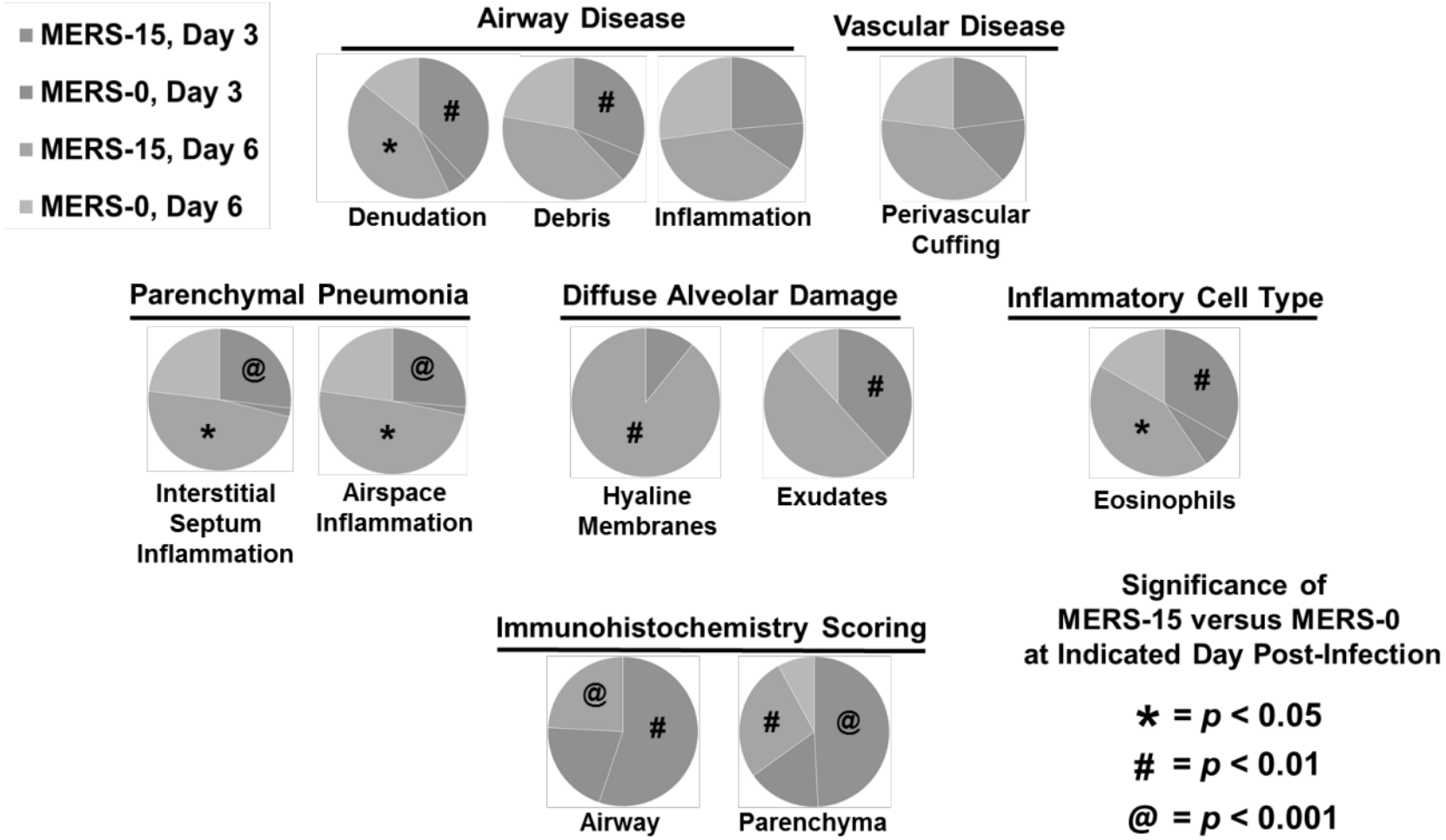
Supplemental Figure 5. Mouse-adapted MERS-15 virus does not infect the brain. (a) Viral titers from brain tissue were determined by plaque assays at day 3 and 6 p.i. for 288-330$^{+/+}$ + MERS-15 (day 3, *n* = 4 and day 6, *n* = 4), or MERS-0 (day 3, *n* = 4 and day 6, *n* = 4); 288-330$^{+/-}$ + MERS-15 (day 3, *n* = 4 and day 6 *n* = 4); and, C57Bl/6J wt + MERS-15 (day 3, *n* = 4 and day 6, *n* = 3). The limit of detection (LOD) is indicated. (b) Lung and brain RNA was examined by RT-PCR to determine if MERS-15 RNA could be detected in the lung and brains of 288-330$^{+/+}$ and C57Bl/6J wt mice at days 3 and 6 p.i. Mouse DPP4 and GAPDH were examined as controls (bp = base pair size markers in red).

Supplemental Figure 6



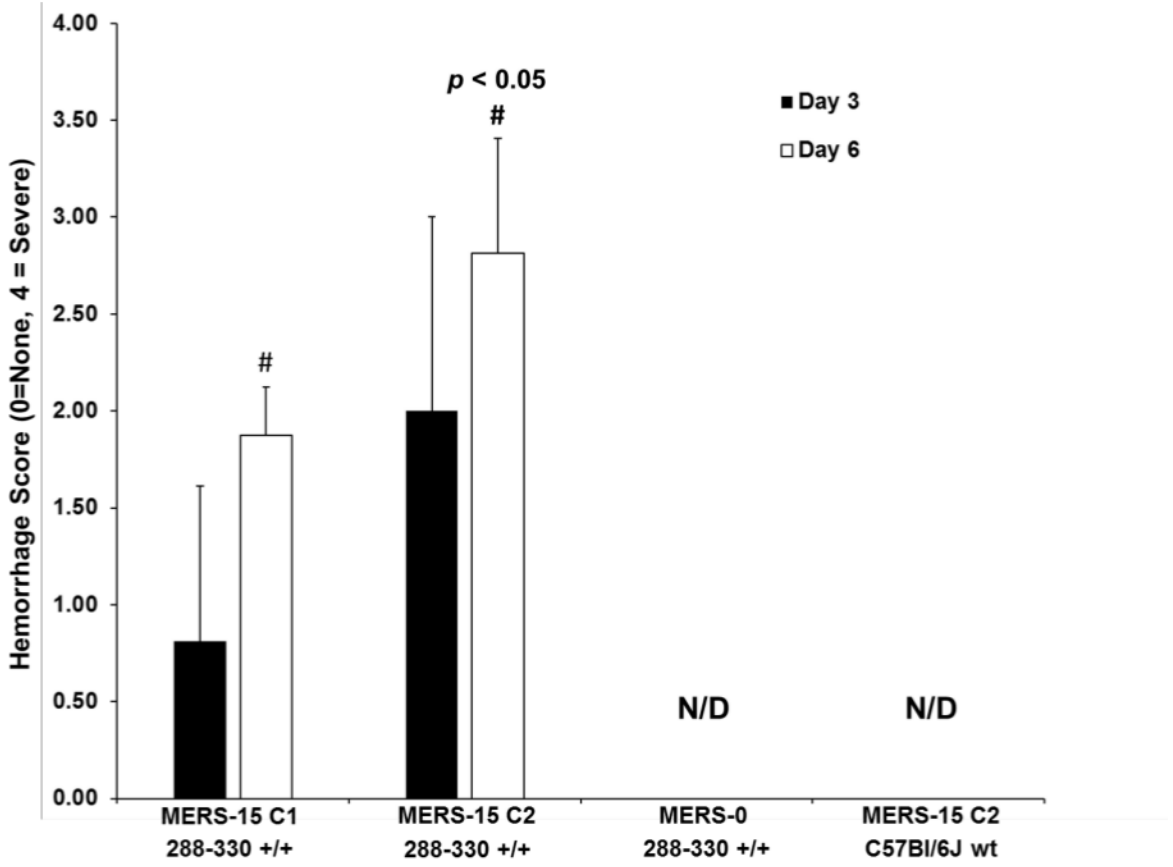
Supplemental Figure 6. Mouse-adapted MERS-15 cause's hemorrhage in mouse lungs. (a) Image of a hemorrhaged left lung lobe from a 288-330$^{+/+}$ mouse infected with MERS-15 (left) compared to left lung lobe of a C57Bl/6J wt mouse infected with MERS-15 (right). Image is representative of at least 3 lungs. (b) The severity of hemorrhage was scored for 288-330$^{+/+}$ + MERS-15 (day 3, $n = 4$ and day 6, $n = 3$), or MERS-0 (day 3, $n = 5$ and day 6, $n = 5$); 288-330$^{+/-}$ + MERS-15 (day 3, $n = 5$ and day 6, $n = 5$); and, C57Bl/6J wt + MERS-15 (day 3, $n = 5$ and day 6, $n = 5$). Lungs were scored from 0 (no hemorrhage) to 4 (severe hemorrhaging in all lobes of the lung). N/D is none detectable. Student *t*-test was used to demonstrate significant differences between days 3 and 6 p.i. for 288-330$^{+/+}$ (# is $p < 0.05$) and 288-330$^{+/-}$ (* is $p < 0.05$) mice infected with MERS-15. Error bars are +SD.

Supplemental Figure 7



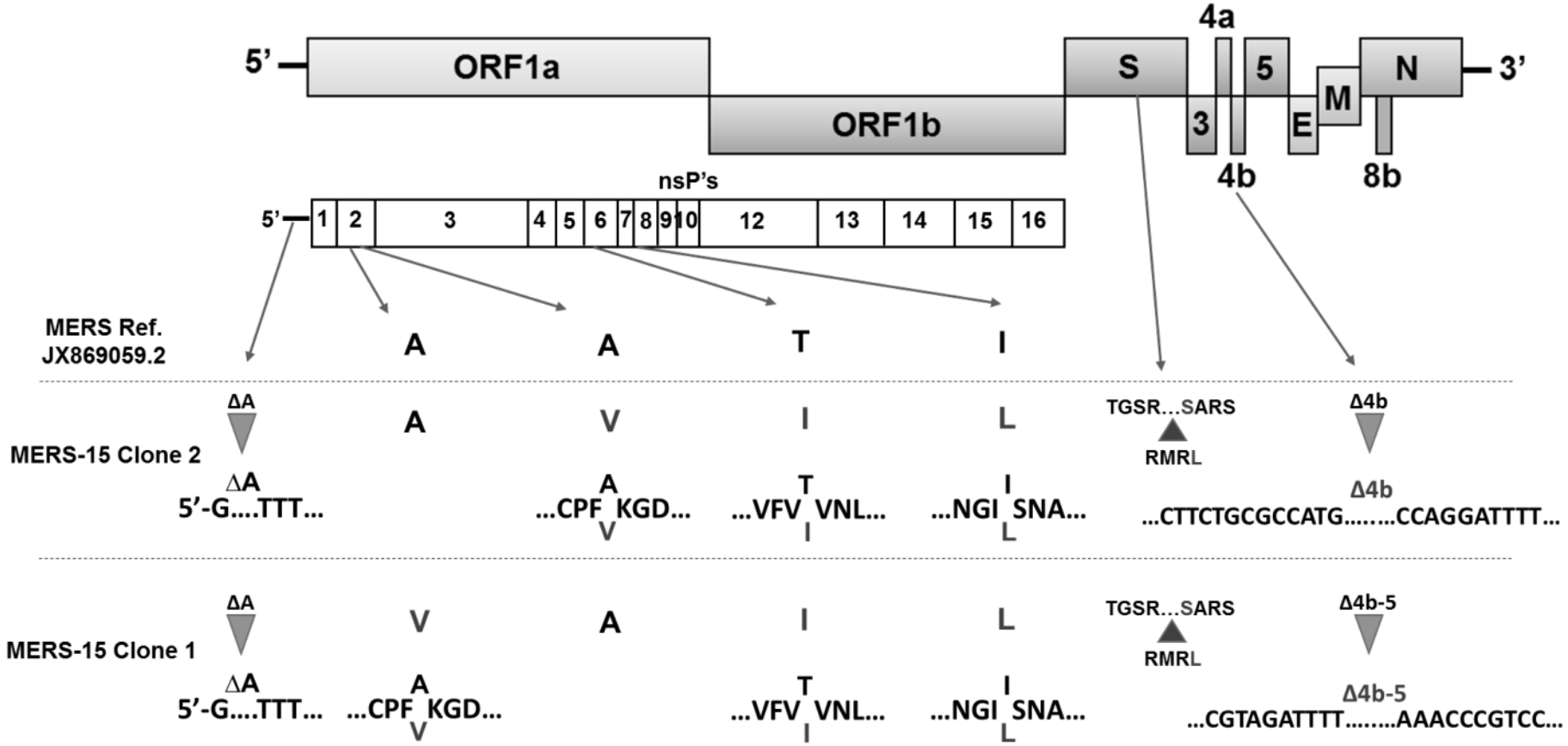
Supplemental Figure 7. Quantitation of histopathological findings for MERS-15 compared to MERS-0 at Days 3 and 6 post-infection. Data are represented as pie graphs with MERS-15/Day 3 (blue), MERS-0/Day 3 (orange), MERS-15/Day 6 (gray), and MERS-0/Day 6 (yellow). Each indication was scored, blinded on a scale of 0 – 3, where 0 = none, 1 = mild, 2 = moderate, and 3 = severe. Average scores are represented on the pie graphs with significance indicated by * = $p < 0.05$; # = $p < 0.01$; and @ = $p < 0.001$. Student *t*-test was used to compare the respective indication at days 3 and 6 p.i. for 288-330$^{+/+}$ mice infected with MERS-15 (day 3, $n = 5$ and day 6, $n = 5$) compared to those infected with MERS-0 (day 3, $n = 5$ and day 6, $n = 5$).

Supplemental Figure 8



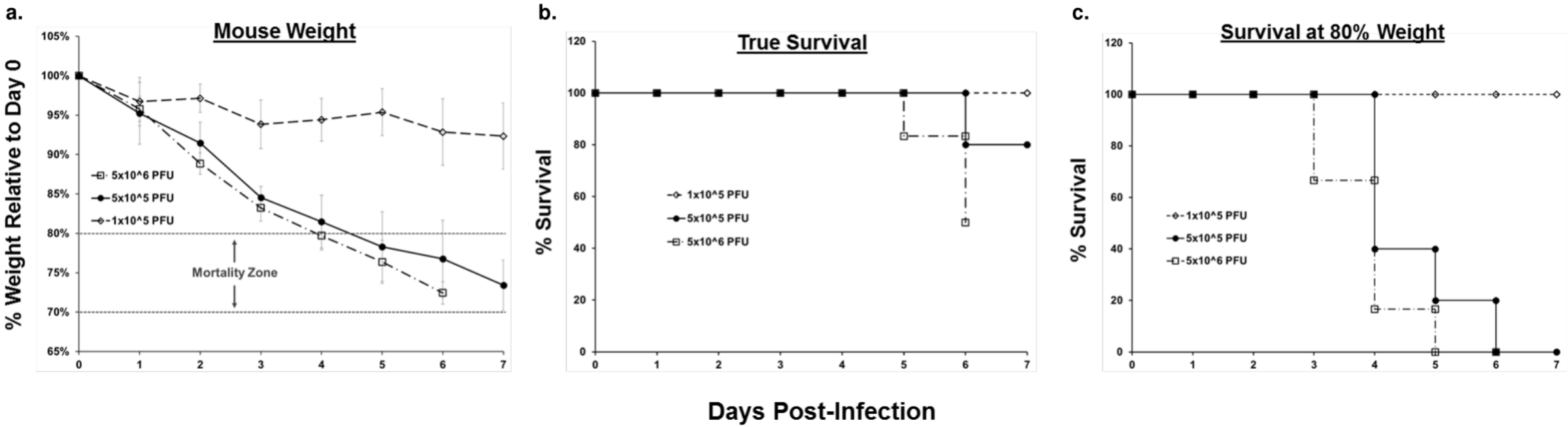
Supplemental Figure 8. Mouse-adapted MERS-15 clone 2 causes more severe hemorrhage than MERS-15 clone 1. Severity of hemorrhage was scored for 288-330$^{+/+}$ + MERS-15 C1 (day 3, n = 4 and day 6, n = 4), MERS-15 C2 (day 3, n = 3 and day 6, n = 7), or MERS-0 (day 3, n = 3 and day 6, n = 3); and, C57Bl/6J wt + MERS-15 C2 (day 3, n = 3 and day 6, n = 3). Lung hemorrhage was scored at day 3 and day 6 p.i. Lungs are given a score from 0 (no hemorrhage) to 4 (severe hemorrhaging in all lobes of the lung). N/D is none detectable. Student t-test was used to compare hemorrhage scores at day 6 post-infection for 288-330$^{+/+}$ + MERS-15 C1, and MERS-15 C2 (# is $p < 0.05$). Error bars are +SD.

Supplemental Figure 9



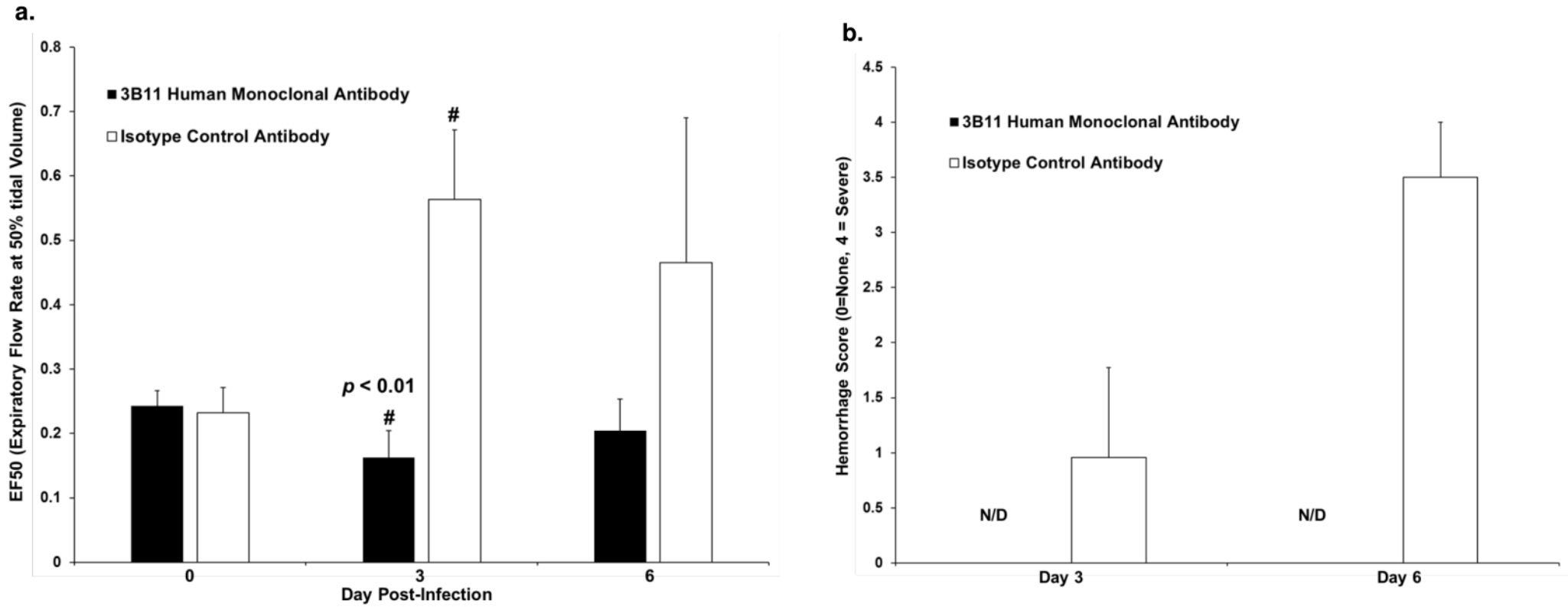
Supplemental Figure 9. Mutations identified in MERS-15 clone 2 and MERS-15 clone 1 compared to the MERS-CoV reference sequence, JX869059.2. A deletion of the second nucleotide in the 5'UTR is shown with a blue arrow head. Amino acid changes in nsP2, nsP6, and nsP8 are shown in red. The original RMR insertion with the S to L change in the S2 domain of the spike protein with the red arrow head. A deletion in the accessory gene Orf4b is shown with the blue arrow head with the sequence encoding the borders of the deletion shown.

Supplemental Figure 10



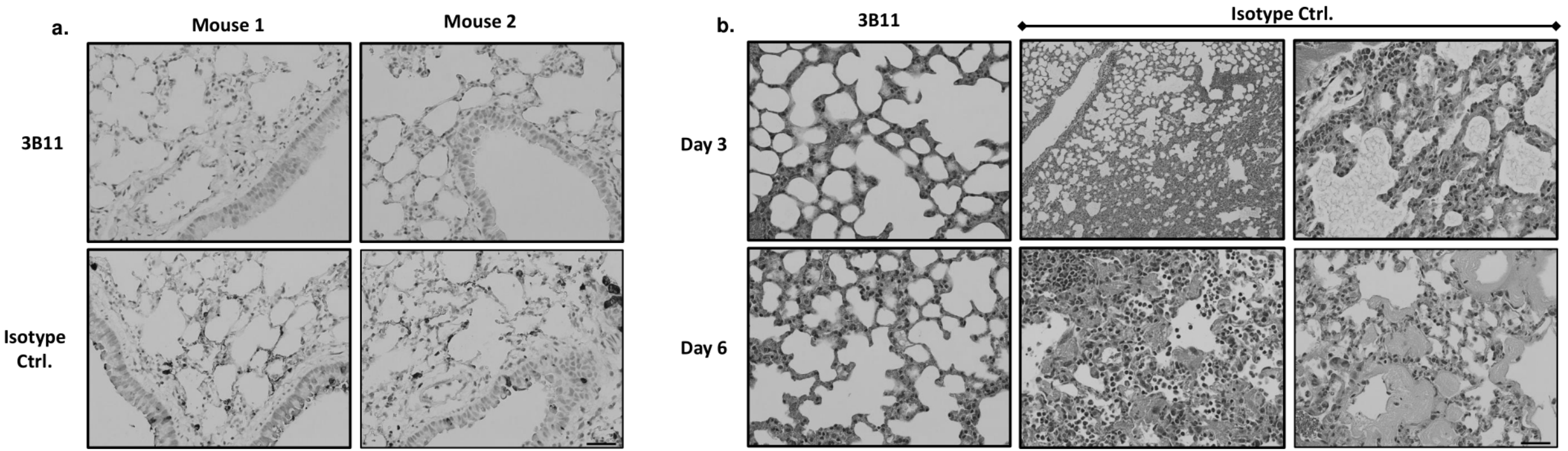
Supplemental Figure 10. An infectious clone generated with all the changes in the MERS-15 clone 2 recapitulates signs of disease. Infectious clone MERSma1 causes weight loss (a) and mortality (b & c) at both $5x10^6$ PFU ($n = 6$) and $5x10^5$ PFU ($n = 5$). Survival comparisons demonstrate dramatic differences between True survival (defined by death in cage) (b) compared to the standard in the field where mice are considered dead at an 80% humane weight cut-off (c). Based on 20-30% humane weight loss the mortality zone (red) is shown. Error bars are +/- SD.

Supplemental Figure 11



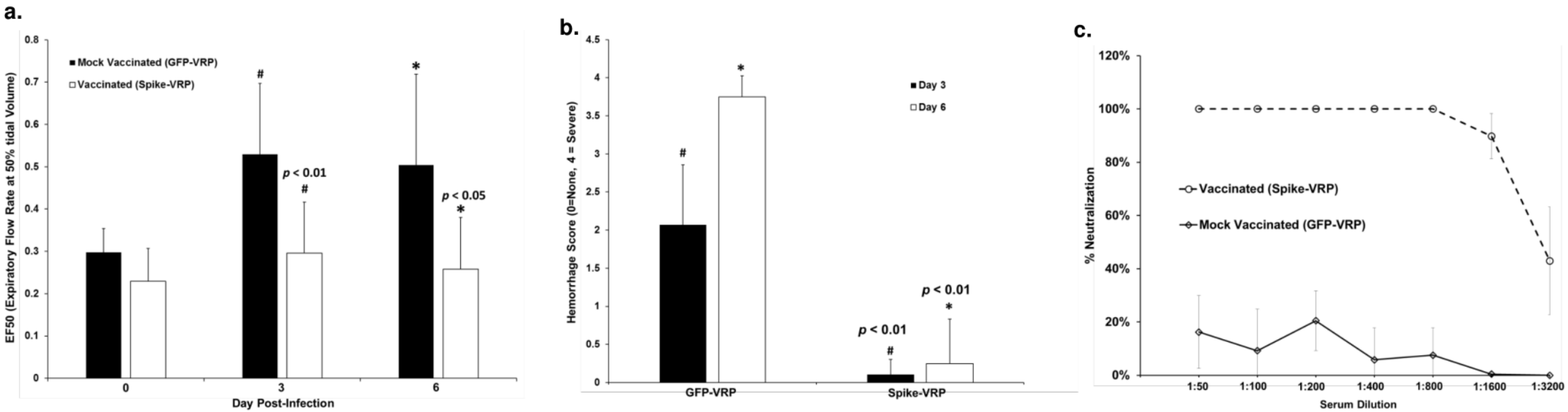
Supplemental Figure 11. 3B11 hmAB protects 288-330$^{+/+}$ mice from loss of respiratory function and hemorrhage. (a) Lung function was assessed by measuring expiratory flow rate at 50% tidal volume (EF50) at 0, 3, and 6 days p.i. for mice receiving 3B11 (day 0, 3, and 6, $n = 6$) or isotype control antibody (days 0 and 3, $n = 6$, day 6, $n = 3$). Data are represented as averages of the lung parameter measured +SD. Student t-test was used to compare lung function of mice receiving 3B11 human monoclonal antibody with the isotype control antibody at day 3 (# is $p < 0.01$) post-infection. (b) Lung hemorrhage was scored for mice administered 3B11 (days 3 and 6, $n = 6$) and isotype control antibody (day 3, $n = 6$ and day 6, $n = 6$) at days 3 and 6 p.i. Lungs were given a score from 0 (no hemorrhage) to 4 (severe hemorrhaging in all lobes of the lung). There was no detectable (N/D) hemorrhaging for mice receiving 3B11 human monoclonal antibody. Error bars are +SD.

Supplemental Figure 12



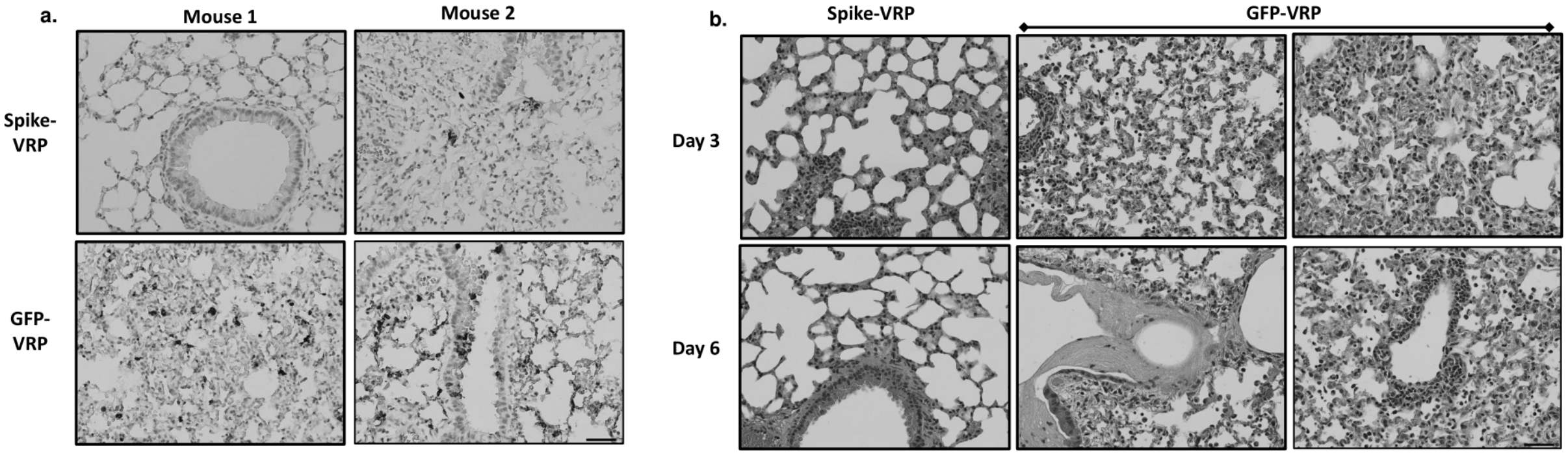
Supplemental Figure 12. 3B11 human monoclonal antibody protects from MERS-CoV-induced lung pathology. (a) IHC of lung sections at 3 days post-challenge from two different mice, treated with either 3B11 or isotype control antibodies. Infected cells are identified by IHC using anti-MERS nucleocapsid serum. (b) Pathology of lungs from 288-330$^{+/+}$ mice infected with MERS-15 C2 was assessed by H&E at days 3 and 6 post-infection. Images demonstrate severe inflammation, edema, and hyaline membrane formation in mice treated with the isotype control antibody, but absent in mice that received 3B11 hmAB. All images are at 10X or 40X magnification. Images are representative of at least 3 samples. Scale bars in lower right panels are 1mm.

Supplemental Figure 13



Supplemental Figure 13. Spike-VRP vaccination protects 288-330$^{+/+}$ mice. (a) Lung function was assessed by EF50 at 0, 3, and 6 days p.i. for mice receiving either GFP-VRP (days 0 and 3, $n = 12$ and day 6, $n = 6$) or Spike-VRP (days 0, 3, and 6, $n = 12$). Error bars are +SD. Student *t*-test was used to compare lung function of mice receiving GFP-VRP with Spike-VRP at day 3 (# is $p < 0.01$) and day 6 (* is $p < 0.05$) p.i. (b) Lung hemorrhage was scored for mice vaccinated with GFP-VRP (day 3, $n = 7$ and day 6, $n = 6$) or Spike-VRP (day 3, $n = 7$ and day 6, $n = 12$) at day 3 and day 6 p.i. Student *t*-test was used to compare lung hemorrhaging of mice receiving GFP-VRP with Spike-VRP at day 3 (# is $p < 0.01$) and day 6 (* is $p < 0.01$) p.i. Error bars are +SD. (c) Neutralization of MERS-15 C2 was analyzed using pre-challenge serum from spike-VRP ($n = 5$) vaccinated and GFP-VRP ($n = 5$) mock vaccinated mice. Neutralization was assessed at 2-fold dilutions within the range of 1:50 – 1:3200. Percent neutralization is indicated +/- SD.

Supplemental Figure 14



Supplemental Figure 14. Spike-VRP vaccination protects from MERS-CoV-induced lung pathology. (a) IHC of lung sections at 3 days post-challenge from two different mice. Infected cells are identified by IHC using anti-MERS nucleocapsid serum. (b) Pathology of lungs from 288-330$^{+/+}$ mice infected with MERS-15 C2 was assessed by H&E at days 3 and 6 post-infection. Images demonstrate severe inflammation, perivascular cuffing, and hyaline membrane formation only in mice that received GFP-VRP control. All images are at 40X magnification. Images are representative of at least 3 samples. Scale bars in lower right panels are 1mm.

From: Cockrell, Adam
Sent: Wed, 3 Aug 2016 21:20:12 +0000
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph; Deborah Butler; Neil Pearson; Feng Wang
Subject: RE: GSK A57 Study
Attachments: Time line for Drug study.pdf

Hi everyone. It was good to meet everyone in the gsk group.

In putting together the time line (attached to email) I had some additional thoughts.

1) There are two slides. The first is the initial time line that we discussed on the phone. The second slide takes into account the fact that the half-life of drug is really short, therefore we can adjust the drug delivery time line to bracket the initial viral delivery to be -6 hours and +6 hours if you guys would prefer. This would shorten the study on the back end by 6 hours, which should be of no consequence regarding the data we will capture.
2) This is just a thought, and not sure if this is a viable possibility given the half-life of the drug, but we could eliminate any confounding issues with repeated anesthetic administration if there was an option to deliver drug by the IP route. Thoughts?

That said I look forward to working with everyone.

Best Regards,
Adam

From: Jeff Pouliot (b)(6)
Sent: Wednesday, August 03, 2016 2:13 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6) Cockrell, Adam (b)(6) Feng Wang (b)(6)
Subject: RE: GSK A57 Study

Thank you all for the productive discussion. We look forward to working together.

I've added one person to the email list above. Please include Feng Wang on the experimental planning communications.

Best,

Jeff

Jeffrey Pouliot, Ph.D.
Investigator
Biology Host Defense DPU

R&D Infectious Disease

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Tel (b)(6)

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, August 03, 2016 1:59 PM
To: 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph; Deborah Butler; Neil Pearson; Jeff Pouliot; 'Cockrell, Adam'
Subject: GSK A57 Study

EXTERNAL

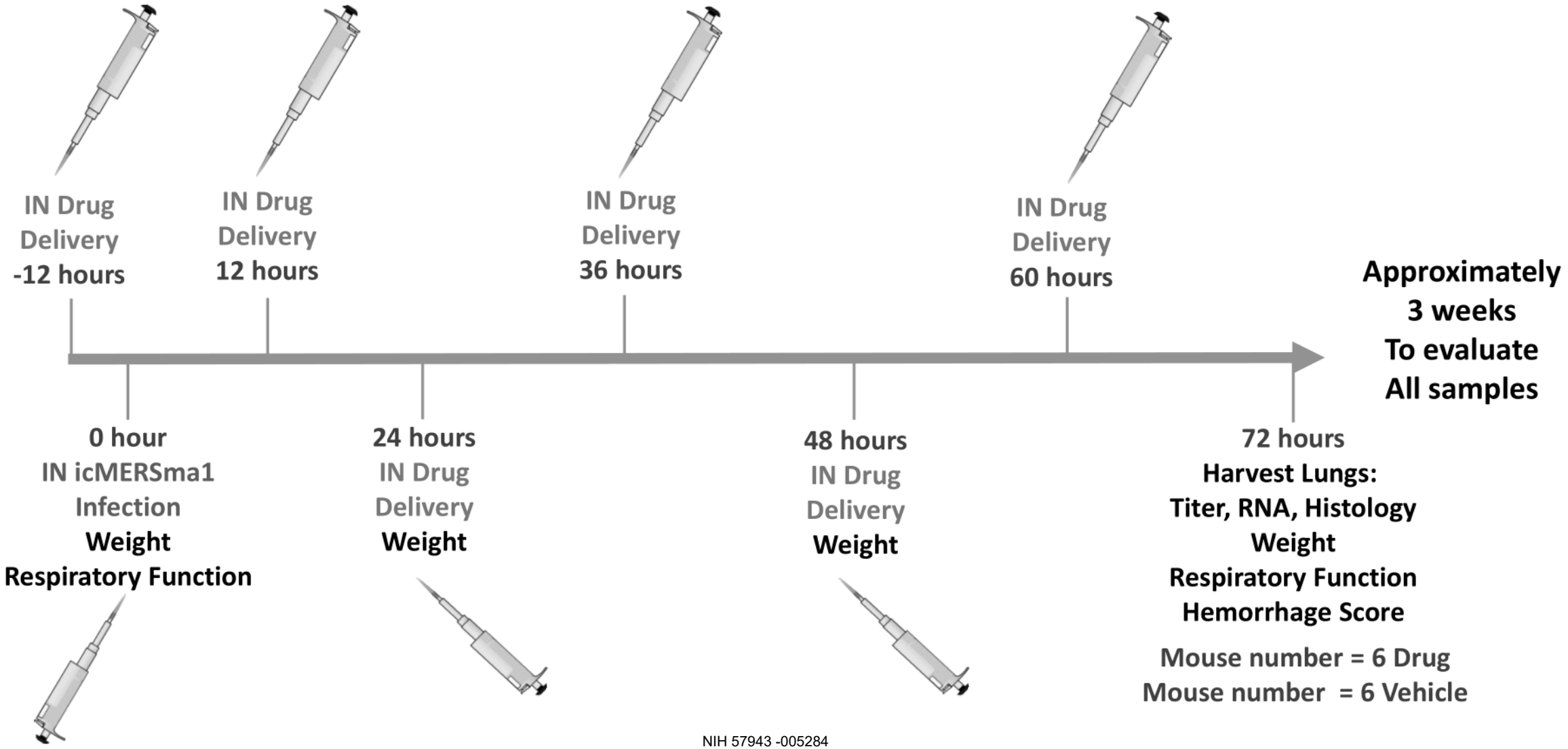
Hi Everyone,
Thanks for your time today, it was a productive call. Please feel free to email directly to work out the protocol/dosing and other study details, but be sure to CC everyone on this message.

Erik

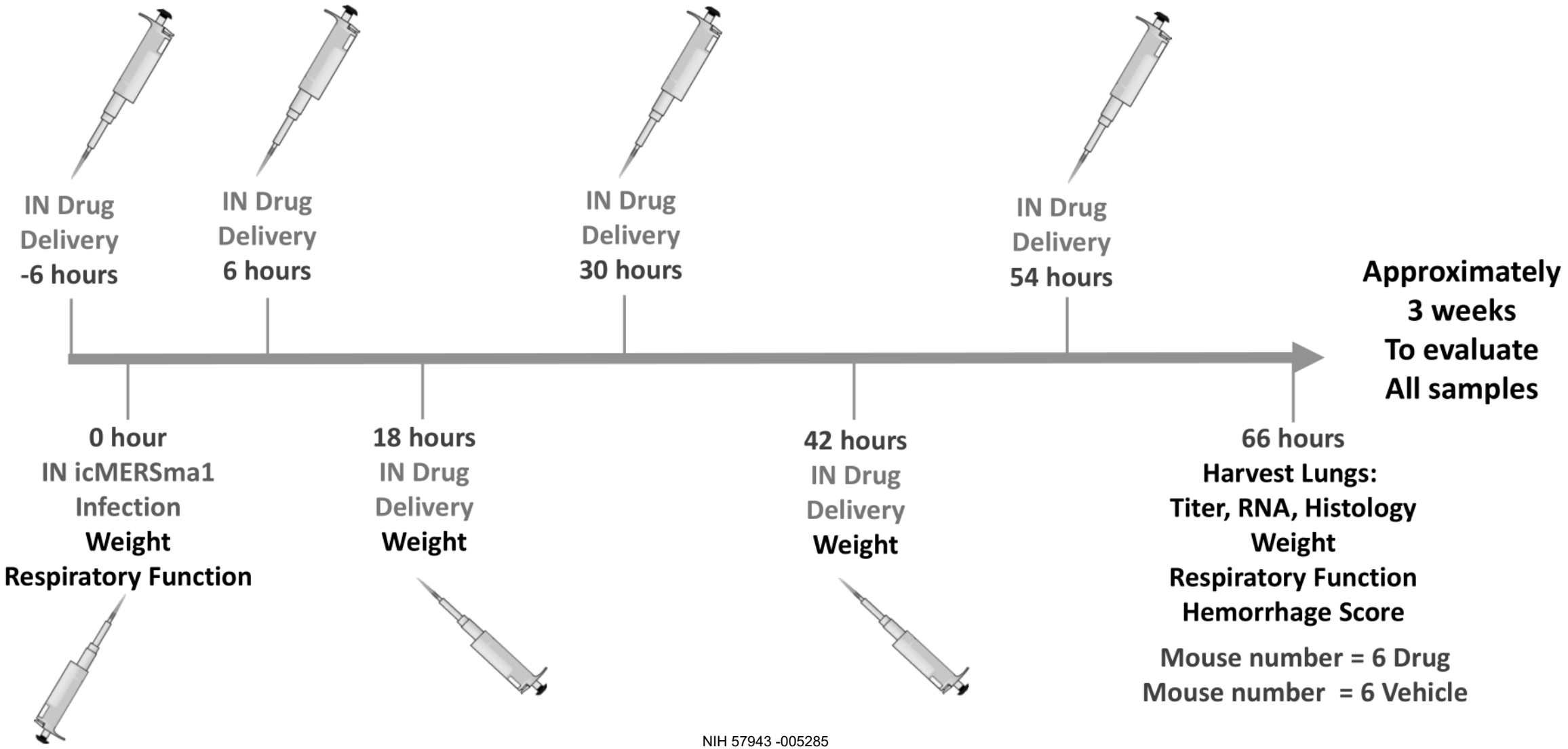
Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email: (b)(6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

Drug Study with GSK (High Dose Study Delivered Every 12 hours)



Drug Study with GSK (High Dose Study Delivered Every 12 hours)



From: Baric, Ralph
Sent: Mon, 30 Nov 2015 14:05:39 +0000
To: Maria Zambon;(b)(6) Baric, Toni C
Cc: Stemmy, Erik (NIH/NIAID) [E]; Cockrell, Adam
Subject: RE: UPDATE MERS MABS
Attachments: Baric-MERS-CoV-Model Description-Confidential.pdf

---

From: Maria Zambon (b)(6)
Sent: Sunday, November 29, 2015 7:31 AM
To: (b)(6) Baric, Toni C
Cc: Stemmy, Erik (NIH/NIAID) [E]; Cockrell, Adam; Baric, Ralph S
Subject: RE: UPDATE MERS MABS

Colleagues,

Is there material in regards the mouse model set up by ralph Baric to be shared before this meeting tomorrow (as suggested in some of the earlier correspondance). I will be doing the phone call externally, so would appreciate an early view of any data, as I am not sure whether I will have good email access during the day tomorrow

thanks

Maria Zambon
Director, Reference Microbiology
Deputy Director, NIS
National Infection Service
Public Health England
(b)(6)
Tel: (b)(6)
www.gov.uk/phe Follow us on Twitter@PHE.uk
Protecting and improving the nation's health

From: (b)(6)
Sent: 10 November 2015 17:32
To: Baric, Toni C
Cc: Stemmy, Erik (NIH/NIAID) [E]; Maria Zambon; Cockrell, Adam; Ralph Baric; Robin Gopal
Subject: Re: UPDATE MERS MABS

Dear Toni,

This is fine. I can provide the call-in number.

Here it is:

UK: 0808 234 88 76
Switzerland: 0800 329 329
USA: +1 866 591 43 61 (or +1 888 50 333 35)

Participant access code: (b)(6)

Best regards,

(b)(6)

Il giorno 10 nov 2015, alle ore 17:52, Baric, Toni C (b)(6) ha scritto:

Hi Everyone,
Let's set this call for Nov 30 at 9 am EST/ 2pm UK time. Does this work? Also, does someone have a call-in number or should I set this up?
Thank you,
Toni

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Thursday, November 05, 2015 9:30 AM
To: Baric, Toni C; Maria Zambon; Cockrell, Adam; (b)(6)
Cc: Baric, Ralph S; Robin Gopal
Subject: RE: UPDATE MERS MABS

My preference would be for 11/30. I can do any time before 1pm EST.

Erik

From: Baric, Toni C (b)(6)
Sent: Thursday, November 05, 2015 9:26 AM
To: Maria Zambon (b)(6) Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Baric, Ralph (b)(6) Robin Gopal (b)(6)
Subject: RE: UPDATE MERS MABS

Hi Group,

Let's we revisit the following dates:
11/30 9-10 am EST or after 10 am EST
12/2 before 2 pm EST.

Please let me know the day and time range that works for all of you, keeping in mind that Maria will be calling in from UK.
Thanks
Toni

From: Maria Zambon (b)(6)
Sent: Wednesday, November 04, 2015 5:49 PM
To: Cockrell, Adam; Baric, Toni C; Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
Cc: Baric, Ralph S; Robin Gopal
Subject: RE: UPDATE MERS MABS

Hello,

Sorry if late to the party. I have already sent back a note saying the Monday of this week would work for me. Unfortunately I will be in Hng Kong the 17th to 20th, so would suggest we try earlier if we can

maria

Maria Zambon
Director, Reference Microbiology
Deputy Director, NIS
National Infection Service
Public Health England
(b)(6)
www.gov.uk/phe Follow us on Twitter@PHE.uk
Protecting and improving the nation's health

From: Cockrell, Adam (b)(6)
Sent: 04 November 2015 20:21
To: Baric, Toni C; Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
Cc: Baric, Ralph S; Maria Zambon; Robin Gopal
Subject: RE: UPDATE MERS MABS

That sounds good for me.
Thanks,
Adam

From: Baric, Toni C
Sent: Wednesday, November 04, 2015 3:11 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Baric, Ralph S (b)(6) Maria Zambon (b)(6) Cockrell,

Adam (b)(6) Robin Gopal (b)(6)
Subject: RE: UPDATE MERS MABS

11/20 sounds good. How about 10 am? If this works for everyone, please let me know. Otherwise, please suggest a time before 1 pm that suits or a different day.
Thank you,
Toni

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, November 04, 2015 3:07 PM
To: (b)(6) Baric, Toni C
Cc: Baric, Ralph S; Maria Zambon; Cockrell, Adam; Robin Gopal
Subject: RE: UPDATE MERS MABS

On 11/20 I can do any time before 1pm EST. Can we aim for that date?

Erik

From: (b)(6)
Sent: Wednesday, November 4, 2015 3:02 PM
To: Baric, Toni C (b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Baric, Ralph (b)(6) Maria Zambon (b)(6) Cockrell, Adam (b)(6) Robin Gopal (b)(6)
Subject: Re: UPDATE MERS MABS

Dear Toni,

I am available on all dates with the exception of 12/4.

Best regards,

(b)(6)

Il giorno 04 nov 2015, alle ore 20:25, Baric, Toni C (b)(6) ha scritto:

How about the following:

Friday 11/20 Ralph is open all day. Then the next day is 11/30 –after 11, Wednesday 12/2 before 2 and all day on 12/4.

Best regards,
Toni

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, November 04, 2015 2:13 PM
To: Baric, Toni C; Baric, Ralph S; Maria Zambon; Cockrell, Adam
Cc: (b)(6) Robin Gopal
Subject: RE: UPDATE MERS MABS

I am leaving for a meeting in Riyadh on 11/12, so we'll have to schedule a call after I return on 11/16.

Erik

From: Baric, Toni C (b)(6)
Sent: Wednesday, November 4, 2015 12:06 PM
To: Baric, Ralph (b)(6) Maria Zambon (b)(6) Cockrell, Adam (b)(6)
Cc: (b)(6) Robin Gopal (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Subject: RE: UPDATE MERS MABS

Hi Maria,
Ralph is available on 11/12 after 3:30 and between 11:30-3 on Friday 11/13
Best regards,
Toni

From: Baric, Ralph S
Sent: Tuesday, November 03, 2015 4:11 PM
To: Maria Zambon; Cockrell, Adam; Baric, Toni C
Cc: (b)(6) Robin Gopal; Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: UPDATE MERS MABS

Hi Maria, We have recently received 20mg of pure antibody from (b)(6) and have support by Erik Stemmy to perform your studies in the mouse model. Initially, we will evaluate protection prior to infection. We currently don't have approval to use sharps for therapeutic intervention postinfection, but are in the process of putting in the paperwork to administer drug postinfection. I recommend a two phase study, first prior to infection to demonstrate efficacy and then drug dose at day 1 or 2 postinfection (single dose?). We likely need to set up a time to discuss the experiments. We will also share the model details at that time. Toni can assist. We also are planning on doing the protection study in early December, post infection study would likely be jan at best. Adam is a key contact person for discussion. Hope you are doing well. It's a pleasure to work with you again. Thoughts? ralph

---

From: Maria Zambon (b)(6)
Sent: Friday, October 23, 2015 3:52 PM
To: Baric, Ralph S
Cc: (b)(6) Robin Gopal
Subject: UPDATE MERS MABS

Dear Ralph,

Greetings , we have not corresponded for a while…I think another pesky virus (Ebola) has caused a bit of a diversion for all of us. (b)(6) has mentioned that you have developed a new animal model for MERS which is transgenic, and is very sensitive. This is just a brief note to explore the possibility of extending mouse model work for LCA60. We have submitted a proposal to the Medical Research Council (MRC) in the UK to take LCA60 into a Phase 1 clinical study. This proposal included the costs for Phase 1 scale up to GMP and also a Phase 1 Pk study in healthy volunteers, and is a large proposal.

The response from the MRC has been favourable, but they are requesting strengthening of the pre-clinical package in the proposal to try and give more indication of how the Mab could be used. We would appreciate your advice/collaboration in this

(1) Could we propose more work in your mouse model to extend understanding of prophylaxis duration and the window for treatment. I am thinking about extending the time points post infection at which Mab is given and also refining knowledge of the duration of protection if given before challenge. One of the questions we are asked to address is to what are the parameters under which this might be used clinically. Currently the data we hold is more of a YES/NO format, rather than a considered model approach to window of treatment opportunity.
(2) If you thought some more work was feasible, would this be possible without provision of funding from us under existing NIH contracts, or would you require additional funding, and if so, what would that be . (NB could we also slip in some work on LCA57, the non neutralising Mab that we have got ?). We would be pleased to include you as a co-applicant for MRC funding, subject to MRC rules for overseas applicants, but the full proposal application cannot be submitted before March, meaning that you might well have already done the work before we could provide any funding
(3) What is your advice about whether the animal model you have developed is suitable for use…Suggestions welcome

Grateful for a rapid response

Maria Zambon
Director, Reference Microbiology
Deputy Director, NIS
National Infection Service
Public Health England
(b)(6)
www.gov.uk/phe Follow us on Twitter@PHE.uk
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(b)(4)

of the Freedom of Information and Privacy Act

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Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

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Withheld pursuant to exemption

(b)(4)

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Page 391 of 455

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(b)(4)

of the Freedom of Information and Privacy Act

From: Cockrell, Adam
Sent: Thu, 8 Sep 2016 16:18:37 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Baric, Ralph
Subject: FW: GSK A57 Study
Attachments: MTR -UNC-Sept 8 2016.docx

Hi Erik,

We were provided this MTA regarding the drugs we received from Feng at GSK. Just wanted to clear it with you first that we are responsible for signing and returning this to Feng.
Thanks,
Adam

From: Feng Wang (b)(6)
Sent: Thursday, September 08, 2016 10:52 AM
To: Cockrell, Adam (b)(6)
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study

Hi Adam/Yount,

We shipped another 3 bottles (~ 1mg per bottle) of the test compound to you yesterday. Additional vehicle (i.e. 0.5%Tween80) is also on the way. All together, you should have total 8 bottles of the test compound. Please fill in the actual compound weight and email me back the signed material transfer form as attached for the acknowledgment of compound receiving.
How is your formulation testing going? Acting cautiously, we will recommend freshly preparing the formulation for each dose administration as original discussed. Below is a brief reminder of the formulation procedure:

(1) Aliquot enough volume of vehicle in 5 replicates and store them at 4-8°C until use. Use one aliquot for each dose preparation.
(2) Wait until the compound bottle and the vehicle equilibrating to room temperature. Gently stir or mix the vehicle. Add the exact volume of vehicle to the bottle for a formulation concentration of 0.5mg/mL
(3) Sonicate or vertex or stir on a slightly warm plate (< 37°C) for a couple minutes until a clear solution is obtained
(4) Dose each mouse with a fixed 50µL of the above formulation. (please also record the mouse weight)

Thanks and look forward to the study!
feng
Feng Wang
Investigator
Host Defense DPU
RD Infectious Disease R&D

GSK

1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(4)
Tel (b)(4)

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From: Cockrell, Adam (b)(4)
Sent: Thursday, September 01, 2016 4:04 PM
To: Feng Wang; Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study

EXTERNAL

Hi Feng,

Thanks for the resuspension info.

I was previously informed that the drug is highly unstable, therefore I would have to resuspend the drug prior to every administration. There are five administrations therefore I would need all five bottles you send for the experiment.

That is why I requested a couple extra vials. Please let me know if I can use one vial for more than one administration.

Thanks,
Adam

From: Feng Wang (b)(4)
Sent: Thursday, September 01, 2016 3:30 PM
To: Cockrell, Adam (b)(4) Jeff Pouliot (b)(4) Stemmy, Erik (NIH/NIAID) [E] (b)(4) 'Leyva-Grado, Victor' (b)(4) 'Umerah, Nina' (b)(4) Baric, Ralph S (b)(4) Deborah Butler (b)(4) Neil Pearson (b)(4)
Cc: Yount, Boyd L Jr (b)(4)
Subject: RE: GSK A57 Study

Hi Adam,

Each bottle would provide more than enough formulations required for one day (BID) dosing. So, for the whole study, you only need three bottles. You could use the 4th bottle for your formulation test and the last bottle as a backup.

Here is the calculation:
To achieve a 1mg/kg IN dose with fixed 50uL dose volume, you need a dose solution of 0.5mg/mL assuming a typical mouse weight of 0.025g. So for one day BID dosing of 6 mice, you only need 0.3mg test compound.

To prepare a dose solution of 0.5mg/mL. You just need to take the weight information from the bottle and calculate the volume of 0.5%Tween 80 needed, and then add that exact volume of vehicle to the bottle. After a couple min sonication or mixing on a warm hotplate, a clear solution will be obtained.

Let me know if you have more questions. We still have time to ship more materials as needed.

Thanks,
feng

Feng Wang
Investigator
Host Defense DPU
RD Infectious Disease R&D

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Tel (b)(4)

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From: Cockrell, Adam (b)(4)
Sent: Thursday, September 01, 2016 3:02 PM
To: Feng Wang; Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study

EXTERNAL

Thanks Feng.

However, this does not include a sample for me to practice the resuspension of the drug prior to treatment. Can you provide at least one additional sample, and maybe an extra in the event something happens during resuspension?

Also, please provide exact instructions for resuspension with the vehicle that was sent previously.

Thanks,
Adam

From: Feng Wang (b)(4)
Sent: Thursday, September 01, 2016 2:55 PM
To: Cockrell, Adam (b)(4) Jeff Pouliot (b)(4) Stemmy, Erik (NIH/NIAID) [E] (b)(4) 'Leyva-Grado, Victor' (b)(4) 'Umerah, Nina' (b)(4) Baric, Ralph S (b)(4) Deborah Butler (b)(4) Neil Pearson (b)(4)
Cc: Yount, Boyd L Jr (b)(4)
Subject: RE: GSK A57 Study

Hi Adam/Boyd,

Just an update, the test compound (labeled as GSKXXX) in five replicates are shipped out today and should arrive at UNC tomorrow. Once received, please store them in 4-8°C. There should be ~1.2mg in each bottle.

Best wishes,
feng

Feng Wang
Investigator
Host Defense DPU
RD Infectious Disease R&D

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(4)
Tel (b)(4)

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From: Cockrell, Adam (b)(4)
Sent: Tuesday, August 30, 2016 10:41 AM
To: Feng Wang; Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study

EXTERNAL

Hi Feng,

I received the vehicle this morning. However, the address on the package had it shipped to a lab in a different building in the pharmacy department. Fortunately, they were able to find our number and let us know.

Also, I stored it at 4C, but it was shipped at ambient temperature.

I will test the formulation late next week when I return.

For shipping of the test compound please use the following address:

Boyd Yount/Adam Cockrell
UNC-CH
135 Dauer Drive
Hooker Bldg./Room 3105
Chapel Hill, NC
27599
Phone# (b)(4)

Best Regards,
Adam

From: Feng Wang (b)(4)
Sent: Tuesday, August 30, 2016 9:39 AM
To: Cockrell, Adam (b)(4) Jeff Pouliot (b)(4) Stemmy, Erik (NIH/NIAID) [E] (b)(4) 'Leyva-Grado, Victor' (b)(4) 'Umerah, Nina' (b)(4) Baric, Ralph S (b)(4) Deborah Butler (b)(4) Neil Pearson (b)(4)
Cc: Yount, Boyd L Jr (b)(4)
Subject: RE: GSK A57 Study

Hi Adam,

We shipped out study vehicle (i.e. 0.5%Tween80) yesterday and should arrive at your lab today. Please watch out and store it at 4-8°C. Due to some paper work delay, I do not think that the test compound will arrive before you leave for vacation. Is it possible that your coworker could do the formulation test in your absence? In addition, the test compound should also be stored at 4-8°C prior to use.

Thanks,
feng

Feng Wang
Investigator

Host Defense DPU
RD Infectious Disease R&D

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(4)
Tel (b)(4)

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From: Cockrell, Adam (b)(4)
Sent: Monday, August 29, 2016 9:25 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study

EXTERNAL

Hi Jeff,

Contact numbers are (b)(4) (Adam) and (b)(4) (Boyd)

Thanks,

Adam

From: Jeff Pouliot (b)(4)
Sent: Friday, August 26, 2016 4:09 PM
To: Cockrell, Adam (b)(4) Stemmy, Erik (NIH/NIAID) [E] (b)(4)
'Leyva-Grado, Victor' (b)(4) 'Umerah, Nina' (b)(4)
Baric, Ralph S (b)(4) Deborah Butler (b)(4) Neil Pearson
(b)(4) Feng Wang (b)(4)
Cc: Yount, Boyd L Jr (b)(4)
Subject: RE: GSK A57 Study

Hi Adam

Thank you very much. Can you supply a contact phone number for shipping?

We will send the 0.5% Tween in saline with our compound. Everything should arrive by midweek.

Best,

Jeff

From: Cockrell, Adam (b)(4)
Sent: Friday, August 26, 2016 10:54 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang; Barb Carter
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study

EXTERNAL

Hi Jeff,

Thanks for the update. I have addressed your questions below in red.

I will be out of town September 1st-september 7th, but Boyd Yount will be available to receive the package if I'm not here. Please advise on any special storage conditions.

Would it be possible for you ship a sample for early arrival next week, with all the components, so that I can test out the resuspension of the drug?

Also, I have attached a copy of the study as we discussed. As you suggested I eliminated the time point for drug delivery 6 hours prior to infection.

Best Regards,
Adam

From: Jeff Pouliot (b)(4)
Sent: Thursday, August 25, 2016 6:38 PM
To: Cockrell, Adam (b)(4) Stemmy, Erik (NIH/NIAID) [E] (b)(4) 'Leyva-Grado, Victor' (b)(4) 'Umerah, Nina' (b)(4) Baric, Ralph S (b)(4) Deborah Butler (b)(4) Neil Pearson (b)(4) Feng Wang (b)(4) Barb Carter (b)(4)
Subject: RE: GSK A57 Study

Dear Adam,

We'd like to update you on the status of the test compound shipping for the study and your formulation pre-work. We have the patent nearly completed and will be able to send the compound early next week,

targeting shipping for Tuesday 8/30 with arrival by the end of the week. Please let us know if this does not agree with your planned work schedule. We also have a few shipping questions to be certain everything goes smoothly:

- Can you advise on the planned start date for the in vivo study? If you need compound on the morning of September 6 we will try to send it earlier in the week to reduce the chance of shipping delays. I have reserved time in our BSL3 facility to initiate the experiment on Monday September 12th. Therefore, we would need to have the compound by Friday September 9th.

- Will your shipping group be receiving packages next Thurs-Fri (Sep 1-2)? If I am not here when the package arrives Boyd Yount in the lab will be available to receive the package. Please advise on any special storage conditions. I have included Boyd on this email.

- Could you please confirm the shipping address we should use for the test compound? Adam Cockrell/Boyd Yount, UNC-CH, 135 Dauer Dr., Chapel Hill, NC, 27599

- Do you have 0.5% Tween-80 in saline available for the formulation or should we plan to ship some? It would be simpler if you had some on hand as it necessitates a second package, but we're happy to arrange it if you prefer. I would prefer that the GSK group provides everything relevant to the drug.

Best,

Jeff

From: Cockrell, Adam (b)(4)
Sent: Sunday, August 14, 2016 10:48 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Subject: RE: GSK A57 Study

EXTERNAL

Thanks Jeff,

Sounds great!

Adam

From: Jeff Pouliot (b)(4)
Sent: Saturday, August 13, 2016 5:27 PM
To: Cockrell, Adam (b)(4) Stemmy, Erik (NIH/NIAID) [E] (b)(4)
'Leyva-Grado, Victor' (b)(4) 'Umerah, Nina' (b)(4)

Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson
(b)(6) Feng Wang (b)(6)
Subject: RE: GSK A57 Study

Hi Adam,

We can send you a sample as soon as legal tells us the patent is filed. This should take roughly another week, so we should be able to get the sample to you by the end of two weeks. We will let you know if there are any unexpected delays.

Thanks for the info on dose groups. We can plan in more detail once the pilot run is complete.

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Saturday, August 13, 2016 7:43 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Subject: RE: GSK A57 Study

EXTERNAL

Thanks Jeff,

Would you guys mind sending me a sample of the drug (exactly how I will receive it for the mouse studies) in the next week, or two, so that I can validate the resuspension process in my hands?

If we see efficacy with the initial study, I believe 2-3 dose groups, with a 24 hour delivery window, would be feasible.

Thanks,
Adam

From: Jeff Pouliot (b)(6)
Sent: Thursday, August 11, 2016 3:45 PM
To: Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6)
Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson
(b)(6) Feng Wang (b)(6)
Subject: RE: GSK A57 Study

Dear Adam,

You should be able to formulate the compound either way. It should easily go into solution in 3-5 min with a 37C water bath. Otherwise, you can vortex and leave it on a heated plate (low setting, warm) with stirring for a couple minutes.

We suggested a 24h dosing schedule for the first study, but your counterproposal of BID dosing to have the greatest chance of efficacy was a good one. A 12-hour doing schedule for the initial study is fine.

For the follow-up study we can modify dosing to qd from 6-hours post infection, presuming the initial results are robust. We can plan this in more detail once the initial test is complete. To help us think it through, though, can you let us know if it is technically feasible to run 2-3 dose groups in parallel?

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Wednesday, August 10, 2016 6:39 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Subject: RE: GSK A57 Study

EXTERNAL

Dear Jeff,

Please see responses to comments/questions below.

Regards,
Adam

From: Jeff Pouliot (b)(6)
Sent: Tuesday, August 09, 2016 5:51 PM
To: Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6) Feng Wang (b)(6)
Subject: RE: GSK A57 Study

Dear Adam,

Thanks for the note. Your research plan nicely reflects our discussion last week. We have some information below to fill in the details and a few questions for you.

- The predosing of compound is not needed as these are direct acting antivirals. In addition, only a suboptimal amount of compound would remain at the time of infection given the short T1/2 of this compound. A therapeutic model with the first dose following infection is our preferred choice. Is this acceptable? Starting with a therapeutic dose at 6 hours post-infection sounds great.
- BID dosing starting at 6 hours post infection seems the better plan. Do you know how long robust viral replication continues in an untreated test subject? Our model exhibits robust replication through day 6 post-infection with peak replication at days 2-3.
- We recommend intranasal dosing at 1 mg/kg, 50 uL volume per mouse, at a concentration of 0.5 mg/mL. This should deliver a compound concentration at Tmax of 100x EC50 to the lung. IN sounds good.
- We will plan to ship you the compound as dry powder. We're exploring stability but until we have firm data we can't guarantee that a solution prepared here would be stable long enough for the experiment. You will need to suspend by brief sonication in a dosing solution of 0.5% Tween-80 in saline. Is this acceptable? This is acceptable, however can you please define sonication? Is a water sonicator necessary for this? Or, will vortexing suffice? Does this compound readily go into solution? The 12 hour dosing schedule is quite rigorous, especially in a BSL3, therefore I am trying to get an understanding of how much additional time I will have to spend suspending the drug prior to each 12 hour administration.

We would like also to think ahead to the second round of the experiment. Presuming the outcome shows positive results, we propose a similar experiment at successive 3-fold lower drug concentrations to clarify the PK/PD relationship. If the follow up allows more than one dose group, we would dose at 0.3 mg/kg and 0.1 mg/kg (30x and 10x EC50). Does this sound reasonable to you? A dosing experiment sounds reasonable. Provided the initial study is successful, In follow-up experiments we discussed moving to a 6-7 day time course. In doing this I will have to move to delivering the drug every 24 hours. Is this reasonable to you? Would you prefer that the initial study use a 24 hour repeated dosing time course? The 24 hour time course would begin after the initial delivery of the drug at 6 hours post-infection.

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Wednesday, August 03, 2016 5:20 PM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Subject: RE: GSK A57 Study

EXTERNAL

Hi everyone. It was good to meet everyone in the gsk group.

In putting together the time line (attached to email) I had some additional thoughts.

1) There are two slides. The first is the initial time line that we discussed on the phone. The second slide takes into account the fact that the half-life of drug is really short, therefore we can adjust the drug delivery time line to bracket the initial viral delivery to be -6 hours and +6 hours if you guys would prefer. This would shorten the study on the back end by 6 hours, which should be of no consequence regarding the data we will capture.
2) This is just a thought, and not sure if this is a viable possibility given the half-life of the drug, but we could eliminate any confounding issues with repeated anesthetic administration if there was an option to deliver drug by the IP route. Thoughts?

That said I look forward to working with everyone.

Best Regards,
Adam

From: Jeff Pouliot (b)(6)
Sent: Wednesday, August 03, 2016 2:13 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6) Cockrell, Adam (b)(6) Feng Wang (b)(6)
Subject: RE: GSK A57 Study

Thank you all for the productive discussion. We look forward to working together.

I've added one person to the email list above. Please include Feng Wang on the experimental planning communications.

Best,

Jeff

Jeffrey Pouliot, Ph.D.
Investigator
Biology Host Defense DPU
R&D Infectious Disease

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, August 03, 2016 1:59 PM
To: 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph; Deborah Butler; Neil Pearson; Jeff Pouliot; 'Cockrell, Adam'
Subject: GSK A57 Study

EXTERNAL

Hi Everyone,
Thanks for your time today, it was a productive call. Please feel free to email directly to work out the protocol/dosing and other study details, but be sure to CC everyone on this message.

Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email: (b)(6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

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SCHEDULE C
MATERIAL TRANSFER RECORD

GlaxoSmithKline LLC
("Providing Party")

to
University of North Carolina at Chapel Hill
("Receiving Party")

The Material described below is supplied by the Providing Party to the Receiving Party subject to the terms and conditions of the Task Order/Statement of Work between **University of North Carolina at Chapel Hill via NIAD** ("Receiving Party") and **GlaxoSmithKline** ("Providing Party") dated 09/25/2012 ("Agreement"). Duplicate originals of this form shall be executed and one fully-executed form shall be given to the Providing Party and one to the Receiving Party.

Description of Material: See below

In signing below, the Study contacts acknowledge that they understand and will abide by the terms and conditions under which the Material is provided.

(Signature) GlaxoSmithKline (Providing Party)

Date Material Sent/Provided to Receiving Party

(Signature) UNC at Chapel Hill (Receiving Party)

Date Material Received by Receiving Party

Compound Number	MW	Weight (mg)
GSKXXX	(b)(4)	1.13
GSKXXX		1.24
GSKXXX		1.22
GSKXXX		1.09
GSKXXX		1.32
GSKXXX		
GSKXXX		
GSKXXX		

From: Cockrell, Adam
Sent: Thu, 8 Sep 2016 17:28:53 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Baric, Ralph; Leyva-Grado, Victor; Umerah, Nina
Subject: RE: GSK A57 Study
Attachments: MTR -UNC-Sept 8 2016.docx

Thanks Erik.

How should we proceed? I have attached the document with the completed list on the second page. We received 8 individual aliquots of the drug.

We currently have the drugs in hand and I planned to move forward with the experiment on Monday, 09-12-16. Do we proceed with experiments?...or do we need to wait for this to be signed?

Adam

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Thursday, September 08, 2016 1:21 PM
To: Cockrell, Adam (b)(6)
Cc: Baric, Ralph S (b)(6) Leyva-Grado, Victor (b)(6)
Umerah, Nina (b)(6)
Subject: RE: GSK A57 Study

Hi Adam,
OA says that it should be up to MSSM since they're your prime contractor.

Erik

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Thursday, September 08, 2016 12:30 PM
To: 'Cockrell, Adam' (b)(6)
Cc: Baric, Ralph (b)(6) Leyva-Grado, Victor (b)(6)
'Umerah, Nina' (b)(6)
Subject: RE: GSK A57 Study

Hi Adam,
Let me check with OA on this, as it's not usually something that happens with these studies. It might also have to go through MSSM since they're the prime contractor. I'll let you know what OA says.

Erik

From: Cockrell, Adam (b)(6)
Sent: Thursday, September 08, 2016 12:19 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)

Cc: Baric, Ralph (b)(6)
Subject: FW: GSK A57 Study

Hi Erik,

We were provided this MTA regarding the drugs we received from Feng at GSK. Just wanted to clear it with you first that we are responsible for signing and returning this to Feng.
Thanks,
Adam

From: Feng Wang (b)(6)
Sent: Thursday, September 08, 2016 10:52 AM
To: Cockrell, Adam (b)(6)
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study

Hi Adam/Yount,

We shipped another 3 bottles (~ 1mg per bottle) of the test compound to you yesterday. Additional vehicle (i.e. 0.5%Tween80) is also on the way. All together, you should have total 8 bottles of the test compound. Please fill in the actual compound weight and email me back the signed material transfer form as attached for the acknowledgment of compound receiving.
How is your formulation testing going? Acting cautiously, we will recommend freshly preparing the formulation for each dose administration as original discussed. Below is a brief reminder of the formulation procedure:

(1) Aliquot enough volume of vehicle in 5 replicates and store them at 4-8°C until use. Use one aliquot for each dose preparation.
(2) Wait until the compound bottle and the vehicle equilibrating to room temperature. Gently stir or mix the vehicle. Add the exact volume of vehicle to the bottle for a formulation concentration of 0.5mg/mL
(3) Sonicate or vertex or stir on a slightly warm plate (< 37°C) for a couple minutes until a clear solution is obtained
(4) Dose each mouse with a fixed 50µL of the above formulation. (please also record the mouse weight)

Thanks and look forward to the study!
feng

Feng Wang
Investigator
Host Defense DPU
RD Infectious Disease R&D

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(6)

Tel (b)(6)

gsk.com | Twitter | YouTube | Facebook | Flickr

From: Cockrell, Adam (b)(6)
Sent: Thursday, September 01, 2016 4:04 PM
To: Feng Wang; Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study

EXTERNAL

Hi Feng,

Thanks for the resuspension info.

I was previously informed that the drug is highly unstable, therefore I would have to resuspend the drug prior to every administration. There are five administrations therefore I would need all five bottles you send for the experiment.

That is why I requested a couple extra vials. Please let me know if I can use one vial for more than one administration.

Thanks,
Adam

From: Feng Wang (b)(6)
Sent: Thursday, September 01, 2016 3:30 PM
To: Cockrell, Adam (b)(6) Jeff Pouliot (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6)
Cc: Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study

Hi Adam,

Each bottle would provide more than enough formulations required for one day (BID) dosing. So, for the whole study, you only need three bottles. You could use the 4th bottle for your formulation test and the last bottle as a backup.
Here is the calculation:

To achieve a 1mg/kg IN dose with fixed 50uL dose volume, you need a dose solution of 0.5mg/mL assuming a typical mouse weight of 0.025g. So for one day BID dosing of 6 mice, you only need 0.3mg test compound.

To prepare a dose solution of 0.5mg/mL. You just need to take the weight information from the bottle and calculate the volume of 0.5%Tween 80 needed, and then add that exact volume of vehicle to the bottle. After a couple min sonication or mixing on a warm hotplate, a clear solution will be obtained.

Let me know if you have more questions. We still have time to ship more materials as needed.

Thanks,
feng

Feng Wang
Investigator
Host Defense DPU
RD Infectious Disease R&D

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(6)
Tel (b)(6)

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From: Cockrell, Adam (b)(6)
Sent: Thursday, September 01, 2016 3:02 PM
To: Feng Wang; Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study

EXTERNAL

Thanks Feng.

However, this does not include a sample for me to practice the resuspension of the drug prior to treatment. Can you provide at least one additional sample, and maybe an extra in the event something happens during resuspension?

Also, please provide exact instructions for resuspension with the vehicle that was sent previously.

Thanks,

Adam

From: Feng Wang (b)(6)
Sent: Thursday, September 01, 2016 2:55 PM
To: Cockrell, Adam (b)(6) Jeff Pouliot (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6)
Cc: Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study

Hi Adam/Boyd,

Just an update, the test compound (labeled as GSKXXX) in five replicates are shipped out today and should arrive at UNC tomorrow. Once received, please store them in 4-8°C. There should be ~1.2mg in each bottle.

Best wishes,
feng

Feng Wang
Investigator
Host Defense DPU
RD Infectious Disease R&D

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(6)
Tel (b)(6)

gsk.com | Twitter | YouTube | Facebook | Flickr

From: Cockrell, Adam (b)(6)
Sent: Tuesday, August 30, 2016 10:41 AM
To: Feng Wang; Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study

EXTERNAL

Hi Feng,

I received the vehicle this morning. However, the address on the package had it shipped to a lab in a different building in the pharmacy department. Fortunately, they were able to find our number and let us know.

Also, I stored it at 4C, but it was shipped at ambient temperature.

I will test the formulation late next week when I return.

For shipping of the test compound please use the following address:

Boyd Yount/Adam Cockrell
UNC-CH
135 Dauer Drive
Hooker Bldg./Room 3105
Chapel Hill, NC
27599
Phone (b)(6)

Best Regards,
Adam

From: Feng Wang (b)(6)
Sent: Tuesday, August 30, 2016 9:39 AM
To: Cockrell, Adam (b)(6) Jeff Pouliot (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6)
Cc: Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study

Hi Adam,

We shipped out study vehicle (i.e. 0.5%Tween80) yesterday and should arrive at your lab today. Please watch out and store it at 4-8°C. Due to some paper work delay, I do not think that the test compound will arrive before you leave for vacation. Is it possible that your coworker could do the formulation test in your absence? In addition, the test compound should also be stored at 4-8°C prior to use.

Thanks,
feng

Feng Wang
Investigator
Host Defense DPU

RD Infectious Disease R&D

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(6)
Tel (b)(6)

gsk.com | Twitter | YouTube | Facebook | Flickr

From: Cockrell, Adam (b)(6)
Sent: Monday, August 29, 2016 9:25 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study

EXTERNAL

Hi Jeff,

Contact numbers are (b)(6) (Adam) and (b)(6) (Boyd)

Thanks,

Adam

From: Jeff Pouliot (b)(6)
Sent: Friday, August 26, 2016 4:09 PM
To: Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6) Feng Wang (b)(6)
Cc: Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study

Hi Adam

Thank you very much. Can you supply a contact phone number for shipping?

We will send the 0.5% Tween in saline with our compound. Everything should arrive by midweek.

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Friday, August 26, 2016 10:54 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang; Barb Carter
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study

EXTERNAL

Hi Jeff,

Thanks for the update. I have addressed your questions below in red.

I will be out of town September 1st-september 7th, but Boyd Yount will be available to receive the package if I'm not here. Please advise on any special storage conditions.

Would it be possible for you ship a sample for early arrival next week, with all the components, so that I can test out the resuspension of the drug?

Also, I have attached a copy of the study as we discussed. As you suggested I eliminated the time point for drug delivery 6 hours prior to infection.

Best Regards,
Adam

From: Jeff Pouliot (b)(6)
Sent: Thursday, August 25, 2016 6:38 PM
To: Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6)
Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson
(b)(6) Feng Wang (b)(6) Barb Carter
(b)(6)
Subject: RE: GSK A57 Study

Dear Adam,

We'd like to update you on the status of the test compound shipping for the study and your formulation pre-work. We have the patent nearly completed and will be able to send the compound early next week,

targeting shipping for Tuesday 8/30 with arrival by the end of the week. Please let us know if this does not agree with your planned work schedule. We also have a few shipping questions to be certain everything goes smoothly:

- Can you advise on the planned start date for the in vivo study? If you need compound on the morning of September 6 we will try to send it earlier in the week to reduce the chance of shipping delays. I have reserved time in our BSL3 facility to initiate the experiment on Monday September 12th. Therefore, we would need to have the compound by Friday September 9th.

- Will your shipping group be receiving packages next Thurs-Fri (Sep 1-2)? If I am not here when the package arrives Boyd Yount in the lab will be available to receive the package. Please advise on any special storage conditions. I have included Boyd on this email.

- Could you please confirm the shipping address we should use for the test compound? Adam Cockrell/Boyd Yount, UNC-CH, 135 Dauer Dr., Chapel Hill, NC, 27599

- Do you have 0.5% Tween-80 in saline available for the formulation or should we plan to ship some? It would be simpler if you had some on hand as it necessitates a second package, but we're happy to arrange it if you prefer. I would prefer that the GSK group provides everything relevant to the drug.

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Sunday, August 14, 2016 10:48 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Subject: RE: GSK A57 Study

EXTERNAL

Thanks Jeff,

Sounds great!

Adam

From: Jeff Pouliot (b)(6)
Sent: Saturday, August 13, 2016 5:27 PM
To: Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6)

Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson
(b)(6) Feng Wang (b)(6)
Subject: RE: GSK A57 Study

Hi Adam,

We can send you a sample as soon as legal tells us the patent is filed. This should take roughly another week, so we should be able to get the sample to you by the end of two weeks. We will let you know if there are any unexpected delays.

Thanks for the info on dose groups. We can plan in more detail once the pilot run is complete.

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Saturday, August 13, 2016 7:43 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Subject: RE: GSK A57 Study

EXTERNAL

Thanks Jeff,

Would you guys mind sending me a sample of the drug (exactly how I will receive it for the mouse studies) in the next week, or two, so that I can validate the resuspension process in my hands?

If we see efficacy with the initial study, I believe 2-3 dose groups, with a 24 hour delivery window, would be feasible.

Thanks,
Adam

From: Jeff Pouliot (b)(6)
Sent: Thursday, August 11, 2016 3:45 PM
To: Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6)
Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson
(b)(6) Feng Wang (b)(6)
Subject: RE: GSK A57 Study

Dear Adam,

You should be able to formulate the compound either way. It should easily go into solution in 3-5 min with a 37C water bath. Otherwise, you can vortex and leave it on a heated plate (low setting, warm) with stirring for a couple minutes.

We suggested a 24h dosing schedule for the first study, but your counterproposal of BID dosing to have the greatest chance of efficacy was a good one. A 12-hour doing schedule for the initial study is fine.

For the follow-up study we can modify dosing to qd from 6-hours post infection, presuming the initial results are robust. We can plan this in more detail once the initial test is complete. To help us think it through, though, can you let us know if it is technically feasible to run 2-3 dose groups in parallel?

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Wednesday, August 10, 2016 6:39 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Subject: RE: GSK A57 Study

EXTERNAL

Dear Jeff,

Please see responses to comments/questions below.

Regards,
Adam

From: Jeff Pouliot (b)(6)
Sent: Tuesday, August 09, 2016 5:51 PM
To: Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6)
Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson
(b)(6) Feng Wang (b)(6)
Subject: RE: GSK A57 Study

Dear Adam,

Thanks for the note. Your research plan nicely reflects our discussion last week. We have some information below to fill in the details and a few questions for you.

- The predosing of compound is not needed as these are direct acting antivirals. In addition, only a suboptimal amount of compound would remain at the time of infection given the short T1/2 of this compound. A therapeutic model with the first dose following infection is our preferred choice. Is this acceptable? Starting with a therapeutic dose at 6 hours post-infection sounds great.
- BID dosing starting at 6 hours post infection seems the better plan. Do you know how long robust viral replication continues in an untreated test subject? Our model exhibits robust replication through day 6 post-infection with peak replication at days 2-3.
- We recommend intranasal dosing at 1 mg/kg, 50 uL volume per mouse, at a concentration of 0.5 mg/mL. This should deliver a compound concentration at Tmax of 100x EC50 to the lung. IN sounds good.
- We will plan to ship you the compound as dry powder. We're exploring stability but until we have firm data we can't guarantee that a solution prepared here would be stable long enough for the experiment. You will need to suspend by brief sonication in a dosing solution of 0.5% Tween-80 in saline. Is this acceptable? This is acceptable, however can you please define sonication? Is a water sonicator necessary for this? Or, will vortexing suffice? Does this compound readily go into solution? The 12 hour dosing schedule is quite rigorous, especially in a BSL3, therefore I am trying to get an understanding of how much additional time I will have to spend suspending the drug prior to each 12 hour administration.

We would like also to think ahead to the second round of the experiment. Presuming the outcome shows positive results, we propose a similar experiment at successive 3-fold lower drug concentrations to clarify the PK/PD relationship. If the follow up allows more than one dose group, we would dose at 0.3 mg/kg and 0.1 mg/kg (30x and 10x EC50). Does this sound reasonable to you? A dosing experiment sounds reasonable. Provided the initial study is successful, In follow-up experiments we discussed moving to a 6-7 day time course. In doing this I will have to move to delivering the drug every 24 hours. Is this reasonable to you? Would you prefer that the initial study use a 24 hour repeated dosing time course? The 24 hour time course would begin after the initial delivery of the drug at 6 hours post-infection.

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Wednesday, August 03, 2016 5:20 PM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Subject: RE: GSK A57 Study

EXTERNAL

Hi everyone. It was good to meet everyone in the gsk group.

In putting together the time line (attached to email) I had some additional thoughts.

1) There are two slides. The first is the initial time line that we discussed on the phone. The second slide takes into account the fact that the half-life of drug is really short, therefore we can adjust the drug delivery time line to bracket the initial viral delivery to be -6 hours and +6 hours if you guys would prefer. This would shorten the study on the back end by 6 hours, which should be of no consequence regarding the data we will capture.
2) This is just a thought, and not sure if this is a viable possibility given the half-life of the drug, but we could eliminate any confounding issues with repeated anesthetic administration if there was an option to deliver drug by the IP route. Thoughts?

That said I look forward to working with everyone.

Best Regards,
Adam

From: Jeff Pouliot (b)(6)
Sent: Wednesday, August 03, 2016 2:13 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6) Cockrell, Adam (b)(6) Feng Wang (b)(6)
Subject: RE: GSK A57 Study

Thank you all for the productive discussion. We look forward to working together.

I've added one person to the email list above. Please include Feng Wang on the experimental planning communications.

Best,

Jeff

Jeffrey Pouliot, Ph.D.
Investigator
Biology Host Defense DPU
R&D Infectious Disease

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(6)
Tel (b)(6)

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, August 03, 2016 1:59 PM
To: 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph; Deborah Butler; Neil Pearson; Jeff Pouliot; 'Cockrell, Adam'
Subject: GSK A57 Study

EXTERNAL

Hi Everyone,
Thanks for your time today, it was a productive call. Please feel free to email directly to work out the protocol/dosing and other study details, but be sure to CC everyone on this message.

Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email: (b)(6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

GSK monitors email communications sent to and from GSK in order to protect GSK, our employees, customers, suppliers and business partners, from cyber threats and loss of GSK Information. GSK monitoring is conducted with appropriate confidentiality controls and in accordance with local laws and after appropriate consultation.

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SCHEDULE C
MATERIAL TRANSFER RECORD

GlaxoSmithKline LLC
("Providing Party")

to
University of North Carolina at Chapel Hill
("Receiving Party")

The Material described below is supplied by the Providing Party to the Receiving Party subject to the terms and conditions of the Task Order/Statement of Work between **University of North Carolina at Chapel Hill via NIAD** ("Receiving Party") and **GlaxoSmithKline** ("Providing Party") dated 09/25/2012 ("Agreement"). Duplicate originals of this form shall be executed and one fully-executed form shall be given to the Providing Party and one to the Receiving Party.

Description of Material: See below

In signing below, the Study contacts acknowledge that they understand and will abide by the terms and conditions under which the Material is provided.

_____
(Signature) GlaxoSmithKline (Providing Party)

_____
Date Material Sent/Provided to Receiving Party

_____
(Signature) UNC at Chapel Hill (Receiving Party)

_____
Date Material Received by Receiving Party

Compound Number	MW	Weight (mg)
GSKXXX	(b)(4)	1.13
GSKXXX		1.24
GSKXXX		1.22
GSKXXX		1.09
GSKXXX		1.32
GSKXXX		0.97
GSKXXX		0.98
GSKXXX		0.94

From: Cockrell, Adam
Sent: Fri, 26 Aug 2016 14:53:31 +0000
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph; Deborah Butler; Neil Pearson; Feng Wang; Barb Carter
Cc: Yount, Boyd L Jr
Subject: RE: GSK A57 Study
Attachments: Time line for GSK study 082616.pdf

Hi Jeff,

Thanks for the update. I have addressed your questions below in red.

I will be out of town September 1st-september 7th, but Boyd Yount will be available to receive the package if I'm not here. Please advise on any special storage conditions.

Would it be possible for you ship a sample for early arrival next week, with all the components, so that I can test out the resuspension of the drug?

Also, I have attached a copy of the study as we discussed. As you suggested I eliminated the time point for drug delivery 6 hours prior to infection.

Best Regards,
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Subject: RE: GSK A57 Study

EXTERNAL

Thanks Jeff,

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Adam

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Subject: RE: GSK A57 Study

Dear Adam,

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Best,

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From: Cockrell, Adam (b)(6)
Sent: Wednesday, August 10, 2016 6:39 AM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Subject: RE: GSK A57 Study

EXTERNAL

Dear Jeff,

Please see responses to comments/questions below.

Regards,
Adam

From: Jeff Pouliot (b)(6)
Sent: Tuesday, August 09, 2016 5:51 PM
To: Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6) Feng Wang (b)(6)
Subject: RE: GSK A57 Study

Dear Adam,

Thanks for the note. Your research plan nicely reflects our discussion last week. We have some information below to fill in the details and a few questions for you.

- The predosing of compound is not needed as these are direct acting antivirals. In addition, only a suboptimal amount of compound would remain at the time of infection given the short T1/2 of this compound. A therapeutic model with the first dose following infection is our preferred

choice. Is this acceptable? Starting with a therapeutic dose at 6 hours post-infection sounds great.

- BID dosing starting at 6 hours post infection seems the better plan. Do you know how long robust viral replication continues in an untreated test subject? Our model exhibits robust replication through day 6 post-infection with peak replication at days 2-3.
- We recommend intranasal dosing at 1 mg/kg, 50 uL volume per mouse, at a concentration of 0.5 mg/mL. This should deliver a compound concentration at Tmax of 100x EC50 to the lung. IN sounds good.
- We will plan to ship you the compound as dry powder. We're exploring stability but until we have firm data we can't guarantee that a solution prepared here would be stable long enough for the experiment. You will need to suspend by brief sonication in a dosing solution of 0.5% Tween-80 in saline. Is this acceptable? This is acceptable, however can you please define sonication? Is a water sonicator necessary for this? Or, will vortexing suffice? Does this compound readily go into solution? The 12 hour dosing schedule is quite rigorous, especially in a BSL3, therefore I am trying to get an understanding of how much additional time I will have to spend suspending the drug prior to each 12 hour administration.

We would like also to think ahead to the second round of the experiment. Presuming the outcome shows positive results, we propose a similar experiment at successive 3-fold lower drug concentrations to clarify the PK/PD relationship. If the follow up allows more than one dose group, we would dose at 0.3 mg/kg and 0.1 mg/kg (30x and 10x EC50). Does this sound reasonable to you? A dosing experiment sounds reasonable. Provided the initial study is successful, In follow-up experiments we discussed moving to a 6-7 day time course. In doing this I will have to move to delivering the drug every 24 hours. Is this reasonable to you? Would you prefer that the initial study use a 24 hour repeated dosing time course? The 24 hour time course would begin after the initial delivery of the drug at 6 hours post-infection.

Best,

Jeff

From: Cockrell, Adam (b)(6)
Sent: Wednesday, August 03, 2016 5:20 PM
To: Jeff Pouliot; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph S; Deborah Butler; Neil Pearson; Feng Wang
Subject: RE: GSK A57 Study

EXTERNAL

Hi everyone. It was good to meet everyone in the gsk group.

In putting together the time line (attached to email) I had some additional thoughts.

1) There are two slides. The first is the initial time line that we discussed on the phone. The second slide takes into account the fact that the half-life of drug is really short, therefore we can adjust the drug delivery time line to bracket the initial viral delivery to be -6 hours and +6 hours if you

guys would prefer. This would shorten the study on the back end by 6 hours, which should be of no consequence regarding the data we will capture.

2) This is just a thought, and not sure if this is a viable possibility given the half-life of the drug, but we could eliminate any confounding issues with repeated anesthetic administration if there was an option to deliver drug by the IP route. Thoughts?

That said I look forward to working with everyone.

Best Regards,
Adam

From: Jeff Pouliot (b)(6)
Sent: Wednesday, August 03, 2016 2:13 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6) 'Leyva-Grado, Victor' (b)(6) (b)(6) 'Umerah, Nina' (b)(6) Baric, Ralph S (b)(6) Deborah Butler (b)(6) Neil Pearson (b)(6) Cockrell, Adam (b)(6) Feng Wang (b)(6)
Subject: RE: GSK A57 Study

Thank you all for the productive discussion. We look forward to working together.

I've added one person to the email list above. Please include Feng Wang on the experimental planning communications.

Best,

Jeff

Jeffrey Pouliot, Ph.D.
Investigator
Biology Host Defense DPU
R&D Infectious Disease

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(6)
Tel (b)(6)

gsk.com | Twitter | YouTube | Facebook | Flickr

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, August 03, 2016 1:59 PM
To: 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph; Deborah Butler; Neil Pearson; Jeff Pouliot; 'Cockrell, Adam'
Subject: GSK A57 Study

EXTERNAL

Hi Everyone,
Thanks for your time today, it was a productive call. Please feel free to email directly to work out the protocol/dosing and other study details, but be sure to CC everyone on this message.

Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email: (b)(6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.
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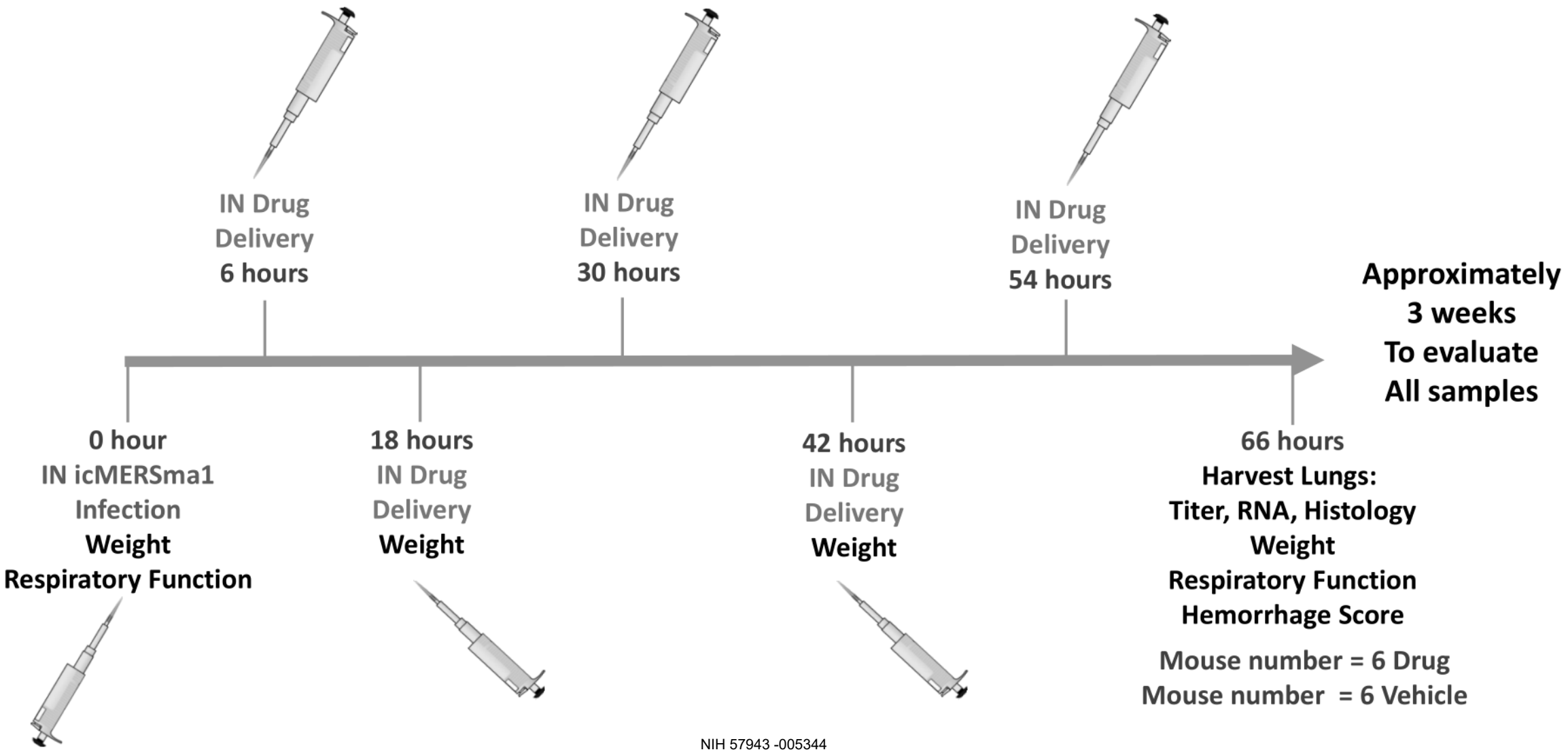
Drug Study with GSK (High Dose Study Delivered Every 12 hours)



From: Erlandson, Karl (OS/ASPR)
Sent: Tue, 27 Oct 2015 15:17:41 +0000
To: Stemmy, Erik (NIH/NIAID) [E]; Subbarao, Kanta (NIH/NIAID) [E]; Baric, Ralph; Munster, Vincent (NIH/NIAID) [E]; Dreier, Thomas (OS/ASPR/BARDA); Hensley, Lisa (NIH/NIAID) [E]; Spiro, David (NIH/FIC) [E]
Cc: 'Baric, Toni C'
Subject: RE: MERS Animal Model SAG
Attachments: ke-MERS Model Standardization Workshop Draft Sessions 10-2-2015.docx

Hi Erik,

I also think it would be good to talk this over. I've made a few comments that could be used in the discussion.

Karl

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Friday, October 23, 2015 8:15 AM
To: Subbarao, Kanta (NIH/NIAID) [E]; Baric, Ralph; Munster, Vincent (NIH/NIAID) [E]; Dreier, Thomas (OS/ASPR/BARDA); Erlandson, Karl (OS/ASPR); Hensley, Lisa (NIH/NIAID) [E]; Spiro, David (NIH/NIAID) [E]
Cc: 'Baric, Toni C'
Subject: RE: MERS Animal Model SAG

Hi Everyone,
I haven't received any comments back on the updated agenda. If it is easier for the group I can have a shot at suggesting organizers for the sessions and we can discuss the agenda by phone. I would appreciate it if you could either send me your feedback, or let me know if scheduling a phone call would be easier, by Tuesday 10/27.

Thanks!
Erik

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Tuesday, October 13, 2015 8:58 AM
To: Subbarao, Kanta (NIH/NIAID) [E] (b)(6) Baric, Ralph (b)(6) Munster, Vincent (NIH/NIAID) [E] (b)(6) Dreier, Thomas (OS/ASPR/BARDA) <Thomas.Dreier@hhs.gov>; Erlandson, Karl (OS/ASPR) <Karl.Erlandson@hhs.gov>; Hensley, Lisa (NIH/NIAID) [E] (b)(6) Spiro, David (NIH/NIAID) [E] (b)(6)
Cc: 'Baric, Toni C' (b)(6)
Subject: RE: MERS Animal Model SAG

Hi Everyone,
Just a friendly reminder soliciting your feedback on the updated agenda draft attached again here. Also, I have looked into availability of the large conference room in our Fishers Lane building and come up with some potentials dates (listed below.) Could you please let me know if there are any that should be

off the table due to conflicts, any that might be good to use to piggy back on other meetings, or any other preferences you have? I would ideally like to reserve the room in the next week.

Thanks!
Erik

Current Room Availability:
January: 18-19, 20-21
February: 10-11, 15-18, 22-23, 22-25, 29-March 1
March: 9-10, 28-31

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Friday, October 02, 2015 12:55 PM
To: Subbarao, Kanta (NIH/NIAID) [E] (b)(6) Baric, Ralph (b)(6) Munster, Vincent (NIH/NIAID) [E] (b)(6) Dreier, Thomas (OS/ASPR/BARDA) <Thomas.Dreier@hhs.gov>; Erlandson, Karl (OS/ASPR) <Karl.Erlandson@hhs.gov>; Hensley, Lisa (NIH/NIAID) [E] (b)(6) Spiro, David (NIH/NIAID) [E] (b)(6)
Cc: 'Baric, Toni C' (b)(6)
Subject: MERS Animal Model SAG

Hi Everyone,

Thank you for your insightful discussion during our call on the 21st. David and I have incorporated your comments in the attached document. In particular we have expanded the agenda to a rough outline of a two day workshop, and would appreciate any feedback you have on the proposed session organization and topics. One other thing we'd like to ask is for volunteers to choose a session to chair. We anticipate the session chairs will take the lead in setting the format for the session, suggesting speakers, and leading the session during the workshop.

If possible we'd like to ask for your feedback on this draft agenda and deliverables on or before Oct 14th. Please let me know If you have any questions.

Many thanks!
Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email: (b)(6)

****

Overarching themes and questions to be addressed by the workshop include:

1. What is the current status of the various models in development (e.g. mouse, rabbit, mink, Rhesus macaque, common marmosetNHP, camel)
    a. What are the biological similarities/differences, pros/cons between the models?
    b. Is there a better, or more appropriate, model for MCM development?
    c. Are different models needed for vaccine vs therapeutic development?
2. Is there a need for standardization of viral challenge, such as: viral strain; route of inoculation, etc?
3. What endpoints are currently possible/desirable that can be linked to human clinical signs/symptoms?
4. What are current gaps/hurdles in advancing model development?
5. What are potential regulatory pathways for promising MCM candidates?

Deliverables from the Workshop

1. Workshop summary/journal publication
2. Establish standardization guidelines for MERS reagents
3. Determine the availability of reagents (mice, viral isolates, etc) and outline a path forward for access to reagents (What/is there a role for USG?) in

DRAFT Workshop Sessions

Session 1: Virology and Epidemiology (15-20 minute lectures)

A. Global status, including case study of Korean outbreak
B. Pathology update of human cases from KSA and/or ROK
C. Viral update, including differences between KSA vs ROK cases

Break

Session 2: Model Summary (20-25 minute lectures on each model; in depth science)

A. Large NHP animal models (NHP, camel)
B. Camelids (camel, alpaca)
C. Small animal models (mice, rabbit, mink?)
D. Experience with vaccines in MERS-CoV animal models
E. Experience with small molecule or biologics in MERS-CoV animal models

Break

Session 3: Panel Discussion of Models (facilitated with questions for discussion)

A. Differences/Similarities
B. Pros/Cons
C. Characteristics (challenge strain, route of inoculation, etc)
D. Need for cross-model comparison, reproducibility.
E. Use of models: MCM development vs studying viral pathology
F. Other potential models deterimined by bioinformatics, etc?
G. Potential of transmission model?

End of day 1

Recap of Session 3 Discussion (10-15 minutes)

Session 4: Lessons Learned (15-20 minute presentations)

A. Lessons Learned from SARS model development
B. Lessons Learned from SARS MCM development
C. BARDA experience licensing products under animal model

Commented [KE1]: It would be good to have a talk on the latent/relapse patient who recently died in ROK. This is an especially important issue in the Ebola field at the moment and may be an emerging issue for MERS-CoV (do we have to look for MERS-CoV in immune privileged sites?).

Commented [KE2]: These should be separated.

Commented [KE3]: We should clearly separate MCM topics so the first section focuses on the models themselves. It may be better to set aside MCM/animal talks on day 2. We could invite a few companies to discuss their development pathway/animal model experience (maybe 10-15 minute talks?)

Commented [KE4]: We can talk about this, but Animal Rule will not likely be needed for MERS-CoV as there are prevalent human cases recurring on a seasonal basis.

Break

Session 5: MCM Development (mix of short presentation/discussion)

A. Regulatory Questions:
    a. How to use models to advance MCMs to clinical trial
    b. What data are required (tox, efficacy, etc)
B. Public health preparedness and need for Human vs animal MCMs
C. Potential/need for broad activity against other CoVs
D. Desired vs available clinical endpoints/outcomes to advance development (e.g. Viral load reduction, weight loss, Standard lung pathology score, Mortality, Respiratory function)

Break

Summary/Path Forward

A. Gaps/hurdles
B. Goals/milestones necessary in models to advance MCM development

From: Cockrell, Adam
Sent: Tue, 12 Apr 2016 13:52:48 +0000
To: Stu Greenberg; Stemmy, Erik (NIH/NIAID) [E]
Cc: Baric, Ralph
Subject: RE: Progress
Attachments: OstriGen Summary.pdf

Hi Stu,

So far the accumulated data indicates that the anti-MERS IgY antibody does not confer protection from severe respiratory disease induced by MERS-CoV in our model, when delivered prophylactically at 12 hours prior to infection. See attachment.

Titer data will be ready in the next 1.5 weeks.

Processing of tissues for IHC and H&E will take another 2-3 weeks once the samples have been submitted for processing. However, based on our experience, we anticipate that the pathology will substantiate the observed disease assessed by the parameters in the summary.

Best Regards,

Adam

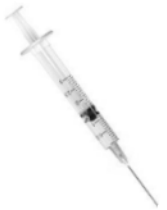
From: Stu Greenberg (b)(6)
Sent: Monday, April 11, 2016 11:22 AM
To: Cockrell, Adam (b)(6)
Subject: Progress

Hi Adam,

Can you give me a quick summary on how the mouse study is going? I would like to update my Japanese colleagues.

Regards,
Stu Greenberg
(b)(6)

Prophylactic Study at 500µg dose of antibodies delivered IP
(Start Date/Finish Date Will Be Determined When We have validation of Shipping)



IP Ab Delivery -12 hours

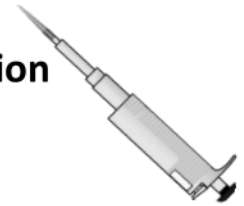
Weight Day 1

Mouse number = 6 MERS-CoV Ostrich Therapeutic
Mouse number = 6 Isotype Ctrl. Ostrich
Harvest Lungs:
Titer, RNA, Histology
Weight
Respiratory Function
Hemorrhage Score
Day 3

Weight Day 5

Approximately 1-2 months To evaluate All samples

0 Day IN MERS-15 Clone 2 Infection
Weight
Respiratory Function

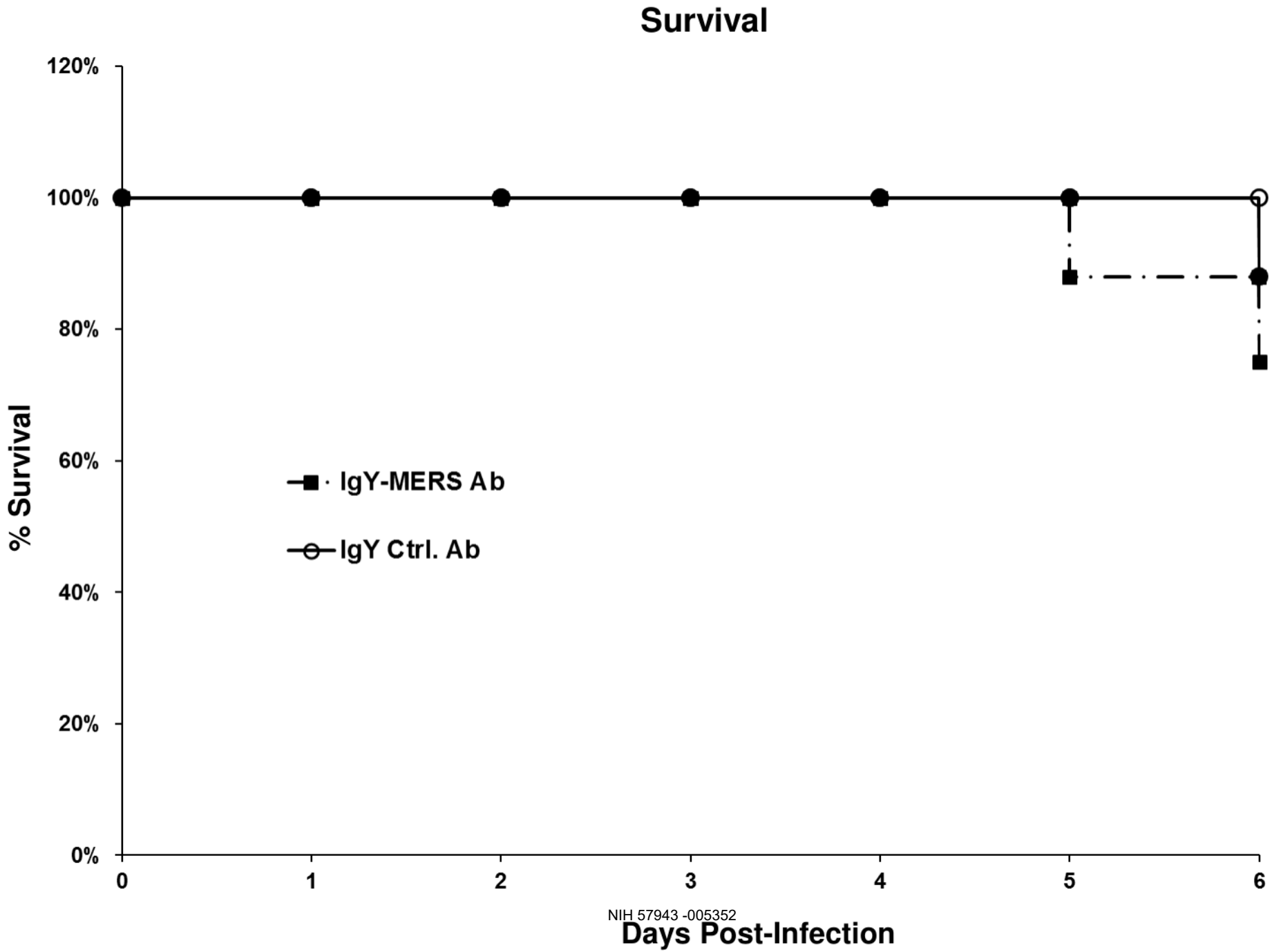


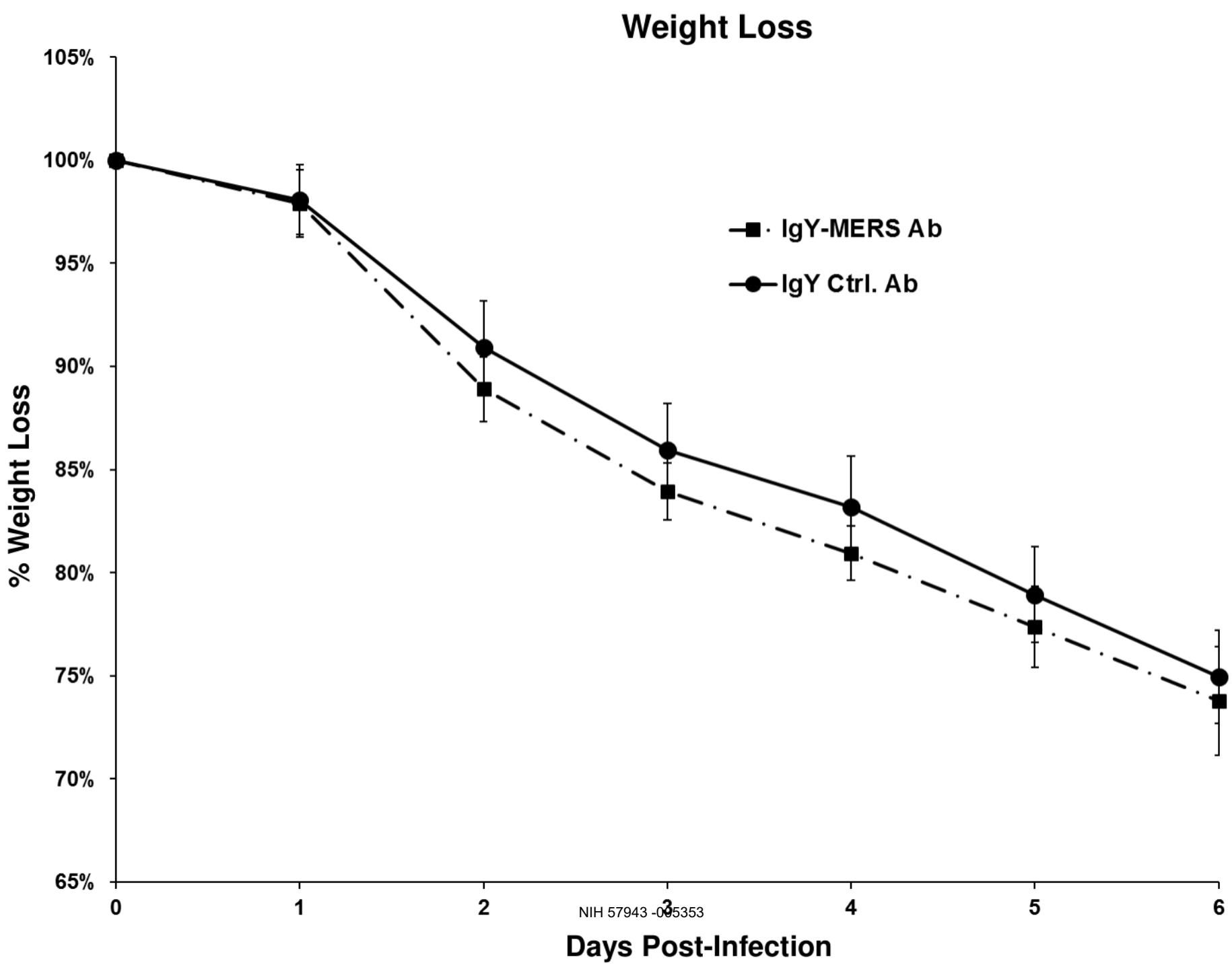
Day 2 Weight

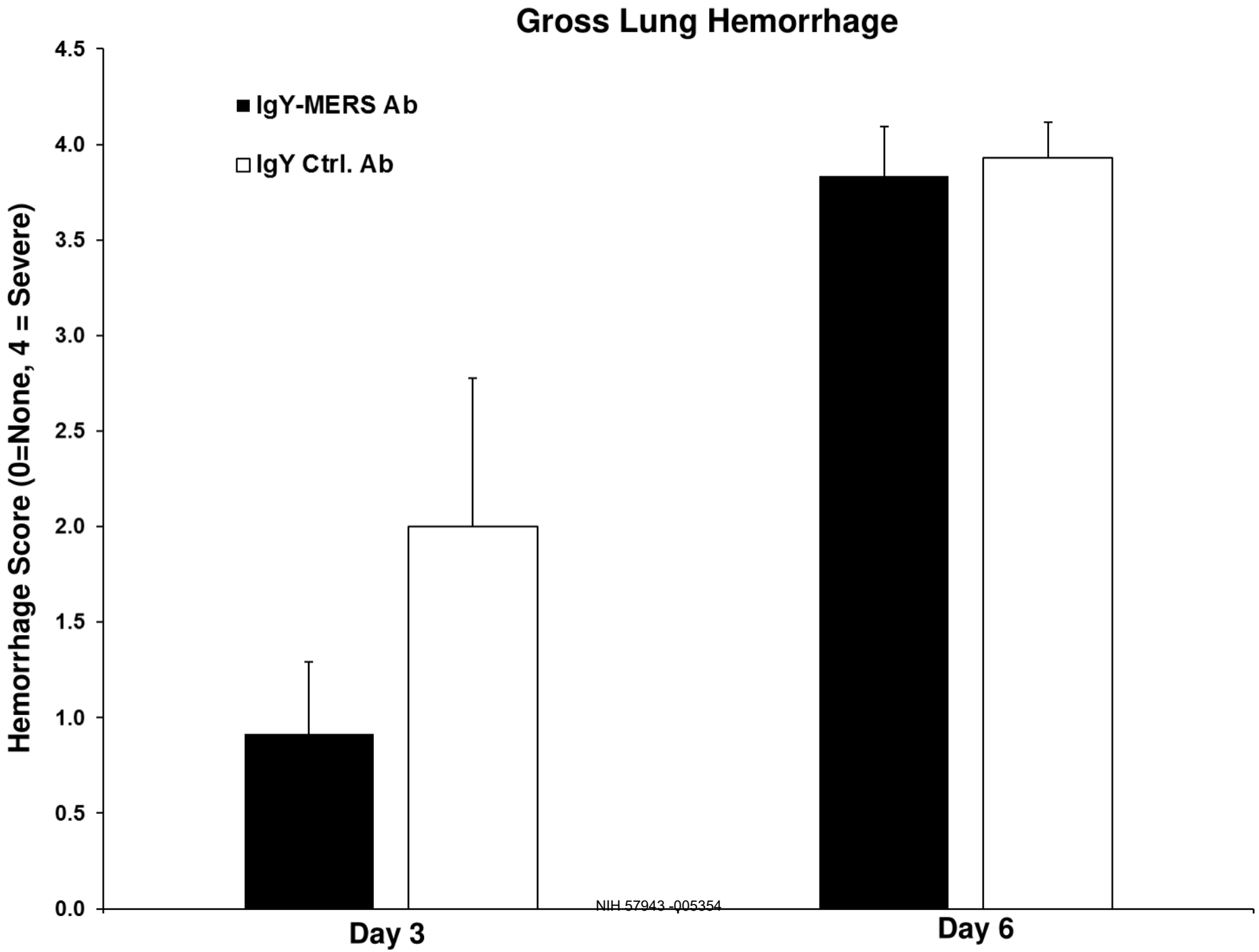
Day 4 Weight

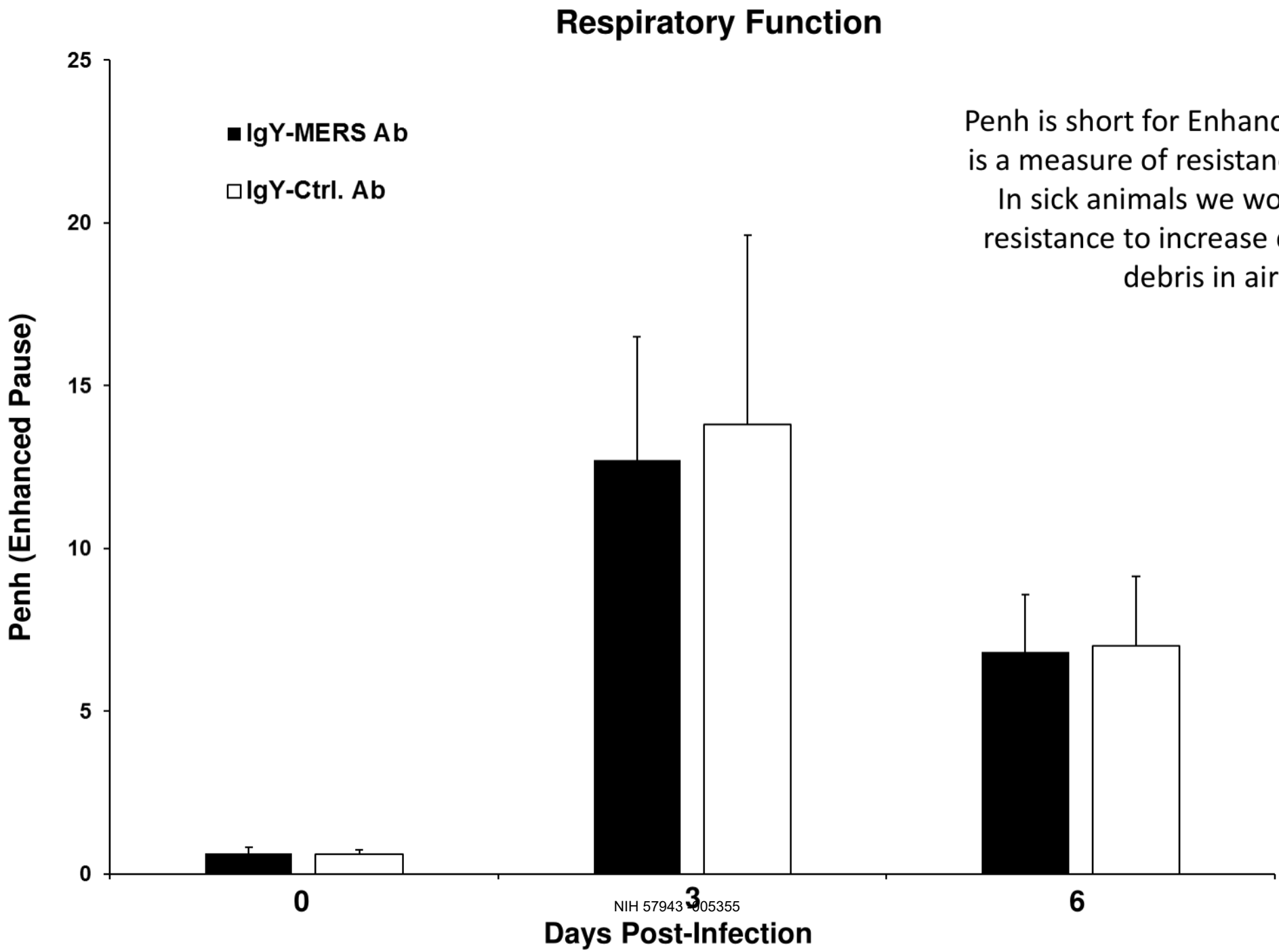
Day 6
Harvest Lungs:
Titer, RNA, Histology
Weight
Respiratory Function
Hemorrhage Score

Mouse number = 8 MERS-CoV Ostrich Therapeutic
Mouse number = 8 Isotype Ctrl. Ostrich

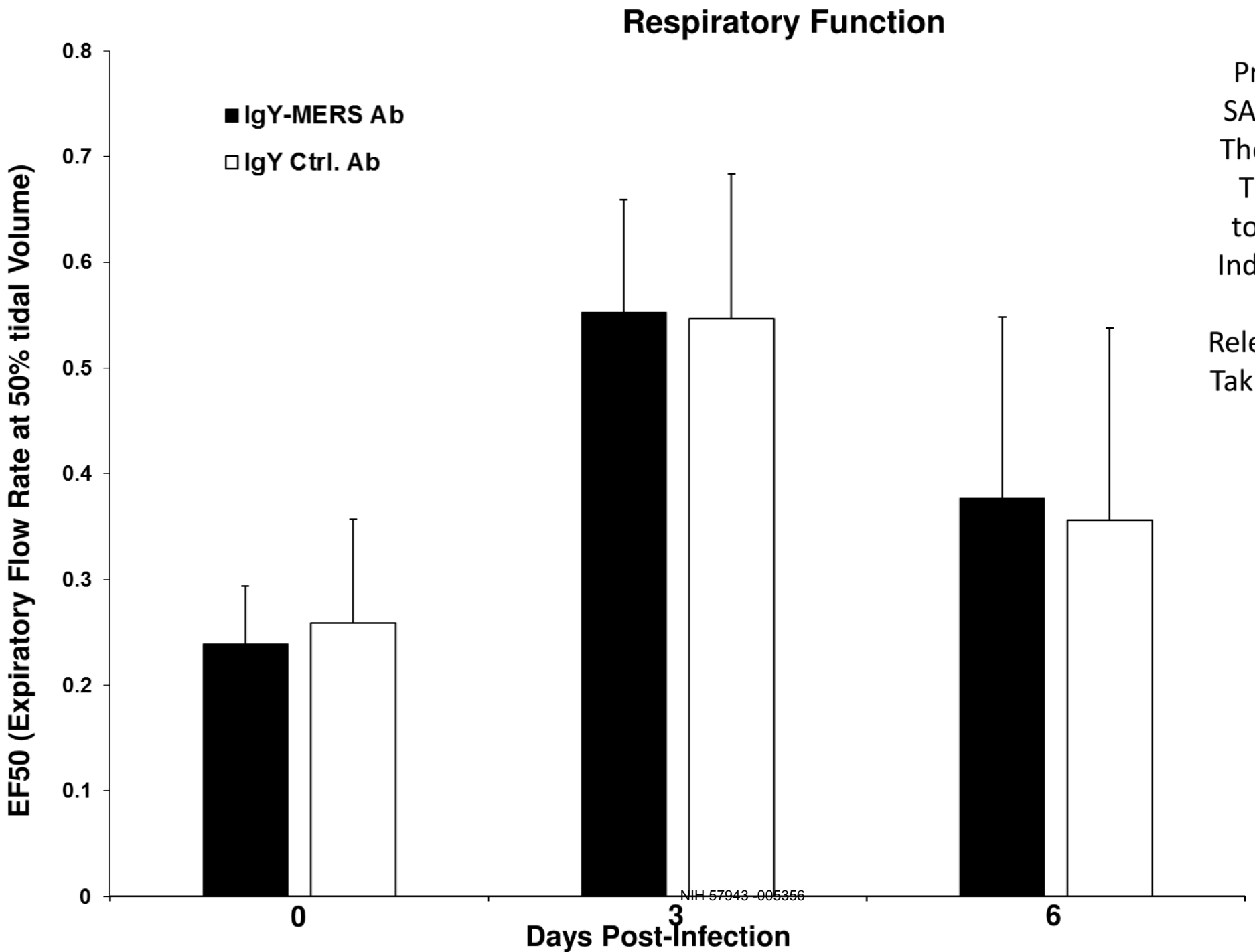

Survival
120%
100%
80%
60%
40%
20%
0%
% Survival
IgY-MERS Ab
IgY Ctrl. Ab
0
1
2
3
4
5
6
Days Post-Infection


Weight Loss
105%
100%
95%
90%
85%
80%
75%
70%
65%
% Weight Loss
IgY-MERS Ab
IgY Ctrl. Ab
0
1
2
3
4
5
6
Days Post-Infection


Gross Lung Hemorrhage
IgY-MERS Ab
IgY Ctrl. Ab
Hemorrhage Score (0=None, 4 = Severe)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Day 3
Day 6
NIH 57943 -005354



Penh is short for Enhanced Pause, which is a measure of resistance in the airway. In sick animals we would anticipate resistance to increase due to possible debris in airway.



Similar to what was Previously observed with SARS infected mice, where They appear to be exhaling The breath more rapidly to 50% volume. This is an Indication that the majority Of breadth is rapidly Released with the remainder Taking a long time similar to A wheeze in humans.

From: Cockrell, Adam
Sent: Fri, 21 Apr 2017 21:16:01 +0000
To: Keith Wycoff
Cc: Stemmy, Erik (NIH/NIAID) [E]; Baric, Ralph
Subject: RE: Study with Planet Biotech.
Attachments: Report for Planet Biotech.pdf

Hi Keith,

I attached the report again here. I agree with you it is difficult to know if the treated mice would have kept losing weight, or not.

Best,
Adam

From: Keith Wycoff (b)(6)
Sent: Friday, April 21, 2017 3:11 PM
To: Cockrell, Adam (b)(6)
Subject: Re: Study with Planet Biotech.

Hi Adam,

Thank you for sending me the report on your results. This seems pretty promising, though it leads me to wonder what might have happened after 6 days. Somehow I have lost the email you sent. Could you please send it again?

Thanks,
Keith

On Feb 28, 2017, at 7:41 PM, Cockrell, Adam (b)(6) wrote:

Hi Keith.

Yes. Homozygous mice develop severe disease and death at high virus dose ($5x10^6$ PFU). Whereas, the heterozygous mice still get sick (weight loss and hemorrhaging), but do not die. MERS replicates to higher titers in homozygous mice, which is most likely due to availability of more receptor. Our current evidence indicates less severe disease in heterozygous mice. This comparison is in figure 2 of the Nature Micro manuscript.

We will only be using homozygous mice for the study with your soluble DPP4 protein.

Best,
Adam

From: Keith Wycoff (b)(6)
Sent: Tuesday, February 28, 2017 9:59 PM
To: Cockrell, Adam (b)(6)
Subject: Re: Study with Planet Biotech.

Hi Adam,

A quick question for you. Does it make any difference whether your mice are homozygous or hemizygous for the human DPP4 transgene?

Thanks,
Keith

On Feb 24, 2017, at 12:27 PM, Cockrell, Adam (b)(6) wrote:

Probably not until late March.

Best,
Adam

From: Keith Wycoff (b)(6)
Sent: Friday, February 24, 2017 3:25 PM
To: Cockrell, Adam
Subject: Re: Study with Planet Biotech.

Hi Adam,

That's great. Let me know if any problems crop up. At what point do you expect to have interim/preliminary efficacy data that you can share with us?

Thanks,
Keith

On Feb 24, 2017, at 12:08 PM, Cockrell, Adam (b)(6) wrote:

Hi Keith,

We received the package.

Best,
Adam

From: Keith Wycoff (b)(6)
Sent: Thursday, February 23, 2017 5:22 PM
To: Cockrell, Adam (b)(6)
Subject: Re: Study with Planet Biotech.

Hi Adam,

I called FedEx and they claim it was delayed because of flooding in San Jose. That doesn't square with the fact that it's been in Raleigh, NC since 5:36 this morning. In any case, they told me it will be delivered first thing tomorrow morning.

Keith

On Feb 23, 2017, at 2:12 PM, Cockrell, Adam (b)(6) wrote:

Hi Keith,

No sign of FedEx today.

Adam

From: Keith Wycoff (b)(6)
Sent: Thursday, February 23, 2017 1:33 PM
To: Cockrell, Adam (b)(6)
Subject: Re: Study with Planet Biotech.

We sent it frozen, so I recommend storage at -80C until ready to use. My suggestion is to put the tubes you'll need in the fridge the day before needed. Once thawed you can keep it at 4C. It has been sterile filtered.

Keith

On Feb 23, 2017, at 10:27 AM, Cockrell, Adam (b)(6) wrote:

Thanks Keith,

Is storage at 4C?

Adam

From: Keith Wycoff (b)(6)
Sent: Thursday, February 23, 2017 1:22 PM
To: Cockrell, Adam (b)(6)
Subject: Re: Study with Planet Biotech.

Hi Adam,

We decided to send twice the amount of protein promised, just in case of a spill or if you need to repeat the experiment. Should arrive by 3:00 today. The FedEx tracking number is 7784 9010 2170.

Keith

On Feb 20, 2017, at 7:36 AM, Cockrell, Adam (b)(6) wrote:

Thanks Keith,

That sounds great.

Adam

From: Keith Wycoff (b)(6)
Sent: Monday, February 20, 2017 10:18 AM
To: Cockrell, Adam (b)(6)
Cc: Erik [E] Stemmy (b)(6) Leyva-Grado, Victor (b)(6)
Baric, Ralph S (b)(6)
Subject: Re: Study with Planet Biotech.

OK, so you will need a minimum of 12 mg of protein at 2 mg/ml. To account for potential losses of volume, I will send 15 mg. I will also send 7.5 ml of control (PBS). Does that sound good?

Thanks,
Keith

On Feb 20, 2017, at 7:12 AM, Cockrell, Adam (b)(6) wrote:

Hi Keith,

You are correct. We are definitely going with 400ug/mouse. I just did not change it on the outline. Corrected outline is attached.

Thanks,
Adam

From: Keith Wycoff (b)(6)
Sent: Monday, February 20, 2017 10:09 AM
To: Cockrell, Adam (b)(6)
Cc: Erik [E] Stemmy (b)(6) Leyva-Grado, Victor (b)(6)
Baric, Ralph S (b)(6)
Subject: Re: Study with Planet Biotech.

Hi Adam,

I just want to make sure I understand the doses, and thus how much drug we need to supply. Our original discussion contemplated administering an amount, on a molar basis, equivalent to antibodies you have tested before. I had understood that 250 µg of antibody had been administered, and due to the greater molar mass of our protein the equivalent amount of DPP4-Fc would be 380 µg, which you suggested rounding up to 400 µg. Did you intend to divide the 400 µg into two doses (of 200 µg each) or administer two doses of 400 µg each? In any case, the numbers differ from the two 250 µg doses on the study design you sent. Please confirm how much protein you want to administer at -12 and +12 hours. Also, please confirm that you wanted the concentration to be 2 mg/ml (if that is the case).

Thanks,
Keith

On Feb 20, 2017, at 6:40 AM, Cockrell, Adam (b)(6) wrote:

Hi everyone,

We have approval to begin the study with Planet Biotech. I would like to schedule this to begin on Friday March 10. I have included the study time line in this email just as a reminder. Also, I bumped the mouse numbers for the Day 6 time point to 20 (10 for each of the therapeutic and control). Want to make sure that we have enough mice by day 6.

Keith: The address to send S2320-Gal-SF, and control, to is as follows:

Attn: Adam Cockrell
University of North Carolina at Chapel Hill
Department of Epidemiology/#4635
135 Dauer Dr.
Room 3105 MHRC
Chapel Hill, NC
27599

Phone number is below.

Adam Cockrell
Research Associate
Department of Epidemiology
University of North Carolina at Chapel Hill
Chapel Hill, NC, 27599
Lab Phone: (b)(6)
Office Phone: (b)(6)

<Timeline for initial study.pdf>

<Timeline for initial study.pdf>

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Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 452 of 455

Withheld pursuant to exemption

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Page 453 of 455

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Page 454 of 455

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From: Munster, Vincent (NIH/NIAID) [E]
Sent: Mon, 9 Nov 2015 17:37:58 -0500
To: Stemmy, Erik (NIH/NIAID) [E]; Hensley, Lisa (NIH/NIAID) [E]; Erlandson, Karl (OS/ASPR); Munster, Vincent (NIH/NIAID) [E]; Subbarao, Kanta (NIH/NIAID) [E]; Baric, Ralph; Dreier, Thomas (OS/ASPR/BARDA); Spiro, David (NIH/NIAID) [E]
Subject: Re: MERS Animal Model SAG

Hi Erik,

For the Alpaca/Camels I would ask Richard Bowen from the Colorado State University (b)(6) I'm doing camels/Alpaca experiments with him,

Cheers,

Vincent Munster
Chief Virus Ecology Unit
Rocky Mountain Laboratories, NIAID/NIH
903 South 4th street
Hamilton, MT, 59840, USA
(b)(6)

From: "Stemmy, Erik (NIH/NIAID) [E]" (b)(6)
Date: Monday, November 9, 2015 at 8:32 AM
To: "Hensley, Lisa (NIH/NIAID) [E]" (b)(6) "Erlandson, Karl (OS/ASPR)" (b)(6) "Munster, Vincent (NIH/NIAID) [E]" (b)(6) "Subbarao, Kanta (NIH/NIAID) [E]" (b)(6) "Baric, Ralph" (b)(6) "Dreier, Thomas (OS/ASPR/BARDA)" (b)(6) David Spiro (b)(6)
Subject: RE: MERS Animal Model SAG

Hi Everyone,
Thank you again for your time last week. I have attached an updated version of the agenda that I think incorporates the discussion and speaker suggestions. I would appreciate it if you could please review and send me any updates by Monday Nov 16th. In particular there are a few highlighted areas that we still need to identify a potential speaker.

Please let me know if you have any questions.
Erik

From: Hensley, Lisa (NIH/NIAID) [E]
Sent: Monday, November 2, 2015 9:30 AM
To: Erlandson, Karl (OS/ASPR) (b)(6) Munster, Vincent (NIH/NIAID) [E] (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) Subbarao, Kanta (NIH/NIAID) [E] (b)(6) Baric, Ralph (b)(6) Dreier, Thomas (OS/ASPR/BARDA) (b)(6) Spiro, David (NIH/NIAID) [E] (b)(6)
Cc: (b)(6)
Subject: Re: MERS Animal Model SAG

Waiting for the leader to admit
Lisa Hensley

Sent from b berry

From: Erlandson, Karl (OS/ASPR)
Sent: Monday, November 02, 2015 09:21 AM
To: Munster, Vincent (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Subbarao, Kanta (NIH/NIAID) [E]; Baric, Ralph; Munster, Vincent (NIH/NIAID) [E]; Dreier, Thomas (OS/ASPR/BARDA); Hensley, Lisa (NIH/NIAID) [E]; Spiro, David (NIH/NIAID) [E]
Cc: 'Baric, Toni C' (b)(6)
Subject: RE: MERS Animal Model SAG

(301) 761-5000 (NIAID)
Code: (b)(6)

From: Munster, Vincent (NIH/NIAID) [E] (b)(6)
Sent: Monday, November 02, 2015 9:11 AM
To: Stemmy, Erik (NIH/NIAID) [E]; Erlandson, Karl (OS/ASPR); Subbarao, Kanta (NIH/NIAID) [E]; Baric, Ralph; Munster, Vincent (NIH/NIAID) [E]; Dreier, Thomas (OS/ASPR/BARDA); Hensley, Lisa (NIH/NIAID) [E]; Spiro, David (NIH/NIAID) [E]
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Trying to get onto the call?

Any ideas?

From: "Stemmy, Erik (NIH/NIAID) [E]" (b)(6)
Date: Wednesday, October 28, 2015 at 12:01 PM
To: "Erlandson, Karl (OS/ASPR)" (b)(6) "Subbarao, Kanta (NIH/NIAID) [E]" (b)(6) "Baric, Ralph" (b)(6) "Munster, Vincent (NIH/NIAID) [E]" (b)(6) "Dreier, Thomas (OS/ASPR/BARDA)" (b)(6) "Hensley, Lisa (NIH/NIAID) [E]" (b)(6) David Spiro (b)(6)
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Thanks!
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http://doodle.com/poll/ (b)(6)

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I also think it would be good to talk this over. I've made a few comments that could be used in the discussion.

Karl

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To: Subbarao, Kanta (NIH/NIAID) [E] (b)(6) Baric, Ralph (b)(6) Munster, Vincent (NIH/NIAID) [E] (b)(6) Dreier, Thomas (OS/ASPR/BARDA) (b)(6) Erlandson, Karl (OS/ASPR) (b)(6) Hensley, Lisa (NIH/NIAID) [E] (b)(6) Spiro, David (NIH/NIAID) [E] (b)(6)
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If possible we'd like to ask for your feedback on this draft agenda and deliverables on or before Oct 14th. Please let me know If you have any questions.

Many thanks!
Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email:

****

From: Hensley, Lisa (NIH/NIAID) [E]
Sent: Mon, 2 Nov 2015 09:29:55 -0500
To: Erlandson, Karl (OS/ASPR); Munster, Vincent (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Subbarao, Kanta (NIH/NIAID) [E]; Baric, Ralph; Dreier, Thomas (OS/ASPR/BARDA); Spiro, David (NIH/NIAID) [E]
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All

Thanks

I still can't get on
Lisa Hensley

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Erik

Erik J. Stemmy, Ph.D.

Program Officer
Respiratory Diseases Branch
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5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
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Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

****

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Fri, 30 Oct 2015 16:45:30 +0000
To: Stemmy, Erik (NIH/NIAID) [E]; Subbarao, Kanta (NIH/NIAID) [E]; Baric, Ralph; Munster, Vincent (NIH/NIAID) [E]; Dreier, Thomas (OS/ASPR/BARDA); Erlandson, Karl (OS/ASPR); Hensley, Lisa (NIH/NIAID) [E]; Spiro, David (NIH/NIAID) [E]; 'Baric, Toni C'
Subject: MERS Model Workshop Call
Attachments: MERS Model Standardization Workshop Draft Sessions 10-30-2015.docx

Hi Everyone,
This seemed to be the best time for most folks, so I've arrange the Lync call. Please use the link and/or dial in below to join on Monday. Brief agenda below, as well as a sessions draft with comments included.

Erik

Call Agenda:

1. Dates for meeting (tentatively reserved Feb 29th-March 1st)
2. Volunteers to chair individual sessions
3. Discussion of sessions

.....

→

Join Lync Meeting

Join by phone
(b)(6) (NIAID) English (United States)
Find a local number

Conference ID: (b)(6)

Forgot your dial-in PIN? | Help

[!OC([1033])!]

.....

Overarching themes and questions to be addressed by the workshop include:

1. What is the current status of the various models in development (e.g. mouse, rabbit, mink, Rhesus macaque, common marmosetNHP, camel)
    a. What are the biological similarities/differences, pros/cons between the models?
    b. Is there a better, or more appropriate, model for MCM development?
    c. Are different models needed for vaccine vs therapeutic development?
2. Is there a need for standardization of viral challenge, such as: viral strain; route of inoculation, etc?
3. What endpoints are currently possible/desirable that can be linked to human clinical signs/symptoms?
4. What are current gaps/hurdles in advancing model development?
5. What are potential regulatory pathways for promising MCM candidates?

Deliverables from the Workshop

1. Workshop summary/journal publication
2. Establish standardization guidelines for MERS reagents
3. Determine the availability of reagents (mice, viral isolates, etc) and outline a path forward for access to reagents (What/is there a role for USG?) in

DRAFT Workshop Sessions

Session 1: Virology and Epidemiology (15-20 minute lectures)

A. Global status, including case study of Korean outbreak
B. Pathology update of human cases from KSA and/or ROK
C. Viral update, including differences between KSA vs ROK cases

Break

Session 2: Model Summary (20-25 minute lectures on each model; in depth science)

A. Large NHP animal models (NHP, camel)
B. Camelids (camel, alpaca)
C. Small animal models (mice, rabbit, mink?)
D. Experience with vaccines in MERS-CoV animal models
E. Experience with small molecule or biologics in MERS-CoV animal models

Break

Session 3: Panel Discussion of Models (facilitated with questions for discussion)

A. Differences/Similarities
B. Pros/Cons
C. Characteristics (challenge strain, route of inoculation, etc)
D. Need for cross-model comparison, reproducibility.
E. Use of models: MCM development vs studying viral pathology
F. Other potential models deterimined by bioinformatics, etc?
G. Potential of transmission model?

End of day 1

Recap of Session 3 Discussion (10-15 minutes)

Session 4: Lessons Learned (15-20 minute presentations)

A. Lessons Learned from SARS model development
B. Lessons Learned from SARS MCM development
C. BARDA experience licensing products under animal model

Commented [KE1]: It would be good to have a talk on the latent/relapse patient who recently died in ROK. This is an especially important issue in the Ebola field at the moment and may be an emerging issue for MERS-CoV (do we have to look for MERS-CoV in immune privileged sites?).

Commented [KE2]: These should be separated.

Commented [KE3]: We should clearly separate MCM topics so the first section focuses on the models themselves. It may be better to set aside MCM/animal talks on day 2. We could invite a few companies to discuss their development pathway/animal model experience (maybe 10-15 minute talks?)

Commented [KE4]: We can talk about this, but Animal Rule will not likely be needed for MERS-CoV as there are prevalent human cases recurring on a seasonal basis.

Break

Session 5: MCM Development (mix of short presentation/discussion)

A. Regulatory Questions:
    a. How to use models to advance MCMs to clinical trial
    b. What data are required (tox, efficacy, etc)
B. Public health preparedness and need for Human vs animal MCMs
C. Potential/need for broad activity against other CoVs
D. Desired vs available clinical endpoints/outcomes to advance development (e.g. Viral load reduction, weight loss, Standard lung pathology score, Mortality, Respiratory function)

Break

Summary/Path Forward

A. Gaps/hurdles
B. Goals/milestones necessary in models to advance MCM development

From: Aviles, Natalie (OS/ASPR/BARDA) (CTR)
Sent: Tue, 7 Jul 2015 20:07:46 +0000
To: Swerdlow, David (CDC/OID/NCIRD); Gerber, Sue (CDC/CGH/DGHA); Haynes, Lia (CDC/OID/NCIRD); Erdman, Dean (CDC/OID/NCIRD); Pallansch, Mark A. (CDC/OID/NCIRD); (b)(6) (b)(6) (b)(6) Chaitram, Jasmine (CDC/OID/NCEZID); Carter, Wendy (FDA/CDER); Bavari, Sina; Miele, Peter (FDA/CDER); O'Rear, Julian (FDA/CDER); Deming, Damon (FDA/CDER); Merlin, Toby (CDC/OID/NCEZID); Feldmann, Heinrich (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Marinissen, Maria (OS/ASPR/OPP); Annelli, Joseph; DiEuliis, Diane (OS/ASPR/OPP); Robinson, Robin (OS/ASPR/BARDA); Maher, Carmen (FDA/OC); Hensley, Lisa (NIH/NIAID) [E]; Kelley, Cynthia (FDA/CBER); Spiro, David (NIH/NIAID) [E]; Fisher, Robert (FDA/OC); Hojvat, Sally A (FDA/CDRH); Beigel, John (NIH) [C]; Murray, Jeffrey S (FDA/CDER); Olinger, Gene (NIH/NIAID) [C]; (b)(6) (b)(6) (b)(6) ; Baric, Ralph; Denison, Mark (NIH); (b)(6) (b)(6) (b)(6) (b)(6) Thomas, Stephen J; Cho, David S (CBER) (FDA/CBER); (b)(6) 'Roberts, Rosemary (FDA/CDER)'; (b)(6) (b)(6) Roberts, Rosemary (FDA/CDER); Gerber, Susan I. (CDC/OID/NCIRD); (b)(6) (b)(6) (b)(6) Zoon, Kathryn (NIH/NIAID) [E]; Bowen, Richard; (b)(6) Subbarao, Kanta (NIH/NIAID) [E]; (b)(6) (b)(6) Ferro, Philip (OS/ASPR/IO); Wathen, Lynne (OS/ASPR/BARDA); (b)(6) (b)(6) (b)(6) 'Gay, Cyril'; Sutton, Troy (NIH/NIAID) [F]; Houser, Katherine (NIH/NIAID) [F]; Czako, Rita (NIH/NIAID) [F]; Gretebeck, Lisa (NIH/CC/OD) [F]; Vogel, Leatrice (NIH/NIAID) [E]; Lamirande, Elaine (NIH/NIAID) [E]; (b)(6) 'Elliott Fineman'; 'Olinger, Gene G'; 'Dan Adams'; Erlandson, Karl (OS/ASPR); 'Baric, Toni C'; 'Sven Andreasson'; 'Jensen, Victoria M. (CTR)'; 'Zhu, Quan'; 'Tang, Xianchun,Phd'; (b)(6) 'Cheryl Kofford'; Ohara, Michael (OS/ASPR/BARDA); Sciarretta, Kimberly (OS/ASPR/BARDA)
Cc: Lurie, Nicole (OS/ASPR/IO); Korch, George (OS/ASPR/IO); Donabedian, Armen (OS/ASPR/BARDA); Bright, Rick (OS/ASPR/BARDA); Uyeki, Timothy M. (CDC/OID/NCIRD); Spiro, David (NIH/NIAID) [E]; Ohara, Michael (OS/ASPR/BARDA); Erlandson, Karl (OS/ASPR); Underwood, Lauren (HHS/ASPR/IO); Weinberger, Collin (OS/ASPR/IO) (CTR)
Subject: MERS-CoV Stakeholders Workshop Report
Attachments: MERS CoV 2015 Stakeholders Workshop Report Final July 2015.pdf

Good afternoon Colleagues,

Thank you for your patience as we assembled the findings and discussions from the MERS-CoV Stakeholders Workshop. Please find attached the final report, "Middle East Respiratory Syndrome Coronavirus: Status Update and Findings from April 2015 Medical Countermeasures Assessment."

Very respectfully,

Natalie (Hanrion) Aviles
Project Manager
HHS/ASPR/IO – Special Projects
Detailed from Office of Portfolio Management BARDA
Conceptual MindWorks, Inc (contractor)
Blackberry: (b)(6)
Office
Email: (b)(6)
www.medicalcountermeasures.gov

Middle East Respiratory Syndrome Coronavirus

Status Update and Findings from April 2015 Medical Countermeasures Assessment

Executive Summary

Human cases of severe illness due to Middle East Respiratory Syndrome Coronavirus (MERS-CoV) have continued to persist in the Arabian Peninsula since first recognition in 2012, and have been exported sporadically to other countries via human travel. While much of the world focused attention on the outbreak of Ebola virus which spread to three countries in West Africa and resulted in tens of thousands of critically ill patients and a high death toll during much of 2014 and into 2015, reported cases of MERS-CoV appeared to once again increase in prevalence in the region in the early spring prompting a concern that the disease was being ignored while attention was shifted to Ebola. Against this backdrop, the Office of the Assistant Secretary for Preparedness and Response convened a meeting in early April, 2015 of subject matter experts and external stakeholders to review current status of information on candidate products for prophylaxis, diagnosis or treatment of human disease. This meeting was timely in that during late May, there was a notable geographic exportation of the virus to Asia (South Korea) from the Arabian Gulf countries which has resulted in dozens of cases and a handful of deaths all within 30 days of introduction.

This report is constructed on the framework of an earlier Ebola review of U.S. government experts convened in June, 2014. This current report adds or updates findings and adjusts recommendations based on knowledge or progress gained since June, 2014. The charge to that original working group was to review: 1) the current situation of MERS-CoV, 2) the conceptual framework for potential use of MERS-CoV countermeasures, 3) the development of MERS-CoV animal models, 4) the status of potential therapeutic and vaccine countermeasures, 5) regulatory issues for therapeutics and candidate vaccines to start clinical trials and 6) estimated timelines for potential countermeasures to begin clinical trials. The April, 2015 Stakeholder's meeting focused again on these issues, as well as providing additional focus on diagnostic development and updates on human epidemiology and clinical experience.

Although progress has been achieved to understand the basic biology of MERS-CoV to date, pre-clinical development and research on potential MERS-CoV MCMs remains preliminary and significant challenges exist before any medical countermeasures are ready for human clinical trials. Limited funding is available through NIAID for preclinical and early clinical development for MERS-CoV MCMs. Animal model development had also steadily advanced with the availability of transgenic mouse models, described below, for early product candidate screening and modeling of human-like pathogenesis. However,

candidate medical products were only marginally advanced between June, 2014 and April, 2015, and this was largely based on efforts that had already been funded by NIH, or were undertaken by industry at their own expense.

Major factors hindering progress in pre-clinical development of MERS-CoV MCMs are: lack of established animal models for human disease from MERS-CoV infection, limited availability of non-human primates (e.g. common marmosets), and limited funding for preclinical development of MERS-CoV MCMs. Addressing these gaps in a timely manner will facilitate availability of potential MERS-CoV MCMs for human clinical trials.

For diagnostics, the CDC has developed an FDA emergency use authorized test for MERS-CoV that is distributed worldwide to public health, DoD and WHO laboratories. However, no commercial assays are currently available to accommodate any surge in the number of cases of MERS–CoV infection. The biggest challenge for the commercial companies has been the ability to obtain specimens from infected and recovered MERS-CoV patients to aid in the validation of the performance of their diagnostic devices.

HHS assembled a cross-government working group and convened an internal portfolio review and external stakeholder workshop to assess the current status of potential medical countermeasures for MERS-CoV. In these assessments, the group suggested the following actions to advance diagnostic assay development for MERS-CoV infection, preclinical development of MERS-CoV MCMs, initiate human clinical trials, and explore alternative interventions for the control of MERS-CoV:

- Standardize use of animal models for studying disease pathogenesis and for evaluation of potential medical countermeasures by convening a scientific meeting, workshop or establishing a MERS-CoV animal models working group to review all available data from animal models and virus challenge strains in development to identify gaps and standardize the most promising animal models and MERS-CoV strains.

- Establish a clinical specimen and assay validation panel working group to develop a strategy for point of care MERS-CoV diagnostics and prioritize the creation of validation panels for use by commercial partners in MERS-CoV diagnostic development.

- Ensure that there are sufficient laboratory populations of non-human primates needed to support preclinical research. The goal of this research is to advance human clinical trials of antivirals, immunotherapeutics and vaccines, with a priority on initiating trials of therapeutics.

- Accelerate product development of the most promising currently available therapeutic candidates, to include an immunotherapy and a small molecule antiviral drug compound.

- Prioritize studies to further the scientific understanding and control of MERS-CoV disease in humans and animals, including vaccination studies in camels.

- Develop a standardized clinical trial protocol and partner with an effective clinical trials network within the Gulf Coast Countries (GCC) to assist in evaluating safety and effectiveness of therapeutic candidates.

I. Introduction

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) was first isolated from a patient who died of severe respiratory illness in Saudi Arabia during June 2012.[1] Earlier human cases of MERS-CoV infection were identified retrospectively by serological testing of patients from a nosocomial outbreak of severe respiratory illness in Jordan in April 2012.[2] While the animal reservoir(s) for MERS-CoV is not conclusively established, molecular evidence has implicated Egyptian tomb bats,[3] and virological and serological data suggest that dromedary camels have been infected with MERS-CoV in several countries, as early as 1992.[4,5] MERS-CoV has been detected in respiratory, fecal, and milk specimens from dromedary camels in the Arabian Peninsula.[6] MERS-CoV is a betacoronavirus and closely related to Severe Acute Respiratory Syndrome-associated coronavirus (SARS-CoV), which emerged from an animal reservoir (bats) and caused epidemics worldwide during 2003. More than 8000 SARS-CoV cases were reported globally with nearly 10% mortality.

As of mid-June 3, 2015 1321 confirmed MERS-CoV cases with 442 deaths (37% mortality) had been reported to the World Health Organization (WHO); median age is approximately 49 years (range 9 months to 99 years), and 66% of cases have been male. Most MERS-CoV cases (>85%) have been identified in Saudi Arabia. While there are many unanswered questions about MERS-CoV epidemiology, both zoonotic transmission and human-to-human transmission have been reported, including large outbreaks in healthcare facilities.[7] Cases have also occurred in pilgrims visiting Saudi Arabia. However, sustained human-to-human MERS-CoV transmission has not been documented. At this time 25 countries have reported locally acquired or exported cases from the Arabian Peninsula, including two U.S. cases identified during May 2014 in healthcare personnel who developed illness after working in Saudi Arabia (KSA).[8] One case reported this May by the Republic of Korea in a traveler to KSA, Qatar, UAE and Bahrain has led to the identification an of over 175 additional confirmed cases through contact tracings in multiple health facilities and among family members. Although only limited clinical data are available to date, a wide spectrum of illness has been identified from MERS-CoV infection, including asymptomatic infection, mild upper respiratory tract symptoms, pneumonia, respiratory failure, acute respiratory distress syndrome, and multi-organ failure. Clinical management of MERS-CoV patients is focused upon supportive care of complications. Recommended public health prevention and control measures include avoiding camel exposure (including raw camel milk), isolation and implementation of infection prevention and control measures with appropriate personal protective equipment for symptomatic cases, and monitoring of exposed close contacts.

Human infections with MERS-CoV are expected to continue in the Arabian Peninsula because of the prevalence of MERS-CoV in camels and the cultural importance of camels (food, milk, racing purposes) in the region. The situation for MERS-CoV is different than for the SARS-CoV outbreak in 2003 in which culling of some infected animals (e.g. civet cats) was done, and no outbreaks have been identified after 2003. In contrast, MERS-CoV infections of humans have continued to occur for more than two years, and culling of camels is impractical. However, the potential for MERS-CoV mutations that could facilitate sustained community transmission and global dissemination cannot be predicted, and the potential for additional imported cases and outbreaks of MERS-CoV in the U.S. is unknown. No vaccines or specific treatments exist for human infections with SARS-CoV, MERS-CoV, or other coronaviruses.

II. MERS-CoV MCM Stakeholder Participants

The participants who assisted in the June 2014 report under the auspices of the ASPR are listed in Appendix 1. Workgroups were developed to aid in recommendations with representatives from across the U.S. Government. The April, 2015 outreach included both members of the U.S. government from an expanded list of agencies, as well as outreach to academic, international and commercial partners. The attendance list is provided in Appendix 2.

III. Epidemiology and Surveillance

As of early June, 2015 there were 25 countries reporting MERS-CoV cases, primarily still in the Arabian Peninsula. A recent significant episode of spread from the UAE countries by one individual to the Korean peninsula has taken place including involvement of tertiary cases, now including 6 deaths and at least 86 infected individuals within the span of four weeks. While the workshop had taken place several months in advance of this recent wave of activity and expansion of the global incidence of MERS-CoV, there is still a dearth of reports of clinical cases studies and treatment options are still limited, outside of aggressive patient management. There are parallels to be drawn here with our continued experiences with Ebola in the West Africa setting, which is still ongoing in several countries, especially in the occurrence of nosocomial spread, lack of well-defined patient management protocols, sluggish international response, rudimentary or lacking clinical trials networks established in affected countries, and lack of specific therapeutics.

The majority of cases still occur in Saudi Arabia with only recent apparent increased cooperation between the Health Ministry of that Kingdom, and sharing with the rest of the world. As of April, there had been twenty different hospitals in KSA experiencing nosocomial transmission. Only a handful of cases have been identified from the surrounding countries, and we lack significant epidemiological investigation to determine what may be contributing to the potential cause(s) of that observation. There is however apparently little human to human spread in the general community. The rate of asymptomatic or mildly symptomatic cases is not well established, although some sero-

surveys in at-risk occupational groups working with camels (animal handlers, slaughterhouse personnel. etc.) do show that there may be a number of mild cases contributing to possible transmission. We need to better understand the transmission dynamics from animal contacts, and whether focus on husbandry practices, animal immunization or spread from alternate animal reservoirs will mitigate zoonotic transmission to humans. MERS-CoV. It is also not apparent why transmission to humans is not seen in Eastern Africa, where large populations of camels reside, and the virus may be circulating. Hypotheses include differences in virus strains, differences in types of at-risk populations, differences in animal handling practices or differences in consumption of animal products between these regions. WHO has begun to recommend that at-risk groups avoid contact with camels (or camel products) for 16 weeks to determine whether it would result in a decrease in the number of severe cases.

There is a strong observational correlation between MERS-CoV case mortality and age (> 60 years), diabetes, renal deficiency and hypertension as risk factors. However, deaths have been observed in patients that do not present with these risk factors.

Nosocomial spread has once again been identified as an important and preventable component of illness and death. There is a tremendous need to greatly improve early identification of cases and to incorporate better infection control practices in managing early screening and treatment of hospitalized patients. Recent experiences in KSA have demonstrated that strict adherence to infection control have reduced nosocomial spread such that for the last 85 cases managed in hospital settings, there has been no spread within the hospital setting. Furthermore, case fatality rates have been observed to drop in this setting to 13% with enhanced patient management, including attention to renal issues.

Exportation of cases from the current endemic geographic region is still problematic. There is still a significant length of time between identification of these imported cases and recognition of etiology, such that a recent case in Germany took more than three weeks to identify and led to the need for contact tracing of over 250 individuals. It is imperative to identify travel history for patients reporting with respiratory symptoms during early admission to hospital, even in advanced health care systems elsewhere in the world.

Finally, ongoing studies are needed to identify any changes in the viral genome that lead to increased potential for sustained human-to-human chain of transmission of the virus. Current limited epidemiological and genomic investigations of this suggest that sustained transmission is not yet a common feature of the viruses circulating at this time.

IV. Strategies for Potential Use of MERS-CoV MCMs

MERS-CoV infection could theoretically be prevented by vaccination (active immunization) or by prophylaxis (antiviral chemoprophylaxis or passive immunoprophylaxis) of persons at increased risk of infection in affected countries such as camel workers and healthcare personnel or those at higher risk for more severe disease,

including the elderly or persons with chronic medical conditions. In addition, MERS-CoV vaccines could also be developed for animals and used for vaccination of camels in the Arabian Peninsula and in source countries for camel exports (e.g. Horn of Africa region) to the region. Drugs with activity against MERS-CoV (e.g. antivirals, immunotherapeutics) or that target the host immune response could be used for treatment of illness caused by MERS-CoV infection. Proof-of-concept data from *in-vivo* studies in experimentally infected animals is needed to prove a product's potential efficacy. These studies can also inform the mechanism for selection of available MCM candidates before human clinical trials can be initiated.

V. Animal Models

Preclinical development of MERS-CoV MCMs has been hindered by the lack of an established animal model that recapitulates severe human disease from MERS-CoV infection including a clear understanding of virus tropism beyond the respiratory tract in humans, transmission dynamics and routes of infection. The availability of reagents to analyze the animal model proteins and genes involved in pathogenesis and response is essential. The favored animal model should also ideally be easy to use. Animal model availability, price and the degree of risk associated with using the model are all important.

Both small animal and non-human primate (NHP) models are useful to test potential MCMs for efficacy (Appendix 3). Studies have demonstrated that a number of small animals commonly used as laboratory animal models (e.g. mice, hamsters, ferrets) are not susceptible to MERS-CoV. As a result multiple tactics are being taken to develop small animal models including but not limited to: testing of nontraditional species (e.g. mink), passage of virus in animals, engineering of animal models through rational design (e.g. transgenic animals that express human DPP4, the MERS-CoV receptor[9]). However, screening of MERS-CoV MCMs in small animal models is now possible.

NHP models that are under development include rhesus macaques and common marmosets. Major gaps for the NHP models include: characterization of the different MERS-CoV strains, determining and standardizing the optimal viral challenge dose, volume, route of exposure, and the ability for sequential sampling in marmosets and determining clinical relevant symptoms. Overall, common marmosets appear to be better suited than rhesus macaques for therapeutic studies designed to target severe disease given the slightly slower onset of illness and the longer duration and severity of disease. However, the limited supply and availability of common marmosets in the U.S. is currently a major barrier to the development of MERS-CoV MCMs.

Large animal models in development include camels and camelids such as alpacas. These models may be important to help understand the virology and immunology of MERS-CoV infection in dromedary camels since MERS-CoV infections have been documented in camels in multiple countries.

VI. In –vitro Diagnostic Devices

On May 29, 2013 The HHS Secretary declared a Potential Public Health emergency regarding MERS–CoV infection that could have a significant potential to affect national security or the health and security of US citizens living abroad. Circumstances were deemed to exist to justify emergency use authorization (EUA) of in-vitro diagnostic tests to diagnose the infection. The CDC submitted a request for an EUA on May 29, 2013 with data to support the performance of their molecular assay to detect the MERS-CoV virus in several sample types. They were granted on June 5, 2013, authorization for use of the test by trained technicians in qualified laboratories for testing symptomatic patients

On June 10, 2014 the EUA was expanded to include the ability to test asymptomatic contacts after a US returning traveler case. This assay has been made available to multiple public health, DoD and WHO laboratories worldwide but is limited in terms of being able to scale up reagents to support a possible surge in the numbers of infected cases. The current coverage is therefore insufficient if no alternative FDA reviewed commercial tests are available

The lack of such assays is likely due to the availability of samples from infected and recovered patients. A small working group has been formed to make available panels of specimens that could be sourced, stored and made available to diagnostic assay developers to help validate the performance of their devices and support an EUA. This model has been used previously to generate panels of difficult to source specimens for Ebola virus diagnostic test developers. Funding such a project will be a major challenge that needs to be explored

VII. Therapeutics

There are no candidate products that have been specifically evaluated for treatment of MERS-CoV patients in well controlled clinical studies. Potential therapeutics for MERS-CoV patients include assessment of available approved drugs with non-specific properties such as immunomodulators (e.g. corticosteroids, intravenous immunoglobulin, interferons), small molecule drugs with broad antiviral activity, approved small molecule drugs for other diseases that are being screened for specific activity against MERS-CoV (termed "re-purposing"), and development of new therapies (immunotherapeutics, other inhibitors) with specific activity against MERS-CoV (Appendices 4a and 4b). There has been discussion elsewhere regarding the use of convalescent serum as a potential therapeutic approach. A small number of MERS-CoV patients have been treated with interferon α 2b and ribavirin, but not in the context of a clinical trial, so clinical benefit is unknown.

Most immunotherapeutics in development are monoclonal antibodies that have specific neutralizing activity against the MERS-CoV spike protein. Challenges to development of immunotherapeutics include ensuring absence of antibody-dependent

disease enhancement and reducing the risk of generation of escape mutant viruses (that would not be susceptible to treatment). Platforms to rapidly produce fully human monoclonal antibodies in cell culture or polyclonal antibodies using animal systems have been developed. Preliminary immunoprophylaxis or treatment studies in MERS-CoV-infected humanized mice and NHPs using fully human monoclonal or polyclonal antibodies are in-progress or planned. The United States Government (USG) needs to develop a prioritization strategy to inform which of the potential early stage candidates should be considered for accelerated development.

VIII. Vaccine candidates

Human vaccination

Development of MERS-CoV candidate vaccines was initiated by NIH's National Institute for Allergy and Infectious Diseases (NIAID), academic investigators, and several companies (Appendix 5a). Most vaccine development approaches are still in pre-IND evaluation in animal models. They have generally targeted the spike protein of MERS-CoV and are recombinant subunit, DNA or virus particle vector vaccines. One live-attenuated MERS-CoV candidate vaccine is in early development. Preliminary studies for several MERS-CoV vaccine candidates have been initiated and demonstrate immunogenicity; two have progressed to NHP challenge. One vaccine company (Inovio) is preparing to submit an IND and anticipates start of a Phase 1 study in 2015.

A concern that must be addressed in the development of MERS-CoV vaccines is the potential for causing enhanced disease upon virus challenge, such as what was observed with a SARS-CoV candidate vaccine upon SARS-CoV challenge.[10] Additional challenges for evaluation of MERS-CoV vaccines in humans include the lack of a precedent of human coronavirus vaccines and concerns about enhanced disease based on some animal coronavirus vaccines such as feline infectious peritonitis virus, although vaccines against other animal coronaviruses are safe and in use in animals. Again, the relatively early state of development of the potential vaccine candidates requires that the USG identifies a prioritization method to speed access for human clinical trials.

Camel vaccination

Given the cultural importance of camels in the Arabian Peninsula for meat and milk consumption, and racing, prevention of camel-to-camel MERS-CoV transmission and reduction of spread from camels to humans by camel vaccination should be investigated. Experimental MERS-CoV infection studies and vaccine studies in a small number of dromedary camels has been accomplished in a large animal biosafety level 3 (BSL3) facility in the U.S. and overseas (Appendix 5b). Alpacas (new world camelid) are being investigated as a suitable proxy for camels due to the lack of available dromedary camels in the U.S., the high cost of acquiring dromedary camels and their size,. One company,

Novartis A.B. is in discussion with KSA on a camel trial that would lead to licensing for veterinary use.

IX. Regulatory Considerations for MERS-CoV MCMs

FDA recognizes the public health urgency for developing MERS-CoV MCMs and is committed to working with sponsors and US partners to expedite the review of applications, including nonclinical studies and clinical protocols. The Working Group encourages all sponsors, as well as government agencies to engage the appropriate FDA review divisions as early as possible in product development communications, in order to provide feedback on submissions of preliminary data and proposals for clinical trial protocols.

Regulatory considerations for MERS-CoV MCMs should remain focused on a pathway to human clinical trials through submission of U.S. Investigational New Drug (IND) applications. IND submissions should follow requirements set forth in the Code of Federal Regulations, Title 21, Part 312 (21 CFR 312). Several guidance documents exist on the FDA website, http://www.fda.gov/Drugs/GuidancecomplianceRegulatoryInformation/Guidances/default.htm, related to virology, microbiology, pharmacology/toxicology, clinical/medical considerations. At this point, it is premature for FDA to determine the most appropriate approval pathway, as this determination may be product-specific and will need to consider existing product data, proposed intended use and population, and validated surrogate endpoints for efficacy predictive of clinical benefit, if any. Likewise, data needed to consider an authorization for emergency use (EUA), such as dose finding/dose ranging, duration and safety can be obtained, for example, through IND clinical trials. An approach similar to a "Master Protocol" that was developed for testing clinical products during the Ebola outbreak in West Africa should be considered as an approach for evaluating and prioritizing medical countermeasures for MERS-CoV.

Already approved FDA drugs to be re-purposed for a MERS-CoV indication can potentially be "lower-hanging fruit," if 1) the mechanism of action (MOA) for antiviral activity is defined, 2) there is no change to the approved final drug presentation and route of administration, 3) dosing does not exceed the currently approved dose/duration for the currently indicated population and there are adequate pharmacokinetics (PK) data to support this dosing, and finally, 4) the risk/benefit profile is acceptable for the intended population/indication. For example, the risk/benefit profile for an approved drug with an oncology indication may be unacceptable if the drug is re-purposed for a healthy population for MERS-CoV post-exposure prophylaxis.

Data requirements to initiate human trials will depend on the characteristics of the drug product and its intended use against MERS-CoV. As such, sponsors should prioritize drug development based upon the totality of scientific evidence/merit of the drug alone, and not whether the drug has been previously approved.

For diagnostic devices the current EUA pathway serves as a fast track approach to make products available for public health purposes. Once an emergency has been declared over these products have to be submitted to FDA for a more thorough evaluation as 510(k)s.

All sponsors, as well as government agencies, are encouraged to engage the appropriate FDA review divisions as early as possible in product development communications, in order to provide feedback on submissions of preliminary data and proposals for clinical trial protocols.

X. Access to Clinical Information

As of mid- June, 2015 25 countries had reported a total of 1,321 laboratory confirmed MERS-CoV cases, with most cases primarily still in the Arabian Peninsula. A recent significant episode of spread from the UAE countries by one individual to the Korean peninsula has taken place including involvement of tertiary cases, now including 19 deaths and over 162 infected individuals within the span of six weeks.
The rate of asymptomatic or mildly symptomatic cases is not well established.

While there is accumulating data on management of clinical cases with severe presentation, there is still a need for a compilation of data to solidify our understanding of human pathology and pathogenesis from this group of viruses. Efforts have been slow, or perhaps even deliberately delayed in publishing comprehensively on cases, including information on mild, atypical or uncomplicated infections.. The international community should move toward rapidly to address this need, With the expansion of cases into other regions outside of the Middle East, it may be possible to accelerate this deficit.

Knowledge gaps and needs that still exist include accurate information on viral loads during infection, standardization of case reporting and data collection methods, systematic collection of human clinical specimens, prognostic markers, data, tissue and specimen sharing and long-range follow up of patients. Other critical unknowns or areas for increased study include detailed evaluation of co-morbidities affecting clinical care and informing clinical management. Further attention needs to be targeted on clinical immunological dynamics and on host mechanisms for immune clearance of these viruses from tissues. Without an international agreement on protocols and systematic evaluation, we will continue to see haphazard or anecdotal reporting and analysis of disease course and outcome.

International agreement on clinical management has yet to become solidified and evidence-based, peer-reviewed guidelines are needed. International organizations, such as the WHO have been, are developing interim clinical guidance documents and working to set up platforms to address this need. The current understanding on intervention is heavily based on specific ICU experience, and management of acute respiratory distress and septic shock. Suggested management of SARI (severe acute respiratory infection)

patients includes O_2 therapy, collection of lower respiratory tract specimens, immediate empiric antimicrobials for community-acquired pathogens, conservative management of fluids in patients without evidence of sepsis induced tissue hypoperfusion and avoidance of high dose systemic corticosteroids. Close monitoring for progression of sepsis and respiratory distress are strongly urged. Much as was seen with Ebola, high quality supportive care underpins reduction in case fatality rate for these patients.

No specific therapies exist for treatment of MERS-CoV. Anecdotal evidence suggests that early administration of convalescent plasma may be clinically beneficial in treating SARI caused by other viral etiologies, but there is no systematic, well-controlled data collection to support recommendations for this treatment method. Randomized clinical trials will be needed to perform effective evaluation of any candidate therapeutics.

XI. Development of Clinical Trials Networks

One important step in establishment of eventual human clinical trials is the development of a Master Protocol for randomized clinical trials, or some other mechanism to address the multiple possible MCMs that may need access to patient populations. There is uncertainty in estimating timelines for the development of potential MERS-CoV MCMs because of the need to further characterize existing and new animal models, the unknowns of demonstrating a favorable risk–benefit outcome during preclinical testing and limited funding for preclinical and clinical development.

It is essential to develop and partner with an effective clinical trials network within the Gulf Coast Countries (GCC) to assist in evaluating safety and effectiveness of therapeutic candidates. There is no clear organization with which to establish any immediate goals, thus it will likely be necessary to create opportunities in this region. The organization Middle East Clinical Trials Research Association (MECRA) exists as a not-for-profit organization to promote development of clinical research within the North Africa and Middle East region, however there is no evidence that they have provided support or guidance for studies aimed at understanding MERS-CoV issues.

Other possibilities may include direct coordination and development of such a clinical trials network with a leading health care organization in the region. WHO and the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) are collaborating in adapting standardized protocols for controlled clinical trials for MERS-CoV (https://isaric.tghn.org/protocols/severe-acute-respiratory-infection-data-tools/). Other considerations that could impact the ability to plan appropriately for clinical trials includes our reliance on completion of pre-clinical studies. The risk of antibody related immunopathology may delay or interrupt the timeline to conducting human clinical trials of MERS-CoV vaccines and immunotherapeutics. In general, all MERS-CoV MCMs will require pre-clinical toxicology prior to human clinical trials. While animal efficacy data is not technically required for human clinical trials, the Working Group considers such data as

necessary to identify the most promising MCM candidates, justify risk in human volunteers, and inform the design of future clinical studies.

XII. Conclusions

Although pre-clinical development and research on potential MERS-CoV MCMs has achieved appreciable progress to date, such development is preliminary, and significant challenges must be overcome before any MCMs are ready for human clinical trials. Major factors limiting progress in pre-clinical development of MERS-CoV MCMs are: the lack of established animal models for human disease caused by MERS-CoV infection, the shortage of NHPs (e.g. common marmosets), limited funding to support MERS-CoV pre-clinical and clinical product development, availability of well characterized specimens or strains and lack of specifically-identified clinical partners or clinical trials network in the region for evaluation of promising approaches. Addressing these gaps in a timely manner can facilitate availability of potential MERS-CoV vaccines and therapeutics for human clinical trials and the development of in –vitro diagnostic tools.

These considerations favor prioritization of animal model development to facilitate development of MERS-CoV MCMs. Additionally, substantial progress in establishing the infrastructure for pre-clinical and advanced clinical development of MERS-CoV MCMs can serve as a model to enable more timely response to novel coronaviruses of global public health concern in the future.

Gaps and challenges in the pre-clinical development of MERS-CoV MCMs and can be addressed by the following actions with the goal of initiating human clinical trials of therapeutics and vaccines as soon as possible, and with priority on developing therapeutics for MERS-CoV patients:
Current Priorities:

1) Convene a scientific meeting, workshop or working group to review all available MERS-CoV animal model data and virus challenge strains to identify gaps and to support standardization of the most promising animal models and MERS-CoV strains:

   a) Virus stocks as well as cell substrates should be qualified to the extent necessary, including clinical source origin, passage growth and history, testing for adventitious agents.

   b) Viral banks and standard operating procedures (SOPs) for propagation and storage should be produced to share with labs developing MCMs.

   c) Recommendations on best animal species, strains or transgenic animals should be established and discussed with the FDA to accelerate understanding of models that would be presented for any regulatory actions or considerations

d) Assure sufficient supplies of non-human primates and small animals to support pre-clinical development of MERS-CoV therapeutics and vaccine candidates

2) Develop and make available validation panels (exclusivity and inclusivity) to assist diagnostics developers in accelerating medical diagnostic assays and devices for point of care diagnosis of MERS-CoV infections4)

3) Establish partnership with an effective clinical trials network within the Arabian peninsula to assist in evaluating safety and effectiveness of therapeutic candidates.

4) Coordinate epidemiological studies among USG stake-holder organizations (HHS, USAID, DoD and the USDA) to prioritized support the understanding and control of MERS-CoV disease including:

a) Studies at the animal-human interface in the Arabian Peninsula, Northern and East Africa, and other regions,

b) Longitudinal epidemiological, virological, and immunological studies in animals, including camels;

c) Sero-surveys in camel workers (and their close contacts) and studies to identify the source and route of transmission from camels to humans;

d) MERS-CoV vaccination studies in camels.

5) Provide additional resources to existing funding mechanisms to support diagnostics development, pre-clinical research and human clinical trials of antivirals, immunotherapeutics, and vaccines.

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XIV. Appendices

Appendix 1: Working Group on Potential MERS-CoV MCMs Members and Charge

Members

Beigel, John (NIH)
Carter, Wendy (FDA/CDER)
Cho, David S (FDA/CBER)
Choi, Su-Young (FDA/CDER)
Donabedian, Armen M. (HHS/ASPR/BARDA) (co-chair)
Donaldson, Eric (FDA/CDER)
Feldmann, Heinrich (NIH/NIAID)
Graham, Barney (NIH/VRC)
Hensley, Lisa (NIH/NIAID)
Hu-Primmer, Jean (FDA/OC)
Kelley, Cynthia (FDA/CBER)
Miele, Peter (FDA/CDER)
Munster, Vincent (NIH/NIAID)
Spiro, David (NIH/NIAID)
Stemmy, Erik (NIH/NIAID)
Subbarao, Kanta (NIH/NIAID)
Uyeki, Timothy M. (CDC/OID/NCIRD) (co-chair)

(b)(6)

Guests

Bright, Rick (HHS/ASPR/BARDA)
Dreier, Thomas (HHS/ASPR/BARDA)
Erlandson, Karl (OS/ASPR/BARDA)
(b)(6) (DoD/DARPA)
O'Hara, Michael (HHS/ASPR/BARDA)
Phan, Thuy D. (HHS/ASPR/BARDA)
Rippke, Byron E (USDA/APHIS)
Wathen, Michael (HHS/ASPR)
Willis, Melissa (HHS/ASPR/BARDA)

(b)(6)

2014 Working Group Charge

The Working Group will assess potential MERS-CoV therapeutic (antivirals, monoclonals and others) and vaccines countermeasure and report back on initial discussions/findings in 3 weeks.

- inventory of potential therapeutics (and vaccines) under development/investigation and by what groups;
- how far along are they in pre-clinical development (in-vitro, animal studies/mice/non- human primates) and what data are available;

- timelines for animal studies, scale-up, when could human trials be started – phase 1, phase 2, open-label studies;
- regulatory issues for IND, EUA, and clinical studies, safety issues;
- prioritization for clinical trials based upon pre-clinical data and scientific assessment of available data

Appendix 2. List of Participants and Affiliations of the Stakeholder Workshop Attendees, April, 2015.

(b)(6)	Protein Sciences
Joseph Annelli	Novavax Inc.
Natalie Aviles	USDA-APHIS
(b)(6)	HHS/ASPR
Himani Bisht	UNC-Chapel Hill
(b)(6)	Assn of Public Health Laboratories
Rick Bright	Naval Medical Research Center
Bruce Carter	Food and Drug Administration
David Cho	Colorado State University
Gina Conenello	HHS/OS/ASPR/BARDA
(b)(6)	USDA APHIS VS CVB
Rita Czako	FDA
Jon Davis	FDA
Damon Deming	altona Diagnostics
(b)(6)	Armed Forces Health Surveillance Center
Diane DiEuliis	NIAID
Armen Donabedian	ASPR
Eric Donaldson	FDA
(b)(6)	Vanderbilt University School of Medicine
Karl Erlandson	ThermoFisher Scientific
Daniel Feikin	OPP/ASPR
Phil Ferro	ASPR/BARDA
Robert Fisher	FDA
(b)(6)	Kimball Research Institute New York Blood Center
Cyril Gay	Ibis Biosciences and Abbott company
(b)(6)	World Health Organization
Lisa Gretebeck	BARDA
(b)(6)	CDC
Lisa Hensley	HHS/ASPR
(b)(6)	FDA/OCET
Sally Hojvat	US Department of Defense
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	National Institutes of Health
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	AFHSC DoD
	NIAID
	Biological Technologies Office DARPA
	FDA
	NIH
	ASPR/OPP/MCSR
	NIAID IRF
	DHS S&T/NBACC
	BioFire Diagnostics LLC

Cynthia Kelley	FDA/CBER
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George Korch	ASPR HHS
(b)(6)	Regeneron Pharmaceuticals Inc
Elaine Lamirande	NIH/NIAID
(b)(6)	Thermo Fisher Scientific
	Naval Medical Research Center
Carmen Maher	OCET/OCS/OC/FDA
(b)(6)	Association of Public Health Laboratories
Maria Julia Marinissen	Division of International Health Security/ASPR/HHS
(b)(6)	Geneva Foundation/USAMRIID
Peter Miele	FDA
(b)(6)	bioMerieux
Gene Olinger	NIH/NIAID/DCR/IRF-Frederick
(b)(6)	Houston Methodist Hospital
Jules O'Rear	FDA\OMPT\CDER\OND\OAP
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Mark Pallansch	Centers for Disease Control and Prevention
(b)(6)	Greffex Inc.
Robin Robinson	BARDA
(b)(6)	Inovio Pharmaceuticals
	Cepheid
	OASD(Health Affairs)
Kim Sciarretta	BARDA/CBRN
(b)(6)	Regeneron Pharmaceuticals
	Novavax Inc
David Spiro	DMID/NIAID/NIH/HHS
(b)(6)	Greffex Inc
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Troy Sutton	National Institute of Allergy and Infectious Diseases
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Lynne Wathan	HHS/ASPR/BARDA
(b)(6)	University of Pennsylvania
	GlaxoSmithKline
	Planet Biotechnology Inc
	University of Iowa
	Dana-Farber Cancer Institute

Appendix 3. Animal Models in Development

Organization	Species	Notes
The University of Iowa	Mouse expressing human DPP4 (from Adenovirus 5 vector)	Transient and localized expression of DPP4. Mild infection. Funding support provided by NIAID.
University of Texas Medical Branch-Galveston	Mouse Knock-in of human DPP4, constitutive promoter	Expression of DPP-4 throughout the animal (including brain). Relentless weight loss and death within days post infection. Virus in the brain.
Regeneron	Mouse Knock-in of human DPP4, natural promoter	Stable expression of human DPP4 under a natural promotor (e.g. limited to the lung). Viral replication and lung pathology observed. Possible lethal phenotype, no virus in the brain.
Utah State University and Icahn School of Medicine	2 x transgenic mouse models	MCM evaluation. Funding support provided by NIAID.
Rocky Mountain Laboratories, NIAID **Integrated Research Facility, NIAID**	Rhesus Macaque	Macaque DPP4 receptor binds MERS. Infection causes acute localized to widespread pneumonia with transient clinical disease. Similar to mild /moderate human MERS-CoV cases. Advanced medical imaging being used to chart and better characterize disease development as well as to measure potential benefit of MCM. Multiple virus isolates being evaluated. Similar to mild/moderate human MERS-CoV cases.
Rocky Mountain Laboratories, NIAID **Integrated Research Facility, NIAID**	Marmoset	Marmoset DPP4 receptor binds MERS naturally. Multiple routes of infection used. Similar to more severe human MERS-CoV cases. Lethality is ~20%. Advanced medical imaging being used to chart and better characterize disease development as well as to measure potential benefit of MCM. Multiple virus isolates being evaluated. Disease course was more severe than rhesus but no deaths were observed with either isolate tested.
Rocky Mountain Laboratories, NIAID	Dromedary Camels	Infection studies in a small number of dromedary camels are underway in a large animal BSL3 facility in the U.S.
Lab of Infectious Diseases, NIAID	Rabbit	Transient infection, no clinical signs, passaging 4-5 times. Possible enhancement of disease seen upon second challenge.
AutoImmune Technologies	Mink	Mink epithelial are permissive to MERS-CoV infection. DMID grant.

The first MERS-CoV mouse model achieved transient expression of DPP4 via an adenovirus vector.[1] Several laboratories including some supported by NIAID's Division of Microbiology and Infectious Diseases (DMID), are developing transgenic mouse models, including the development of fully transgenic mice expressing human DPP4 through the Preclinical Services Program using different expression vectors to establish the human MERS-CoV receptor in multiple mouse lines. There are currently at least two founder lines, with initial infection and expression studies ongoing. One humanized mouse model has been published by UTMB, with human DPP4 expressed in every cell. These knock-in mice were susceptible to wild-type MERS-CoV and had a lethal phenotype. However, the virus spread to the brain in this model, an organ not implicated in human disease. At least one large-scale pharmaceutical company (Regeneron) has developed a knock-in mouse in which the human DPP4 gene has been inserted into the mouse genome using a proprietary genetic approach. These mice have been tested for susceptibility to MERS-CoV infection and are permissive to wild-type MERS-CoV, display lung pathology, and some lethality.

A novel mink model is in development by one laboratory (AutoImmune Technologies). A rabbit model of MERS-CoV infection is in development that involves the intranasal installation of the 2012 Erasmus Medical Center (EMC) MERS-CoV strain. Infection results in viral replication in the lower respiratory tract on day 3 post infection (dpi) with moderate multifocal broncho-interstitial pneumonia on days 3 and 5 dpi. Viral antigen is detected by immunohistochemistry in medium caliber airways and type 1 and type 2 pneumocytes. However, no clinical signs and a poor neutralizing antibody response were observed. Further development of the model and adaptation of the virus via serial passage in rabbits is ongoing.

At least two laboratories have been testing rhesus macaques. The models differ in the viral strains tested, routes of inoculation, and infectious doses used. At the IRF, model development using a Jordanian MERS-CoV isolate indicates that rhesus macaques inoculated via the intratracheal route develop a transient respiratory disease that peaks between 4 and 6 dpi based on findings using CT imaging and which clears by 14 dpi. Inoculation using the Jordan MERS-CoV strain results in limited clinical indicators of disease; however, radiographic indicators of disease were present and quantifiable in all groups after inoculation. There is a marked difference in volume of abnormalities and patterns of resolution among all groups.

Model development at RML using the Erasmus MERS-CoV strain also led to transient lower respiratory tract infection in rhesus macaques. In this model, animals were inoculated with the virus via a combination of intratracheal, ocular, oral, and intranasal routes. Clinical signs of disease such as reduced appetite, increased respiration rate, cough,

[1] Zhao J et al. Rapid generation of a mouse model for Middle East respiratory syndrome. Proc Natl Acad Sci U S A. 2014 Apr 1;111(13):4970-5. doi: 10.1073/pnas.1323279111. Epub 2014 Mar 5.

piloerection, and hunched posture were observed within 24 hours of infection, but are variable. Peak disease occurred between 2 and 3 dpi without observed lethality. Radiographic changes showed varying degrees of localized infiltration and interstitial markings. Virus shedding, virus replication in respiratory tissues, gene expression, and cytokine and chemokine profiles peaked early in infection (approximately 3 dpi) and decreased over time. Gross lesions in infected rhesus macaques are moderate. Multifocal, mild to marked interstitial pneumonia, with virus replication occurring mainly in alveolar pneumocytes was observed without evidence of systemic infection.[2]

Lack of consistently observed severe disease in the rhesus macaque model complicates analysis of therapeutic studies. Therefore, at least two laboratories (RML, IRF) have been developing a MERS-CoV infection model using the common marmoset. In contrast to the rhesus macaque, marmosets appear to develop a more severe and protracted respiratory disease. At the RML, inoculation of common marmosets with the EMC MERS-CoV strain via a combination of intratracheal, ocular, oral, and intranasal routes resulted in clinical disease, including reduced appetite, increased respiration, and open mouth breathing within 48 hours. Peak disease occurs between 4 and 6 dpi. Radiographic changes showed varying degrees of localized infiltration ranging from mild to severe congestion. Extensive gross pathology was observed and viral loads in lungs were approximately 1000 times higher than rhesus macaques with evidence of systemic extrapulmonary dissemination. Total RNA sequencing demonstrated the induction of immune and inflammatory pathways. The distribution of lesions in the lungs was increased compared to the rhesus model, but the severity was comparable. Some animals were euthanized due to severe clinical illness (20% fatality rate) and multiple animals had extensive fluids in their lungs at necropsy.

At the IRF, model development using the Jordanian MERS-CoV isolate with a single route of exposure indicate that challenge results in limited clinical indicators of disease such as increased respiration rate and decreased activity and food consumption. However, radiographic indicators of disease were more prominent and longer lasting in marmosets than in rhesus macaques. Testing of the Erasmus isolate produced similar results. While there are many similarities between the marmoset challenge models at RML and IRF laboratories, there are some notable differences that may have an effect on the observed clinical signs and symptoms. The models differ in the doses of the viral challenge, volume of inoculum instilled intratracheally, and source of the marmosets. It should be noted that all studies to date have been hampered by limited numbers of animals. As a result, additional studies will be needed to determine if the observed variability between the IRF and RML and within experiments at each laboratory is a reflection of the numbers of animals used or other variables such as the source of the marmosets, the volume and routes of inoculation or other yet to be determined factors.

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[2] Munster, V.J., de Wit, E., Feldman, H. Pneumonia From Human Coronavirus in a Macaque Model. NEJM 2013, Apr 368:1560-1562.

Some unusual observations that are seen with animal models other than mice are difficulty in recovering the virus from animal tissue homogenates in cell culture, an absence of a robust serologic response and insufficient data on response to re-infection.

Regardless of the animal or strain used, disease outcomes must be correlated to human disease to be useful. Current thought are reductions in viral titer, pathology, or clinical symptoms. However, a clinically relevant change is not known as limited natural history of MERS-CoV in humans has been performed.

Appendix 4. Therapeutics in Development

a) MERS-CoV Small Molecule and Biologics Treatment Candidates

Organization	Drug	Target	MERS Activity	Notes
Rocky Mountain Laboratories	Ribavirin + IFN	Polymerase + Immunomodulator	Active in Rhesus macaques	Approved for Hepatitis C virus. Compassionate use for MERS
University of Hong Kong/Bayer	Interferon B1b	Immunomodulator	Active in cell culture	
Hemispherix Biopharma	Alferon N	Immunomodulator	Active in cell culture	Approved for HPV
Romark Laboratories	Nitazoxanide	Host functions	Active in cell culture	Approved for Cryptospordia and Giardia, not active in all labs
AbbVie	Lopinavir	Protease	Active in cell culture	Approved for HIV
BioCryst Pharmaceuticals	BCX4430	Polymerase	Active in cell culture	
University of Missouri	SSYA10-001	Helicase	Active in cell culture	
Small Business Grant	DPP4 Decoy	Spike/Binding	Binding assay	Funding support provided by NIAID.
Small Business Grant	Peptide Inhibitors	Spike/Fusion	Binding assay	Funding support provided by NIAID.
Loyola University, Chicago, Stritch School of Medicine	Protease Inhibitors	MERS PLpro MERS 3CLpro	Active in cell culture	Funding support provided by NIAID.
Various	FDA Approved Drug Screen Chloroquine and chlorpromazine appear promising	Multiple targets	Active in cell Culture. Chloroquine and chlorpromazine also active in the mouse model	Multiple screening efforts. Funding support for some projects provided by NIAID.
Planet Biotechnology	DPP4-Fc	DPP4-Fc chimera that mimics the MERS-CoV receptor	Active in cell culture	Produced in tobacco

Antiviral drugs under assessment include the combination of interferon (IFN) α2 and ribavirin, an approach used to treat hepatitis C and off-label for other viral indications (orphan status). Screens of other IFN products demonstrated the antiviral activity of IFNβ. *In-vitro* comparisons of MERS antiviral activity (IC50) revealed that IFNβ has greater potency than IFNα2. Additional studies identified *in-vitro* antiviral activity of mycophenolic acid (MPA). MPA has been shown to have antiviral activity and a putative mechanism of action (MOA) has been demonstrated for this drug. To fully explore additional antivirals, other libraries have been screened with *in-vitro* screening tools.

A promising approach for screening has been to target libraries of approved drugs (FDA and European Medicines Agency approval). Repurposing of approved pharmaceutical drugs for new indications presents an attractive alternative to clinicians, researchers, public health agencies, drug developers and funding agencies. Given development times and manufacturing requirements for new products, repurposing of existing drugs might potentially facilitate the response to outbreaks of emerging viruses. Drugs may be developed through traditional approval processes with less risk given their known safety profiles in humans. In emergencies, a physician could prescribe a drug "off-label", as has been done with ribavirin and IFNα2 for hepatitis C. However, the advantages of repurposing drugs assumes that the mechanism of action against MERS-CoV is defined, there is no change in the approved drug formulation, adequate PK data exists for the target population, the anti-MERS-CoV treatment course does not exceed the approved dose/duration, and the safety profile is acceptable for the new indication. For instance, MPA is an immunosuppressive drug used to prevent organ transplant rejection and may not be suitable for treating severe respiratory illness.

In a recent study, a library of 290 compounds was screened for antiviral activity against MERS-CoV and SARS-CoV.[3] Selection of compounds for inclusion in the library was dependent on current or previous FDA approval or advanced clinical development as well as demonstration of antiviral activity *in-vitro* in previous screens performed against other high consequence viruses. Some drugs were included that had a well-defined cellular pathway as a putative target. Overall, 27 compounds with activity against both MERS-CoV and SARS-CoV were identified. The compounds belong to thirteen different classes of pharmaceuticals including; inhibitors of estrogen receptors used for cancer treatment and inhibitors of dopamine receptor used as antipsychotics. Currently these drugs are being evaluated in available small animal models. Studies have also been initiated to understand the biology of the new indications. A second screen of FDA approved drugs was performed in parallel, identifying 2 additional drug candidates. Paralleling the more classic drug discovery efforts are those that are identifying candidate pathways or drugs by characterizing the host responses following MERS-CoV infection. One such effort has been

[3] Dyall J et al. Repurposing of clinically developed drugs for treatment of Middle East Respiratory Coronavirus Infection. Antimicrob Agents Chemother. 2014 May 19. pii: AAC.03036-14. [Epub ahead of print]

studying the kinases in MERS-CoV infected cells. These efforts have mapped critical pathways and have demonstrated that viral infection can be reduced in vitro with approved kinase inhibitors that directly target these pathways.

Studies have begun to evaluate compounds for anti-MERS activity in cell culture and NHP models. IFN-α2b, -β and -αn3 have all exhibited antiviral effect against MERS-CoV in tissue culture. The broad-spectrum antiviral ribavirin also exhibits antiviral effect against MERS-CoV in tissue culture but only at levels that exceed those attainable on standard dosing. At the NIAID Rocky Mountain Laboratories, the broad-spectrum antiviral T-705 did not exhibit an antiviral effect at any of the concentrations tested. When combined, ribavirin and INF-α2b have an antiviral effect *in-vitro* at levels that are clinically achievable. In rhesus macaques the combination of ribavirin and INF-α2b starting 8 hours post-infection led to an overall benefit in all of the assayed parameters. No clinical trials of ribavirin and interferon-α2b for treatment of MERS-CoV patients have been conducted and no conclusions can be made from the limited published data from MERS-CoV patients treated with this combination in Saudi Arabia. Clinically advanced cyclophilin inhibitors have a modest effect against MERS-CoV in tissue culture that is enhanced in the presence of ribavirin. The broad-spectrum viral entry inhibitor griffithsin has a potent antiviral effect in vitro. As a post-exposure therapy in rhesus macaques results were equivocal (there was some reduction observed in viral loads). Studies are also being planned to the evaluation of the broad-spectrum antiviral, nitazoxanide, in the NHP animal model.

Other early stage work on MERS-CoV therapeutics includes studies targeting viral fusion and/or viral proteases. Two potential targets for protease inhibition include MERS-CoV papain-like protease (PLpro) and 3-chymotrypsin-like protease (3CLpro). Recent studies have identified potential small molecule inhibitors with activity against several coronaviruses, including SARS-CoV and MERS-CoV.[4]

[4] Kilianski et al. Assessing activity and inhibition of Middle East Respiratory Coronavirus Papain-Like and 3CLike proteases using Luciferase-based biosensors. J. Virol 2013 87(21):11955-11962.

b) MERS-CoV Immunotherapeutic Treatment Candidates

Organization	Drug	Development Status
	IVIG	In clinic
Kingdom of Saudi Arabia	Convalescent serum	Clinical trials active, not recruiting[5]
NCI	M336, M337, M338	Live MERS-CoV neutralization; NHP studies
University of Hong Kong	MERS-4, MERS-27	Live MERS neutralization
Dana Farber Institute	3B11, 1F8, 3A1, 80R	Live MERS neutralization; NHP studies
University of Minnesota	Mersmab1	Live MERS neutralization
Regeneron	REGN3015, REGN3048	VLP neutralization, efficacy in humanized mice; testing in NHP in July; IND-enabling preclinical tox studies ongoing
Juntendo University	2F9 and YS110	VLP neutralization
Adimab	Anti-Spike	VLP-neutralization
Integrated Biotherpeutics	Anti-Spike	VLP neutralization
Sanford Research	Polyclonal	Mouse and NHP studies; IND enabling preclinical toxicology; Vialed product

Intravenous Immunoglobulin (IVIG) is a blood product containing pooled, polyvalent IgG recovered from the plasma of over a thousand donors. Since IVIG is isolated from the general population, it is not expected to contain MERS-CoV specific antibodies, and the most likely mechanism of action for IVIG is immunomodulation of the host inflammatory response, due to regulatory proteins and molecules present in IVIG. An alternate mechanism of action may involve coronavirus cross-reactive antibodies, which may be present in the pooled IVIG. These antibodies are not likely to neutralize MERS-CoV and would work indirectly via mechanisms such as antibody dependent cellular cytotoxicity or complement-dependent cytotoxicity. Intravenous immunoglobulin (IVIG) is currently available and has been used for the treatment of at least one MERS-CoV patient with unknown clinical benefit.

Convalescent plasma obtained from individuals who have recovered from MERS-CoV infection contains MERS-CoV specific neutralizing antibodies. Currently, there is no availability of convalescent plasma from MERS-CoV patients in the U.S. Convalescent plasma has been recovered from MERS-CoV infected animals (mice and NHPs) and has

[5] https://clinicaltrials.gov/ct2/show/NCT02190799

been tested for efficacy in the Ad-5 hDPP4 mice and in common marmosets and rhesus macaques. Another theoretical source of MERS-CoV convalescent plasma could be persons who receive any MERS-CoV vaccines in clinical trials. A pilot clinical trial of convalescent plasma treatment of MERS-CoV patients (10 patients with convalescent plasma and 10 controls) is ongoing but not recruiting in Saudi Arabia. Convalescent plasma is unlikely to be available in the US until vaccine trials commence.

New techniques for the rapid isolation of MERS-CoV neutralizing monoclonal antibodies (mAbs) were employed by at least eight independent groups, many funded by NIAID, resulting in the discovery of nearly ~14 lead fully human mAbs capable of neutralizing the spike protein of MERS-CoV. The isolated mAbs were tested for neutralization of virus-like particles containing the MERS-CoV spike protein or against live MERS-CoV and had IC_{50}s in the low μg/ml range, allowing for a reasonable human dose. All of the reported antibodies bind to the receptor-binding domain of the MERS-CoV spike protein and neutralize virus by inhibiting the binding of the spike protein to the human DPP4 cellular receptor.

Of the groups who identified multiple mAbs that neutralize the spike protein, many identified antibody pairs that do not compete for the same binding site and could be used in tandem. This approach might reduce the emergence of escape viral mutants. Since until recently there was no small animal model that exhibits severe disease, therapeutic testing of mAbs has been mostly limited to NHPs. The National Cancer Institute (NCI) produced a small batch of their M336 mAb for testing in the common marmoset model. The M336 mAb was given twice (at 4-6 hours post infection then on day 2) at a high concentration (30 mg/kg). Iniital data indicate that 1 of the 3 treated NHPs had lowered viral titer compared to placebo. Further plans at the NCI include investigating infection challenge following immunoprophylaxis, and expansion of production capacity by involving a contract manufacturing organization (CMO). The Dana-Farber Cancer Institute conducted prophylaxis studies of their mAb in rhesus macaques. CT scans suggested efficacy of antibody in reducing lung inflammation. This organization is partnering with two CMOs and has produced one lot (enough for NHP studies and some mice studies) of its lead mAb candidate (3B11) and could produce a GMP lot in 6-8 months under ideal circumstances.

Regeneron produced a humanized mouse expressing the human DPP4 receptor under the natural promoter. This mouse is susceptible to MERS-CoV and treatment or prophylaxis of the mice with the Regeneron mAbs significantly decreased lung titer and lung pathology compared to placebo. Regeneron also has experiments planned in NHPs (July 2015) to test the treatment efficacy of their mAbs. Regeneron has a large capacity and considerable expertise in the manufacture of mAbs and could make a GMP lot in 6-8 months in ideal circumstances. Currently, Regeneron has 100s of grams (enough for 10s of treatments) of non-GMP material which is being used to support IND-enabling preclinical toxicology studies.

There are also companies that can provide antibody discovery services for commercial development. Using various proprietary techniques, mAbs can be developed

targeting any recombinant antigen. If available, affinity matured neutralizing antibodies could also be isolated from the PBMCs of patients who have recovered from MERS-CoV. Expression of the antibodies can then be transferred to standard cell culture lines for large-scale production and purification.

At least one company is pursuing development of MERS-CoV polyclonal antibodies. Sanford Research has produced a transgenic cow expressing fully human B cells from an artificial chromosome (Tc Bovine). Immunization of Tc Bovine with inactivated MERS-CoV virus or purified MERS-CoV spike results in the enrichment of B cells secreting antibodies that can bind and/or neutralize MERS-CoV. Total antibody is extracted from the cows by plasmapheresis. Each cow can produce large amounts of polyclonal antibodies once vaccinated, and a large quantity (200 gallons) of plasma with high titers of neutralizing antibodies has been collected and vialed. The polyclonal preparation has been tested as a therapy or prophylaxis in rhesus macaques and in the adenovirus mouse model. Sanford has produced a pilot GMP Lot, held a Pre-IND meeting with FDA CBER, and is undergoing IND-enabling toxicology studies in preparation for a Phase 1 clinical trial.

Appendix 5. Vaccines in Development

a) MERS-CoV Human Vaccine Candidates

Organization (Vaccine type)	Preclinical Immunogenicity (species)	Preclinical Efficacy (challenge model)	Scale-up Capability/Timing
Novavax (S protein trimer in 40 nm particle; adjuvanted likely)	Mouse-immunogenicity shown (Camel studies being planned)		Yes/≈6 months for manufacture Ph I vaccine
NIAID/VRC (Two candidate vaccine approaches: DNA S prime-S1 protein boost and S1 prime-S1 boost)	Mouse and NHP-immunogenicity shown	NHP (macaque-radiological efficacy shown) (Camel and marmoset studies planned)	Yes, at NIAID
Inovio (DNA expressing S; electroporation device)	Mouse, NHP and camel immunogenicity shown	NHP (viremia, lung pathology and survival)	Yes, via GMP affiliate.
Greffex (full S expressing, fully deleted Adeno packaging vector; RBD constructs being developed)	Mouse immunogenicity shown		
NIAID Supported Extramural Projects (Adeno vector, Recombinant Spike, Live attenuated)	Platform development and mouse immunogenicity	Mouse model development to evaluate vaccine efficacy	

NIAID/NIH, Novavax, Inovio and Greffex have demonstrated immunogenicity in mice using the MERS-CoV Spike (S) protein as an immunogen, through subunit and/or DNA vaccine platforms. The Vaccine Research Center (VRC), NIAID, is evaluating two approaches based on the S protein, an S DNA prime-S1 subunit protein boost regimen and an S1 subunit protein prime-S1 subunit protein boost. Favorable immunogenicity data (generation of high quality neutralizing antibody) have been generated in mice and in NHP

(rhesus macaque) systems, the latter system showing radiological efficacy after virus challenge. The DNA prime-protein boost approach elicited responses to a larger number of epitopes, a more favorable T cell response and better protection. The protein-only approach, however, may be sufficient and would be simpler and more practical. Studies of vaccination followed by virus challenge are planned in marmosets and camels. Inovio has completed NHP challenge studies which have shown protection (viremia, lung pathology and survival data). Inovio has also demonstrated immunogenicity in camels. All four candidate vaccines in development can be scaled up in manufacturing to support phase I human clinical trials. Inovio is currently engaged in preparations for IND submission and start of a Ph1 clinical trial in 2015. For the others, preliminary and probably best-case estimates are that timing for phase I trials may range from 6-18 months, depending upon availability of funding. The Inovio vaccine candidate utilizes a delivery device that facilitates intracellular delivery of DNA by electroporation, and therefore manufacturing and scale-up of this device must be considered for overall production. Greffex utilizes a fully deleted adenoviral packaging vector to deliver S protein-expressing DNA. The company is also developing additional constructs that express partial S protein sequence focusing on the receptor-binding domain (RBD) to possibly better target the protective immune response. Murine immunogenicity studies are scheduled to begin in June.

In addition to the above, a number of MERS-CoV vaccine candidates are being developed with funding/support from NIAID/DMID. These include vaccines based on recombinant S protein receptor binding domain, adenovirus-vectored S protein vaccines and work aimed at developing a modified live vaccine (MLV). One such MLV is deleted genetically of an essential gene (E) and is consequently attenuated, being able to undergo only a single cycle of infection in the host yet providing appropriate immune stimulation theoretically. These extramural projects are at a very early stage of development. To further support vaccine development, VRC/NIAID has developed serological assays (including a panel of 8 pseudotyped lentivirus reporters for assessing neutralizing antibodies) and reagents (including neutralizing murine mAbs with specificities to multiple regions of the S protein). For MERS-CoV vaccine development to progress toward clinical evaluation, additional preclinical data will be necessary along with appropriate financial support.

Camel vaccination

b) MERS-CoV Camel Vaccine Candidates

Organization	Vaccine Type	Camelid Vaccination Studies (PI)
USG/academic institution consortium	S protein nano-particle /Matrix C adjuvant (baculovirus expressed)	Camel vaccination without active challenge, in Saudi Arabia, Egypt, Qatar, and Mongolia; projected start July 2014
USG/academic institution consortium	Inactivated whole virus	See above
Rocky Mountain Laboratories, NIAID	S protein subunit vaccine/Advax adjuvant (baculovirus expressed)	Camel and alpaca vaccination-challenge studies at CSU started in June 2014; analysis in progress
Erasmus University	MVA vectored S protein	In progress, in Qatar, using a camel infection model
Novavax A.B. (Sweden)	S nanoparticles with Matrix C adjuvant	In discussions with KSA Department of Agriculture

Only limited suitable facilities are available for livestock vaccination experiments under high-containment conditions in the US: Colorado State University, USDA (Ames, Iowa) Texas A&M, and Kansas State University. All the facilities/PIs have expressed interest in MERS-CoV livestock studies if funding becomes available. Collaborations for MERS-CoV camel vaccine studies are being explored or planned in the Middle East, East Africa, and Mongolia involving three vaccine candidates. In addition, RML is currently investigating the use of Alpaca's (new world camelid) as a suitable animal model for vaccine studies.

From: Aviles, Natalie (OS/ASPR) (CTR)
Sent: Fri, 10 Apr 2015 13:56:44 +0000
To: Swerdlow, David (CDC/OID/NCIRD); Uyeki, Timothy M. (CDC/DDID/NCIRD/ID); Gerber, Sue (CDC/CGH/DGHA); Haynes, Lia (CDC/DDPHSIS/CPR/OD); Erdman, Dean (CDC/OID/NCIRD); Pallansch, Mark A. (CDC/DDID/NCIRD/DVD); (b)(6) (b)(6) Chaitram, Jasmine (CDC/DDPHSS/CSELS/DLS); Carter, Wendy (FDA/CDER); Bavari, Sina; Miele, Peter (FDA/CDER); O'Rear, Julian (FDA/CDER); Deming, Damon (FDA/CDER); Merlin, Toby (CDC/OID/NCEZID); Feldmann, Heinrich (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Marinissen, Maria (HHS/OS/OGA); Annelli, Joseph; DiEuliis, Diane (HHS/ASPR/OPP); Bright, Rick (HHS/ASPR); Donabedian, Armen (HHS/ASPR/BARDA); Robinson, Robin (HHS/ASPR/BARDA); Maher, Carmen (FDA/OC); Hensley, Lisa (NIH/NIAID) [E]; Kelley, Cynthia (FDA/CBER); Spiro, David (NIH/FIC) [E]; Fisher, Robert (FDA/OC); Hojvat, Sally A (FDA/CDRH); Beigel, John (NIH) [E]; Murray, Jeffrey S (FDA/CDER); Olinger, Gene (NIH/NIAID) [C]; (b)(6) Matthew Frieman; (b)(6) Baric, Ralph; Denison, Mark (NIH); (b)(6) (b)(6) (b)(6) Thomas, Stephen J; Cho, David S (CBER) (FDA/CBER); Stanley Perlman; Roberts, Rosemary (FDA/CDER); (b)(6) (b)(6) Jaffe, Richard (OS/ASPR); (b)(6) Zoon, Kathryn (NIH/NIAID) [V]; Bowen, Richard; (b)(6) Subbarao, Kanta (NIH/NIAID) [E]; (b)(6) (b)(6) Ferro, Philip (HHS/ASPR/IO); Wathen, Lynne (HHS/ASPR/BARDA); (b)(6) (b)(6) (b)(6) Gay, Cyril; Sutton, Troy (NIH/NIAID) [F]; Houser, Katherine (NIH/NIAID) [C]; Czako, Rita (NIH/NIAID) [F]; Gretebeck, Lisa (NIH/CC/OD) [F]; Vogel, Leatrice (NIH/NIAID) [E]; Lamirande, Elaine (NIH/NIAID) [E]; (b)(6) Elliott Fineman'; 'Olinger, Gene G'; 'Dan Adams'; Erlandson, Karl (OS/ASPR/BARDA); 'Baric, Toni C'; 'Sven Andreasson'; 'Jensen, Victoria M. (CTR)'; 'Zhu, Quan'; 'Tang, Xianchun,Phd'; (b)(6) 'Cheryl Kofford'; OHara, Michael (HHS/ASPR/BARDA) (CTR); Sciarretta, Kimberly (OS/ASPR/BARDA)
Cc: Korch, George (OS/ASPR/IO); Davis, Jon (OS/ASPR); Lurie, Nicole (HHS/ASPR/IO); Underwood, Lauren (HHS/ASPR/IO)
Subject: MERS-CoV Stakeholders Workshop - Consolidated Presentations & Feedback Survey
Attachments: MERS-CoV Preparedness Workshop.pdf

Dear Colleagues,

Attached is the consolidated presentation deck from the MERS-CoV Stakeholders Workshop held on April 3, 2015. Please keep in mind that (as mentioned in the workshop) it has been requested these slides/presentations not be shared beyond the immediate recipients of the workshop invitation.

Notes with high points and a report will also be distributed to workshop participants when completed.

In order to improve our future workshops and accommodations, we would appreciate it if you could take 2-4 minutes to complete our online survey. Again—thank you for your participation and great discussion!

Feedback Survey Link:
https://www.surveymonkey.com (b)(6)

Very respectfully,

Natalie (Hanrion) Aviles
Project Manager
DHHS/ASPR/IO – Special Projects
Detailed from Office of Portfolio Management BARDA
Conceptual MindWorks, Inc (contractor)
Blackberry: (b)(6)
Office : (b)(6)
Email: (b)(6)
www.medicalcountermeasures.gov

MERS CoV Stakeholder Workshop

Friday, 3 April 2015

10:00 AM – 2:30 PM (EDT)

ASPR – O'Neill Building
Willow Conference Room (Lower Level)
200 C Street SW
Washington, DC 20024

Welcome &
Opening Remarks

Dr. Nicole Lurie,

Assistant Secretary for Preparedness & Response (ASPR)

Introduction & Purpose

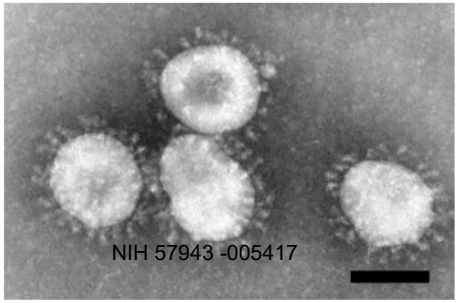
Dr. George Korch,
ASPR, Senior Science Advisor

Introduction to Coronaviruses

Ralph Baric, PhD

No Approved Vaccines or Therapeutics against the SARS-CoV or MERS-CoV;
MERS-CoV: 1090 Cases/444 deaths/2.5 yrs; H5N1: 784 cases/429 deaths/13 years;

Both outbreaks are ongoing



MERS-CoV:

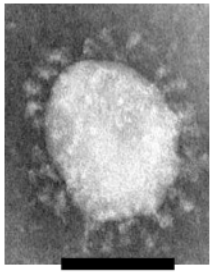
N Protein

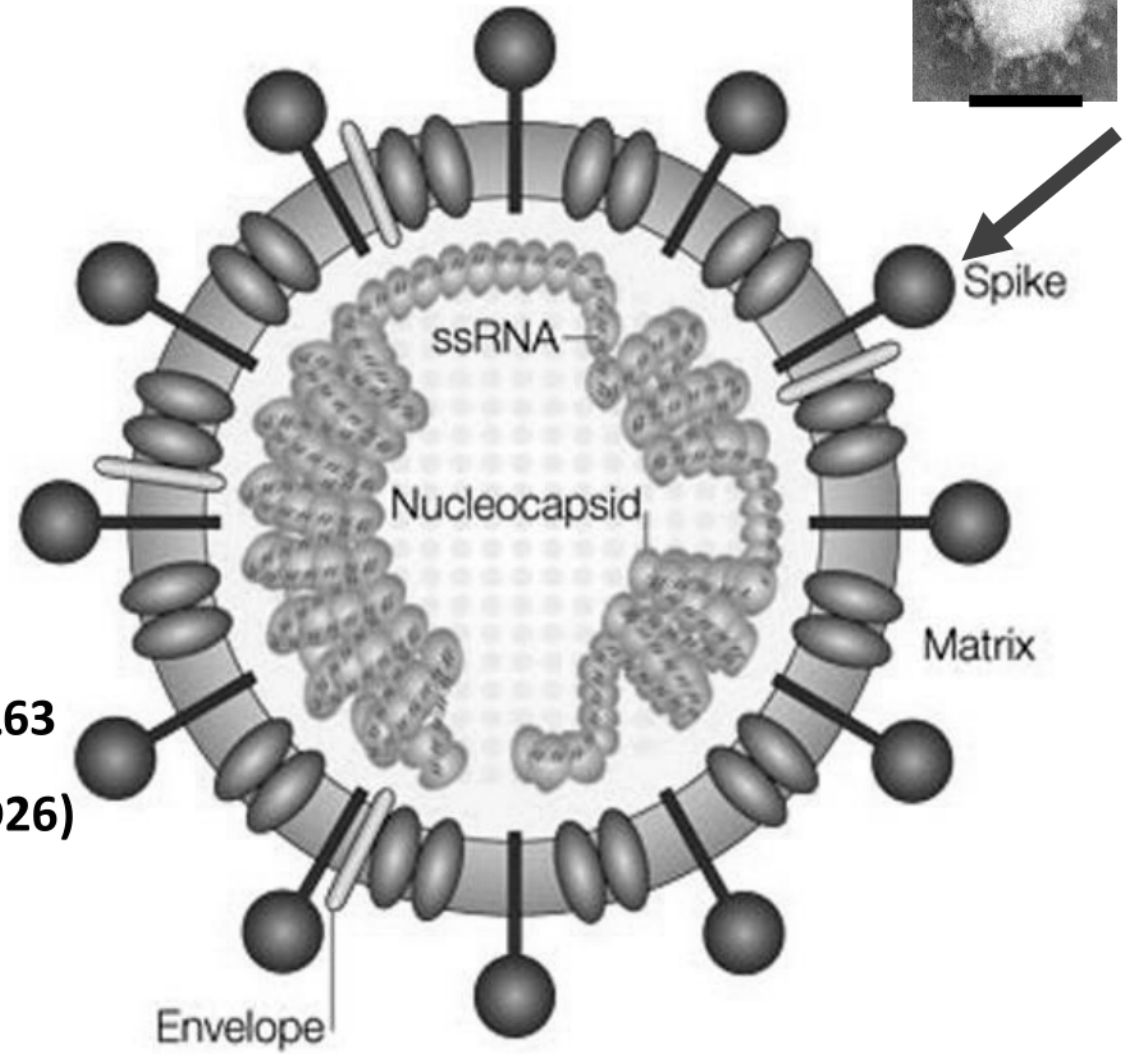
M Glycoprotein

E Protein

S glycoprotein

- **Receptor Binding/entry**
  - **Angiotensin I Converting Enzyme 2 – SARS-CoV + NL63**
  - **Dipeptidyl Peptidase 4 (CD26) (DPP4)—MERS-CoV**
- **Neutralizing epitopes**
- **Major component protective immunity**





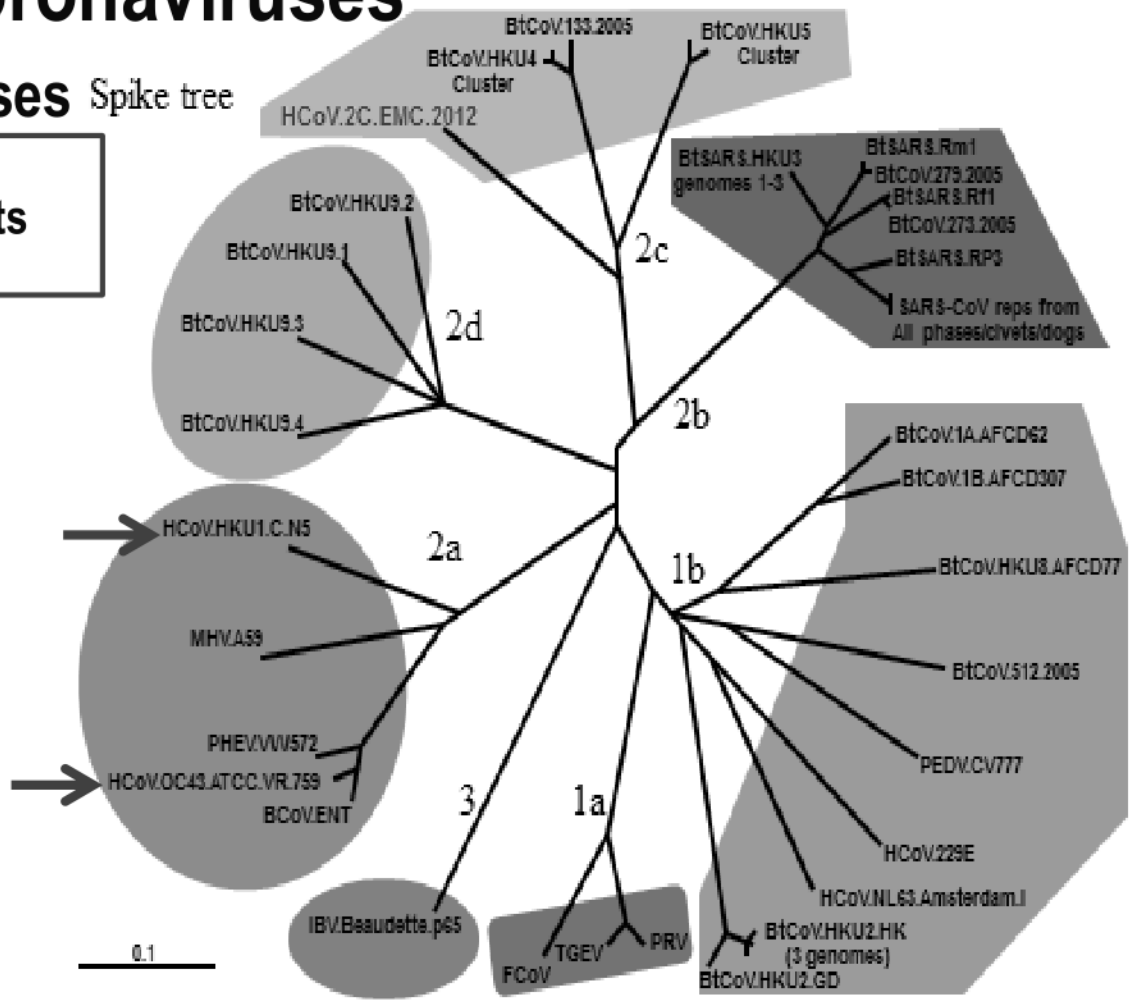
NIH 57943 -005418

Perlman et. al. Nature Reviews Immunology, 2005

Coronavirus Phylogeny

Origins of Human Coronaviruses

- **Five Human Coronaviruses**
  - HCoV-OC43 — **rodents**
  - HCoV-HKU1 — **rodents**
  - **SARS-CoV** — **bats**
  - HCoV-229E
  - HCoV-NL63



Coronavirus Phylogeny

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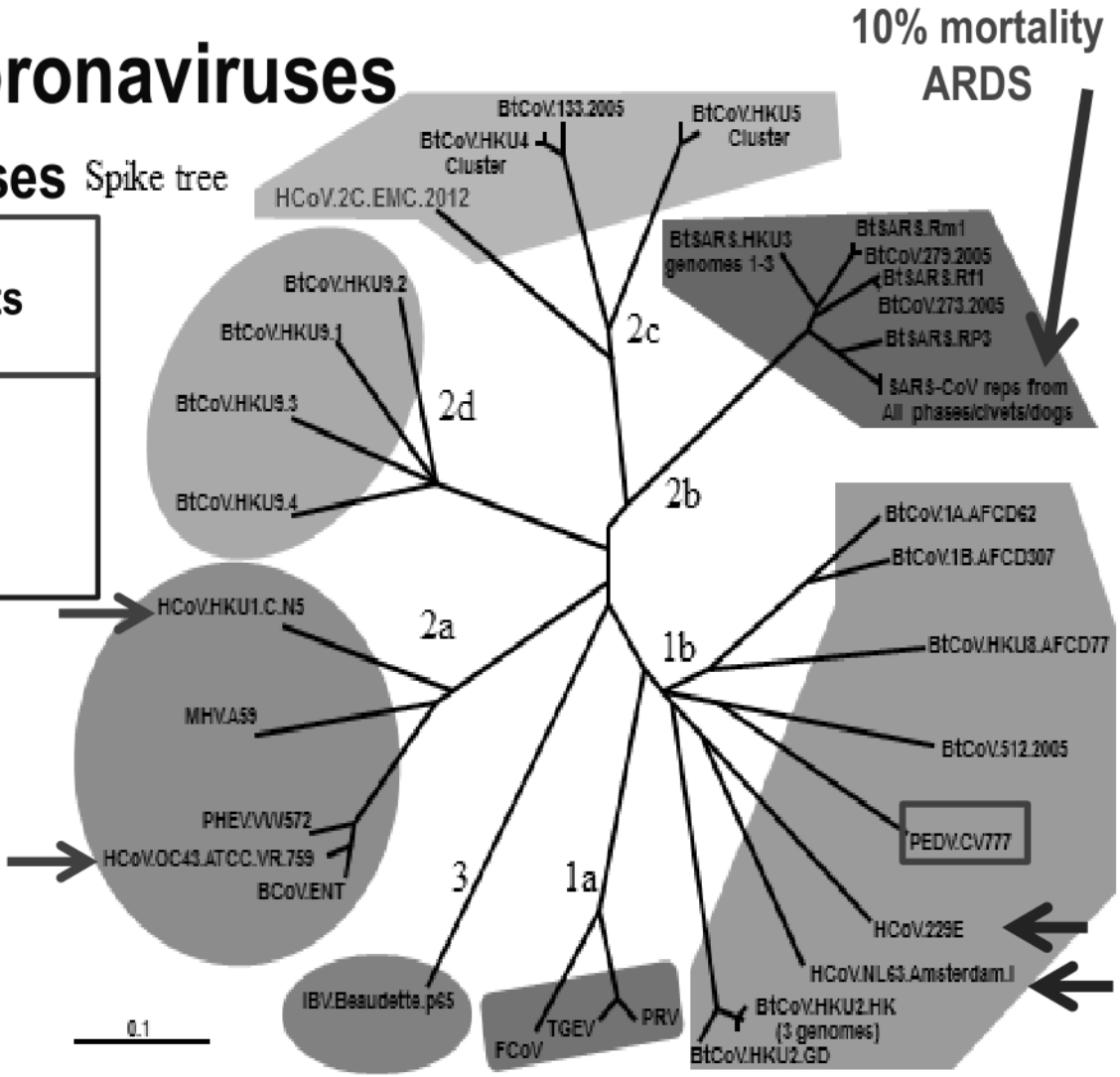
◆ HCoV-229E	
◆ HCoV-NL63	



Coronavirus Phylogeny

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- **Five Human Coronaviruses**
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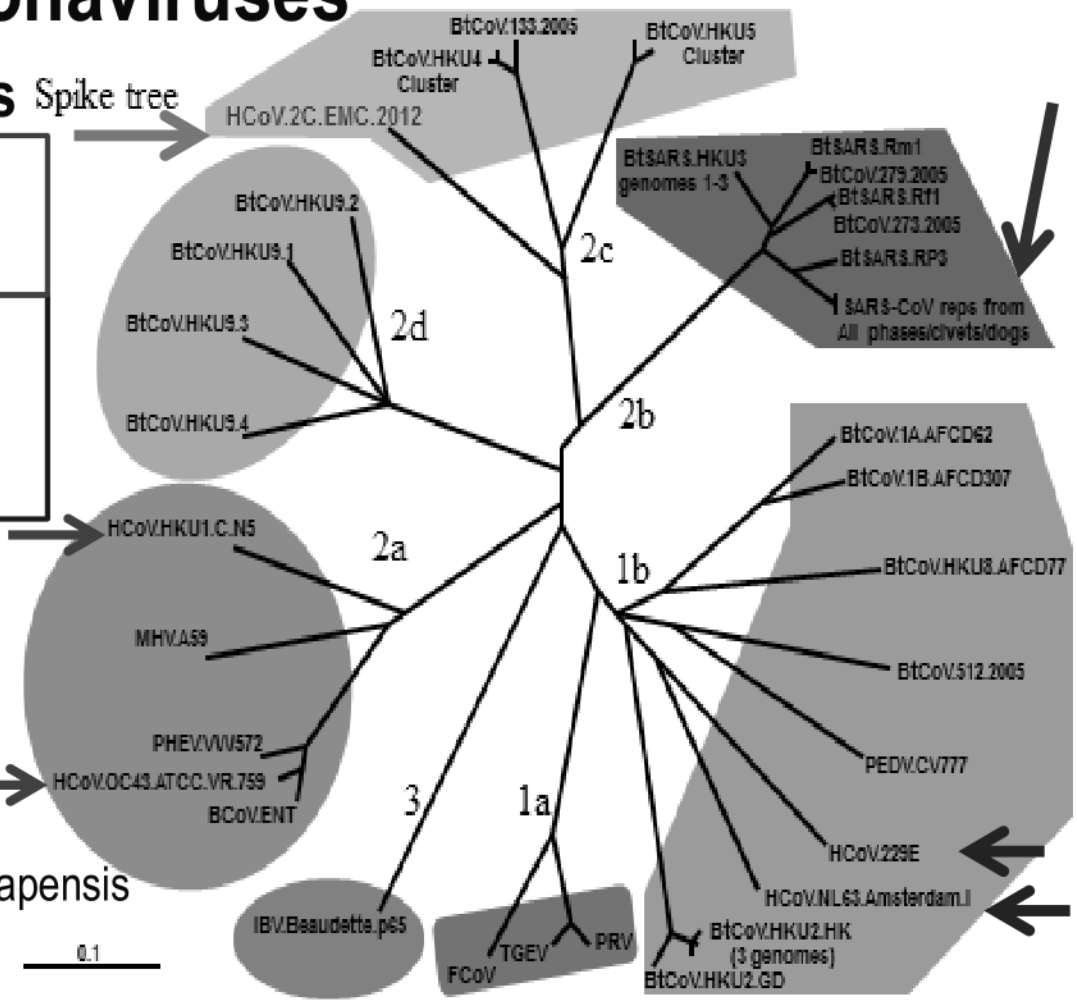
  - **SARS-CoV** — **bats**
  - HCoV-229E — **bats**
  - HCoV-NL63 — **bats**
- **Sixth Human Coronavirus**
  - **Group 2c betacoronavirus**
    - **MERS-CoV**
    - HKU4 and HKU5 (bats)
    - South African Neoromicia capensis bat (**NeoCoV**)(~85%)



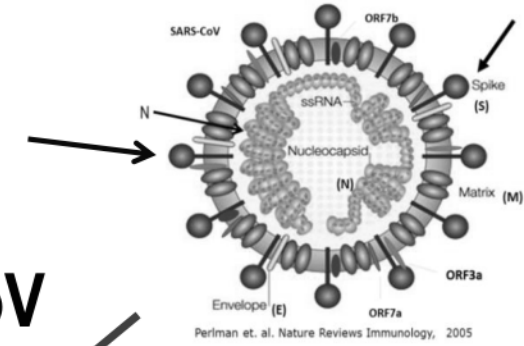
Originated: bats (most likely via intermediate host):

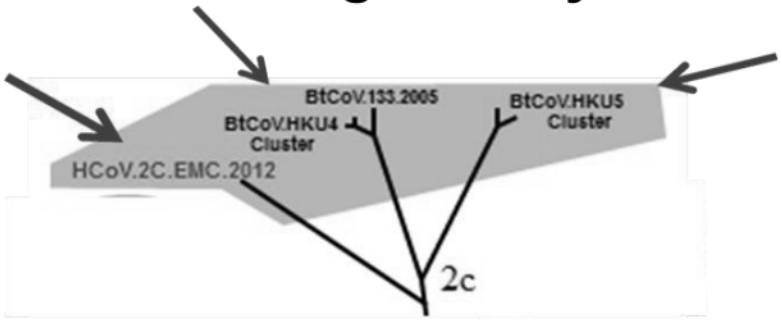
- **SARS/MERS model**

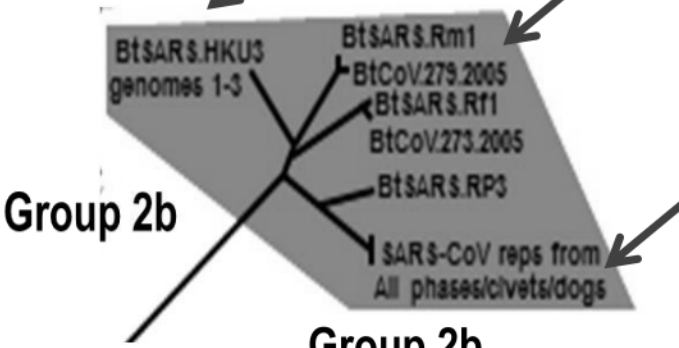
MERS-CoV

Group 2c Betacoronavirus

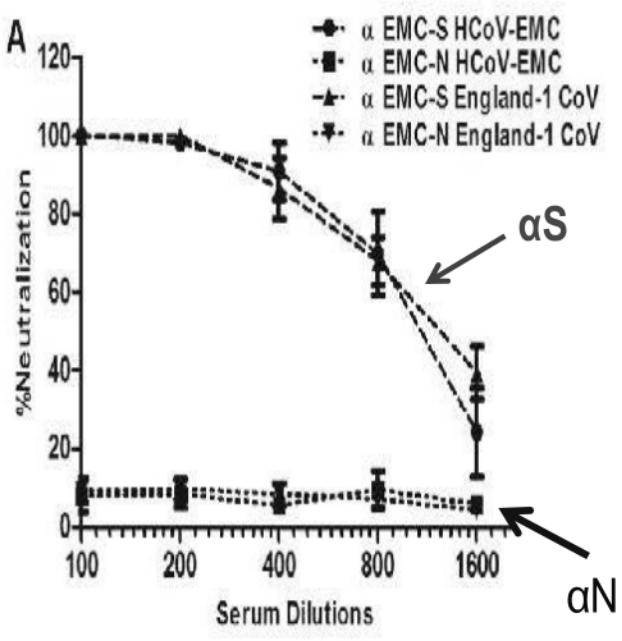
- **MERS-CoV S Antigenically Distinct: other CoV**

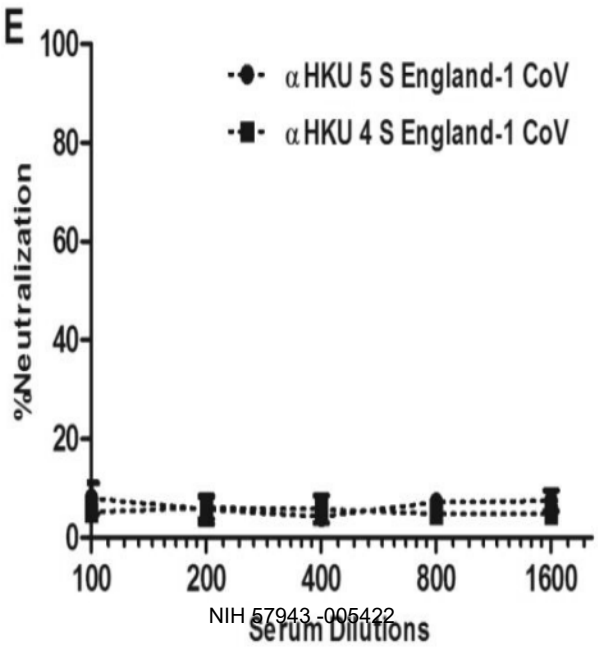


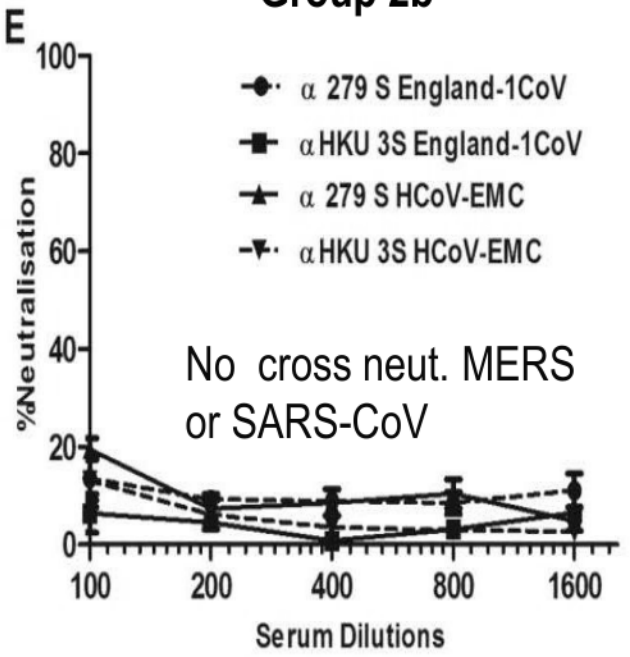




MERS-CoV **Group 2c** **Group 2b**







Agnihothram S et al.., 2014 JID

Emerging CoV

Virus	Species	Emergence
HCoV-NL63	Human	500-800 years
HCoV-229E	Human	200-300 years
HCoV-OC43	Human	~120 years
PEDV	Porcine	~30 years ← 2013 in US
PRRSV	Porcine	~20 years
BCoV	Bovine	~20 years
SARS-CoV	Human	~10 years
MERS-CoV	Human	~2 years

DRIVERS CoV EVOLUTION: RNA Recombination and mutation

MERS-CoV: Origins

Bats

Dromedary Camels
13+ million

Humans

40% mortality, ARDS
Age related mortality
Pandemic Potential?

MERS-CoV Origins

Direct Bat to Human Transmission?

Bats

Dromedary Camels
13+ million

Humans

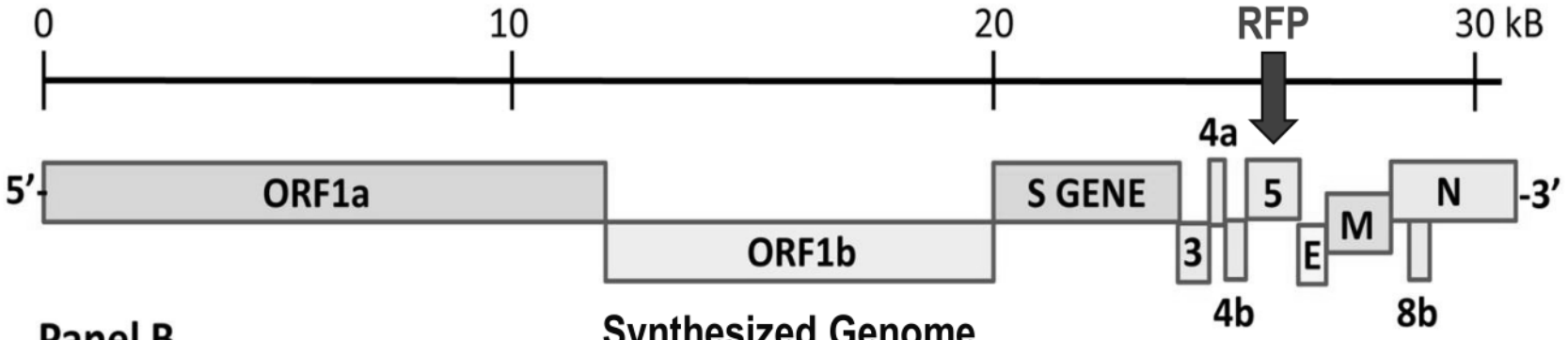
?

Other Intermediates?

Exact Species?
Heterogeneous MERS-like
Other Prepandemic
Strains?

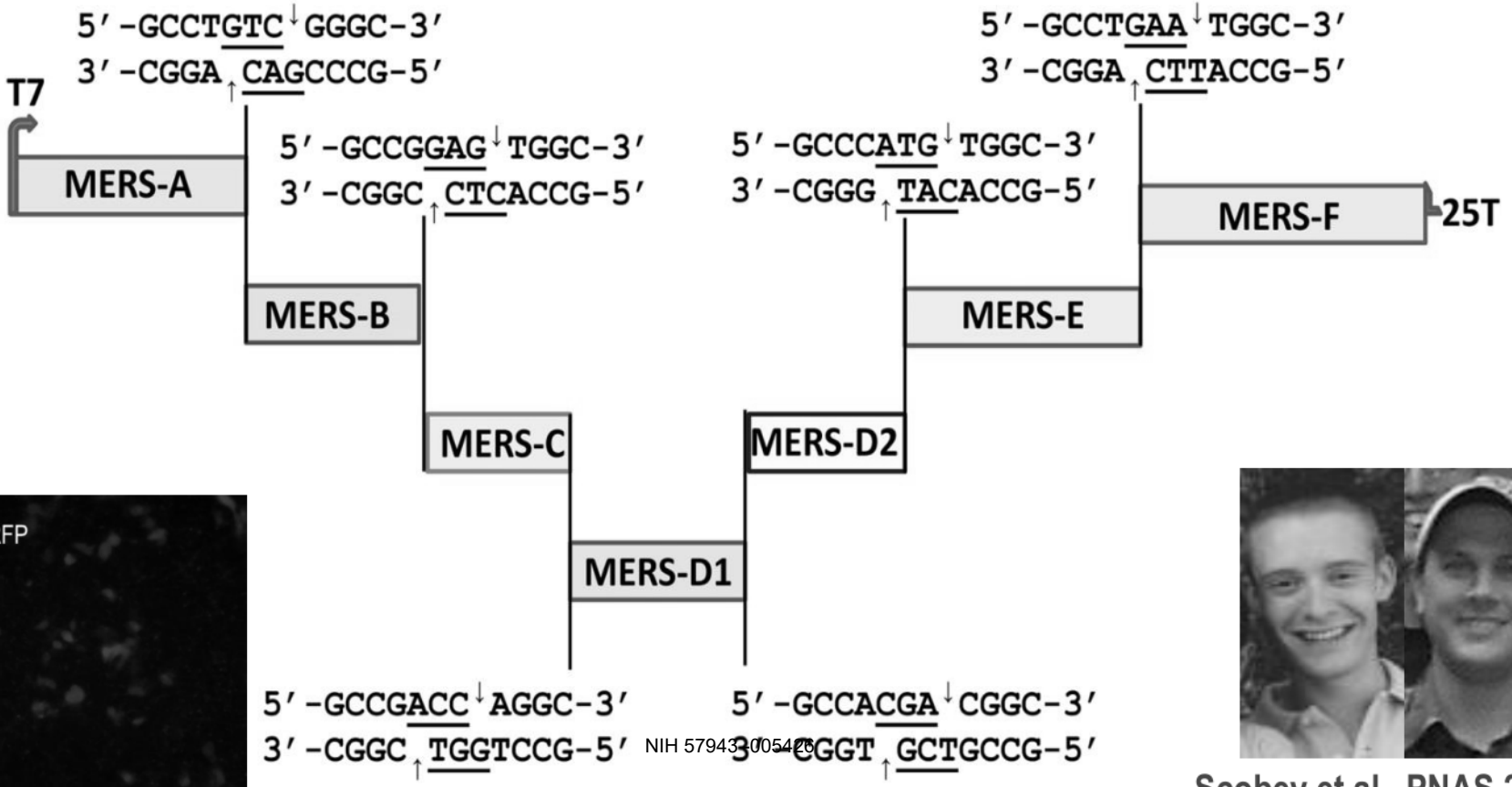
40% mortality, ARDS
Age related mortality
Pandemic Potential?

MERS-CoV Molecular Clone



Panel B

Synthesized Genome



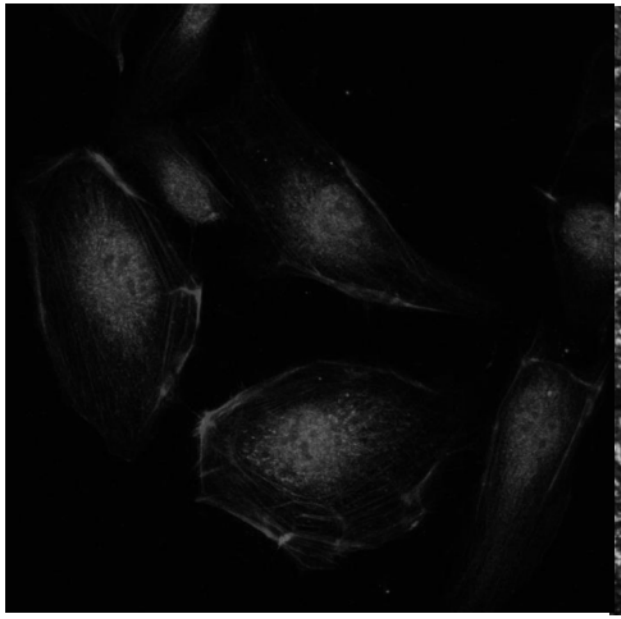
Scobey et al., PNAS 2013

MERS-CoV: Receptor Distribution/Human Lung

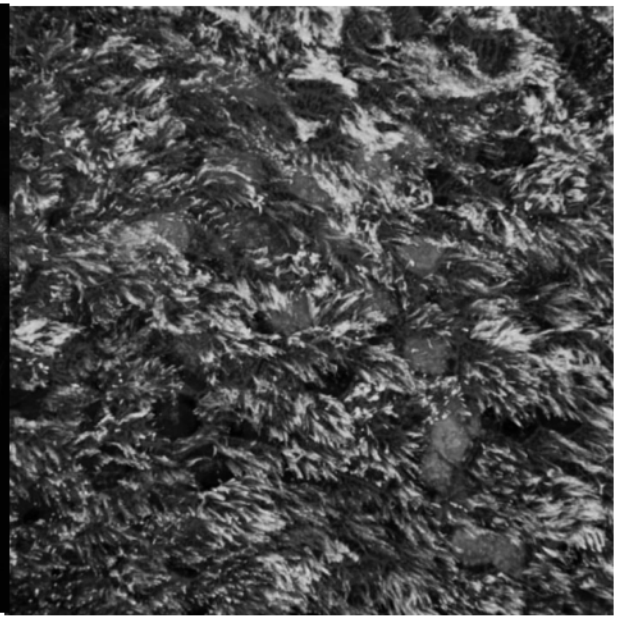
- Acute Respiratory Distress Syndrome
  - Dipeptidyl peptidase 4 (DPP4)-
    - **Receptor:** Nonciliated and ciliated airway epithelial cells, lung microvascular, type II pneumocytes, fibroblasts, etc.

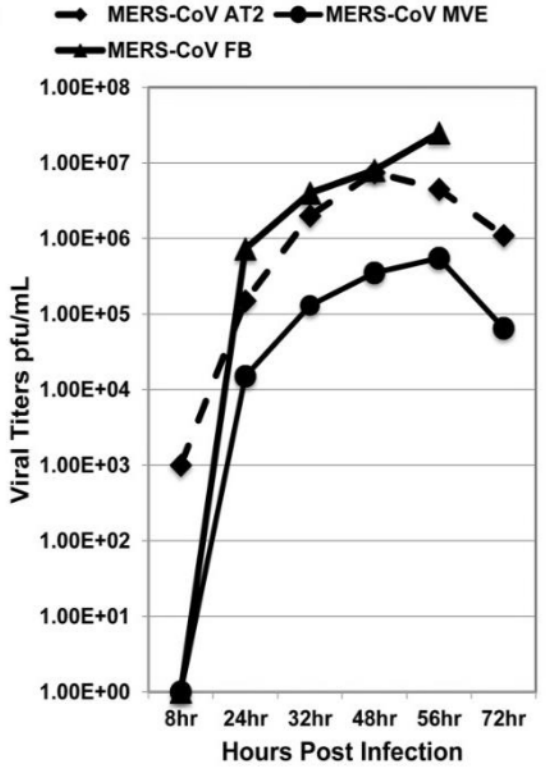
Blue-nucleus
Red-actin
White-cilia
Green-DPP4

Lung Microvascular Endothelial Cells

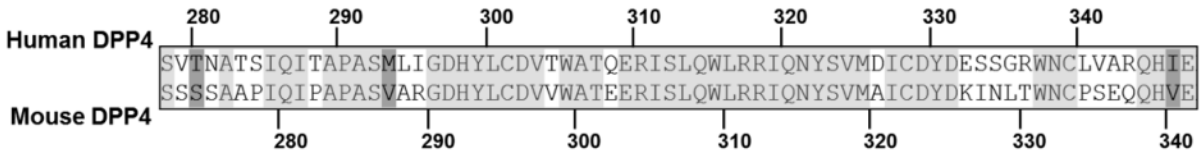


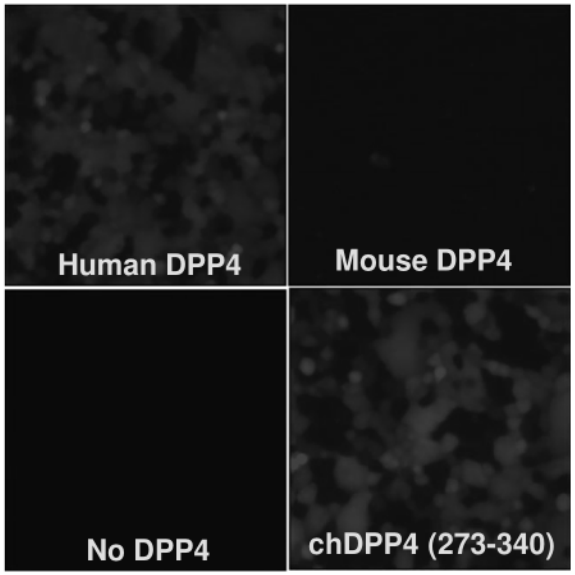
HAE Cultures





DPP4 Species Specificity Determinants

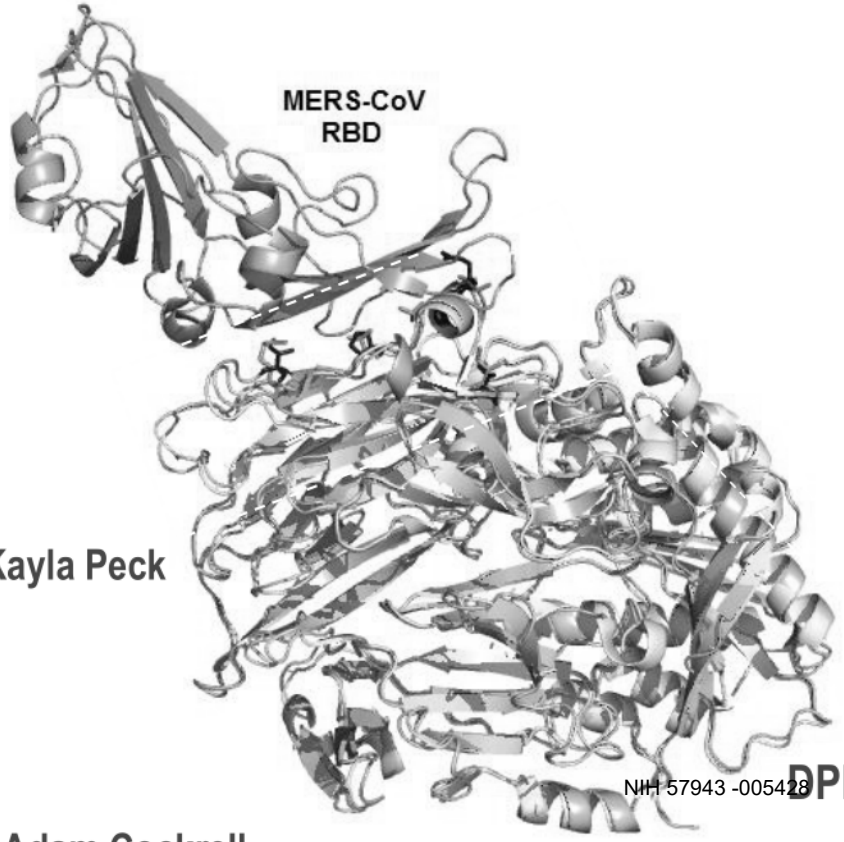


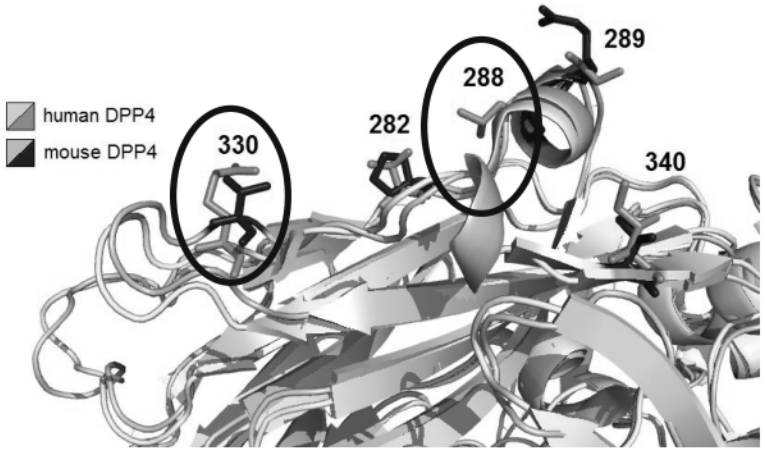


MERS-CoV RFP

Kayla Peck

Adam Cockrell

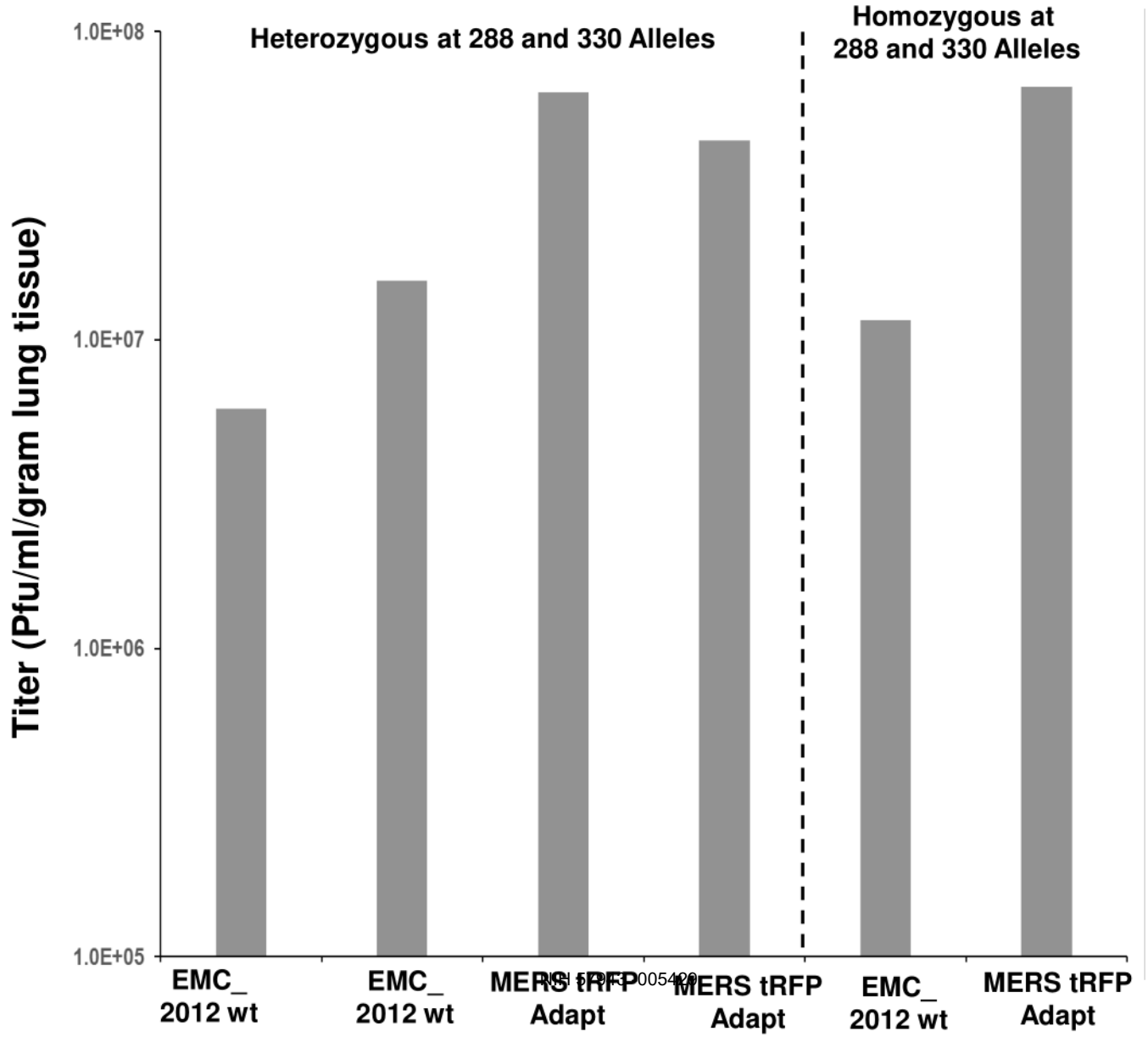




Numbered relative to moue DPP4

Cockrell et al., JVirol 2014

MERS-CoV Replicates in Lungs of Mice Heterozygous and Homozygous for 288L and 330R

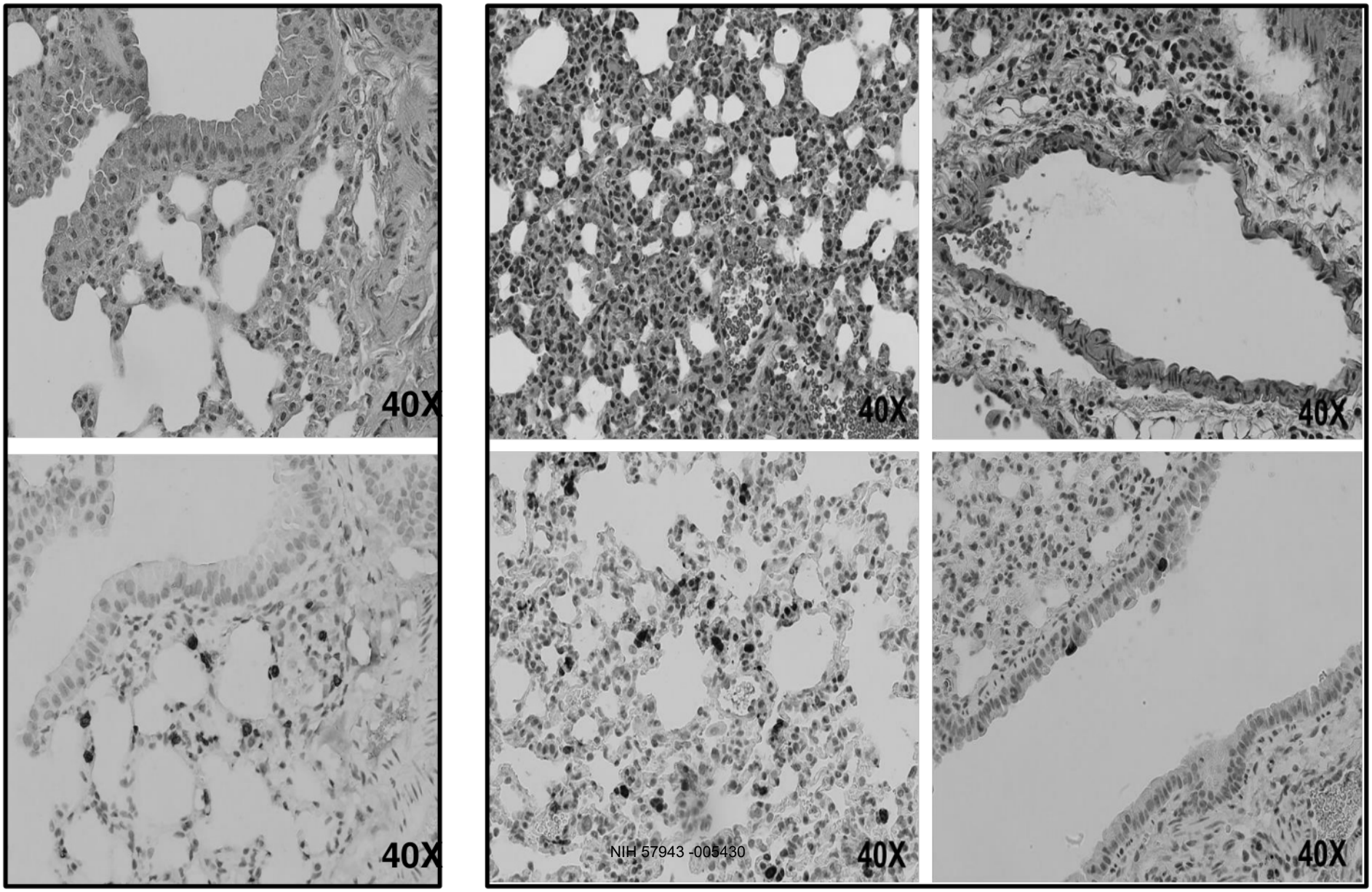


PLoS Pathog 2015;11(12):e1005409

MERS-CoV Replication Occurs in Parenchyma…Near Airways in Early

P1 at 3D p.i.

P13 at 3D p.i.



Vaccine/Therapeutic Challenges

- **Therapeutics: Drugs-Anti-MERS-CoV activity in vitro**
- **No Licensed Vaccines: MERS-CoV**
- **Consider human and animal (camel) vaccines**
- **Vaccines (S) work well in young animals:**
  - Neutralizing antibodies against S protect
  - Most vaccines only evaluated in replication model
    - Robust animal models ⟹ vaccine efficacy/complications
- **Aged Populations: Most Vulnerable (50-70% mortality)**
  - **Vaccination:**
    - Immunosenescence-key target population for MERS-CoV vaccine
    - Neutralizing antibody response drops ~10-15 fold or moew (1 yr mice)
- **Potential for TH2 Immune Pathology (SARS, SARS-like)**
- **Host Genetics and Vaccine Performance**

Epidemiology & Clinical Management

MERS-CoV

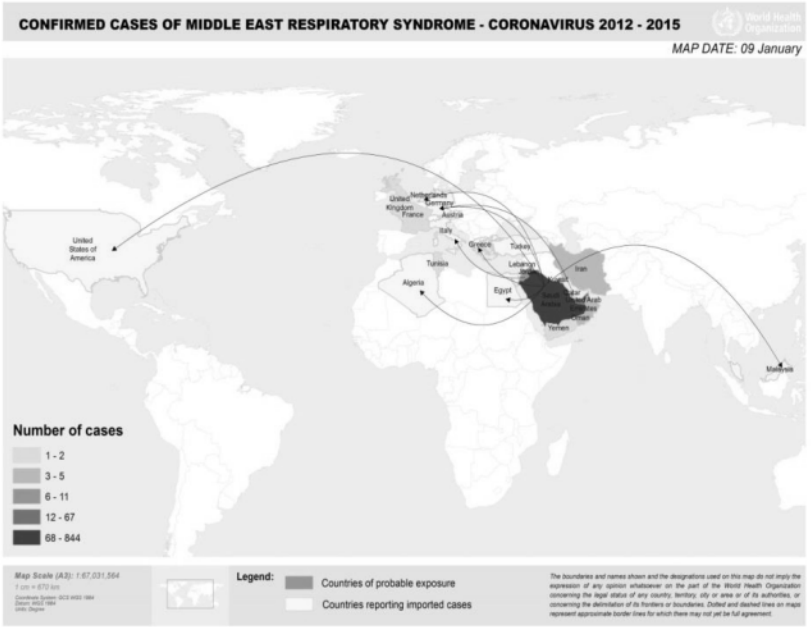
Situational update

03 April 2015

World Health Organization

Middle Eastern Respiratory Syndrome Coronavirus: MERS-CoV

- Novel coronavirus emerged in 2012 in Saudi Arabia
  - Since then more than 1,100 laboratory-confirmed cases reported to WHO
  - Majority (>85%) of the cases reported from KSA
  - 23 countries affected; Exported cases to several European countries, the US and Asia

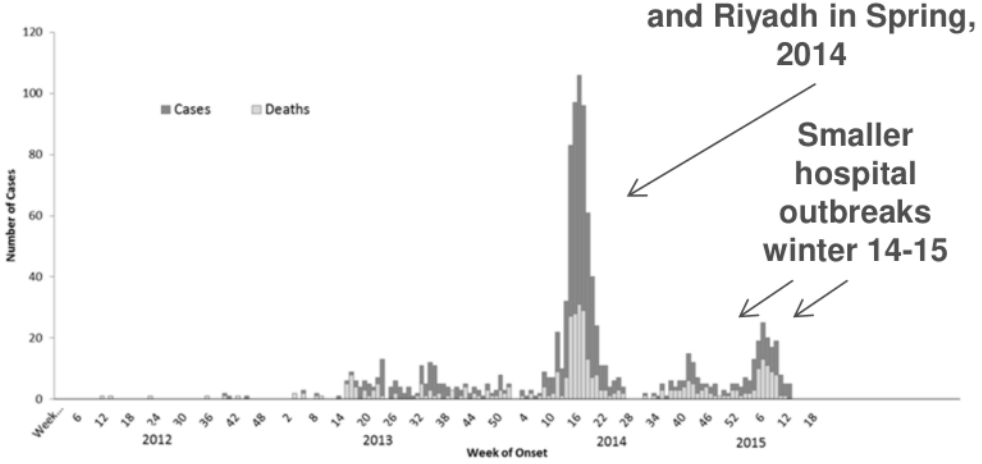


Severity

- Approx 50% of cases are severe/fatal, including >351 reported to have died
- Most severe cases reported in men, over the age of 60, with underlying conditions (e.g., diabetes, obesity, hypertension, renal deficiency)
- Secondary cases are younger, heathier and higher proportion are women
- Many secondary cases are health care workers

Control of MERS-CoV

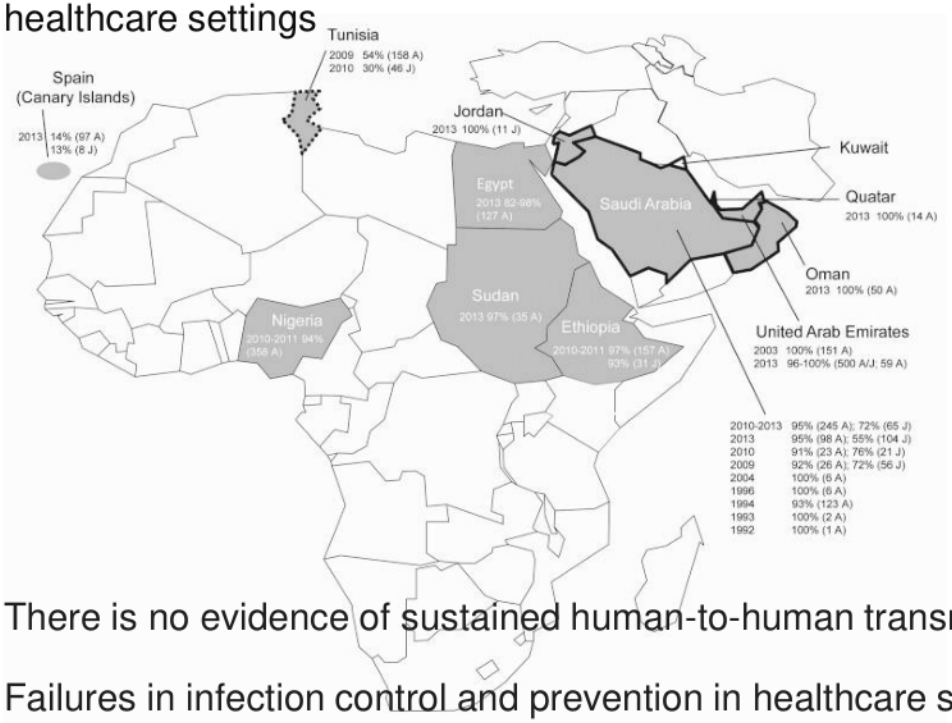
- **Where is it coming from?**
  - **MERS-CoV is phylogenetically related to bat coronaviruses**
  - **MERS-CoV has been circulating in dromedary camels in the Middle East and many parts of Africa for decades**
  - **Believed to be transmitted from camels-to-humans, with subsequent human-to-human spread mainly in health care settings**

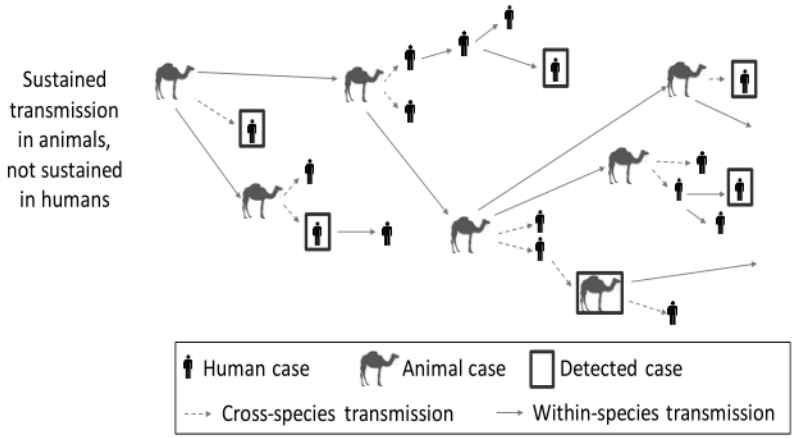


- **How can we stop transmission?**
  - Good surveillance
  - Intensive and joint animal/human investigations for every case
  - Limit contact with camels if in at risk group (>60 year old male with underlying conditions)
  - Stop nosocomial transmission with strict adherence to infection prevention and control practices
  - More research is needed to better understand transmission patterns

Epidemiology

- MERS-CoV is widespread in camel populations in the Middle East and Africa
- Pattern of the epidemic is repeated sporadic introductions of the virus from dromedary camels (and possibly other not-yet identified animals) to people, resulting in limited human-to-human transmission, particularly in healthcare settings

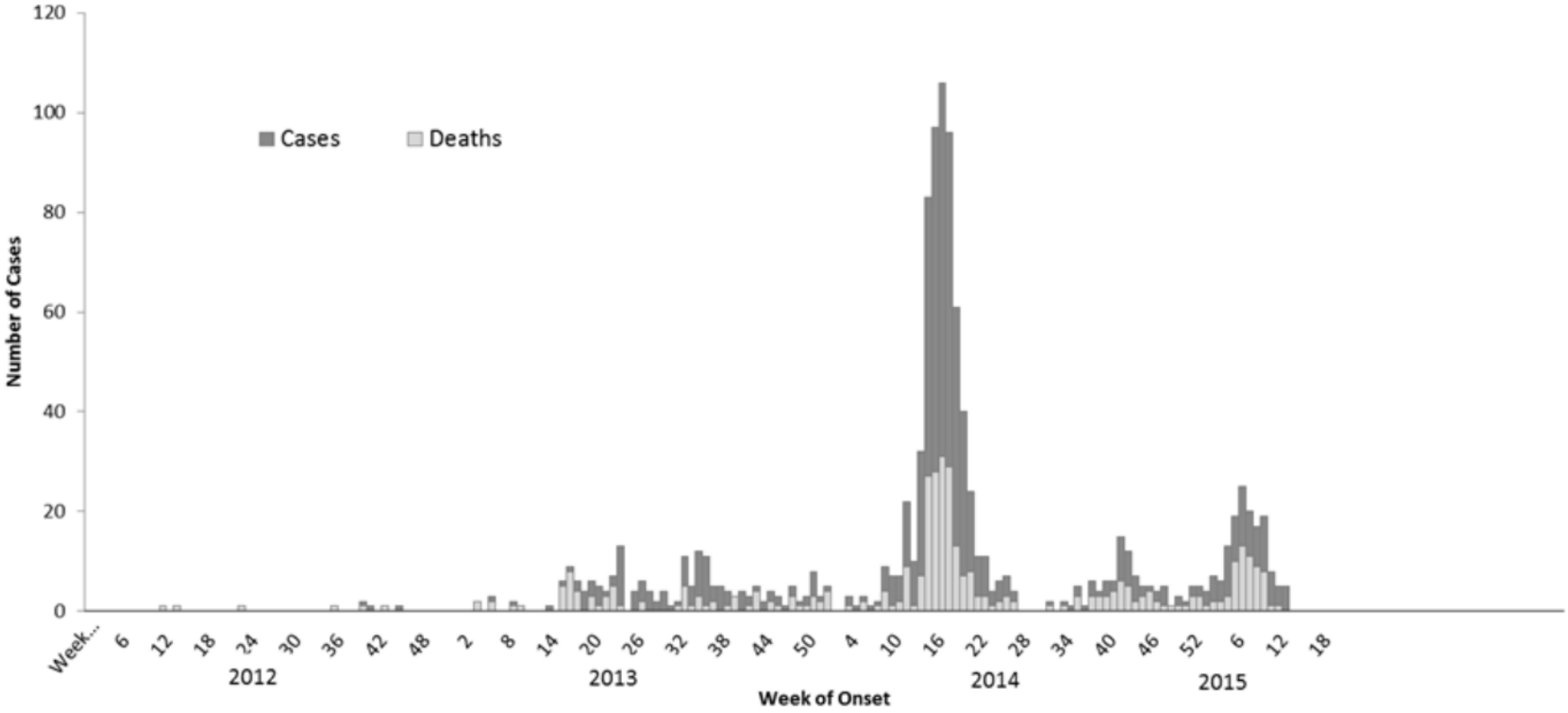




- There is no evidence of sustained human-to-human transmission
- Failures in infection control and prevention in healthcare settings has resulted in large numbers of secondary cases

(L) Reuskin et al EID 2014; (R) Ferguson & Van Kerkhove 2014

Epi curve as of 3rd April 2015



World Health Organization

Summary

- **Cases continue to be reported, primarily in Saudi Arabia**
  - Little H2H community transmission
  - Hospital amplification in Saudi Arabia has continued
  - Exportation to countries outside of the Middle East remain possible
  - Substantial number of very mild cases. I.e. 4-50% sero+ in Slaughterhouse workers
- **Transmission pattern unchanged, profile of affected population unchanged**
- **High level of confidence that H2H transmissibility has not increased**
- **No Hajj associated infections have been reported**

Urgent Needs

- Determine the mode(s) of transmission of the virus from animals to people
  - Tools have been developed, in collaboration with affected countries, to assess risk factors for infection:
    - Case-Control study to assess potential risk factors related to human illness caused by MERS
    - Cross-sectional seroprevalence study of MERS-CoV infection in presumed high risk populations
- Implement preventative measures to interrupt this pattern
  - Primary: decrease circulation in reservoir/secondary hosts, decrease human exposures
  - Secondary: improve IPC in healthcare settings

MERS Coronavirus: The first cases in the United States- How we prepared and responded

David L. Swerdlow, MD
Incident Manager, CDC MERS Coronavirus Response
Associate Director for Science
National Center for Immunization and Respiratory Diseases

April 3, 2015

MERS CoV? Do we still need to be worried?

Ebola

Ebola

AFRICA

SHARE

Q&A

What Are the Chances Ebola Will Spread in the United States?

UPDATED OCT. 3, 2014

Health officials from the Centers for Disease Control and Prevention said they were confident that standard procedures for controlling an infection will contain Ebola in the United States. The C.D.C.

WORLD BRIEFING | AFRICA
Mali: Gunmen Kill 9 U.N. Peacekeepers

Nigeria: Grisly Claim by Boko Haram


A Plan to Use Survivors' Blood for Ebola Treatment in Africa


For Journalists, a Stark Reminder of the Risk in Covering a Deadly Epidemic


U.S. Aid E... Barely Off... Ebola Rag...

U.S.

As U.S. Ebola Fears Widen, Reports of Possible Cases Grow

By MANNY FERNANDEZ and ROBERT PEAR OCT. 4, 2014

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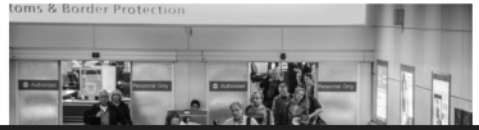
DALLAS — In Washington, a patient who had traveled to Nigeria and who was suspected of having Ebola was placed in isolation at Howard University Hospital on Thursday. In New Haven, two Yale University graduate students plan to sequester themselves when they return this



Ebola

Ebola

Ebola

N.Y. / REGION

New York City Steps Up Preparations to Be Ready for Ebola Cases

By MARC SANTORA OCT. 5, 2014

Email

One week after the first diagnosis of Ebola in a patient in the United States, every person who calls 911 in New York City and relates symptoms such as

TAKE ACTION

MERS CoV: How we prepared and responded

- Domestic preparedness
  - Epidemiology and laboratory
  - Infection control guidance
  - Travelers health recommendations
- Response to US Cases
  - Indiana
  - Florida
- Do we still need to be concerned?

Epidemiology and Laboratory

- Epidemiology tool kit:
  - Case definitions
  - Case investigation forms:
  - Contact investigation tools
    - Household
    - Healthcare
  - Surveillance Plan
- Laboratory
  - Guidelines for laboratory sampling
  - Development of PCR and serology
- Communications activities

Case definitions

Patient under investigation

Fever AND pneumonia or acute respiratory distress syndrome (based on clinical or radiological evidence) AND EITHER:

A. a history of travel from countries in or near the Arabian Peninsula within 14 days before symptom onset, OR

B. close contact with a symptomatic traveler who developed fever and acute respiratory illness (not necessarily pneumonia) within 14 days after traveling from countries in or near the Arabian Peninsula[1] OR

C. a member of a cluster of patients with severe acute respiratory illness (e.g., fever and pneumonia requiring hospitalization) of unknown etiology in which MERS-CoV is being evaluated, in consultation with state and local health departments.

OR

A. Fever AND symptoms of respiratory illness (not necessarily pneumonia; e.g. cough, shortness of breath) AND being in a healthcare facility (as a patient, worker, or visitor) within 14 days before symptom onset in a country or territory in or near the Arabian Peninsula in which recent healthcare-associated cases of MERS have been identified

http://www.cdc.gov/coronavirus/mers/case-def.html

Epidemiology Tool Kit
Patient Under Investigation Form

Middle East Respiratory Syndrome (MERS) Patient Under Investigation (PUI) Short Form

For Patients Under Investigation (PUIs), complete and send this form to eocreport@cdc.gov (subject line: MERS Patient Form) or fax to 770-488-7107. If you have questions, contact the CDC Emergency Operations Center (EOC) at 770-488-7100.

STATE ID:	Today's Date: MM/DD/YY	County:	City:	State:
Interviewer's name:	Phone:		Email:	
Physician's name:	Phone/Pager:			

PUI Definition—Does the patient have: (Please consult CDC website at http://www.cdc.gov/coronavirus/mers/case-def.html)

1. Acute respiratory infection with fever (≥ 38°C, 100.4°F) and cough? ☐ Yes ☐ No ☐ Unknown
2. Clinical or radiographic evidence of pneumonia or acute respiratory distress syndrome (ARDS)? ☐ Yes ☐ No ☐ Unknown
3. Travel from the Arabian Peninsula or neighboring countries* 14 days before illness onset? ☐ Yes ☐ No ☐ Unknown
   If yes, which countries?_____ Date of travel to/from the Middle East: MM/DD/YY | MM/DD/YY

Patient Demographic Information

1. Sex: ☐M ☐F 2. Age:_____ ☐yr ☐mo 3. Residency: ☐US resident ☐non US resident, country:_____

Clinical Presentation, History and Risk Factors

4. Date of symptom onset: MM/DD/YY
5. Symptoms (Check all that apply): ☐ Fever ☐ Dry cough ☐ Productive cough ☐ Chills ☐ Sore throat ☐ Headache ☐ Muscle aches ☐ Shortness of breath ☐ Vomiting ☐ Abdominal pain ☐ Diarrhea ☐ Other_____
6. In the 14 days before symptom onset did the patient have close contact with a recent ill traveler from the Arabian Peninsula or neighboring countries*? ☐ Yes ☐ No ☐ Unknown If yes, which countries?_____
7. Is the patient (Check all that apply): ☐ Health care worker (HCW) ☐ US military ☐ Flight crew ☐ Other_____
8. Concurrent risk factors (Check all that apply): ☐ Immunocompromised ☐Pregnant ☐Unknown ☐Other_____

Clinical Outcomes

9. Is/Was the patient:
   a. Hospitalized? ☐ Yes ☐ No ☐ Unknown If yes, date: MM/YY/DD
   b. Admitted to ICU? ☐ Yes ☐ No ☐ Unknown
   c. Intubated? ☐ Yes ☐ No ☐ Unknown
10. Is/Has patient receiving/received a diagnosis of:
    Pneumonia? ☐ Yes ☐ No ☐ Unknown
    ARDS? ☐ Yes ☐ No ☐ Unknown
    Renal failure? ☐ Yes ☐ No ☐ Unknown
11. Does the patient have a non-MERS etiology for their respiratory illness but has not responded to appropriate therapy? ☐ Yes ☐ No ☐ Unknown
12. Has the patient died? ☐ Yes ☐ No ☐ Unknown

Infection Control

13. When hospitalized, is/was the patient in a:
    a. Negative pressure room? ☐ Yes ☐ No ☐ Unknown
    b. Private room? ☐ Yes ☐ No ☐ Unknown
14. Are/Were surgical masks being used by the patient during transport? ☐ Yes ☐ No ☐ Unknown
15. What personal protective equipment are/were being used by HCW when entering the patient's room (Check all that apply): ☐ Gloves ☐ Gowns ☐ Eye protection (goggles or face shield) ☐ N95/other form of respiratory protection (e.g., PAPR) ☐ Facemask ☐ Unknown

Laboratory Testing

Tests Performed	Results +	–	Pending (Pe)	Not done	Tests Performed	Results +	–	Pending (Pe)	Not done
Influenza ☐A ☐B			☐	☐	Streptococcus pneumoniae			☐	☐
RSV			☐	☐	Legionella pneumophila			☐	☐
Human metapneumovirus			☐	☐	Blood culture If positive_____			☐	☐
Parainfluenza 1-4			☐	☐					
Adenovirus			☐	☐	Other:_____			☐	☐

MERS Testing

Specimen#	ID #	Date collected	State +	–	Pe	Sent to CDC?	Specimen#	ID #	Date collected	State +	–	Pe	Sent to CDC?
NP/OP		MM/DD/YY			☐	☐	PF		MM/DD/YY			☐	☐
Sputum		MM/DD/YY			☐	☐	Stool		MM/DD/YY			☐	☐
BAL		MM/DD/YY			☐	☐	Serum		MM/DD/YY			☐	☐
TA		MM/DD/YY			☐	☐			MM/DD/YY			☐	☐

*Countries considered in the Arabian Peninsula and neighboring include: Bahrain, Iraq, Iran, Israel, Jordan, Kuwait, Lebanon, Oman, Palestinian territories, Qatar, Saudi Arabia, Syria, the United Arab Emirates (UAE), and Yemen.
#NP/OP, Nasopharyngeal/Oropharyngeal swab; BAL, Bronchoalveolar lavage; TA, Tracheal aspirate; PF, Pleural fluid

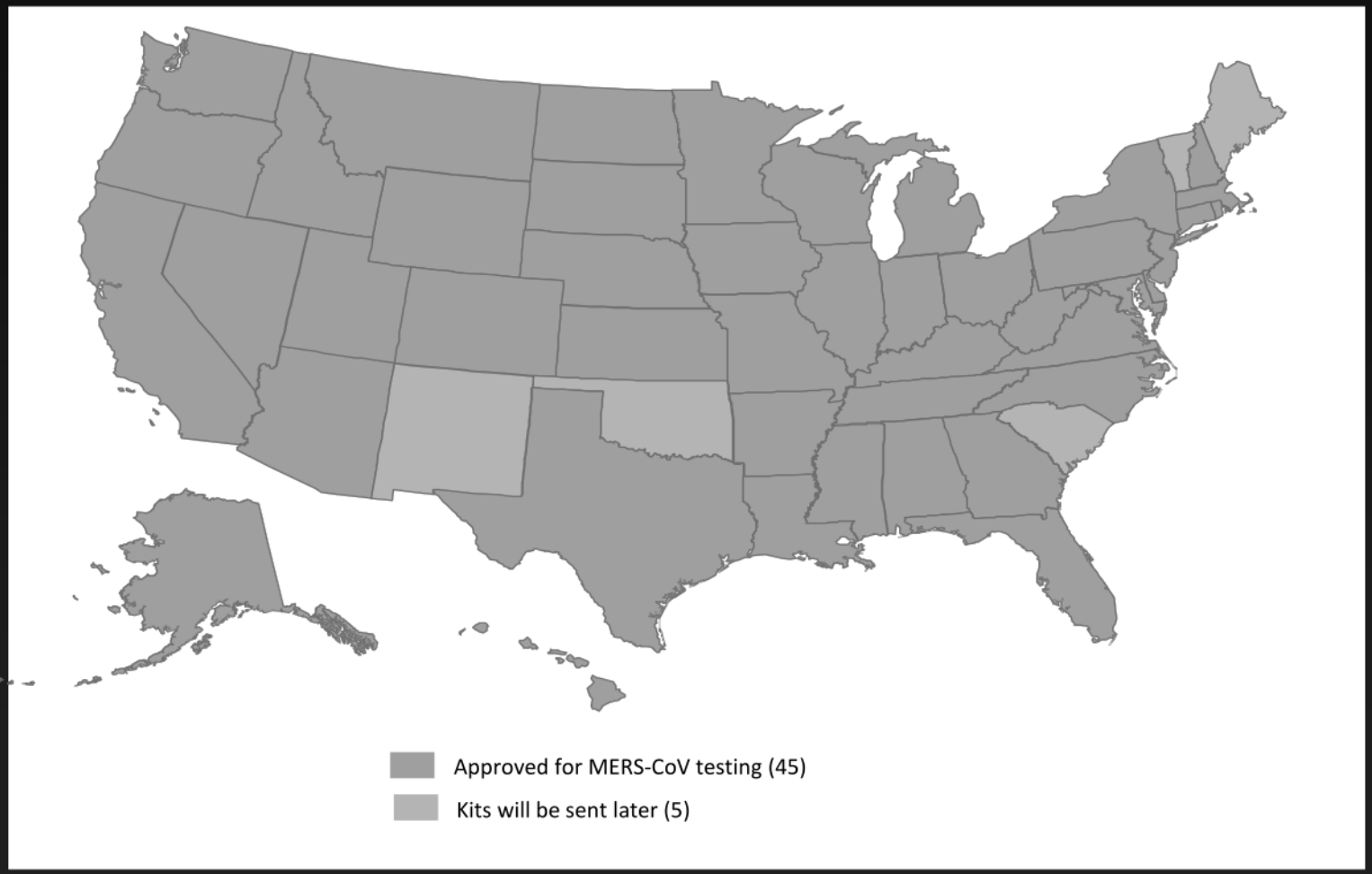
Version 5.5, 7/3/13

NIH 57943 -005445

Guidelines for Collecting and Testing Clinical Specimens

- Test “patients under investigation”
- Consider timing of collection
- Collect multiple specimens
- Lower respiratory (BAL, sputum) preferred
- Also NP/OP, stool, serum (PCR and serology)

Domestic Deployment of CDC MERS-CoV rRT-PCR Assay by LRN



436 patients from 45 states have tested negative

NIH 57943 -005447

Middle East Respiratory Syndrome (MERS)

MERS
FAQs
Healthcare Providers
Interim Guidance
Case Definitions
Infection Prevention and Control
Preparedness
Interim Home Care and Isolation Guidance
Interim Guidance for Preventing MERS-CoV from Spreading in Homes and Communities
Health Departments
Laboratories
Guidance for Travel
Related Materials

Related Links
Coronavirus
SARS

Email page link
Print page

Information for Healthcare Providers

Interim Guidance For Health Professionals
CDC interim guidance for evaluating patients, reporting patients under investigation (PUIs), testing specimens, and conducting investigations.

Case Definitions
CDC case definitions for PUI, close contact, probable case, confirmed case, and clusters of SARI.

Preparedness
Checklists and resources to help healthcare providers and facilities better prepare for the possibility of MERS patients.

Infection Prevention and Control
Interim recommendations for managing hospitalized patients with known or suspected MERS-CoV infection.

Interim Home Care and Isolation Guidance
CDC interim guidance to prevent MERS-CoV from spreading in homes and communities if there is ever a case in the U.S.

Important Links

- Guidelines for Clinical Specimens
- Data Collection

Contact Us:

Centers for Disease Control and Prevention
1600 Clifton Rd
Atlanta, GA 30333

800-CDC-INFO
(800-232-4636)
TTY: (888) 232-6348
Contact CDC-INFO

Infection Prevention and Control Recommendations

- Standard, Contact and Airborne Precautions
  - N95 respirators if available
  - Airborne infection isolation rooms
- Similar recommendations as SARS
  - High mortality
  - Human-to human transmission
  - Unknown modes of transmission
  - No vaccine or chemoprophylaxis

TRAVELERS' HEALTH
TRAVEL SAFE. TRAVEL SMART.

Home
Destinations
Travel Notices
MERS in the Arabian Peninsula
Find a Clinic
Disease Directory
Information Centers
For Travelers
For Clinicians
Travel Industry
Yellow Book
Mobile Apps
RSS Feeds

Disease Directory
Learn more about travel-related diseases.
African Sleeping Sickn
Go

Before you travel make sure you speak with your doctor.

Home > Travel Notices

MERS in the Arabian Peninsula

Warning - Level 3, Avoid Nonessential Travel
Alert - Level 2, Practice Enhanced Precautions
Watch - Level 1, Practice Usual Precautions

Updated: May 21, 2014

What is the Current Situation?

Cases of MERS (Middle East Respiratory Syndrome) have been identified in multiple countries in the Arabian Peninsula.* There have also been cases in several other countries in travelers who have been to the Arabian Peninsula and, in some instances, their close contacts. Two cases have been confirmed in two health care workers living in Saudi Arabia who were visiting the United States. For more information, see CDC's MERS website.

CDC does not recommend that travelers change their plans because of MERS. Most instances of person-to-person spread have occurred in health care workers and other close contacts (such as family members and caregivers) of people sick with MERS. If you are concerned about MERS, you should discuss your travel plans with your doctor.

Countries in or near the Arabian Peninsula

View Larger Map

What is MERS?

MERS is a viral respiratory illness first reported in Saudi Arabia in 2012. It is caused by a virus that is different from any other virus that has been previously found in people. Symptoms of MERS include fever, cough, and shortness of breath. CDC is working with the World Health Organization and other partners to understand the public health risks from this virus.

What can travelers do to prevent MERS?

All travelers

Email page link
Print page
Our Twitter
Our Facebook page
Get email updates

Get email updates
To receive email updates about this page, enter your email address:
What's this? Submit

Contact Us:

Centers for Disease Control and Prevention
1600 Clifton Rd
Atlanta, GA 30333
800-CDC-INFO
(800-232-4636)
TTY:
(888) 232-6348
Contact CDC-INFO

Travel-related MERS-CoV Planning for Hajj

- 3 million pilgrims, about 12,000 from US
- Public health communications for inbound and outbound travelers
  - Electronic messaging on airport monitors
  - Travel notice on CDC Travel website
  - Social messaging– using Travel Tweets and CDC Travel Facebook postings
  - Outreach to Hajj/Umrah travel agencies, business orgs., education, CBOs
- KSA Ministry of Health recommended that elderly, children, pregnant women, and those with chronic diseases, weakened immune systems and terminal illnesses not attend Hajj


HEALTH ADVISORY: MERS
Middle East Respiratory Syndrome
Were you in the Middle East recently?
• Watch for fever with cough or difficulty breathing.
• If you get sick within 14 days of leaving, call a doctor.
• Tell the doctor you traveled.
www.cdc.gov/travel
VERIZON 4G LTE DEVICES THAT WORK AT HOME AND ABROAD. THAT'S POWERFUL.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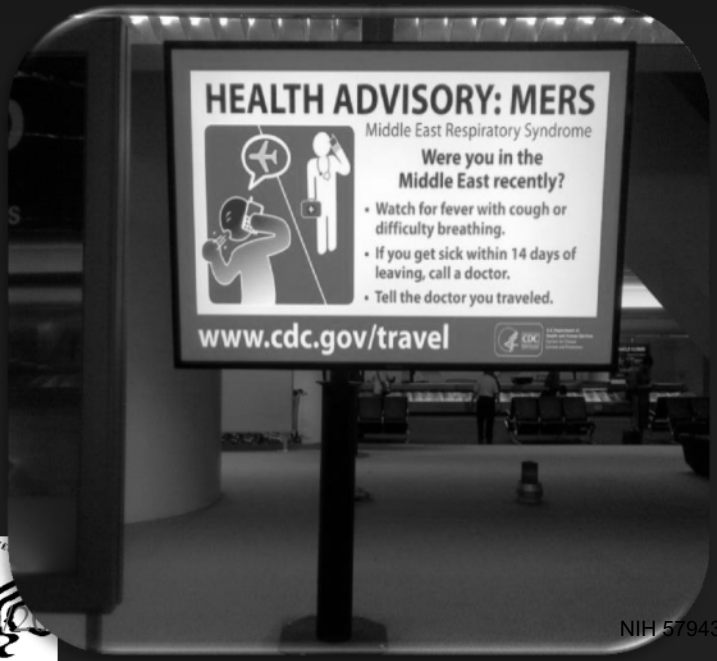
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www.cdc.gov/travel

نصيحة طبية: MERS

متلازمة الشرق الأوسط التنفسية

هل سافرت إلى الشرق الأوسط مؤخرًا؟

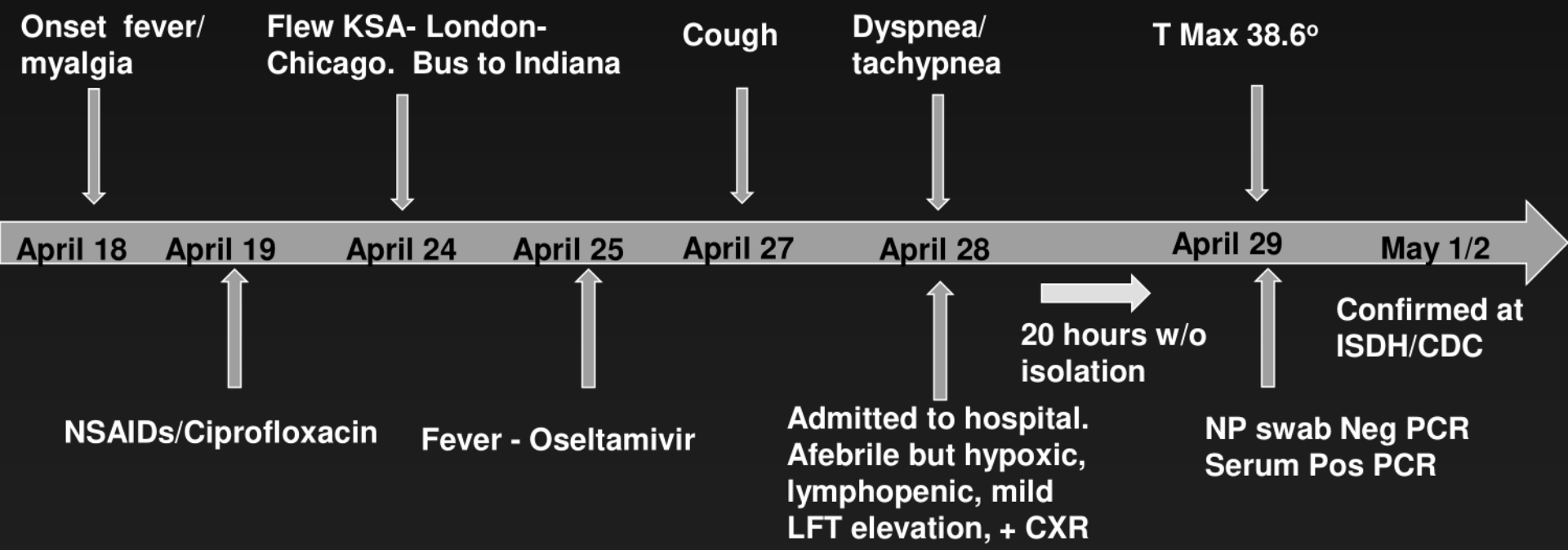
- انتبه لأعراض المرض التالية: الحمى المصحوبة بالسعال أو الصعوبة في التنفس.
- إذا أصبت بالمرض خلال 14 يومًا من مغادرتك، فعليك الاتصال بالطبيب.
- أبلغ الطبيب بسفرك.

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

www.cdc.gov/travel

NIH 57943 -005453

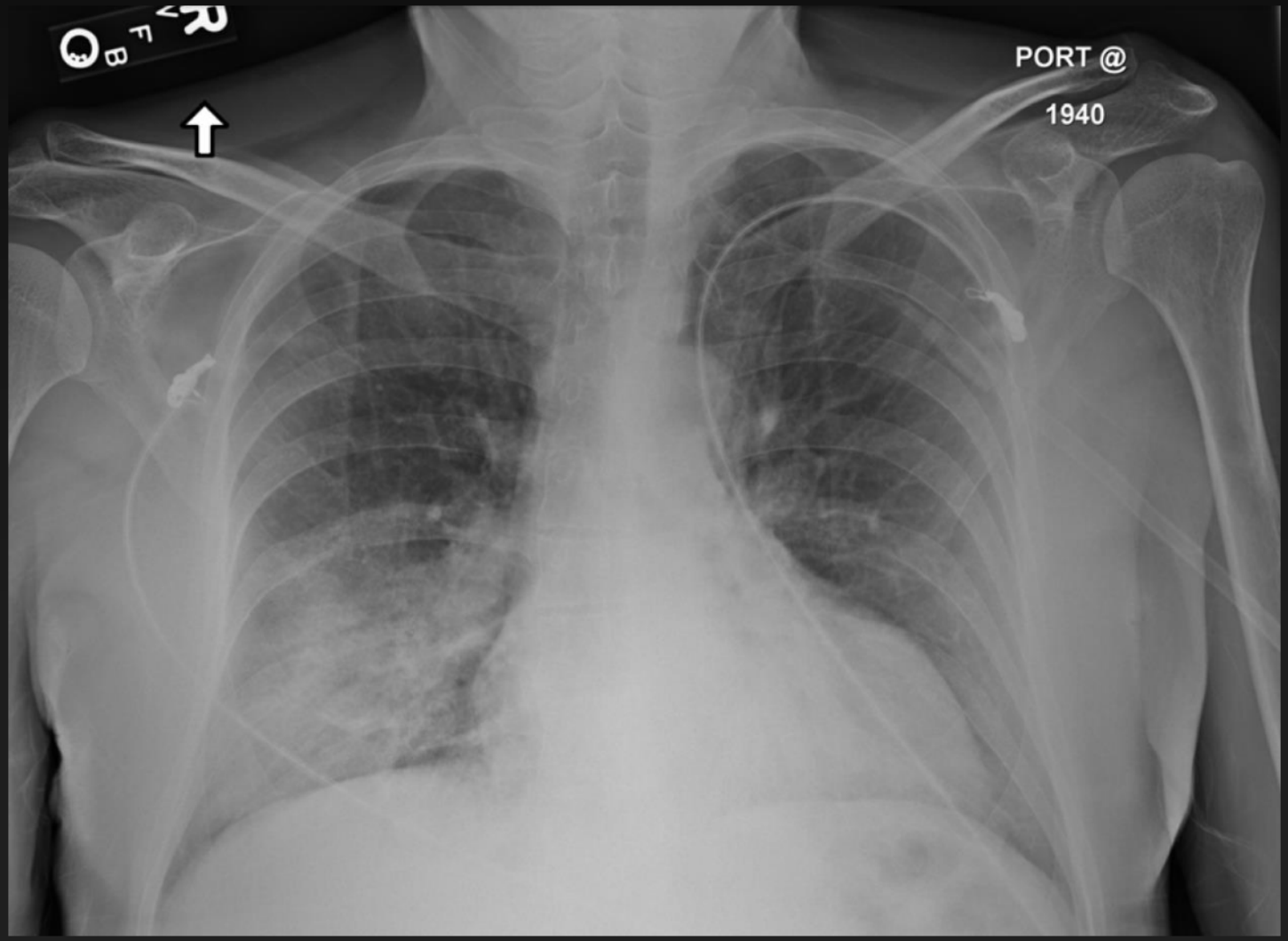
Timeline, first MERS case in the US, Indiana
65 yo male physician living in KSA



Indiana patient

- Hx of HTN and CAD
- Worked at hospital in Riyadh
- Did not recall directly treating MERS patient- but did enter room of intubated patients with respiratory illnesses
- No known family members with MERS infections
- Initial symptoms non-specific and was afebrile
- Initial NP swab was negative

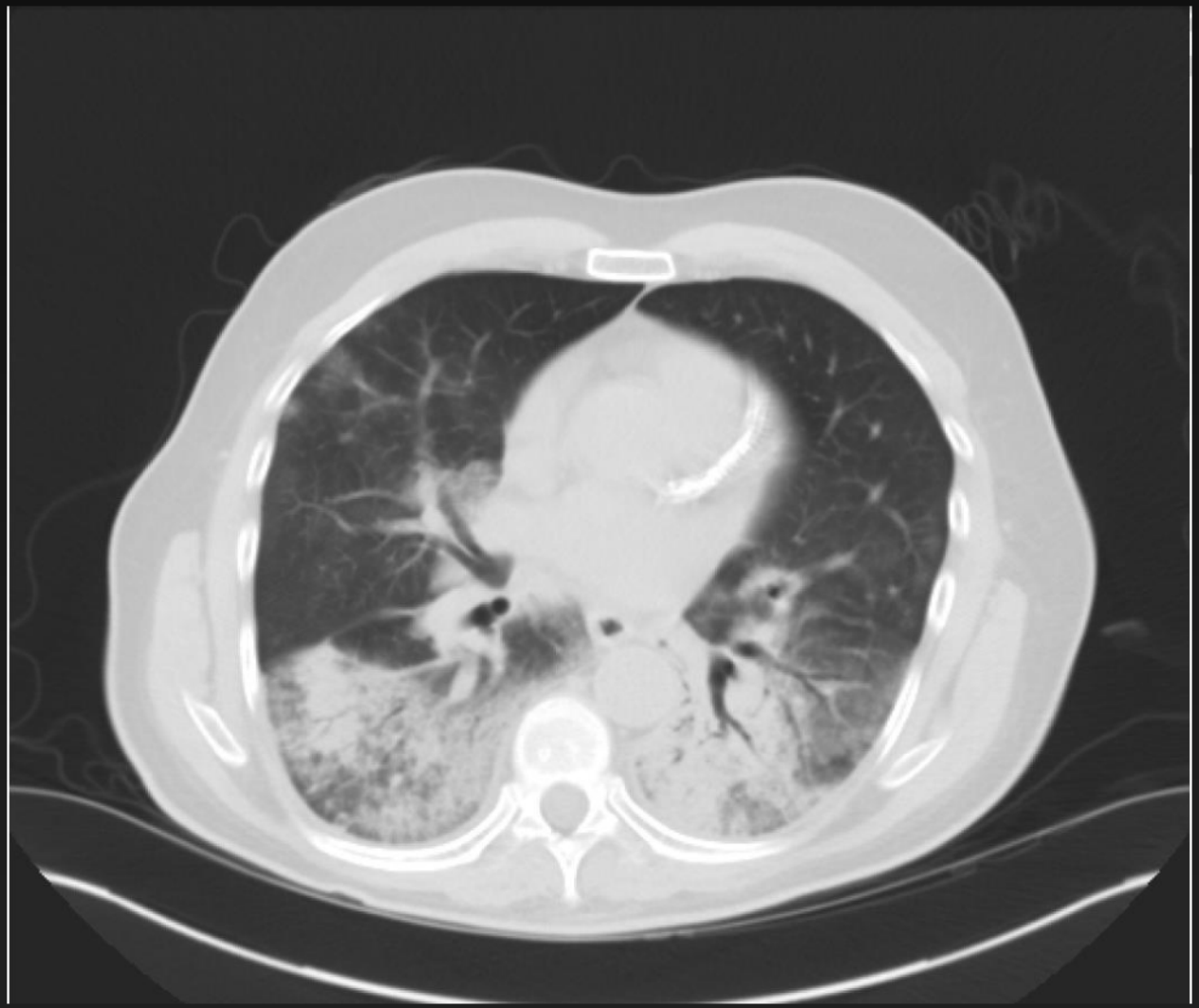
CXR, MERS CoV patient, Indiana



Kapoor M, Pringle K, Kumar A, et al. CID 2014;
doi: 10.1093/cid/ciu635

CDC

CT Scan, MERS CoV patient, Indiana



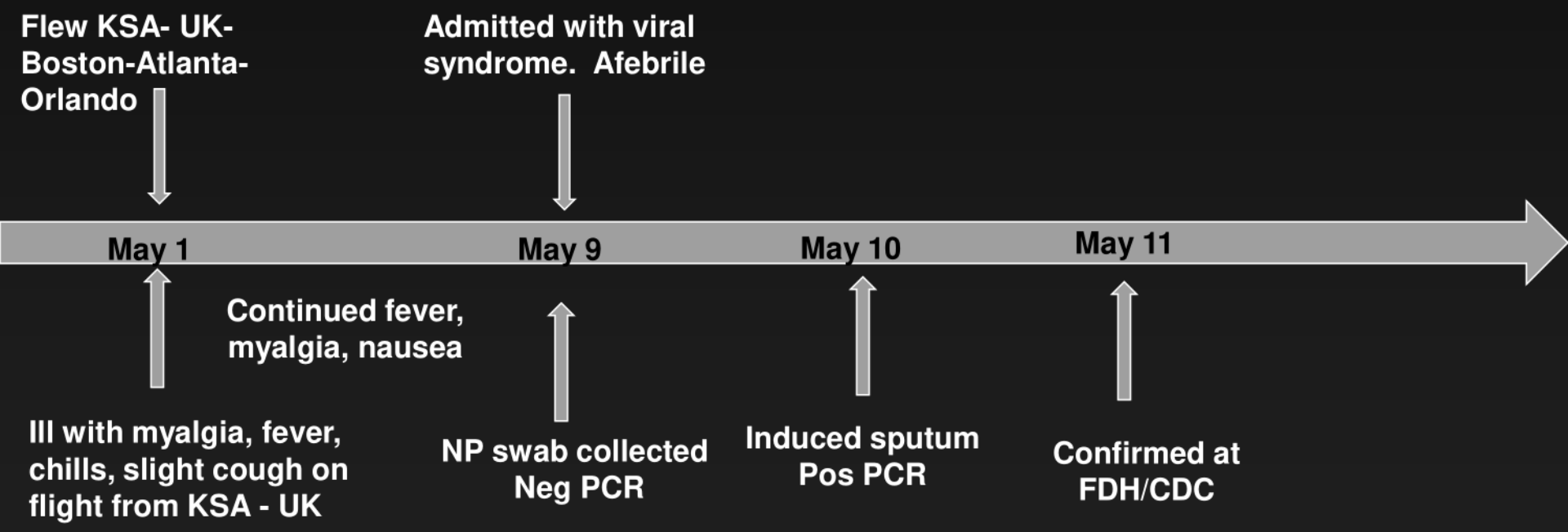
Kapoor M, Pringle K, Kumar A, et al. CID 2014;
doi: 10.1093/cid/ciu635

Contact investigations: Indiana patient

- HCW in hospital (n=53)
  - Monitored temp
  - Serology and PCR tests performed
  - Were furloughed for 14 days
- Household members and business associate (n=7)
  - Voluntary home quarantine, mask outside home
  - Serology and PCR tests performed
- Airplane flights (n=80)
  - Most contacted. Serology
- Bus – 6/10 contacted.

No secondary cases identified

Timeline, second MERS case in the US-Florida HCW, about 40 yo, living in KSA

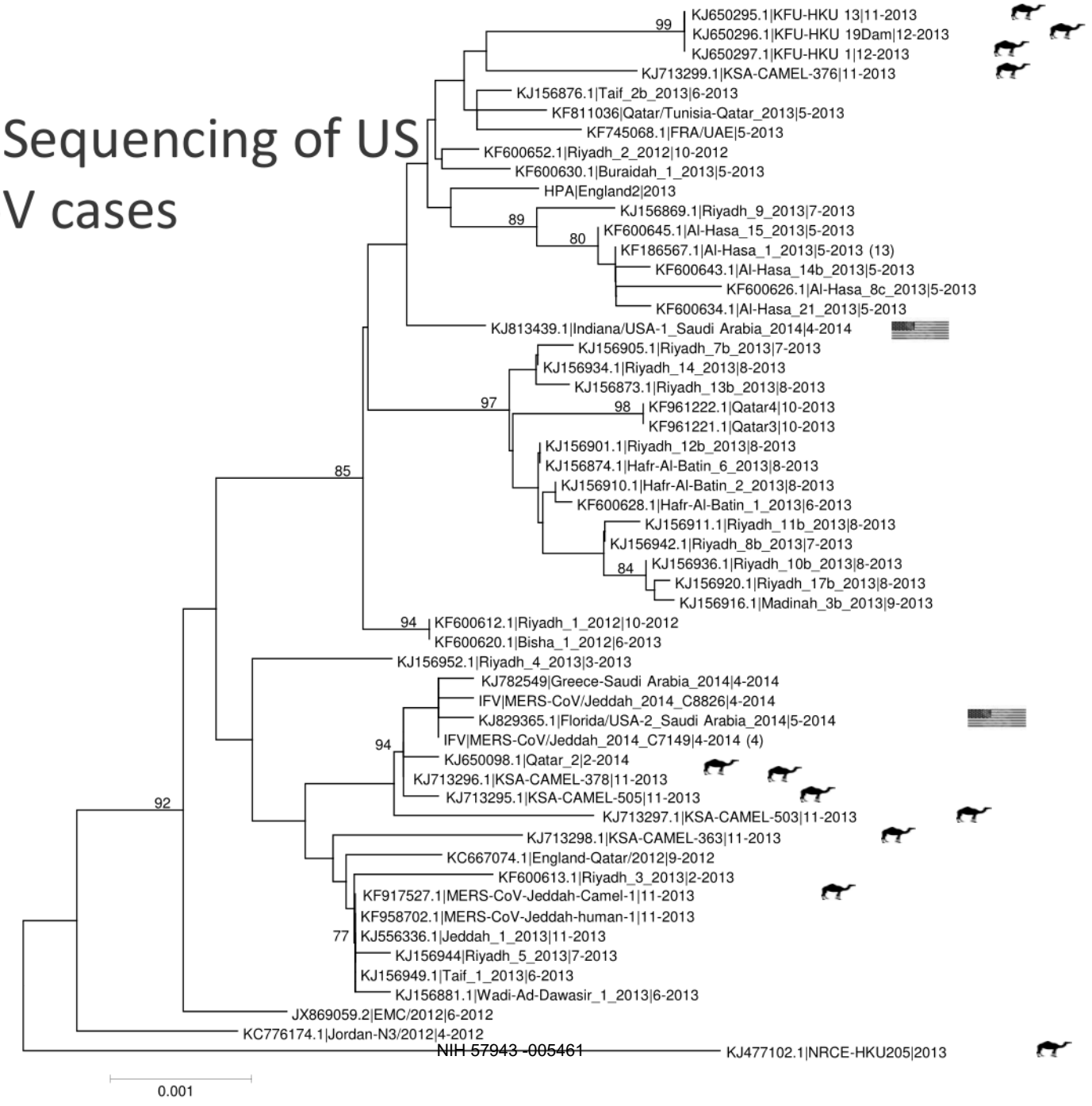


Contact investigation Florida patient

- HCW in hospital (n=20)
- Furloughed
  - Monitored temp
  - Serology and PCR tests performed
- Patients in ER (about 80)
- Household members (n=9)
  - Voluntary home quarantine, mask outside home
  - Serology and PCR tests performed
- Airplane flights (n=523)
  - 97% contacted. Serology

No secondary cases identified

Genome Sequencing of US MERS-CoV cases

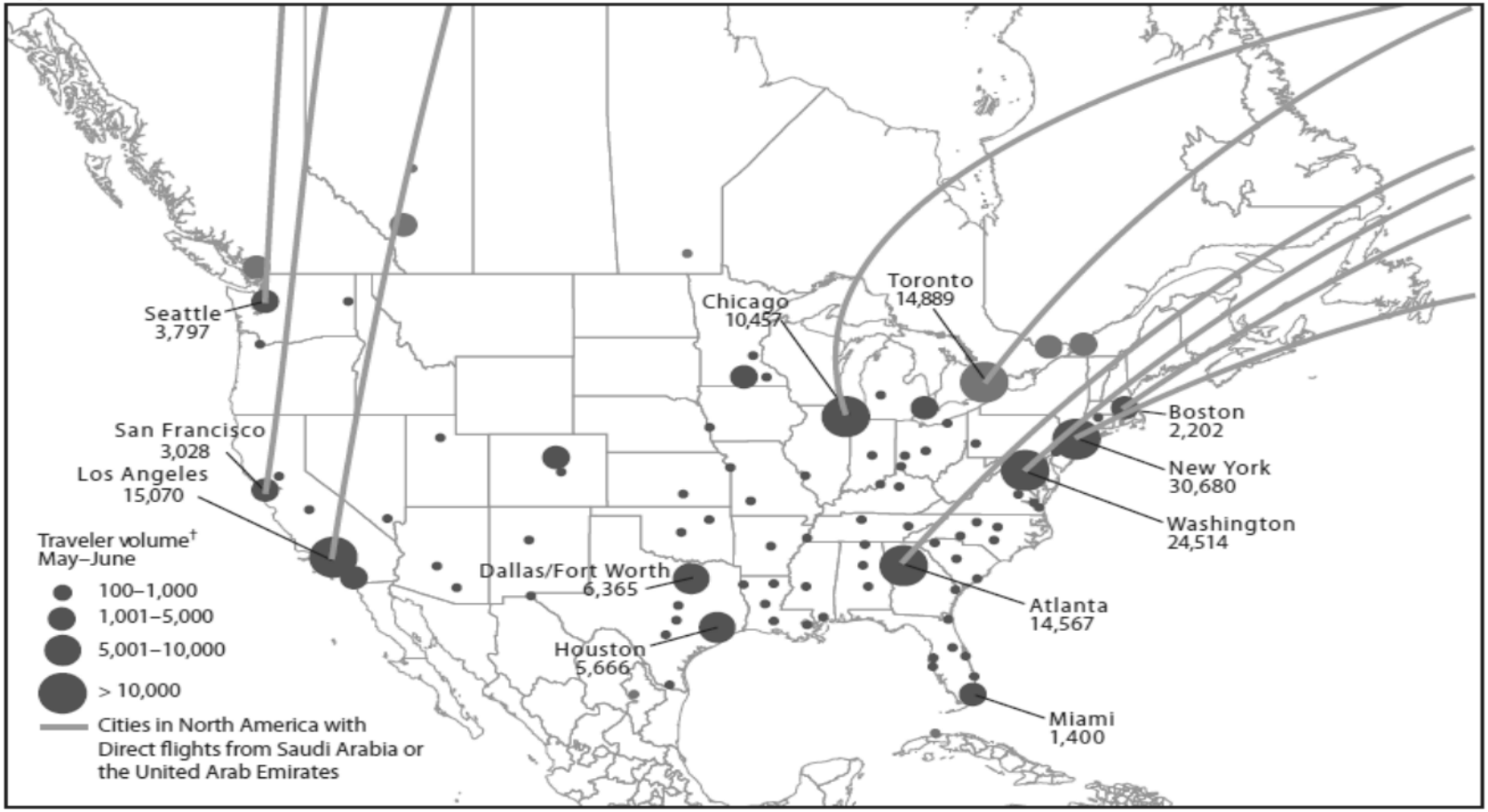


S ORF

MERS CoV-Do we still need to be worried?

- Since August 1, 2014 236 confirmed cases
  - KSA, Qatar (4), Oman (3), UAE (1)
- Recent exportations
  - Austria, Turkey, Jordan, Philippines, Germany
- High mortality
- Likely respiratory transmission
- No treatment
- No vaccine
- Healthcare workers
- Travelers

FIGURE 3. Points of entry and volume of travelers on flights to the United States and Canada from Saudi Arabia and the United Arab Emirates — May–June 2014*



Source: BioMosaic, an analytic tool for integrating demography, migration, and health data developed in collaboration between the University of Toronto, Boston Children's Hospital, and CDC's Division of Global Migration and Quarantine.
* Excludes cities with fewer than 100 travelers from affected areas.
† Based on total number of arrivals at final destination in North America.

Health-care providers should contact their state or local health department if they have any questions.

Infection control. HCP should adhere to recommended infection-control measures, including standard, contact, and as the separation or restriction of activities of an ill person with a contagious disease from those who are well. Additional information on home care and isolation guidance is available at http://www.cdc.gov/coronavirus/mers/hcp/home-care.html.

MERS Coronaviruses (MERS CoV): Value of preparedness

- Epidemiology and laboratory activities facilitated identification of first cases in US
- Infection control guidance may have helped prevent spread
- Advice for travelers to educate travelers going to, and returning from, Arabian Peninsula important
- Need to be vigilant


If you ever find yourself at the Spice Souk,
you'd realize that sadly, there is not much to nosh on
except this fabulous Arabianized Gelateria.
THE FIRST EVER
camel milk gelato
our flavours include
camel milk, saffron, dates,
ferrero, biscuit,
pistachio, hazelnut
FIND US AT THE DUBAI SPICE MARKET

Acknowledgments

Indiana State Dept of Health
Cook County Dept Public Health
Illinois Dept Public Health
Community Hospital, Munster, Indiana
Florida Dept Health
Division of Global Migration and Quarantine, CDC
Division of Viral Diseases, CDC
Division of Healthcare Quality Promotion, CDC

Many, many, others

NIH 57943 -005466

Animal Models

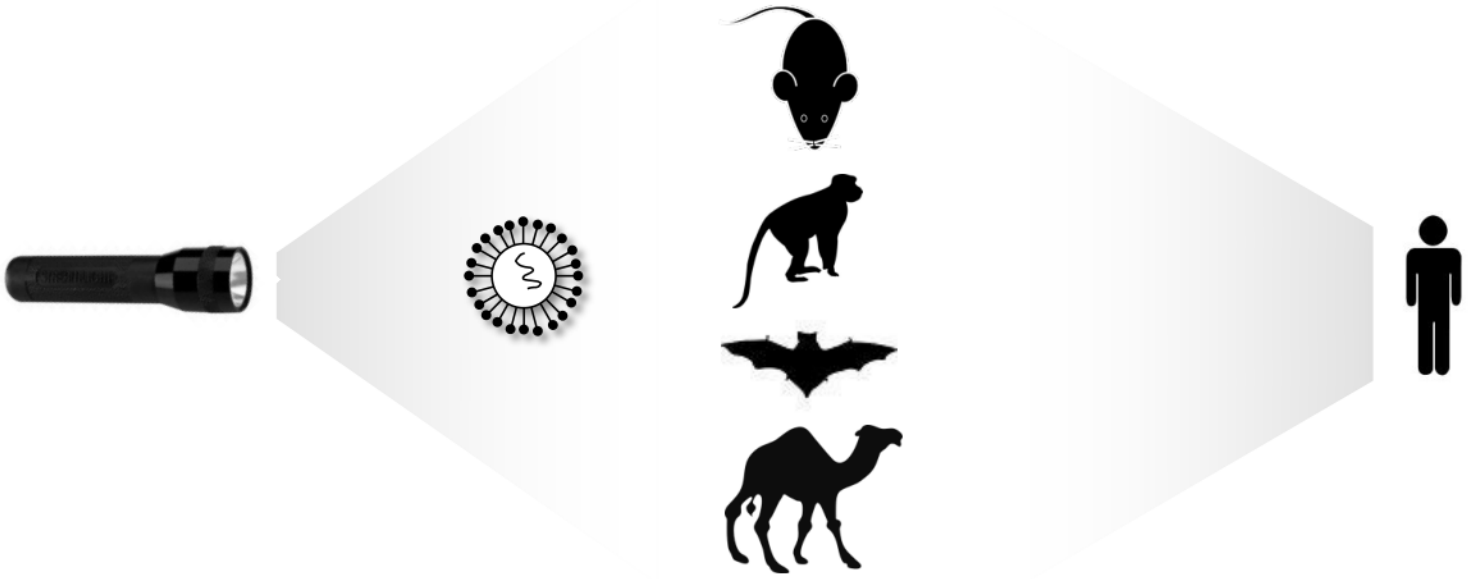
Dr. Lisa Hensley

MERS-CoV Animal Model Progress

Dr. Matthew Frieman, Ph.D.
Associate Professor
University of Maryland School of Medicine
Department of Microbiology and Immunology

(b)(6)

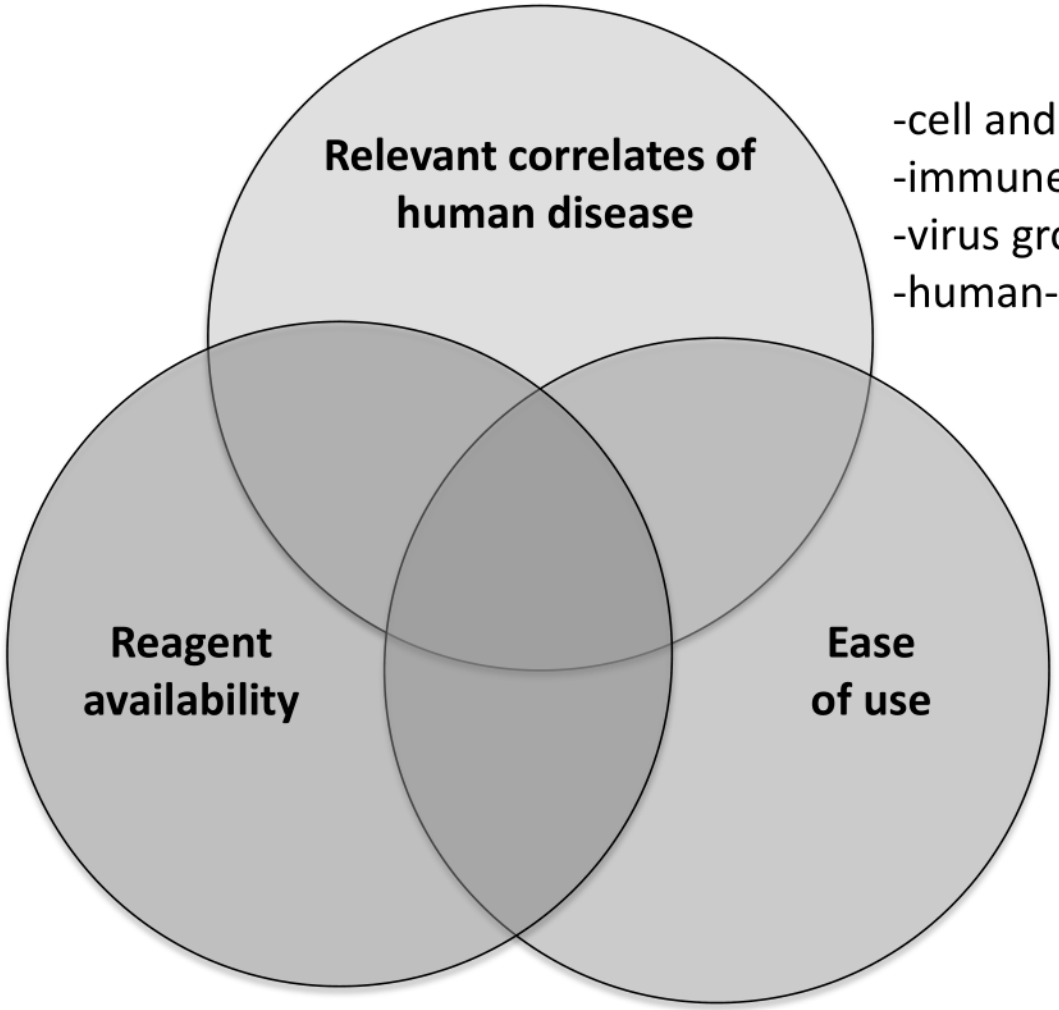
Animal Models should enlighten us about clinical disease



Need animal models needed to inform:

- Pathogenesis
- Anti-viral development
- Vaccine efficacy studies
- Virus tropism
- Host protein interactions
- Immune response

Keys to successful animal models



-cell and organ tropism
-immune response
-virus growth
-human-like pathology

-gene knockouts
-genes/probes
-antibodies

-availability of animals
-price
-BSL3 risk

Questions/Issues for MERS-CoV animal Model

- Lack critical human disease correlates (human tissue etc)
  - What role to comorbidities play?
- Transmission dynamics? Route of infection?
- Are there differences in virus isolates?
- Large vs Small animal model (human disease v. experimental)
- What is the role of DPP4 in disease?
  - Known link to diabetes and diabetes is a common co-factor to MERS
- Once models are established, how to compare?
- Continuous funding/experiment stream
- How to test MCMs across systems?

MERS-CoV Animal Model Progress

Large Animals	Permissive?	Pathology	Reference
Rhesus Macaque	++	mild interstitial pneumonia with focal bronchiolotis	De Wit et al PNAS 2013 Yao et al JID 2014
African Green Monkey	+/-	Minimal lung pathology	Haagmans, pers. comm
Marmoset	+++	Severe lung disease with clinical symptoms and death	Falzarano et al Plos Path 2014
Camel	+++	Significant upper respiratory infection	Adney et al EID 2014

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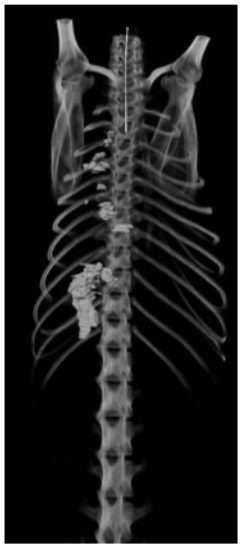
Small Animals			
Rabbit	+	Replication but no histological disease	Haagmans et al JV 2015
Rat/Cotton Rat	-	No disease or replication	Frieman/Subbarao
Syrian Hamster	-	No disease or replication	Sutton et al Virology 2015
Ferret	-	No disease or replication	Sutton et al Virology 2015
Mice	-	No disease or replication	Coleman et al Virology 2013
Various hDPP4/mice	+++	Replication, lung/brain disease, clinical disease in some models	Zhao et al PNAS 2014 Agrawal et al JV 2015 Frieman (unpublished) Baric (unpublished)

MERS-CoV Animal Model Progress

Large Animal Models

-Rhesus Macaque
-Marmoset
-Camel

Rhesus CT with MERS-CoV

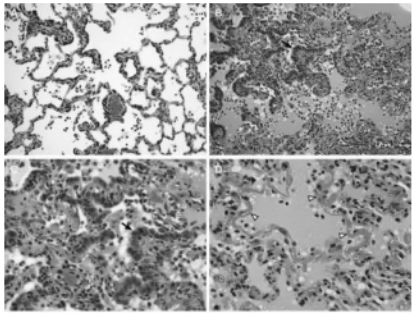


(Hensley/IRF)

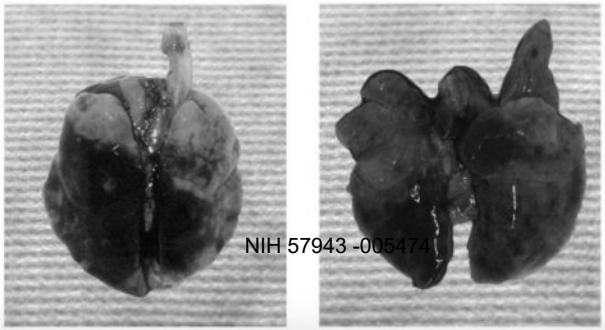
Benefits

-Potential to have human-like disease
-Imaging capability
-Lethal Disease (Marmoset)
-Natural Reservoir (Camel)

Rhesus (de Wit et al)



Marmoset (Falzarano et al)



MERS-CoV Animal Model Progress

Large Animal Models

-Rhesus Macaque
-Marmoset
-Camel

Benefits

-Potential to have human-like disease
-Imaging capability
-Lethal Disease (Marmoset)
-Natural Reservoir (Camel)

Challenges

-Expensive
-Specialized facilities needed
-Limited supply (Marmoset/Camel)
-Inoculation volume large (Rh/Marm)

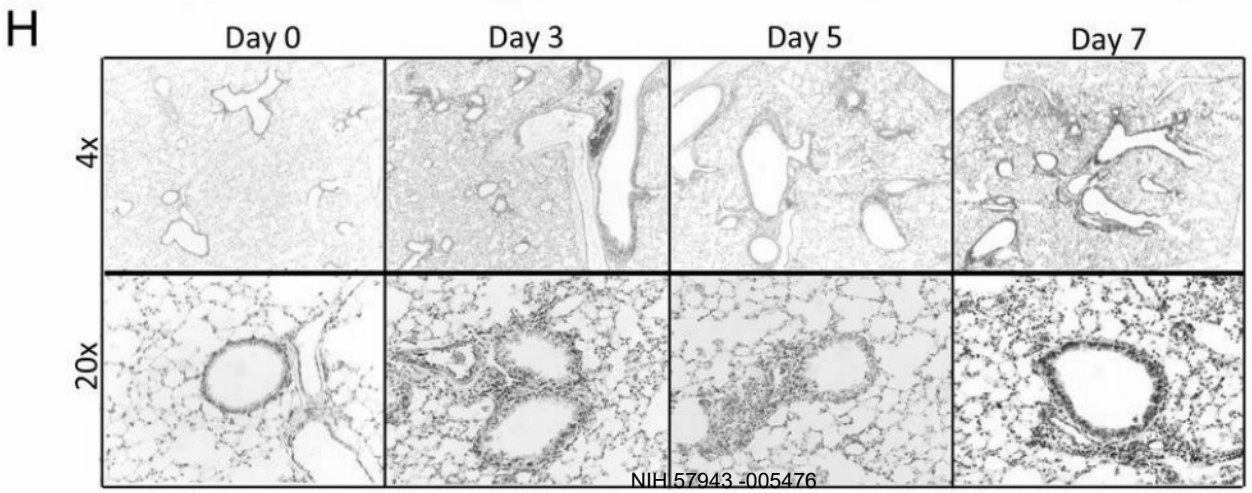
MERS-CoV Animal Model Progress

Small Animal Models

-Rabbit (**Subbarao follow up)**
-hDPP4 expressing Mice

Adenovirus/hDPP4 Mouse Transduction Model (Zhou et al PNAS 2014)

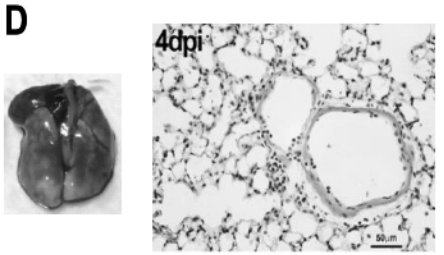
-intranasal inoculation of adenovirus expressing hDPP4
-become permissive, develop pneumonia
-issue: non cell-type specific expression of hDPP4 in Ad infected cells



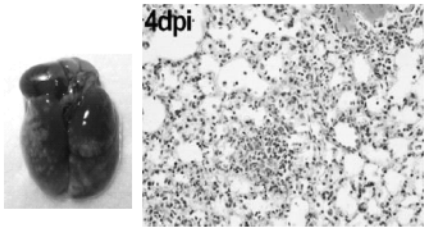
MERS-CoV Animal Model Progress

Constitutive expression of hDPP4 in mice (Agrawal et al JV 2015)

-CAGGS promoter/hDPP4 random insertion mouse
-highly permissive mice, high MERS-CoV RNA/virus in lungs and brain
-significant lung inflammation, mild brain inflammation
->20% weight loss and lethal disease in 5 days
-issue: non cell-type specific expression of hDPP4 in all cells,
brain infection



WT mouse challenged with MERS-CoV

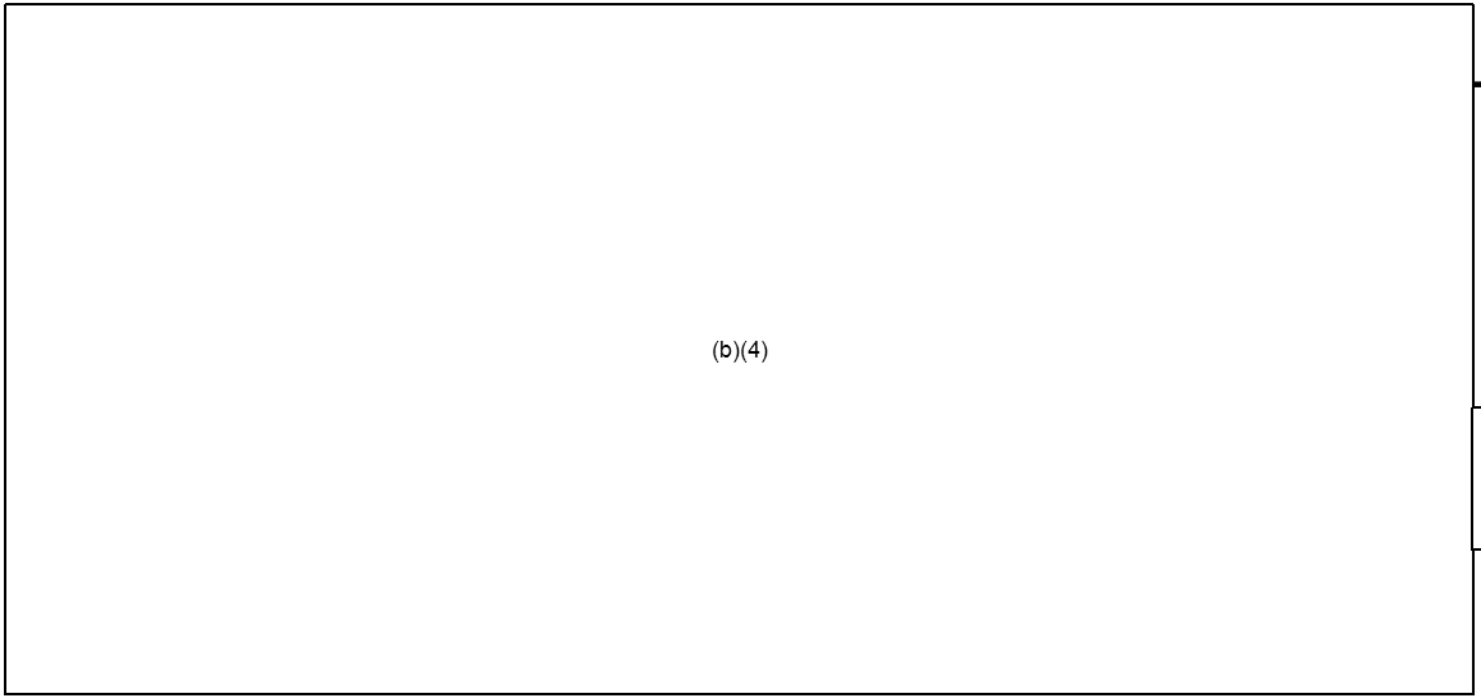


Tg mouse challenged with MERS-CoV

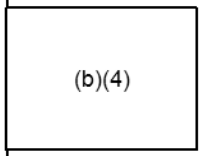
MERS-CoV Animal Model Progress

Small Animal Models

-Rabbit (**Subbarao follow up)**
-hDPP4 expressing Mice



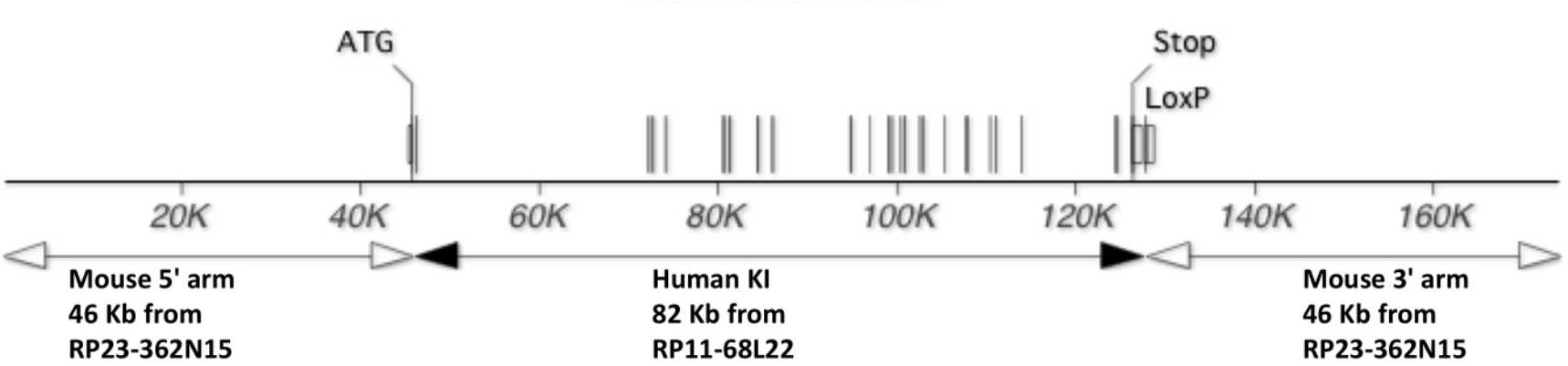
unpublished)



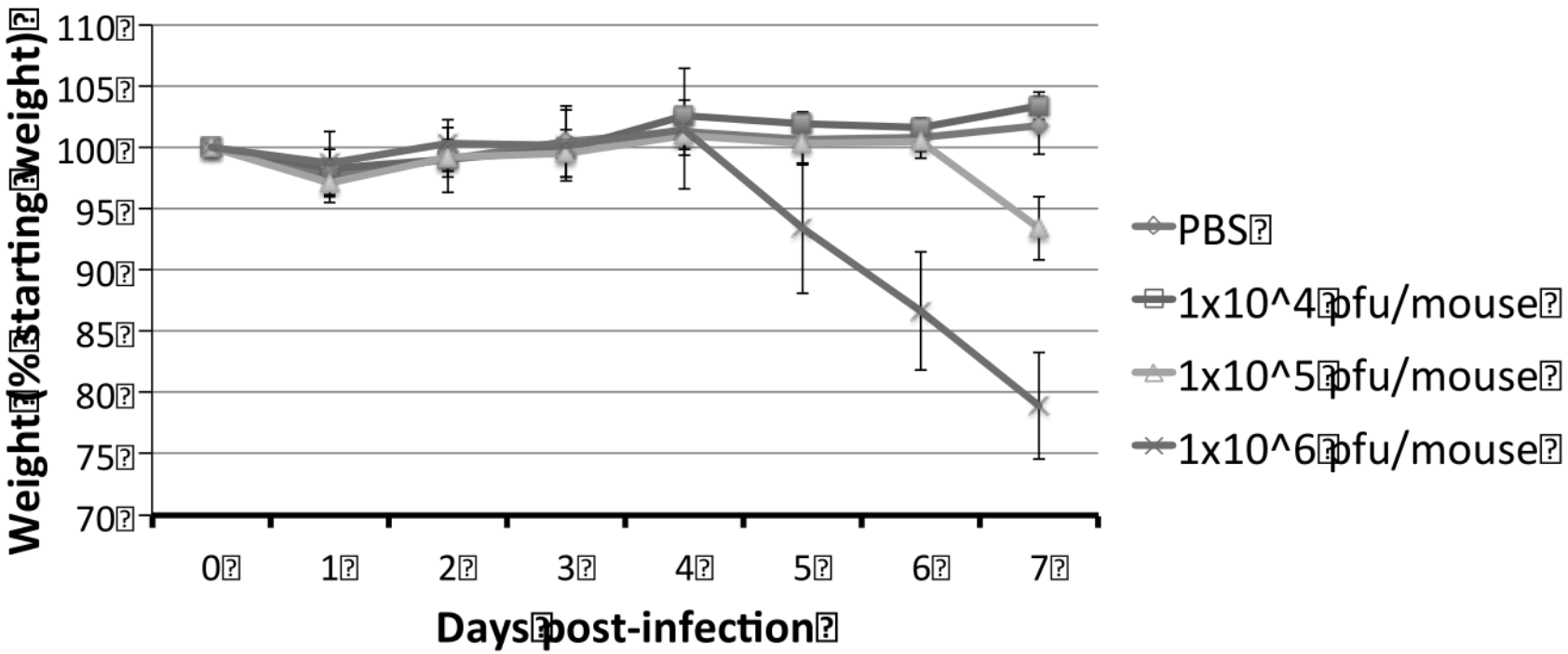
REGENERON

(b)(6)

- Exons 2 to 25 of mouse DPP4 are replaced with the corresponding sequence of human DPP4 (78,824bp replaced by 81,783bp).
- DPP4 humanized mice express fully human DPP4 under the control of the mouse promoter and 3’ UTR.

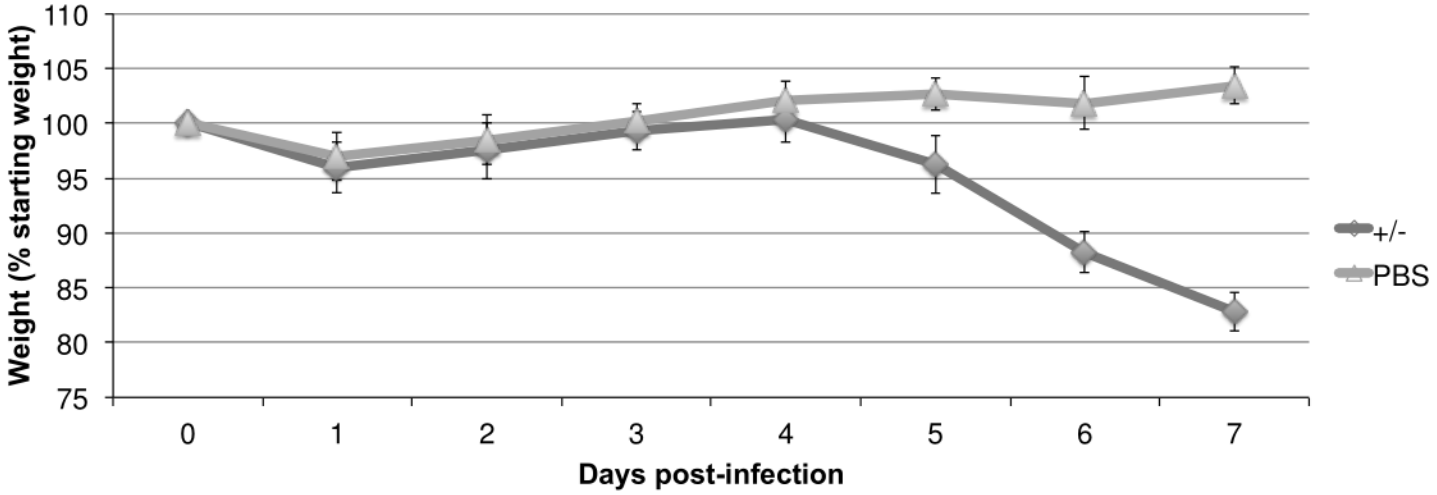


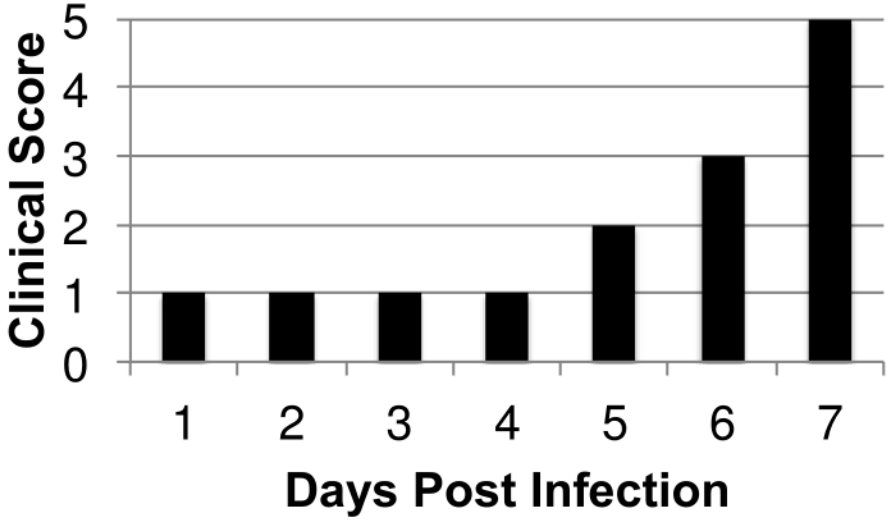
Pathogenesis of MERS-CoV in B6/hDPP4 mice

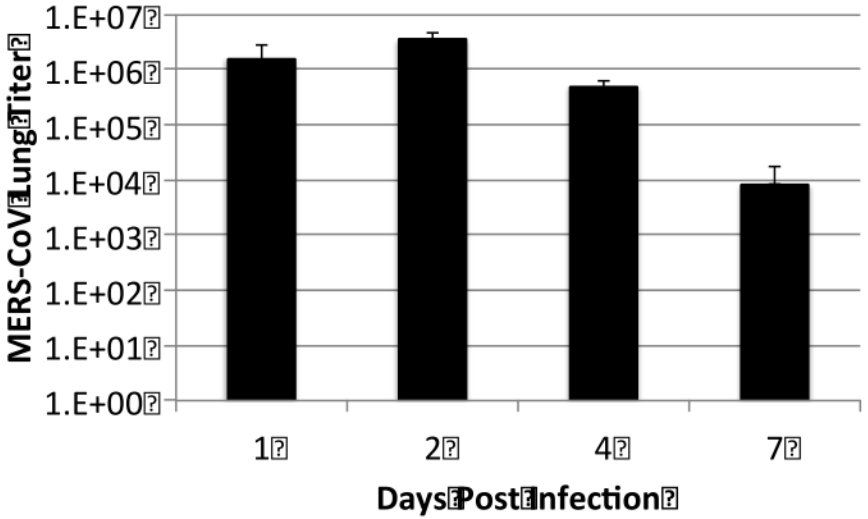


Intranasal infection, MERS-CoV (Jordan strain), 50ul inoculation

Pathogenesis of MERS-CoV in B6/hDPP4 mice



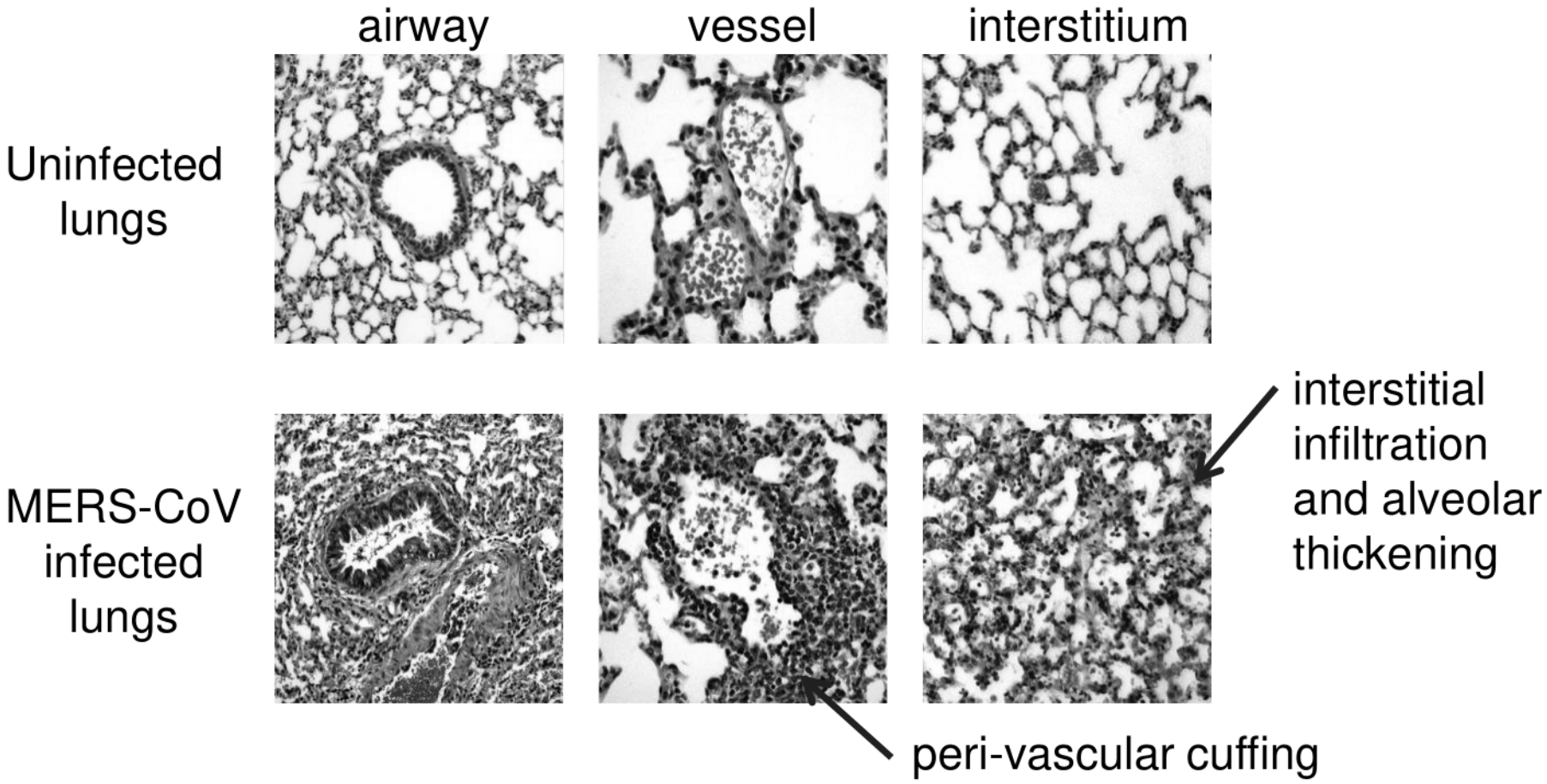




NIH 57943 -005481

**No Brain RNA, Titer

Pathogenesis of MERS-CoV in B6/hDPP4 mice



NIH 57943 -005482

Pascal et al, submitted

Pathogenesis of MERS-CoV in B6/hDPP4 mice

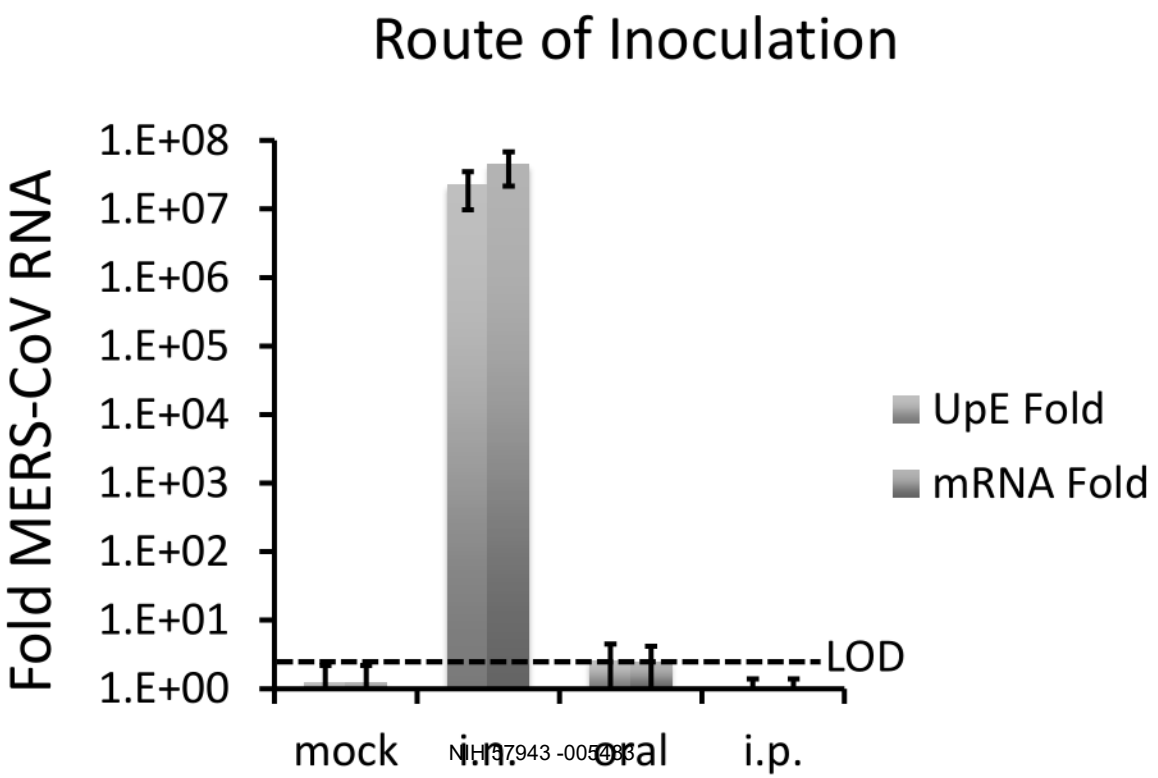
-Using these mice now for:

host response analysis

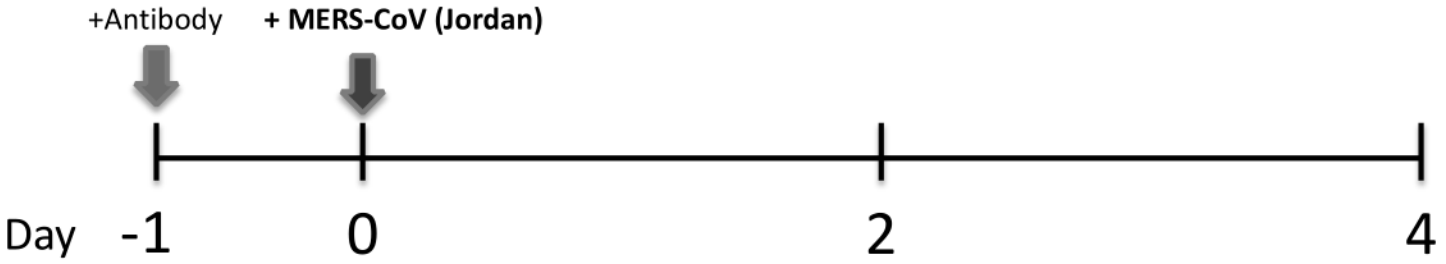
immune response analysis and kinetics

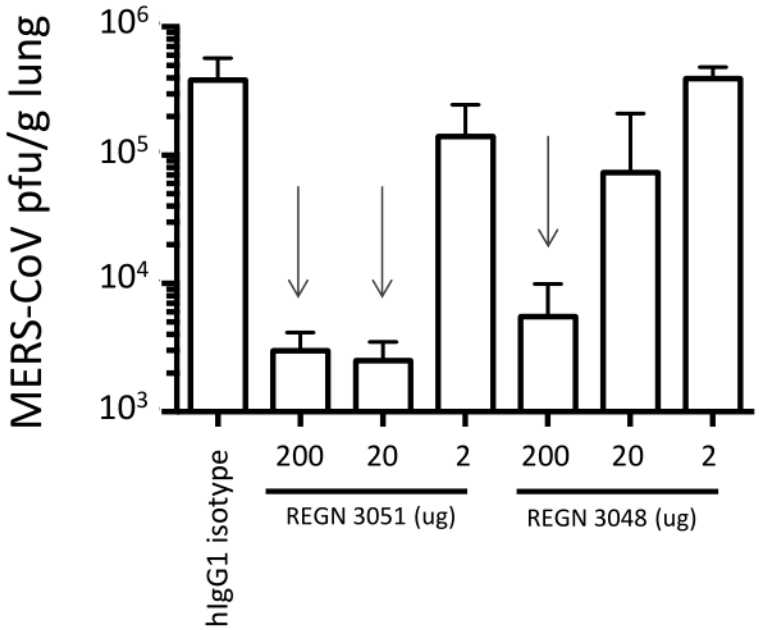
route of inoculation (camel milk hypothesis)

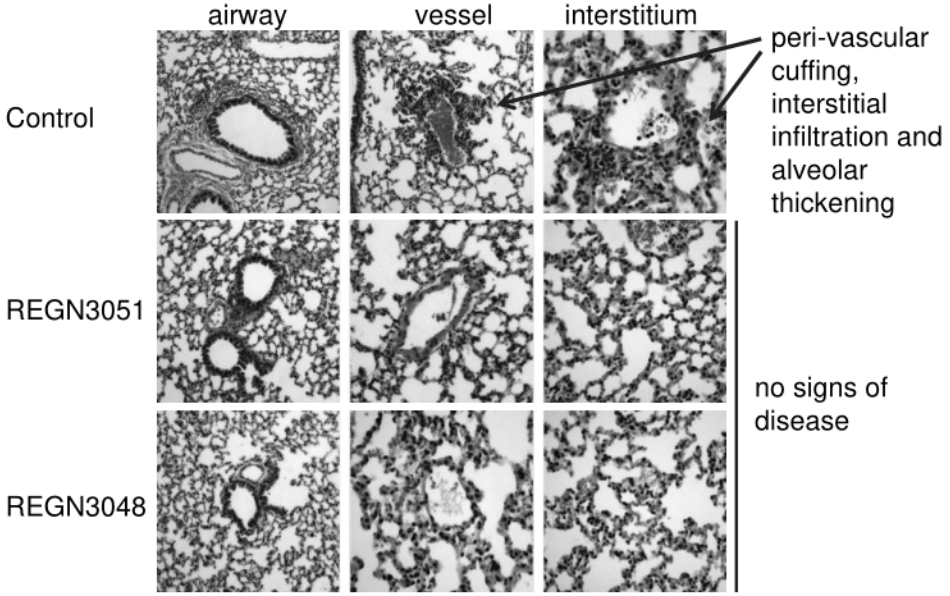
Future countermeasure testing (antibodies, drugs, vaccines, decoy receptors)



Regeneron MERS monoclonals protect B6/hDPP4 mice from MERS-CoV **given 1 day before infection**

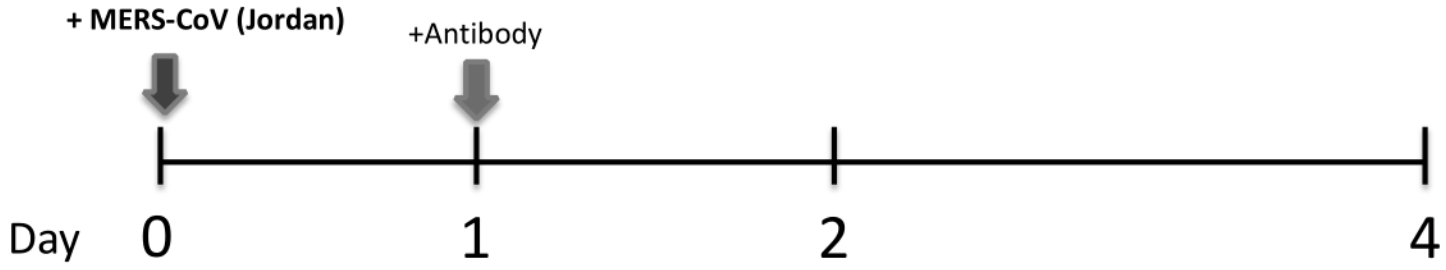


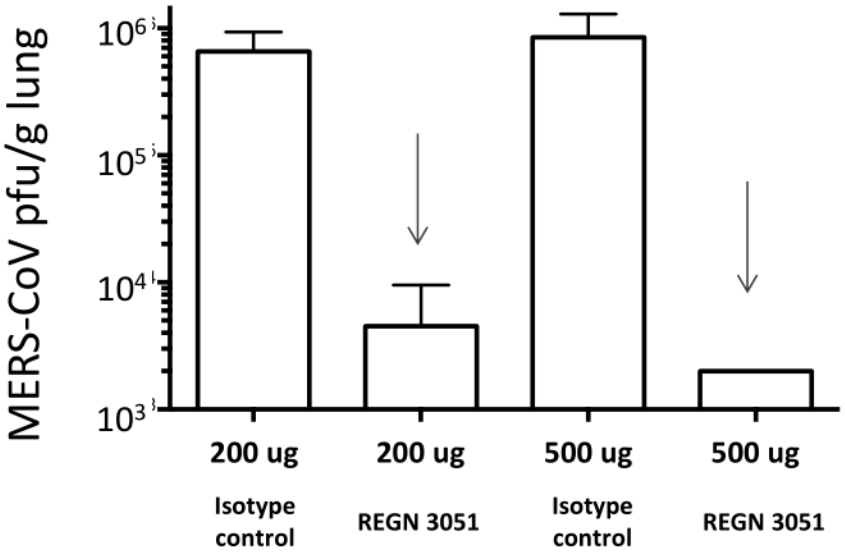


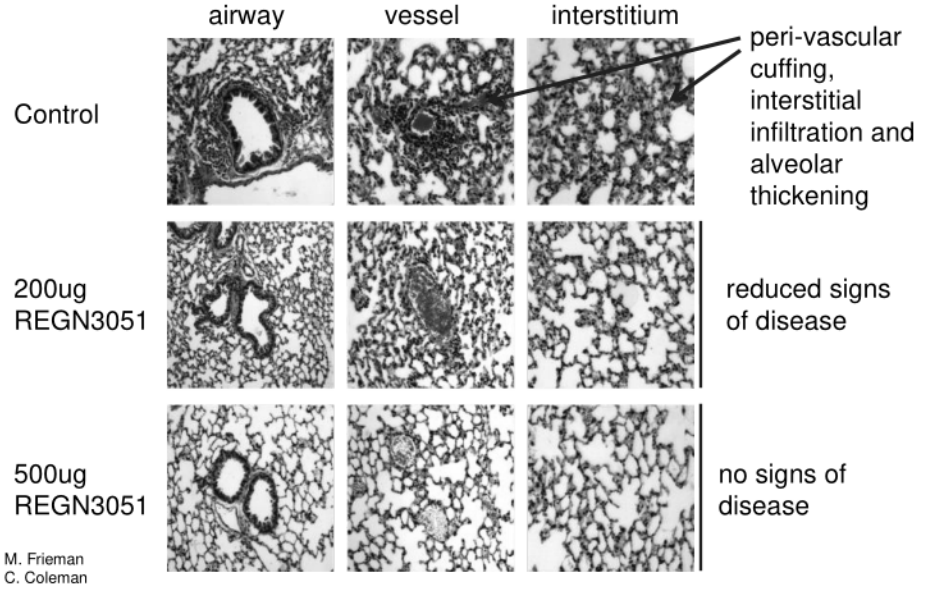


Pascal et al, submitted

Regeneron MERS monoclonals protect B6/hDPP4 mice from MERS-CoV **given 1 day after infection**





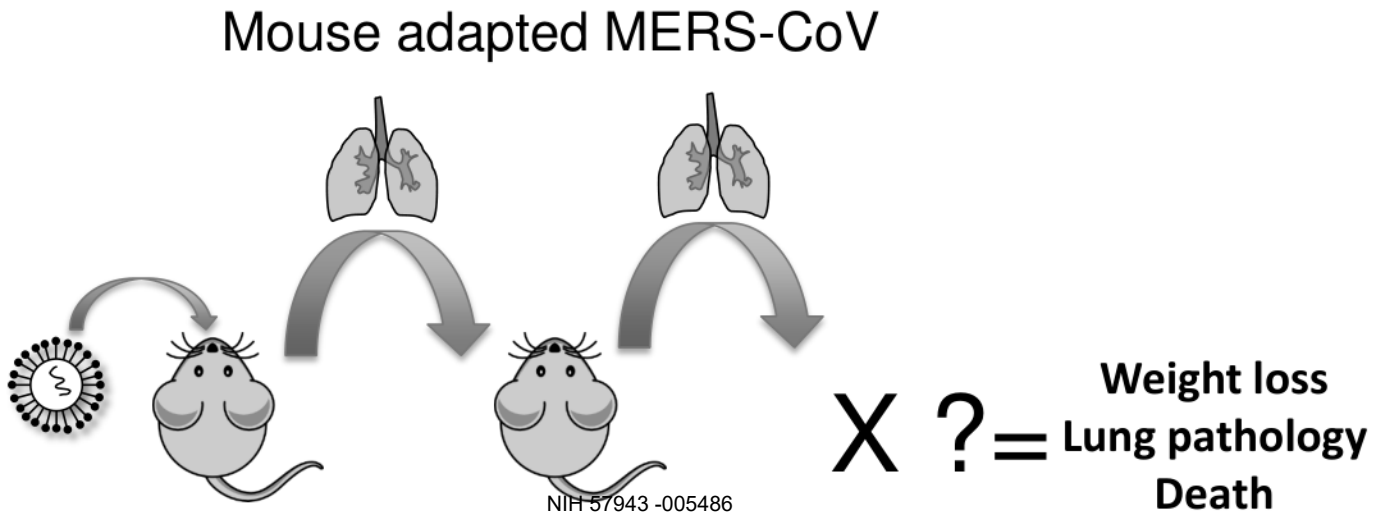


Pascal et al, submitted

Production of a mouse adapted MERS-CoV?

Delayed by the “Pause” in Gain-of-Function experiments with SARS, MERS, Influenza

Benefit: Use single virus in any mouse strain (knockouts, CC mice, etc)
Could compare MERS Spikes to identify pathogenicity differences/evolution
Drawbacks: Can’t compare different MERS-CoV strains without introduction of MA mutations into different infectious clones
mDPP4 conflicts with Spike making passage success difficult



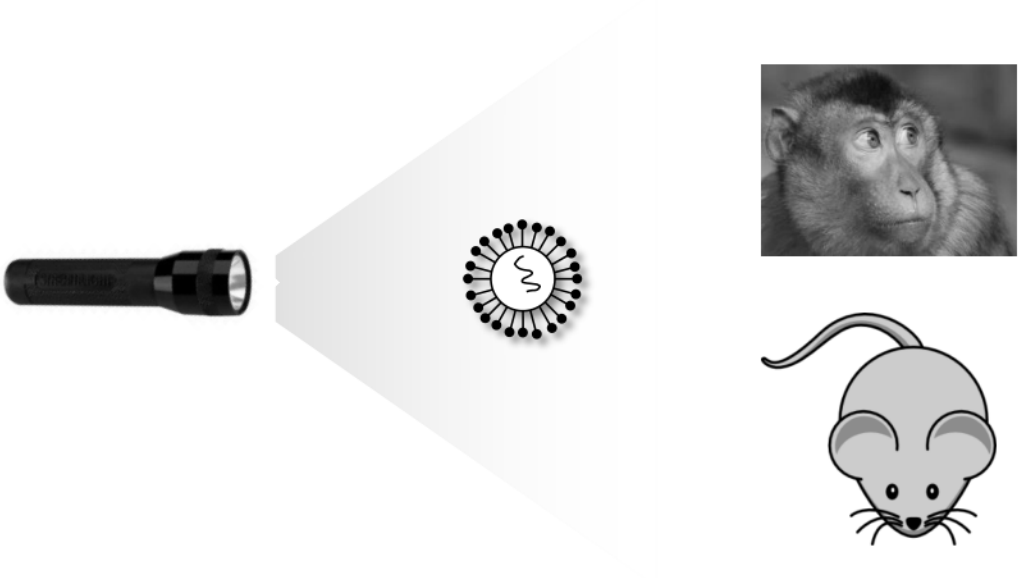
Progress made in MERS animal models

1. Large animal models
    - Rhesus/marmoset are permissive and have disease
    - camels are permissive with URT infection
2. Small animal models
    - Rabbits are mildly permissive but there is hope!
    - Mice are not permissive but can be made so
        - Ad/hDPP4-easily transferable between strains
        - CAGGS/hDPP4-highly sensitive with lethal disease
        - KI/hDPP4 (REGN)-permissive with severe disease
3. Comparisons between SARS and MERS models are not possible
4. Pathogenesis studies underway in all models
5. Testing of MCMs are in progress

Questions Remaining

1. What are we **comparing** the animal models to?
2. What is the **best** animal model for MERS-CoV?
   large animal vs small animal
   natural vs transgenic model
3. Are there significant MERS-CoV **strain** differences on infection?
4. Does inoculation **volume** alter disease pathogenesis/severity?
5. Does **route** of infection matter (4 way infection vs i.n.)?
6. What are the key **differences** between the models developed?
7. What model will be best suited for **future** use?
   -testing antibodies, drugs, vaccines, other MCMs
8. How do we **synergize** data/models/information to speed therapeutic development?

Goal of a MERS-CoV model



Pathophysiology
Immune response
Host Pathways
Countermeasures
Vaccines/Drugs

Acknowledgements

Frieman Lab

(b)(6)

IRF/NIAID

(b)(6)

Peter Jahrling

Lisa Hensley

Reed Johnson

(b)(6)

Mike Holbrook

Zalicus

(b)(6)

Novavax

(b)(6)

Regeneron

(b)(6)

SAB

(b)(6)

MERS reagents

(b)(6)

(Life Tech.)

Funding

NIH/NIAID (E. Stemmy)

Novavax Inc

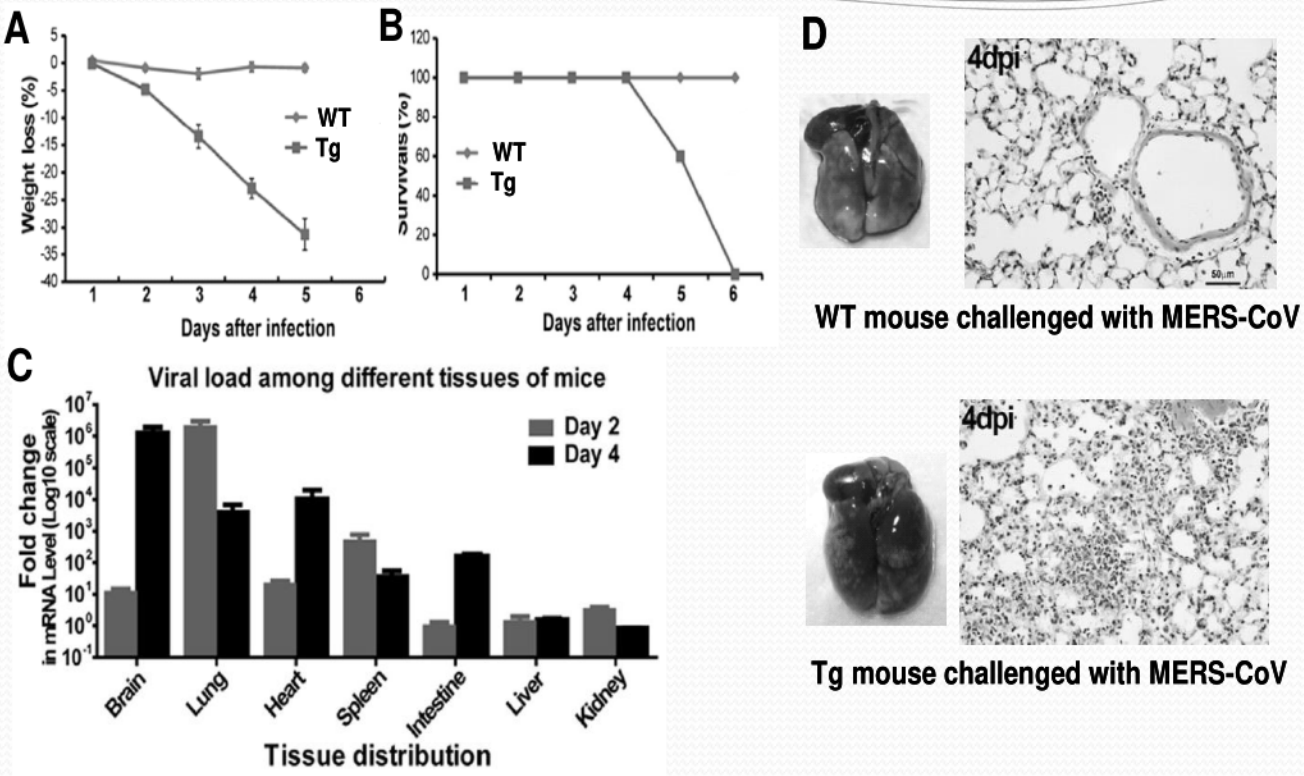
Romark Inc

Unither Virology

Regeneron Inc

National Institute of Allergy and Infectious Diseases

HUMAN CD26/DPP4 TRANSGENIC MOUSE MODEL FOR MERS-COV INFECTION AND DISEASE



Human CD26/DPP4 transgenic mice/high dose (~10^6 $TCID_{50}$) MERS-CoV infection:

- **Permissive to MERS-CoV infection and disease**
- **Systemic infection with lung and brain as the primary targets**
- **Gross and microscopic lesions of the lungs, but not brains**
- **Succumbed to infection with relentless weight loss and 100% mortality within days**

SOME OTHER DATA

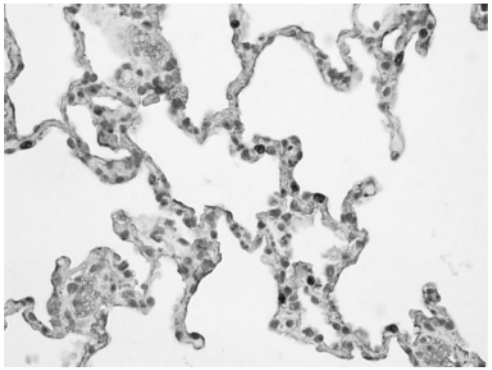
- 50% lethal dose (LD_{50}): ~10 $TCID_{50}$
- 50% infectious dose (ID_{50}): ~ 1 $TCID_{50}$
- All low dose survivors:
  - serum neutralizing antibody to MERS-CoV
  - immune to 100 LD_{50} MERS-CoV challenge

MERS-CoV infection in rabbits

Kanta Subbarao

Laboratory of Infectious Diseases,
NIAID

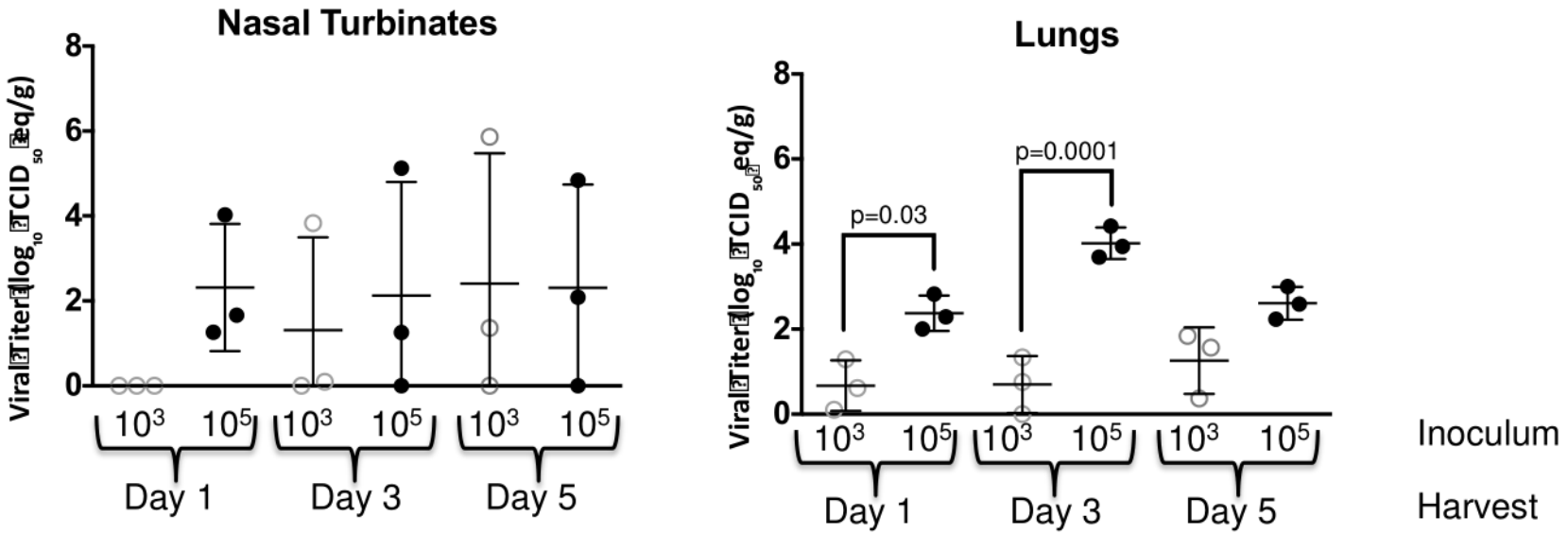
New Zealand White Rabbits

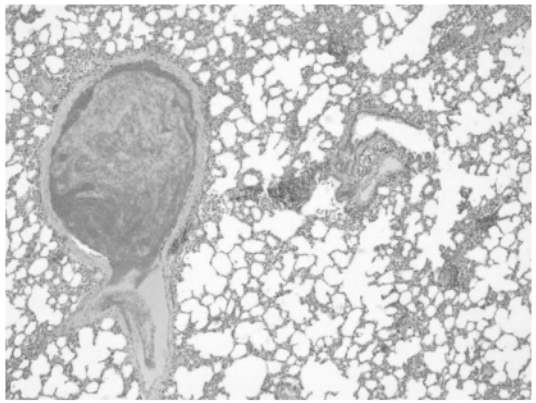


- DPP4/CD26 very similar to humans within 14aa region that interacts with the spike protein of MERS-CoV
- IHC demonstrates CD26 staining in the lungs and kidneys of rabbits

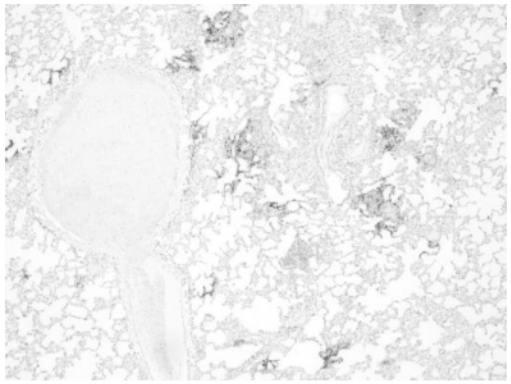
	Amino Acid residue (human DPP4 numbering)													
Species	229	267	286	288	291	294	295	298	317	322	336	341	344	346
Human	N	K	Q	T	A	L	I	H	R	Y	R	V	Q	I
Common Marmoset/Rhesus Macaque	N	K	Q	T	A	L	I	H	R	Y	R	V	Q	I
Rabbit-New Zealand White	**N**	**R**	**Q**	**T**	**A**	**L**	**I**	**H**	**R**	**Y**	**R**	**V**	**Q**	**I**
Camel	N	K	Q	V	A	L	I	H	R	Y	R	V	Q	I
Ferret	N	K	E	T	D	S	T	Y	R	Y	S	E	E	T
Mouse	N	K	Q	P	A	A	R	H	R	Y	T	S	Q	V
Hamster	N	K	Q	T	E	L	T	H	R	Y	T	L	Q	V

Primary infection results in pulmonary infection with inflammation and viral antigen





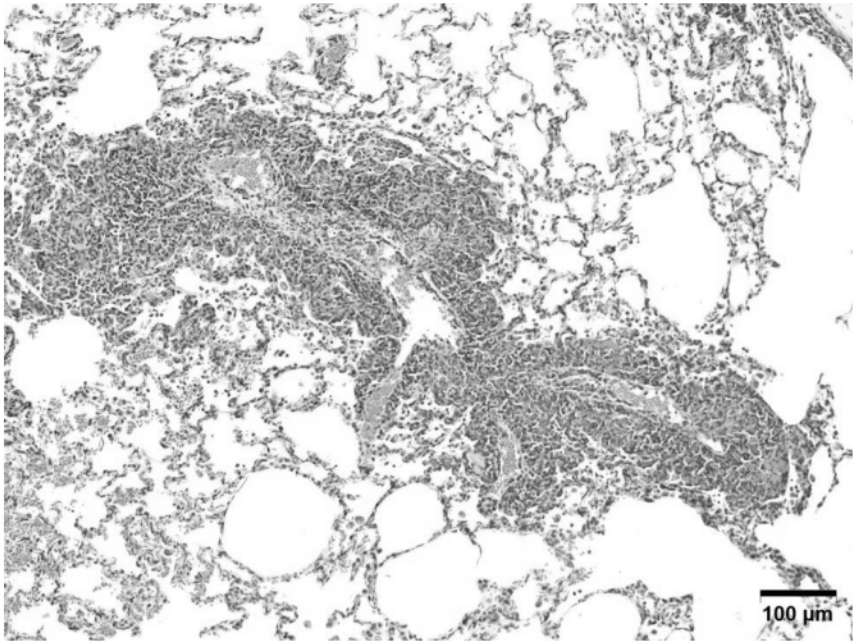
Perivascular and peribronchiolar infiltrates of lymphocytes, heterophils and macrophages

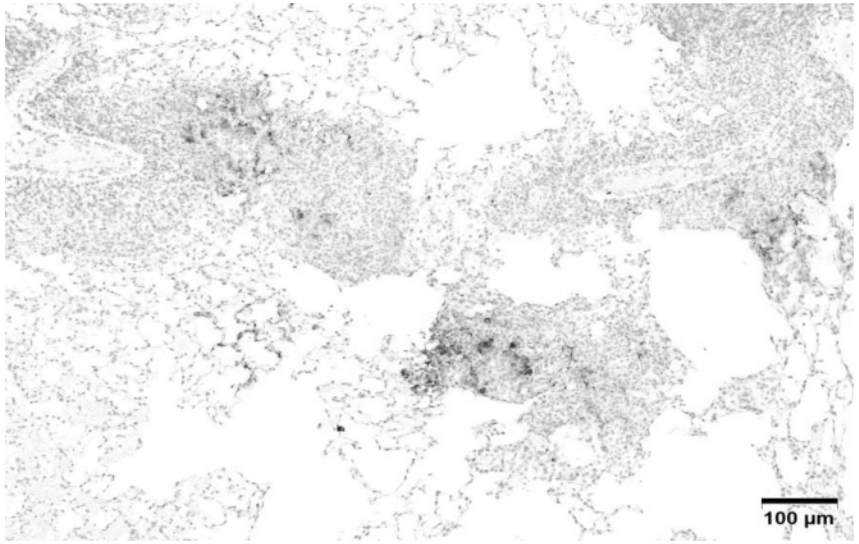


ELISA Ab detected at higher dose; neutralizing Ab not detected

H&E revealed increased inflammation following secondary infection

$10^3/10^5$ $TCID_{50}$ infected rabbit, Day 3, 10x

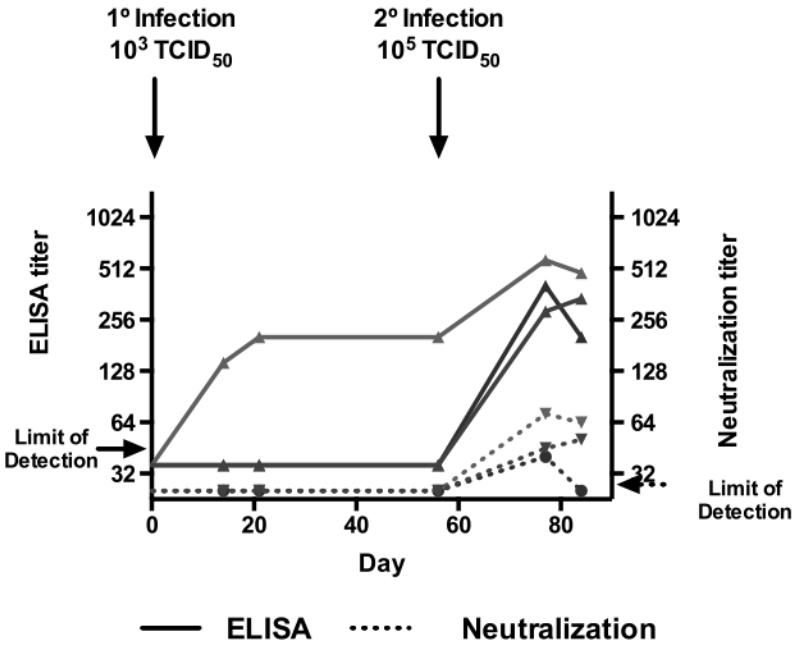


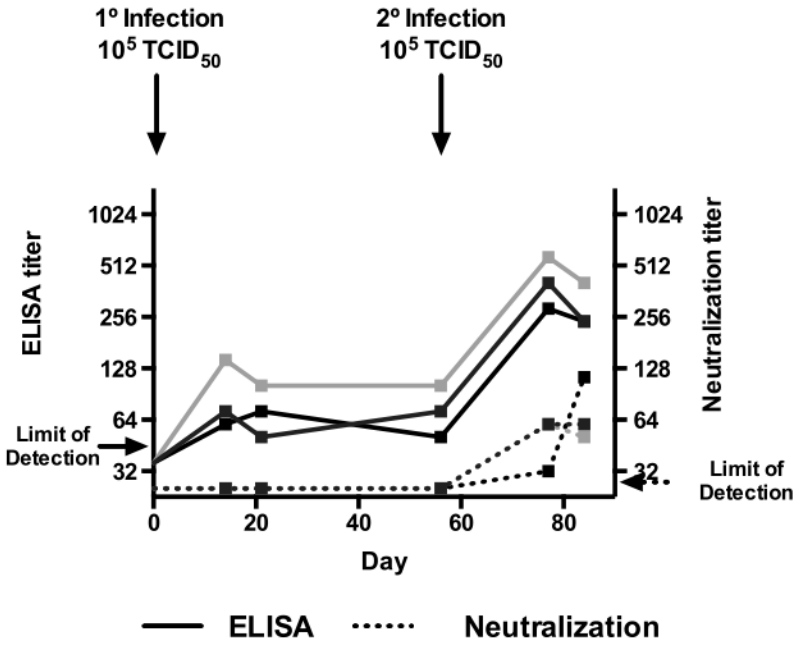


- Increase in pathology following secondary infection
- Foci of inflammatory infiltrates, interstitial congestion, and perivascular cuffing observed

- Antigen burden following secondary infection

Serum Antibody Responses to Primary and Secondary Infection





Summary from studies in rabbits

- Primary infection:
  - vRNA detected in the respiratory tract; lung>Nasal turbinates/trachea; dose response
  - Lungs: viral antigen burden and perivascular inflammation
  - ELISA antibody detected in 1/3 rabbits that received the lower dose and all rabbits that received a higher virus dose
  - Little to no neutralizing antibody elicited
- Secondary infection:
  - vRNA detected in lower respiratory tract
  - Lungs: increased inflammation; most remarkable in rabbits that were previously infected with the lower dose of virus
  - Neutralizing antibody response detected in all rabbits; these rabbits were now protected from challenge with almost no viral antigen and minimal inflammation

Presence of neutralizing antibodies protects from challenge

This was confirmed by IN and IV administration of a human mab against MERS-CoV in rabbits (Ying J Virology 88: 7796-7805, 2014; Dimitrov lab NCI)

Panel Discussion

(25 minutes)

Questions

(b)(5)

Questions

(b)(5)

BREAK

(10 minutes)

United States Department of
Health & Human Services

Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV)

Current Status of Diagnostics

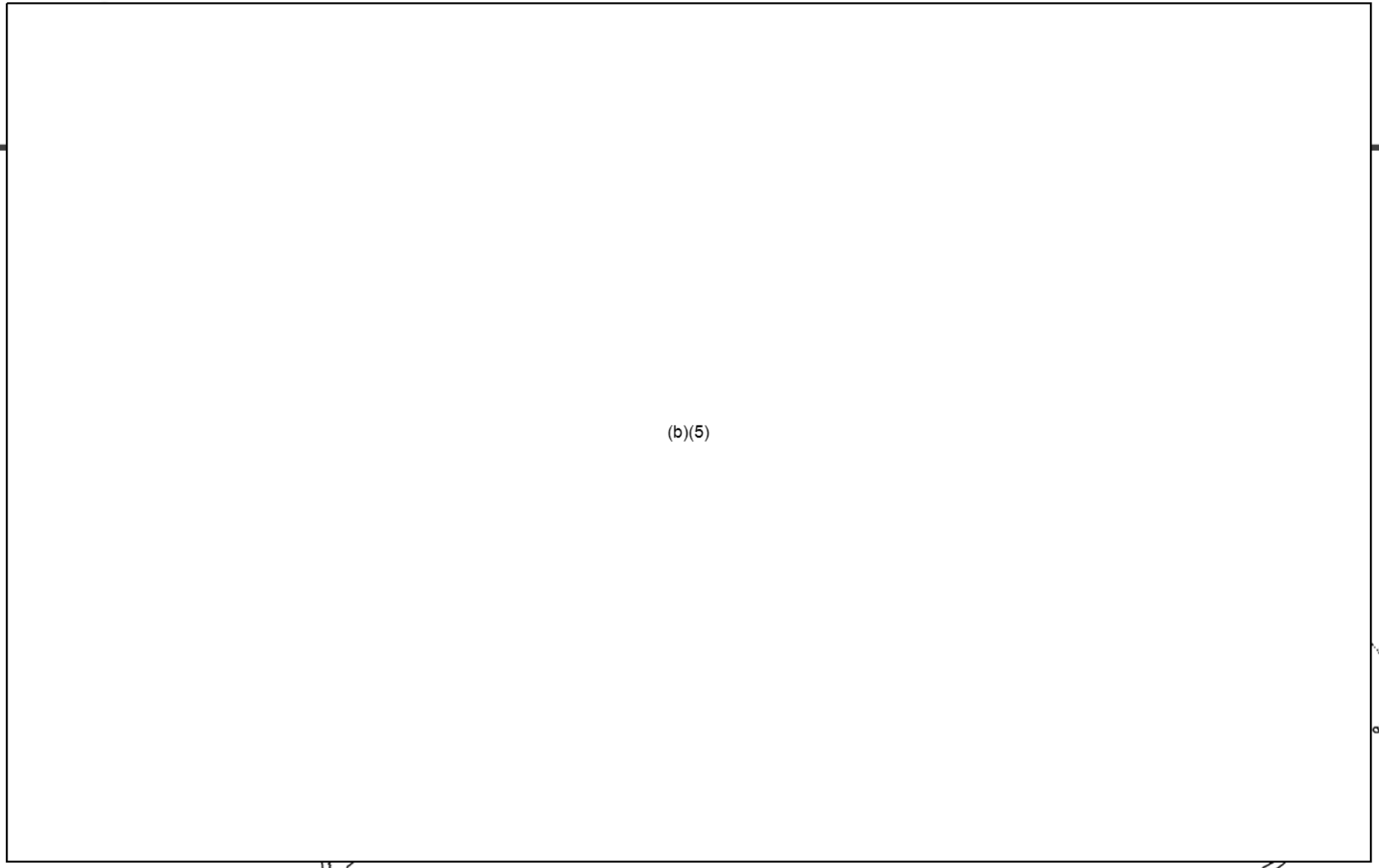
Sally Hojvat FDA

Dean Erdman CDC

(b)(5)

Emergency Use Authorization (EUA)

(b)(5)



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Current Status of Diagnostic Test

(b)(5)

(b)(5)

CDC Laboratory Response to MERS: Real-time RT-PCR Assays

Dean D. Erdman, DrPH

HHS/ASPR MERS-CoV Workshop
April 3, 2015, Washington DC

MERS-CoV Timeline
First Cases

2012

Mar-Apr - SARI outbreak of unknown etiology at Jordanian hospital

Apr - Investigation by Jordan MOH/NAMRU-3 fails to identify pathogen

June 13 - Saudi national with SARI is admitted to hospital in Jeddah, Saudi Arabia; dies on June 24

Sept 3-7 - Qatari national presents with ARI symptoms and is admitted to hospital in Doha, Qatar

Sept 11-12 - Qatari patient medevaced to UK and admitted to ICU

Sept 15 - Report of new CoV identified in the Saudi patient is submitted to ProMED; published **Sept 20**

International Society for Infectious Diseases — ProMED-mail

Published Date: 2012-09-20 15:51:26
Subject: PRO/EDR> Novel coronavirus - Saudi Arabia: human isolate
Archive Number: 20120920.1302733

NOVEL CORONAVIRUS - SAUDI ARABIA: HUMAN ISOLATE
****
A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

Date: Sat 15 Sep 2012
From: Ali Mohamed Zaki <azaki53@hotmail.com> [edited]

A new human coronavirus was isolated from a patient with pneumonia by Dr Ali Mohamed Zaki at the Virology Laboratory of Dr Soliman Fakeeh Hospital Jeddah Saudi Arabia.

The virus was isolated from sputum of a male patient aged 60 years old presenting with pneumonia associated with acute renal failure. The virus grows readily on Vero cells and LLC-MK2 cells producing CPE in the form of rounding and syncetia formation.

[The clinical isolate] was initially tested for influenza virus A, influenza virus B, parainfluenza virus, enterovirus and adenovirus, with negative results. Testing with a pancoronavirus RT-PCR yielded a band at a molecular weight appropriate for a coronavirus. The virus RNA was tested also in Dr. Ron Fouchier's laboratory in the Netherlands and was confirmed to be a new member of the beta group of coronoviruses, closely related to bat coronaviruses. Further analysis is being carried out in the Netherlands.

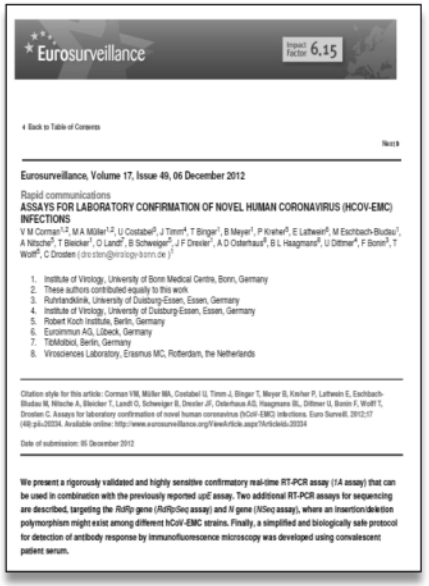
The Virology Laboratory at the Dr Fakeeh Hospital will be happy to collaborate with others in studies of this virus.

--
Ali Mohamed Zaki
Professor of Microbiology
Dr Fakeeh hospital Jeddah Saudi Arabia
<azaki53@hotmail.com>

MERS-CoV Timeline
rRT-PCR Assays

RAPID COMMUNICATIONS

Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction



Eurosurveillance

Impact Factor 6,15

Eurosurveillance, Volume 17, Issue 49, 06 December 2012

Rapid communications

ASSAYS FOR LABORATORY CONFIRMATION OF NOVEL HUMAN CORONAVIRUS (HCOV-EMC) INFECTIONS

2012

Sept 25 - CDC receives MERS-CoV N gene sequence from Erasmus MC

Sept 25-28 - CDC N1, N2, N3 rRT-PCR assays and template oligos synthesized

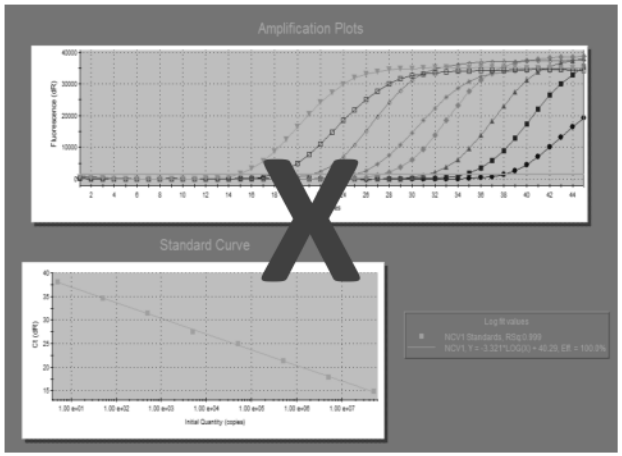
Sept 27 - upE, ORF1b rRT-PCR protocol posted on Eurosurveillance website by Institute of Virology, Bonn MC; ORF1b proves insensitive and is replaced by ORF1a on **Dec 6**

Sept 27 - Full genome sequence posted on GenBank by Erasmus MC

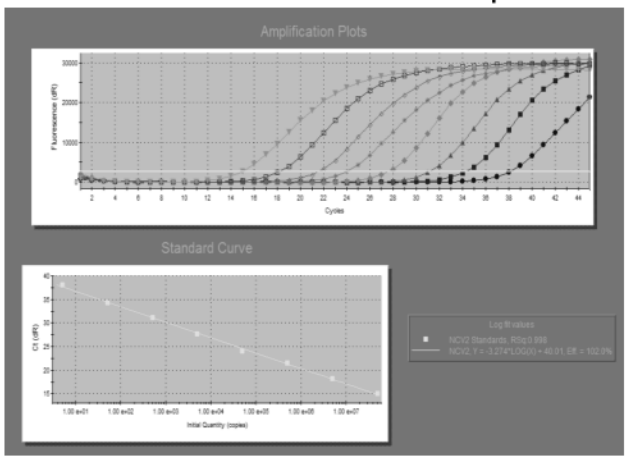
Oct 2-10 - CDC conducts analytical validation of upE, N1, N2, N3 rRT-PCR assay using RNA transcripts

MERS-CoV rRT-PCR Assay
N Gene Signature Plots

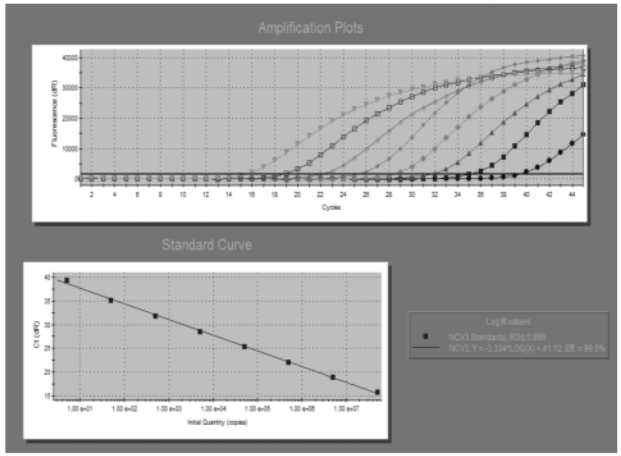
NCV1 standardcurve.mxp



NCV2 standardcurve.mxp



NCV3 standardcurve.mxp



MERS-CoV Timeline
Receipt of Samples and Issuance of EUA

2012

Oct 21 - NAMRU-3 receives rRT-PCR reagents from CDC to retrospectively test samples from Jordan outbreak cluster

Nov - NAMRU-3 reports detection of MERS-CoV by rRT-PCR and culture

Dec 26 - CDC receives first MERS-CoV positive clinical specimens from NAMRU-3 for confirmation

2013

Feb 11 - CDC receives Jordan MERS-CoV isolate from NAMRU-3

Apr 29 - CDC rRT-PCR kits distributed to States by LRN

May 29 - Secretary Sebelius declares that circumstances exist justifying the authorization of emergency use of assay

June 5 - FDA issues emergency use authorization (EUA)

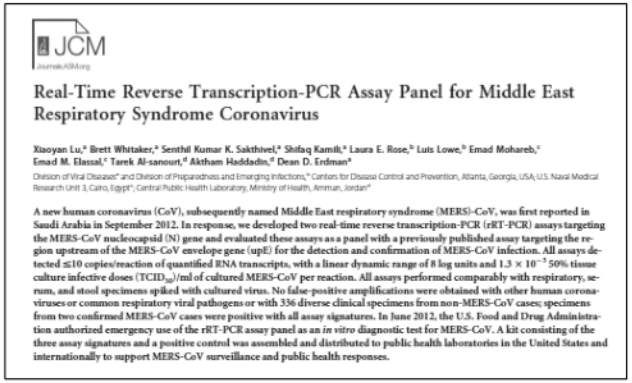
June 7 - States begin testing PT panels for CLIA compliance

MERS-CoV rRT-PCR Assay Development
Major Challenges

- Assay signatures & target conservation
- Assay specificity
- Lack of authentic positive clinical specimens
- Optimal specimen types/timing
- Compatibility with existing platforms

CDC MERS-CoV rRT-PCR Assay
Testing Algorithm

*NOTE: Stool specimens may not give positive RP Assay results. See results interpretation section for guidance.



JCM

Real-Time Reverse Transcription-PCR Assay Panel for Middle East Respiratory Syndrome Coronavirus

Xiaoyan Lu,[a] Brett Whitaker,[a] Senthil Kumar K. Sakthivel,[a] Shifaq Kamili,[a] Laura E. Rose,[b] Luis Lowe,[b] Emad Mohareb,[c] Emad M. Elassal,[c] Tarek Al-sanourt,[d] Aktham Haddadin,[d] Dean D. Erdman[a]

Division of Viral Diseases[a] and Division of Preparedness and Emerging Infections,[b] Centers for Disease Control and Prevention, Atlanta, Georgia, USA; U.S. Naval Medical Research Unit 3, Cairo, Egypt[c]; Central Public Health Laboratory, Ministry of Health, Amman, Jordan[d]

A new human coronavirus (CoV), subsequently named Middle East respiratory syndrome (MERS)-CoV, was first reported in Saudi Arabia in September 2012. In response, we developed two real-time reverse transcription-PCR (rRT-PCR) assays targeting the MERS-CoV nucleocapsid (N) gene and evaluated these assays as a panel with a previously published assay targeting the region upstream of the MERS-CoV envelope gene (upE) for the detection and confirmation of MERS-CoV infection. All assays detected ≤10 copies/reaction of quantified RNA transcripts, with a linear dynamic range of 8 log units and 1.3×10^{-5} 50% tissue culture infective doses ($TCID_{50}$)/ml of cultured MERS-CoV per reaction. All assays performed comparably with respiratory, serum, and stool specimens spiked with cultured virus. No false-positive amplifications were obtained with other human coronaviruses or common respiratory viral pathogens or with 336 diverse clinical specimens from non-MERS-CoV cases; specimens from two confirmed MERS-CoV cases were positive with all assay signatures. In June 2012, the U.S. Food and Drug Administration authorized emergency use of the rRT-PCR assay panel as an *in vitro* diagnostic test for MERS-CoV. A kit consisting of the three assay signatures and a positive control was assembled and distributed to public health laboratories in the United States and internationally to support MERS-CoV surveillance and public health responses.

CDC MERS-CoV rRT-PCR Assay
Clinical Studies

	Specimens from Confirmed Cases (Jordan cluster, positive results expected)					Other Specimens (Negative results expected)				
Specimen type	#	NCV.upE # pos	NCV.N2 # pos	NCV.N3 # pos	Overall # pos	#	NCV.upE # pos	NCV.N2 # pos	NCV.N3 # pos	Overall # pos
NP/OP Swabs	0	-	-	-	-	290	0/290	0/290	0/290	0/290
Sputum	0	-	-	-	-	4	0/4	0/4	0/4	0/4
Broncheal or transtracheal aspirates or washes	1	1/1	1/1	1/1	1/1	23	0/23	0/23	0/23	0/23
Serum	1	1/1	1/1	1/1	1/1	3	0/3	0/3	0/3	0/3
Stool	0	-	-	-	-	7	0/7	0/7	0/7	0/7*

*One stool specimen generated a negative PCR result for RP as well as the NCV primers and probes, thus the result is inconclusive.

Positive percent agreement = 2/2 = 100% (95% CI: 34.2% - 100%)
Negative percent agreement = 326/327 = 99.7% (95% CI: 98.3% - 99.9%)
Overall percent agreement = 328/329 = 99.7% (95% CI: 98.3% - 99.9%)

CDC MERS-CoV rRT-PCR Assay

Analytical Sensitivity (LoD) – Matrix and Extraction Comparison

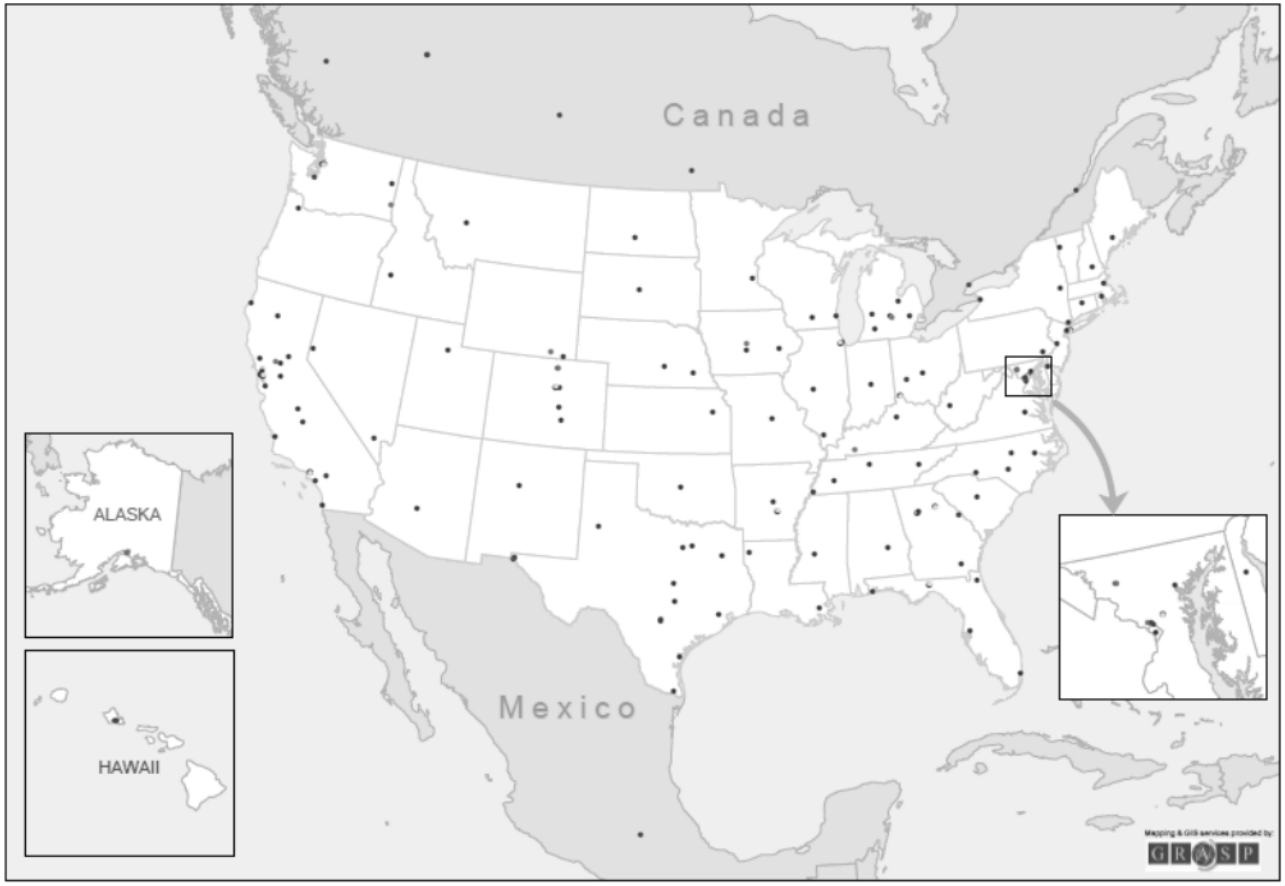
				NCV.upE				NCV.N2				NCV.N3				RP			
Extraction	Dil.	Concentration (TCID50/mL)	Ct	Serum	NP/OP	Sputum	Stool	Serum	NP/OP	Sputum	Stool	Serum	NP/OP	Sputum	Stool	Serum	NP/OP	Sputum	Stool
MagNA Pure Compact Nucleic Acid Isolation Kit I	10^{-4}	1.3×10^{0}	1	25.99	25.79	27.33	32.99	25.01	25.66	26.85	32.16	24.87	23.16	24.54	29.86	32.39	28.06	24.2	neg
			2	26.5	25.73	27.35	33.83	24.9	25.76	26.85	32.04	25.16	23.34	24.46	29.67	32.49	28.24	24.34	neg
			3	25.93	25.8	27.44	NA	24.98	25.69	26.85	NA	25.47	23.35	24.52	30.4	32.7	28.16	24.4	neg
			Ave	26.14	25.78	27.37	33.41	24.96	25.7	26.85	32.1	25.17	23.28	24.51	29.99	32.53	28.15	24.31	NA
			SD	0.31	0.04	0.06	0.59	0.05	0.05	0	0.08	0.3	0.1	0.04	0.38	0.15	0.09	0.1	NA
			Call rate	3/3	3/3	3/3	2/2	3/3	3/3	3/3	2/2	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3
MagNA Pure Compact Nucleic Acid Isolation Kit I	10^{-5}	1.3×10^{-1}	1	31.26	30.76	32.01	neg	30.84	30.88	31.68	neg	30.35	28.37	29.5	34.99	32.24	28.03	24.69	neg
			2	31.8	30.96	32.12	neg	30.67	31.09	31.97	neg	30.56	28.47	29.51	35.13	32.06	28.1	24.7	neg
			3	31.55	30.9	32.24	neg	30.74	31.06	31.95	neg	30.39	28.66	29.72	34.84	32.05	28.13	24.65	neg
			Ave	31.53	30.88	32.12	NA	30.75	31.01	31.87	NA	30.43	28.5	29.58	34.98	32.12	28.09	24.68	NA
			SD	0.27	0.1	0.11	NA	0.09	0.11	0.16	NA	0.11	0.14	0.12	0.15	0.1	0.05	0.03	NA
			Call rate	3/3	3/3	3/3	0/3	3/3	3/3	3/3	0/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3
MagNA Pure Compact Nucleic Acid Isolation Kit I	10^{-6}	1.3×10^{-2}	1	33.86	34.22	34.81	neg	33.24	34.07	34.82	neg	32.98	31.82	32.43	38.75	31.64	28	24.51	neg
			2	33.86	35.61	36.05	neg	32.94	34.51	35.36	neg	32.9	32.54	33.01	39.25	32.03	28.04	24.49	neg
			3	33.86	34.78	34.37	neg	33.42	34.66	35.16	neg	33	31.99	32.73	neg	32.21	28.05	24.57	neg
			Ave	33.86	34.87	35.07	NA	33.2	34.42	35.11	NA	32.96	32.12	32.72	39	31.96	28.03	24.52	NA
			SD	0	0.7	0.87	NA	0.24	0.31	0.27	NA	0.06	0.38	0.29	0.35	0.29	0.03	0.04	NA
			Call rate	3/3	3/3	3/3	0/3	3/3	3/3	3/3	0/3	3/3	3/3	3/3	2/3	3/3	3/3	3/3	0/3
MagNA Pure Compact Nucleic Acid Isolation Kit I	10^{-7}	1.3×10^{-3}	1	37.79	38.35	39.92	neg	39	38.25	37.18	neg	37.66	34.83	35.87	neg	32.23	26.39	24.44	neg
			2	39.06	38.49	38.53	neg	36.9	38.08	37.89	neg	37.55	35.8	35.89	neg	31.99	26.5	24.58	neg
			3	37.88	38.31	37.86	neg	38.68	37.56	39.45	neg	36.75	34.95	35.88	neg	32.24	26.49	24.57	neg
			Ave	38.24	38.39	38.77	NA	38.19	37.96	38.17	NA	37.32	35.2	35.88	NA	32.15	26.46	24.53	NA
			SD	0.71	0.1	1.05	NA	1.13	0.36	1.16	NA	0.5	0.53	0.01	NA	0.14	0.06	0.08	NA
			Call rate	3/3	3/3	3/3	0/3	3/3	3/3	3/3	0/3	3/3	3/3	3/3	0/3	3/3	3/3	3/3	0/3
MagNA Pure Compact Nucleic Acid Isolation Kit I	10^{-8}	1.3×10^{-4}	1	neg	39.07	neg	neg	neg	40.85	neg	neg	neg	neg	neg	neg	32.14	28.05	25.05	neg
			2	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	32.38	28.02	25.08	neg
			3	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	32.35	28.14	25.09	neg
			Ave	NA	39.07	NA	NA	NA	40.85	NA	NA	NA	NA	NA	NA	32.29	28.07	25.07	NA
			SD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.13	0.06	0.02	NA
			Call rate	0/3	1/3	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3	3/3	3/3	3/3	0/3

MagNA Pure Compact (Nucleic Acid Isolation Kit I)

National Center for Emerging Zoonotic and Infectious Diseases
Division of Preparedness and Emerging Infections

Laboratory Preparedness and Response Branch

LRN
LABORATORY RESPONSE NETWORK
PARTNERS IN PREPAREDNESS



Legend

- Public Health (102)
- Military (12)
- Federal (6)
- Food (12)
- Veterinary (6)
- International (15)

LRN Laboratories
March, 2014
n = 153

U.S. Department of Health & Human Services

a A A

U.S. Food and Drug Administration

Protecting and Promoting *Your* Health

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SEARCH

Home | Food | Drugs | Medical Devices | Radiation-Emitting Products | Vaccines, Blood & Biologics | Animal & Veterinary | Cosmetics

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring, MD 20993

June 5, 2013

Thomas R. Frieden, MD, MPH
Director
Centers for Disease Control and Prevention
1600 Clifton Rd, MS D-14
Atlanta, GA 30333

Dear Dr. Frieden:

This letter is in response to your request that the Food and Drug Administration (FDA) issue an Emergency Use Authorization (EUA) for emergency use of the CDC Novel Coronavirus 2012 Real-time RT-PCR Assay for the presumptive detection of Middle East Respiratory Syndrome Coronavirus (MERS-CoV), formerly known as Novel Coronavirus 2012 or NCV-2012, in patients with signs and symptoms of MERS-CoV infection in conjunction with clinical and epidemiological risk factors, pursuant to section 564 of the Federal Food, Drug, and Cosmetic Act (the Act) (21 U.S.C. § 360bbb-3), by qualified laboratories.

On May 29, 2013, pursuant to section 564(b)(1)(C) of the Act (21 U.S.C. § 360bbb-3(b)(1)(C)), the Secretary of the Department of Health and Human Services (HHS) determined that there is a significant potential for a public health emergency that has a significant potential to affect national security or the health and security of United States citizens living abroad, and that involves a biological, chemical, radiological, or nuclear agent or agents, or a disease or condition that may be attributable to such an agent or agents—in this case, Middle East Respiratory Syndrome Coronavirus (MERS-CoV).[1] Pursuant to section 564(b)(1) of the Act (21 U.S.C. § 360bbb-3(b)(1)), and on the basis of such determination, the Secretary of HHS then declared that circumstances exist justifying the authorization of the emergency use of [illegible] for the detection of MERS-CoV, subject to the terms [illegible] 360bbb-3(a).[2]

Having concluded that the criteria for issuance of [illegible] Act (21 U.S.C. § 360bbb-3(c)) are met, I am authorizing [illegible] Coronavirus 2012 Real-time RT-PCR Assay (as described in Section I) for the presumptive detection of MERS-CoV infection in conjunction with clinical and epidemiological risk factors, subject to the terms of this authorization.

I. Criteria for Issuance of Authorization

[1] As amended by the Pandemic and All-Hazards Preparedness Reauthorization Act of 2013 (PAHPRA), section 564(b)(1)(C) of the Act the Secretary may make a determination that there is a significant potential for a public health emergency.

[2] Memorandum, Determination of a Significant Potential for a Public Health Emergency that Affects, or Has a Significant Potential to Affect, National Security or the Health and Security of United States Citizens Living Abroad, and that Involves a Biological Agent or Agents, or a Disease or Condition that May be Attributable to such Agent or Agents; Determination that Circumstances Exist Justifying an Authorization Pursuant to Section 564 of the Federal Food, Drug, and Cosmetic Act (May 29, 2013).

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring, MD 20993

June 10, 2014

Thomas R. Frieden, MD, MPH
Director
Centers for Disease Control and Prevention
1600 Clifton Rd, MS D-14
Atlanta, GA 30333

Dear Dr. Frieden:

On June 5, 2013, based on a request by the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA) issued a letter authorizing the emergency use of the CDC Novel Coronavirus 2012 Real-time RT-PCR Assay for the presumptive detection of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in patients with signs and symptoms of MERS-CoV infection in conjunction with clinical and epidemiological risk factors, pursuant to section 564 of the Federal Food, Drug, and Cosmetic Act (the Act) (21 U.S.C. § 360bbb-3), by qualified laboratories.[1] On May 22, CDC submitted a request for an amendment to the Emergency Use Authorization (EUA). In response to that request, and having concluded that revising the June 5, 2013, EUA is appropriate to protect the public health or safety under section 564(g)(2)(C) of the Act (21 U.S.C. § 360bbb-3(g)(2)(C)), the June 5, 2013, letter authorizing the emergency use of the CDC Novel Coronavirus 2012 Real-time RT-PCR Assay is being reissued in its entirety with the amendments incorporated.[2]

On May 29, 2013, pursuant to section 564(b)(1)(C) of the Act (21 U.S.C. § 360bbb-3(b)(1)(C)), the Secretary of Health and Human Services (HHS) determined that there is a significant potential for a public health emergency that has a significant potential to affect national security or the health and security of United States citizens living abroad, and that involves a biological, [illegible], or a disease or condition that may be [illegible], MERS-CoV.[3] Pursuant to section 564(b)(1) [illegible] the basis of such determination, the Secretary of [illegible] fying the authorization of the emergency use of

[illegible] *ergency Use of an In Vitro Diagnostic for Detection of* [illegible]. 78 Fed. Reg. 42779 (July 17, 2013).

[illegible] expanded use of the CDC Novel Coronavirus 2012 Real-[illegible] tion of genomic RNA from MERS-CoV in clinical [illegible] miological criteria (e.g., contact with a probable or [illegible] locations where MERS-CoV cases were detected, or [illegible] be indicated as part of a public health investigation) [illegible] sociated with MERS-CoV infection. The amendments [illegible] and revisions to the Instructions for Use, product insert,

[illegible] ss Reauthorization Act, Pub. L. No. 113-5, under section [illegible] ation of a public health emergency, or of a significant

Emergency Use Authorization

Skip to list of current EUAs

The Emergency Use Authorization (EUA) authority allows FDA to help strengthen the nation's public health protections against CBRN threats by facilitating the availability and use of MCMs needed during public health emergencies.

Under section 564 of the Federal Food, Drug, and Cosmetic Act (FD&C Act), the FDA Commissioner may allow unapproved medical products or unapproved uses of approved medical products to be used in an emergency to diagnose, treat, or prevent serious or life-threatening diseases or conditions caused by CBRN threat agents when there are no adequate, approved, and available alternatives.

Section 564 of the FD&C Act was amended by the Project Bioshield Act of 2004 and the Pandemic and All-Hazards Preparedness Reauthorization Act of 2013 (PAHPRA), which was enacted in March 2013.

NIH 57943 -005520

MERS-CoV

3 Years Later – We Know More

- 83 (+4 from 2015) genome sequences deposited in GenBank
  - human and camel derived
  - sequences from 2012 - 2015
- Published assays work
- Preferred specimens: LRT, URT, acute serum
- Serology has matured to include multiple assays with more robust performance data

MERS-CoV
Diagnostic Gaps

- Alternative FDA cleared MERS-CoV assays*
- Integrated multi-pathogen assay panels
- Rapid Tests (POC)
- Tests for veterinary applications
- Generic CoV assays

*LRN distributed CDC kits has proven effective for domestic MERS surveillance to date.

MERS-CoV

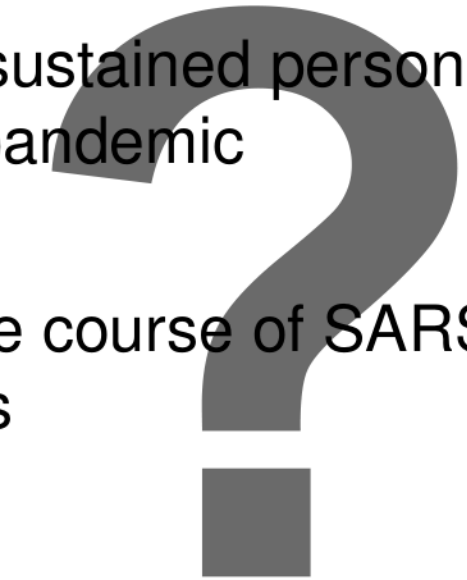
Sample Availability

- MERS-CoV positive samples
  - Mock specimens spiked with inactivated virus
    - CDC proficiency test panel
    - RCPA quality assurance program
    - QCMD external quality assessment program
  - Authentic specimens
    - CDC, Other*
    - Saudi Arabia (?)
- MERS-CoV negative samples
  - CDC Influenza Reagent Resource
    - http://www.influenzareagentresource.org/About/IRR.aspx
  - Biospecimen repositories
    - www.bioserve.com
    - www.cambridgebiosource.com
  - Other

*Few; limited volume; not pristine (frozen and thawed)

MERS-CoV
The Future

- Achieves sustained person-to-person spread – pandemic
- Follows the course of SARS-CoV and disappears
- Status quo



Open Discussion

(15-20 minutes)

Therapeutics

Dr. David Spiro

SAb
BIOTHERAPEUTICS

*Personal Impact + **Global Influence***

sabbiotherapeutics.com

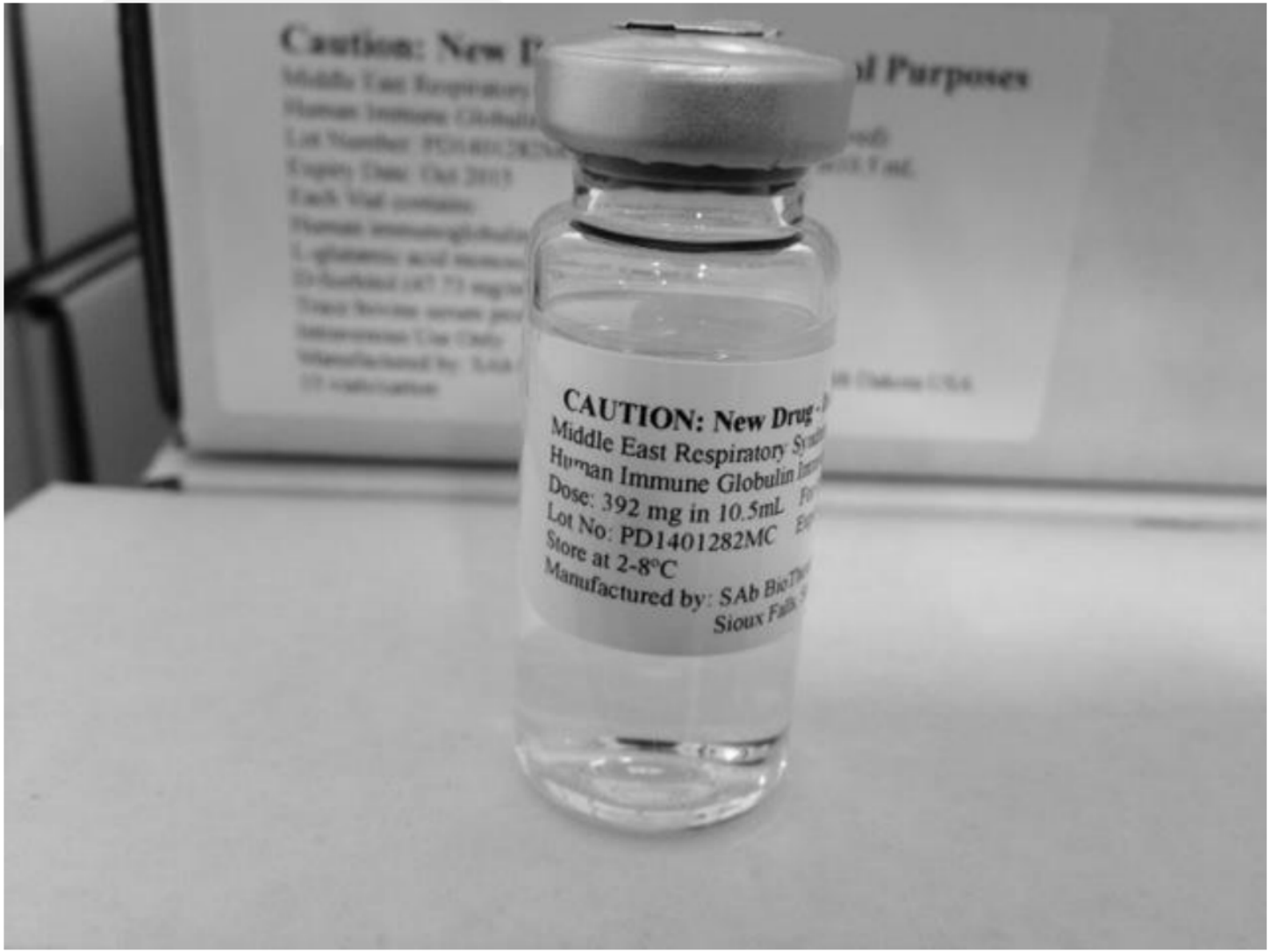
DiversitAb™ Antibody Production Process

Calves produce specific human antibody after immunization

Plasma collection

Pilot manufacturing facilities

- Natural human antibody
- Targets many diseases
- Large quantities
- Short lead time
- No toxicity
- Fast acting
- Patent protection


CAUTION: New Drug
Middle East Respiratory
Human Immune Globulin
Dose: 392 mg in 10.5mL
Lot No: PD1401282MC
Store at 2-8°C
Manufactured by: SAb

Current Status of Product

(b)(4)

(b)(4)

MERS-CoV monoclonal antibodies in a humanized mouse infection model using Regeneron VelociTechnologies: A novel discovery platform for emerging infectious diseases

(b)(6)

Regeneron Pharmaceuticals Inc.

(b)(6)

April 3rd 2015

REGENERON
science to medicine®

(b)(4)

(b)(4)

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REGENERON
science to medicine®

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REGENERON
science to medicine®

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REGENERON
science to medicine®

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(b)(4)

Acknowledgments

Infectious Diseases | Therapeutic Proteins | Protein Expression Science

(b)(6)

VelociGene | University of Maryland | VelocImmune mouse

(b)(6)

REGENERON
science to medicine®

BCX4430

Review of antiviral activity in vitro

BioCryst Pharmaceuticals, Inc.

BCX4430 is an adenosine nucleoside analog that is metabolized

(b)(4)

(b)(4)

NIH 57943 -005549

BioCryst Pharmaceuticals, Inc.

(b)(4)

(b)(4)

(b)(4)

(b)(4)

Page 186 of 263

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

MERS-CoV Therapeutic Development

(b)(6)

Associate Professor

University of Maryland School of Medicine

(b)(6)

MERS-CoV Therapeutic Development

Over the last 12 years, there are **no** "real" drugs that work *in vivo* against SARS-CoV in any animal model of disease

No therapeutics on-the-shelf for future Coronavirus outbreaks including MERS-CoV

Gap in Knowledge needs to be filled:

- **To understand dimensions of viral infection that are key targets for therapeutic development and to identify**
- **To identify current technologies that can be used upon future spread of MERS-CoV or other emerging Coronavirus (or broadly acting anti-virals)**

MERS-CoV Therapeutic Development

Summary of work currently underway in Frieman Laboratory

(b)(4)

Moving forward with therapeutic development and approval

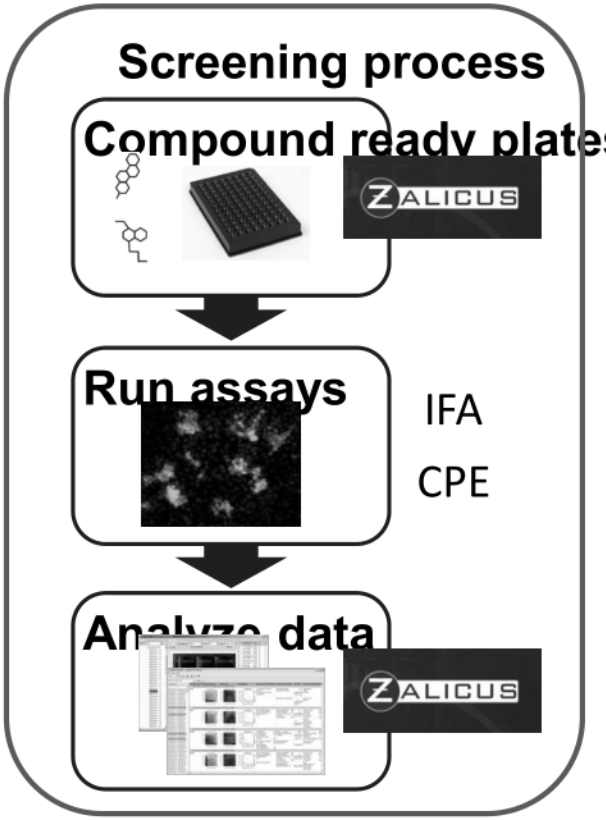
(b)(4)

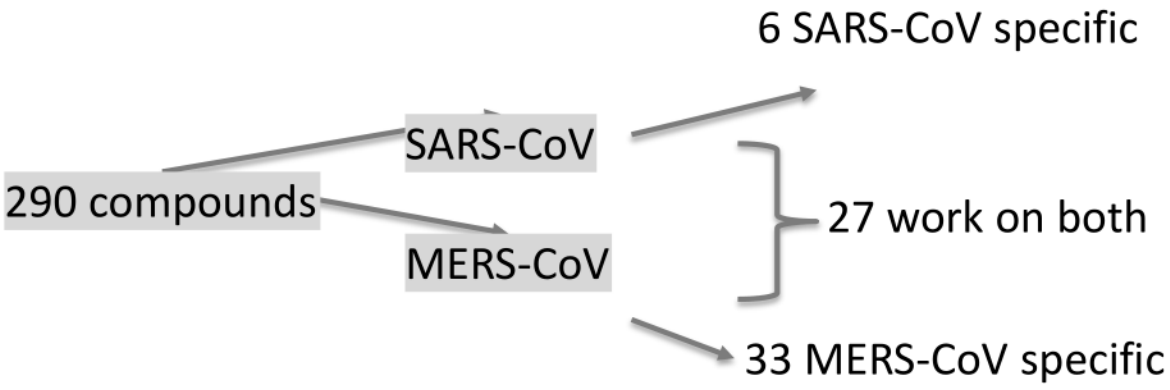
Moving forward with therapeutic development and approval

(b)(4)

FDA approved drug screen against MERS-CoV and SARS-CoV

In collaboration with IRF/NIAID and Zalicus Inc
-screened 290 FDA approved compounds (HALT^{tm} collection)
-assays for Cell viability and viral protein expression (IFA)





Dyall et al, AAC, 2014

How to deal with Repurposed Drugs as MERS-CoV antivirals?

(b)(4)

Discussion Topic

(b)(4)

Discussion Topic

(b)(4)

Discussion Topic

(b)(4)

AUDIENCE COMMENTS

(5-10 minutes)

BREAK

(10 minutes)

United States Department of
Health & Human Services

MERS-CoV Vaccine Candidates

Rick Bright, PhD

(b)(5)

SARS-Coronavirus:

NIAID/DMID Response

(b)(6) **PhD**

Enteric & Hepatic Diseases Branch Chief

(former SARS & Influenza Vaccine PO, RDB)

DMID, NIAID, HHS

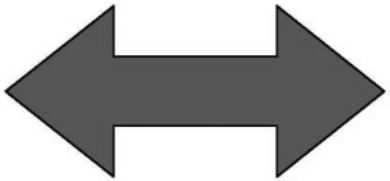
MERS Workshop, BARDA

3 April 2015

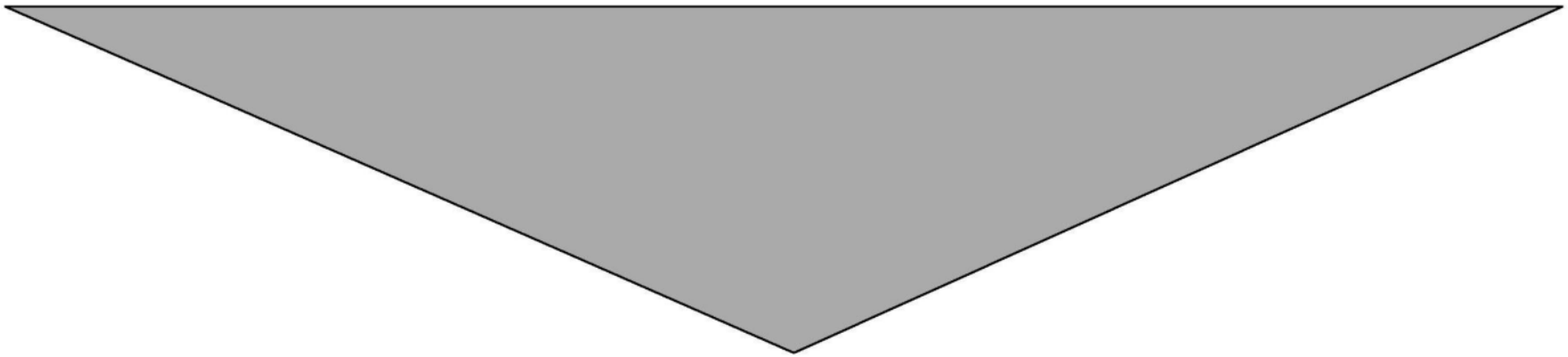
National Institute of Allergy and Infectious Diseases

NIAID Research: A Dual Mandate

Maintain and "grow" a robust basic and applied research portfolio in microbiology, infectious diseases, immunology and immune-mediated diseases



Respond rapidly to new and emerging disease threats

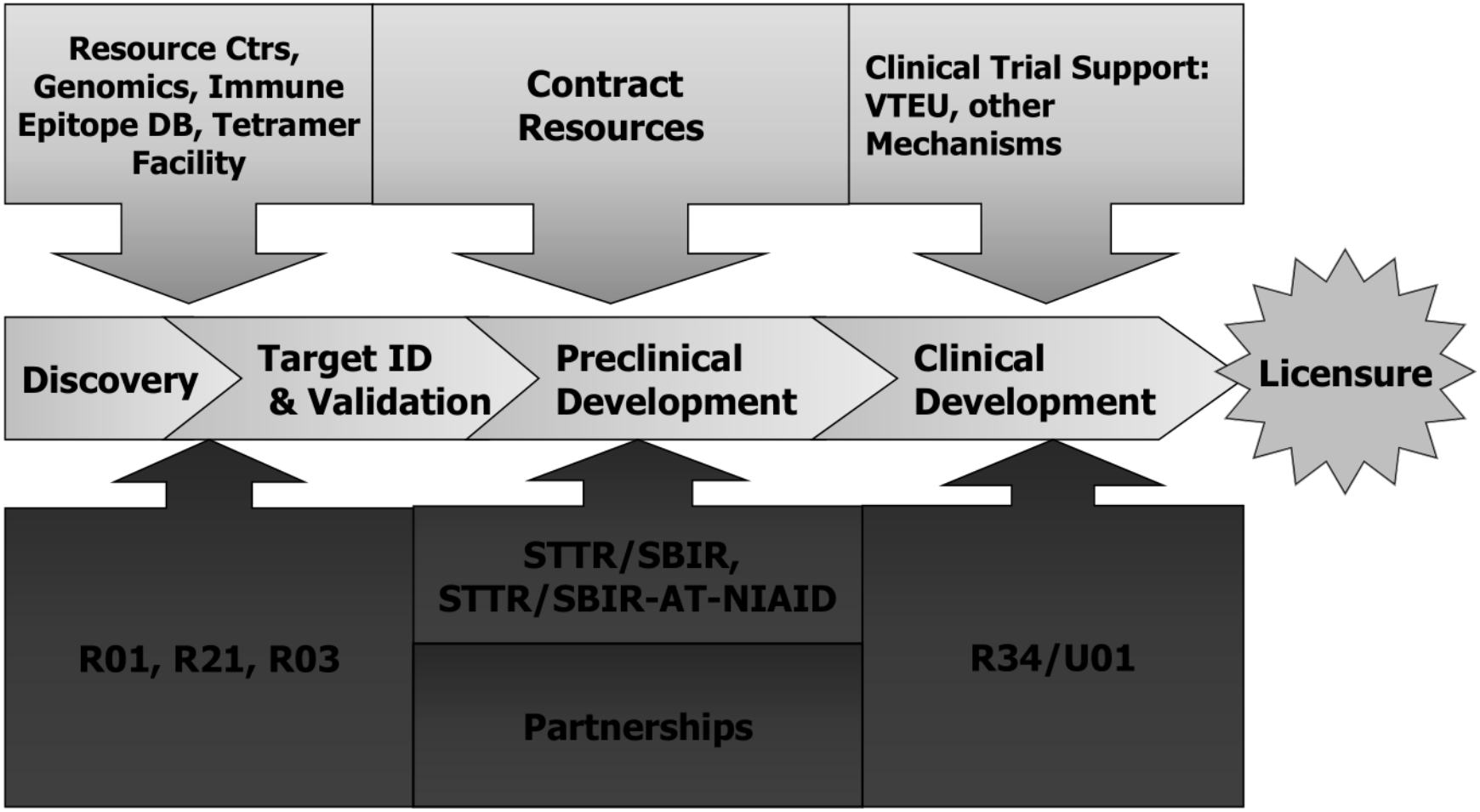


New/Improved Interventions

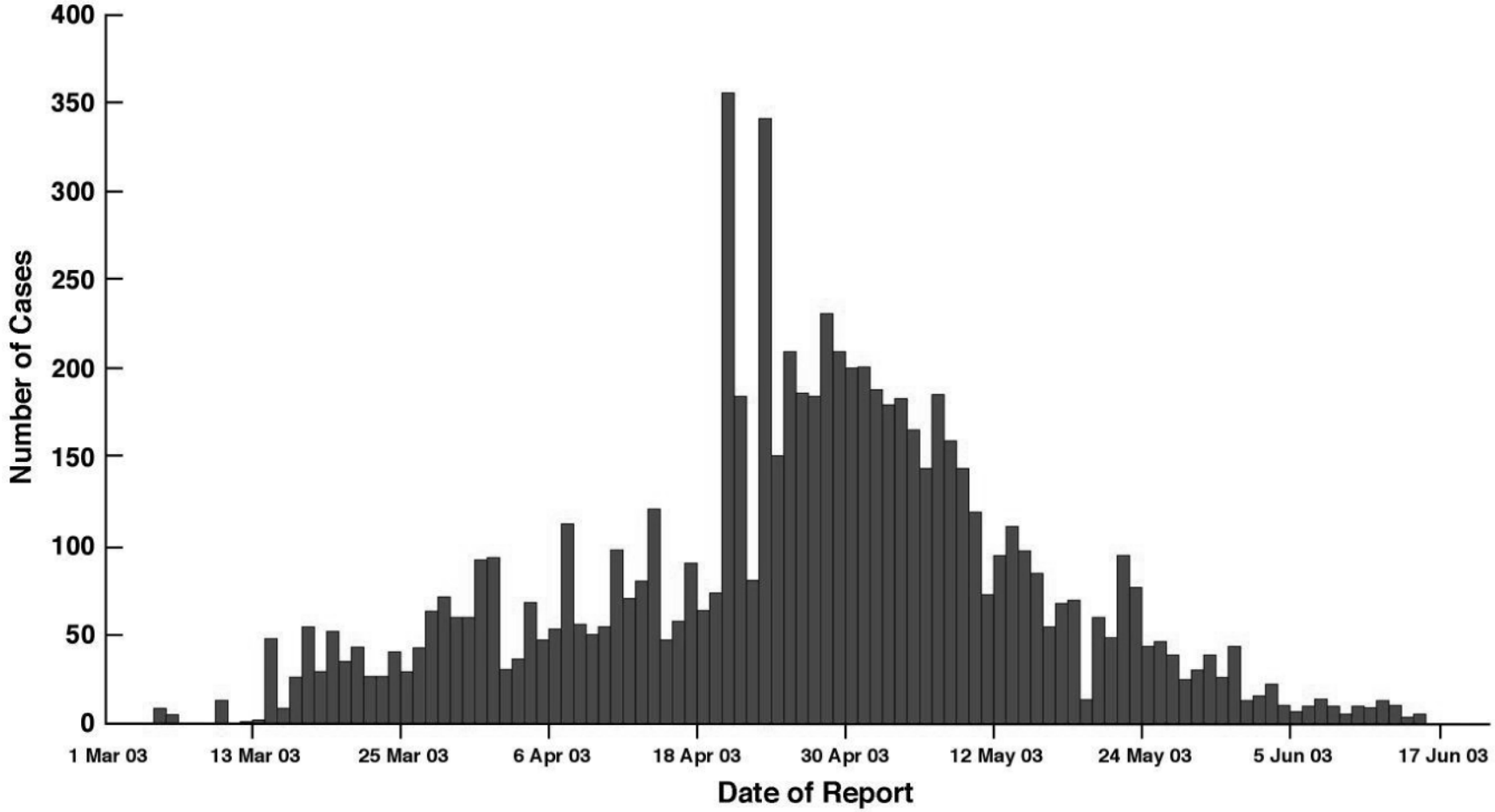
NIH 57943 -005568

Slide source: AS Fauci

DMID/NIAID Support Mechanisms Across the Product Development Pipeline

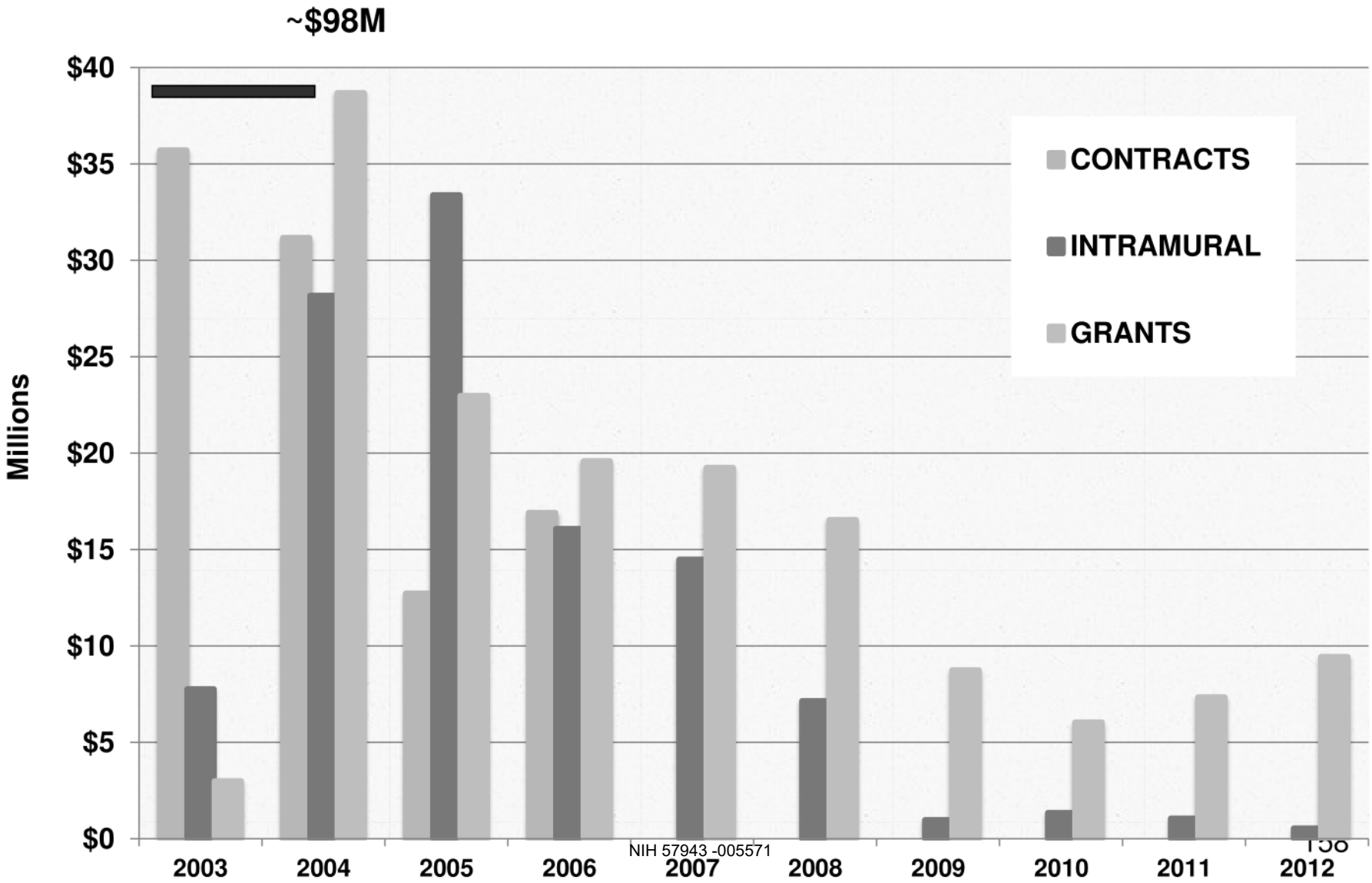


Probable Cases of SARS Worldwide by Date of Report, March 1-June 16, 2003



Source: WHO

NIAID Funding for SARS-CoV



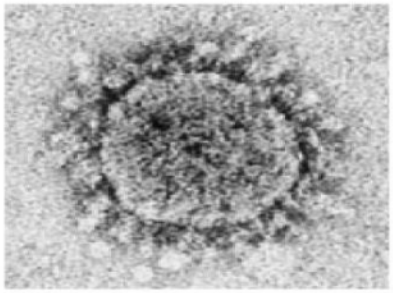
SARS Characterization and Vaccine Development

2003

2004

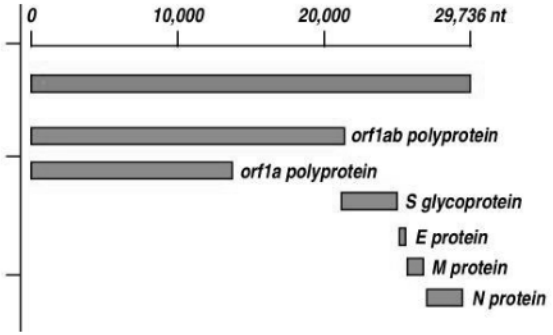
March 24, 2003

SARS CoV Discovered



April 14, 2003

SARS CoV Sequenced



March 31, 2004

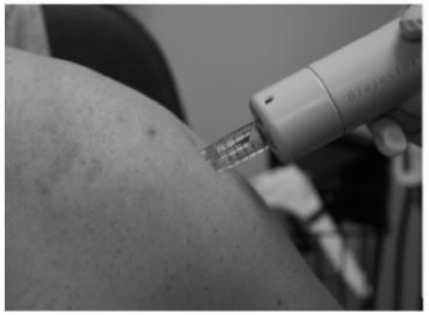
SARS Vaccine Developed

A DNA Vaccine Induces SARS Coronavirus Neutralization and Protective Immunity in Mice

Zhi-yong Yang, Wing-pui Kong, Yue Huang, Anjeanette Roberts, Brian R. Murphy, Kanta Subbarao and Gary J. Nabel

December 13, 2004

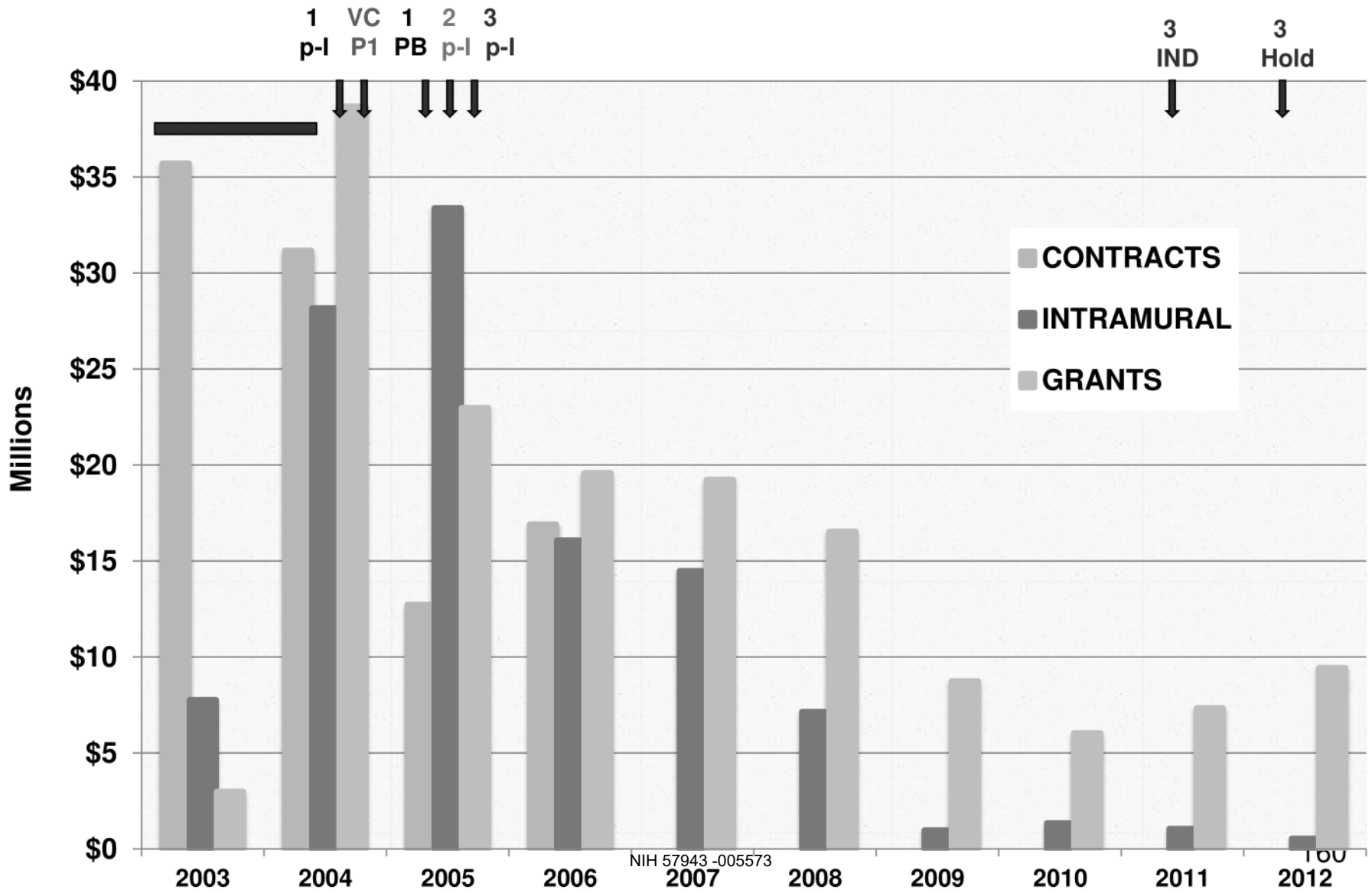
SARS Phase I Clinical Trial Initiated at NIAID VRC



NIH 57943 -005572

Slide source: AS Fauci

SARS-CoV Funding, Vaccine Regulatory

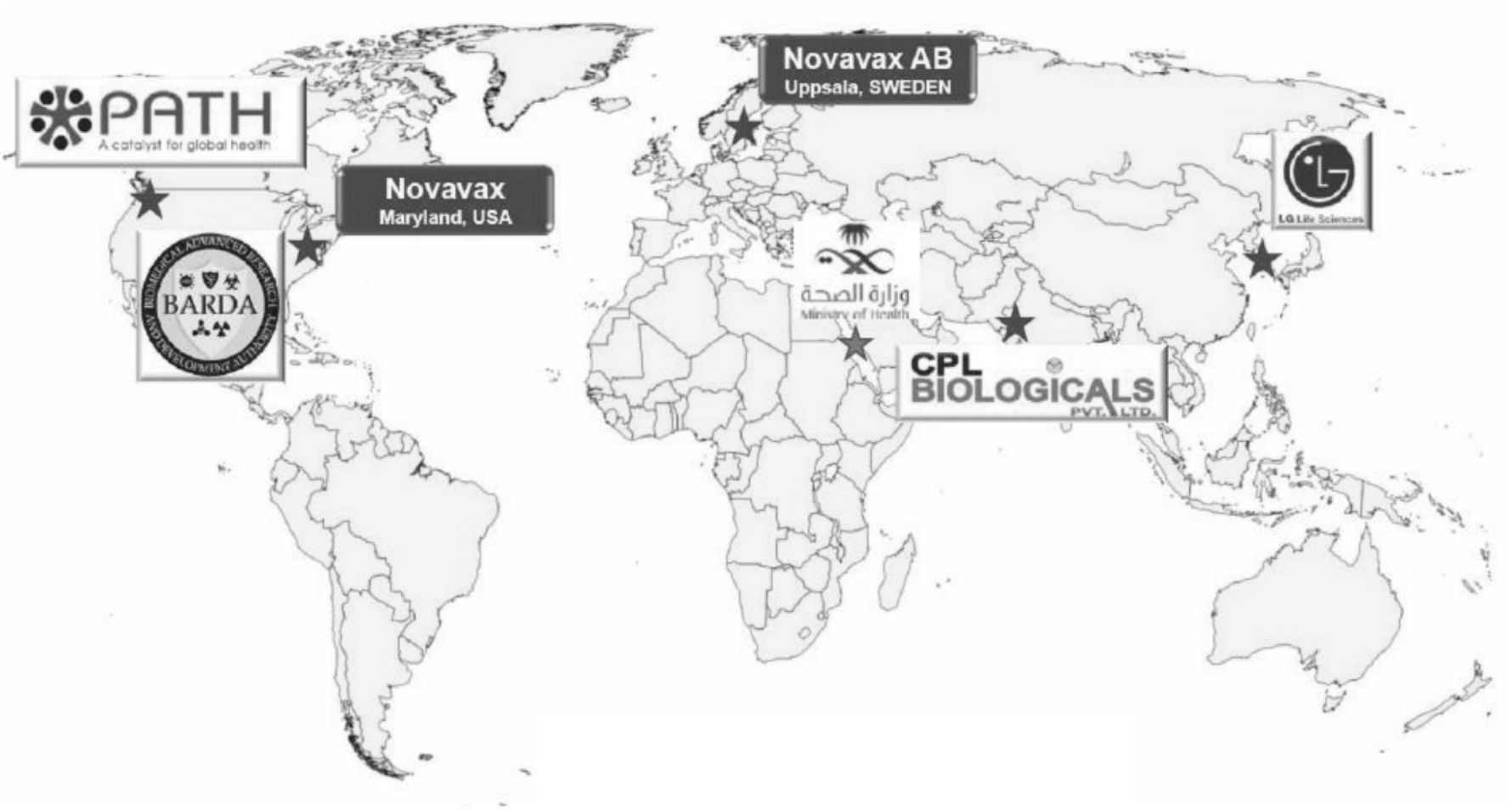


Protection & Immunopathology in SARS-CoV Animal Studies

Animal Model	Vaccine Type	Adjuvant/Protein	Protection	Immunopathology
Mice	Whole virus	alum	Yes	Yes
		---	Yes	Yes
	VLP	alum	Yes	Yes
		---	Yes	Yes
	S Protein	alum	Yes	Yes
		---	Yes	Yes
	VEE Vector	N protein	No	Yes
		S protein	Yes	No
	Vaccinia vector	N protein	No	Yes
		S protein	Yes	?No
Hamsters	Whole virus	ASO1	Yes	No
Ferrets	Whole virus	alum	Yes	Yes
NHP	Whole virus	alum	Yes	Yes

Adapted from Tseng, et al., 2012, PLoS One (7) e35421

Novavax, Inc. Gaithersburg, MD



Recombinant MERS CoV S Nanoparticles

(b)(4)

MERS-CoV Spike Nanoparticle Vaccine

(b)(4)

NOVAVAX

Clinical Approach to a MERS CoV Vaccine Development

(b)(4)

Demonstration of Efficacy

(b)(4)

MERS Vaccine – Regulatory Aspects

(b)(4)

Resources, and Other Considerations

(b)(4)

Status MERS CoV S Nanoparticle Vaccine Development

Category	Tasks	Status (% complete)
Vaccine candidate Master Seed Stock	BV: MERS CoV Al-Hasa-1-2013 Spike (S) Nanoparticle	80%
Process Development	Production process (lab-scale) PD (200 – 1000L)	100% TBD
Analytical Development	Release assays	50%
Pre-clinical Animal Studies	Immunogenicity, challenge	20%
Formulation Development	2 – 8°C stable drug product	80%
Adjuvant	cGMP Matrix-M™	100%
Manufacturing	Sf9 Cell Bank cGMP 200 – 1000L	100% TBD
Pre-IND	Meeting request CBER	TBD
IND	Submission	TBD

Greffex
PROTECT AND HEAL

The GreVAX Vaccine Platform

Adenoviral vector technology overview

(b)(4)

NIH 57943 -005584

The GreVAX Technology

(b)(4)

Engineering of fully deleted GreVac modules

(b)(4)

(b)(4)

GreVAX vaccines

(b)(4)

(b)(4)

GreVAX vaccines MERS-CoV

(b)(4)

GreVAX platform - Applications

(b)(4)

SynCon® MERS-CoV anti-Spike DNA Vaccine

MERS-CoV Preparedness Workshop
HHS/ASPR/BARDA

(b)(6) Ph.D.

COO

Collaboration Partners:

(b)(6) (U. Penn)

(b)(6) (U. Manitoba/PHAC)

(b)(6) (Rocky Mountain Labs/NIAID)

Washington, DC
April 3, 2015

(b)(4)

In vivo Immunogenicity (Murine Model):

(b)(4)

(b)(4)

In vivo

(b)(4)

(b)(4)

In vivo Immunogenicity (NHP Model):

(b)(4)

In vivo Immunogenicity (NHP model):

(b)(4)

(b)(4)

In vivo Challenge (NHP model):

(b)(4)

(b)(4)

Page 232 of 263

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

In vivo Immunogenicity (Camel model):

(b)(4)

Summary & Next Steps for (b)(4)

(b)(4)

(b)(4)

Acknowledgements

Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories

(b)(6)

Special Pathogens Program, University of Manitoba and Public Health Agency of Canada

(b)(6)

Department of Microbiology, University of Washington

(b)(6)

University of Pennsylvania

(b)(6)

USF

(b)(6)

Inovio Pharmaceuticals Inc.,

(b)(6)

AUDIENCE COMMENTS

(5-10 minutes)

United States Department of
Health & Human Services

MERS-CoV Stakeholder Workshop

The Need to Strengthen the Domestic/International Collaboration Interface

Maria Julia Marinissen, PhD
Director, Division of International Health Security (DIHS)
ASPR/OPP

Collaborators:
Mark Pallansch, David Swerdlow, Tim Uyeki, CDC
Carmen Maher, FDA
Rick Bright, ASPR/BARDA
Lauren Barna, Ryan Morhard, ASPR/OPP/DIHS

MERS-CoV and Generic MCM Issues that Require a Domestic/International Solution

(b)(5)

Final Perspectives & Action Items

Dr. George Korch,
ASPR, Senior Science Advisor

Past Recommendations for MERS-CoV Needs

George W. Korch, Ph.D.
Senior Science Advisor
Assistant Secretary Preparedness and Response

April, 2015

Basic Research

- Convert MERS-CoV genetic strains into genetic tools to study the biology of the virus
- Instead of waiting for the full genome sequence of the virus, use the Spike protein to assess antibody protection
- Determine whether antibodies against SARS have cross-reactivity
- Develop monoclonal antibodies to broadly neutralizing sites in the virus
- Understand the innate immune response to MERS-CoV infection in humans
- Develop mouse models to understand MERS-CoV pathogenic mechanisms and gene function and to evaluate vaccine and therapeutic efficacies
- Determine the etiology of the virus, including the animal source and how it is transmitted to humans
- Virus stocks as well as cell substrates should be qualified to the extent necessary, including clinical source origin, passage growth and history, testing for adventitious agents.
- Viral banks and standard operating procedures (SOPs) for propagation and storage should be produced to share with labs developing MCMs.

Epidemiology and Clinical Management

- Sero-surveys in camel workers (and their close contacts) and studies to identify the source and route of transmission from camels to humans.
- Longitudinal epidemiological, virological, and immunological studies in animals, including camels;
- Evaluation of suitability for Veterinary Vaccine strategy (MERS-CoV vaccination studies in camels)

General Needs

- Collaborations between countries, including those most affected by MERS-CoV infections
- Access to more and varied clinical samples and strains in order to broaden the diversity of MERS-CoV specimens.
- Development of a repository that is accessible to researchers to store collections of sera, recombinant proteins, and other key reagents should be considered.
- Identifying the diversity of MERS and other CoVs in the wild and assessing the risks of different CoVs for humans.
- Access to wildlife samples from Saudi Arabia and other countries
- Assure sufficient supplies of non-human primates to support pre-clinical development of MERS-CoV therapeutics and vaccine candidates; and
- Provide additional resources to existing funding mechanisms to support pre-clinical research and to implement human clinical trials of antivirals, immunotherapeutics, and vaccines.
- Support standardization of the most promising animal models and MERS-CoV strains:

Animal Models

- Will human DPP4 cleave in the same way in mice as mouse DPP4?
- How does DPP4 behave in pig cells?
- What co-receptor or protease can be used to develop a transgenic animal model?
- Why is the disease associated with MERS-CoV so much worse in humans than in animals?
- What is the pathogenesis of CoVs in bats?
- Can vaccine data from animal models predict what will happen in humans?
- Longitudinal epidemiological, virological, and immunological studies in animals, including camels;

Vaccines

- Investigating a variety of vaccine approaches including recombinant protein, viral replicons and live attenuated vaccines.
- Developing stocks of anti-MERS-CoV neutralizing antibody to protect health care workers in the event that the outbreak becomes more widespread.
- Conducting proof-of-concept tests of the ability to engineer CoV vaccines by testing a vaccine against transmissible gastroenteritis or porcine epidemic diarrhea CoV in animals.
- Developing a subunit vaccine.
- Using vector-based or recombinant-protein-based platforms to rapidly develop vaccines.
- Developing highly attenuated but immunogenic infectious virus vaccines. Ultimately, developing a vaccine that protects humans from multiple CoVs.

Therapeutics

- Developing a gold standard or performance benchmark for small molecules that have been tested in cell cultures or animal models.
- Developing antivirals that inhibit a broad range of CoVs.
- Using pharmacogenomics and new computational and mathematical methods to design novel host-based therapeutics.
- Computationally designing proteins that interact with viral proteins.
- Developing small-molecule probes to facilitate basic research and drug development.
- Using replicons to study the ability of compounds to inhibit infection.

Diagnostics

- Access to a standardized set of samples to develop diagnostic assays.

From: Korch, George (HHS/ASPR/IO)
Sent: Thu, 2 Apr 2015 14:54:23 +0000
To: (b)(6) Joseph; Aviles, Natalie (OS/ASPR) (CTR); Baric, Ralph; Bavari, Sina; (b)(6) (b)(6) Bisht, Himani (FDA/CDRH); (b)(6) Bright, Rick (HHS/ASPR); (b)(6) Cho, David S (CBER) (FDA/CBER); Conenello, Gina (FDA/CDRH); (b)(6) Czako, Rita (NIH/NIAID) [F]; Davis, Jon (OS/ASPR); Deming, Damon (FDA/CDER); Denison, Mark (NIH); (b)(6) DiEuliis, Diane (HHS/ASPR/OPP); Donabedian, Armen (HHS/ASPR/BARDA); Donaldson, Eric (FDA/CDER); (b)(6) (b)(6) Erlandson, Karl (OS/ASPR); Feikin, Danny (CDC/OID/NCIRD); Ferro, Philip (HHS/ASPR/IO); Fisher, Robert (FDA/OC); (b)(6) (b)(6) Gerber, Susan I. (CDC/OID/NCIRD); (b)(6) (b)(6) Hensley, Lisa (NIH/NIAID) [E]; (b)(6) Hojvat, Sally A (FDA/CDRH); Houser, Katherine (NIH/NIAID) [F]; Jaffe, Richard (OS/ASPR); Jahrling, Peter (NIH/NIAID) [E]; (b)(6) Kelley, Cynthia (FDA/CBER)'; OS Kerr, L; (b)(6) Korch, George (HHS/ASPR/IO); (b)(6) Lamirande, Elaine (NIH/NIAID) [E]; (b)(6) (b)(6) Maher, Carmen (FDA/OC); (b)(6) Marinissen, Maria Julia (HHS/ASPR/OPP); (b)(6) Miele, Peter (FDA/CDER); (b)(6) Olinger, Gene (NIH/NIAID) [C]; (b)(6) O'Rear, Julian (FDA/CDER); (b)(6) Pallansch, Mark A. (CDC/OID/NCIRD); (b)(6) ; Robinson, Robin (HHS/ASPR/BARDA); (b)(6) (b)(6) Sciarretta, Kimberly (OS/BARDA); (b)(6) Spiro, David (NIH/NIAID) [E]; (b)(6) Stemmy, Erik (NIH/NIAID) [E]; Styrt, Barbara (FDA/CDER); Subbarao, Kanta (NIH/NIAID) [E]; (b)(6) Sutton, Troy (NIH/NIAID) [F]; Swerdlow, David (CDC/OID/NCIRD); (b)(6) Thomas, Stephen J; (b)(6) Uyeki, Timothy M. (CDC/OID/NCIRD); Vogel, Leatrice (NIH/NIAID) [E]; Wathen, Lynne (HHS/ASPR/BARDA); (b)(6) (b)(6) (b)(6) Jones, Estella (FDA/OC); MacGill, Tracy (FDA/OC); OHara, Michael (HHS/ASPR/BARDA) (CTR); (b)(6) Cassels, Fred (NIH/NIAID) [E]; (b)(6) Johnson, Reed (NIH/NIAID) [E]; Lurie, Nicole (HHS/ASPR/IO)
Cc: Aviles, Natalie (OS/ASPR) (CTR); Korch, George (HHS/ASPR/IO); Davis, Jon (OS/ASPR); Errett, Nicole (HHS/ASPR/IO) (CTR); Moss, Marcille (HHS/ASPR/IO); Stancil, Audrey A (HHS/ASPR/IO); Underwood, Lauren (HHS/ASPR/IO); DeVore, Christopher (HHS/ASPR/IO) (CTR)
Subject: Agenda and Instructions for the MERS CoV Workshop on 3 April 2015
Attachments: Final Agenda for MERS Stakeholder Workshop (GWK) 8 .docx

Greetings to all Registrants for the upcoming MERS CoV Workshop.

Thank you very much for your interest and availability for this event.

You are receiving this email because you have registered and been confirmed to attend (either online or in-person) the MERS-CoV Stakeholder Workshop for this Friday, April 3, 2015. If you will no longer be attending, please let Jon Davis (b)(6) and Natalie Aviles (b)(6) know. Included in this email are logistics for attending the meeting in person, as well as for those dialing in and participating online.

PLEASE TAKE PARTICULAR NOTICE: Seating capacity is limited for this meeting. You must have specifically registered already via the on-line link to attend the Workshop "in person" to have space within our conference area. **If you responded that you would be attending via "web conference," we unfortunately cannot accommodate your visit "in person".** We are confident however that the web conference mode of attendance will be of high quality and will provide you with the benefits of being physically present.

Date/Time
3 April 2015
10:00AM – 2:30PM (EDT)
Registration will start 9:45, please expect to arrive starting around 9:30 to facilitate getting through Building Security. Escorts will be made available to bring you to the conference facility.

Location
Willow Conference Room (Lower Level)
Thomas P. O'Neill Building
200 C Street SW
Washington, DC 20024

Transportation Directions
If taking the metro to Federal Center SW station (blue/orange line), make a left at the top of the escalators. The O'Neill building is about a block away on the right at the corner of D Street and 3rd Street. If taking the metro to L'Enfant Plaza station (blue/orange/yellow/green), exit via the Maryland Street exit and proceed right down Maryland Avenue, take a right on 6th Street, and take a left straight down C Street. The O'Neill building will be about 2.5 blocks down on your right. If driving, there are a couple of parking garages directly on E Street ranging from $15-20 per day and most of these are cash-only.

Security Instructions
Once inside the O'Neill building, please proceed to the security desk to get signed in if you do not already have access to the HHS building. You will need a photo government issued ID for entry (e.g., driver's license, passport, PIV/CAC card, etc.). Non-US citizens will need to bring their passport. You're allowed to bring items into the building, such as computers and cellphones. When you pass through the security screening, you'll be asked to remove your laptop from the case (if applicable) and scan it through separately (just like you do at the airport). There will be several escorts in the lobby gathering everyone into smaller escort groups. If you do not find one of these escorts, you may contact Natalie Aviles (202-809-4165).

Teleconference & Web Conference
For those who will be dialing-in and/or participating in the web conference, the details are below:

Toll free number: (b)(6)
General Participant/Audience passcode: (b)(6)
PRESENTERS PASSCODE ONLY: (b)(6)

Instant Net Conference Instructions:

1. Join the meeting: https://www.mymeetings.com/nc/join/
2. Enter the required fields (Conference number: (b)(6) Audience passcode: (b)(6)
3. Indicate that you have read the Privacy Policy
4. Click on Proceed

Please make sure that you have the proper plug-ins for MyMeeting installed on your computer. Click on the following link to test and see if you have the proper plug-ins: https://www.mymeetings.com/emeet/join/src/plugins_mm.php. Be sure to select the MyMeeting plug-ins "setup" and follow the prompts.

Meeting Materials

Meeting materials will be forthcoming before the meeting. Hard copies will be provided for those attending in person.

Light Refreshments and Coffee / Tea will be available

We are looking forward to your participation.

With best regards,

George W. Korch Jr., Ph.D.
Senior Science Advisor
Assistant Secretary for Preparedness and Response
Department of Health and Human Services
Washington, D.C.
(b)(6)
202-690-7412 (FAX)

Agenda for MERS CoV Stakeholder Workshop
3 April 2015
Willow Conference Room (LL Level)
O'Neill Congressional Building
200 C Street SW, Washington D.C.

Toll free number (b)(6)
General Participant/Audience passcode: (b)(6)
PRESENTERS ONLY passcode: (b)(6)

1. *Join the webinar meeting at:* (b)(6)
2. *Enter the required fields (Conference number:* (b)(6) *; Audience passcode:* (b)(6)
3. *Indicate that you have read the Privacy Policy*
4. *Click on Proceed*

10:00 – 10:05 Welcome Dr. Nicole Lurie, Assistant Secretary for Preparedness and Response

10:05 - 10:10 Introduction and Purpose (George Korch)

10:10 – 10:20 Introduction to Coronaviruses Virology (b)(5)

10:20 – 10:55 Epidemiology and Clinical Management

a.) Peter Ben Embarek and Nahoko Shindo (20 minutes) – Current International Epidemiological Findings and Case Management

b.) **David Swerdlow (15 minutes) – U.S. Preparedness and Response to Domestic Cases**

10:55 – 11:40 Animal Models (b)(5)

Format: Facilitated panel discussion with 6-7 researchers developing models.

a.) Introductions - (b)(5)
b.) Short Overview on Models (b)(5)
c.) Sharing of Unpublished data (b)(5)
d.) **Panel Discussion with audience participation (25 minutes) guided by Facilitator using questions to prompt panelists/audience**

11:40 – 11:50 Break (10 minutes)

11:50 – 12:20 Diagnostics (b)(5)

a.) Introduction (2 min). FDA's Emergency Use Authorization Regulatory Path- Sally Hojvat , FDA/CDRH/OIR
b.) (8-10 minutes) CDC Laboratory Response to MERS.- Dean Erdman, CDC
c.) (15-20 minutes) Open Discussion: What are the main challenges to developing a MERS Co-V diagnostic?

i. **David Ecker, (Abbott)**
ii. **Elizabeth Holmes (Theranos)**
iii. **Karen Li (ThermoFisher)**
iv. **Others including DoD … work in progress**

12:20 – 1:05 Therapeutics (b)(5)

a) **Format: Five short examples of therapeutics in development followed by group discussion with audience/additional panel members with other perspectives.**
b) **Introduction David Spiro, NIAID/DMID**
c) **Immunotherapeutics**
   i) **(5 minutes)** (b)(5) **SAB (Transchromosomic Bovine IgG)**
   ii) **(5 minutes)** (b)(5) **, Regeneron (REGN3048/3051)**
d) **Small Molecule/Drug (5 mins)** (b)(5) **, BioCryst (BCX4430)**
e) **Other Strategies**
   i) **(5 minutes)** (b)(5) **Planet Biotech (Receptor Decoy)**
   ii) **(5 minutes)** (b)(5) **University of Maryland (Repurposing FDA-approved Drugs)**
f) **Group Discussion (15 minutes) Guided by facilitator to answer/pose questions from community.**
   i) **What scientific gaps need to be filled to facilitate the development of MERS Therapeutics?**
   ii) **What are major obstacles to MERS therapeutics development, and what role can governmental and multi-governmental bodies play?**
   iii) **Are there any lessons learned from MERS (or SARS) therapeutics development?**
g) **Audience Comments (5-10 minutes)**

1:05 - 1:15 Break (10 minutes)

1:15 – 2:00 Vaccines (b)(5)

Format short presentation of lessons from SARS vaccine experience, followed by discussion

a) **Overview from the Internal Portfolio Review -** (b)(5) **(5 minutes)**
b) **Lessons Learned from SARS Vaccine Development –** (b)(5) **(10 minutes)**
c) **Industry State of the Art Presentations (20 minutes)**
   i) **Novavax**
   ii) **Greffex –** (b)(5)
   iii) **Inovio –** (b)(5)
d) **Audience Comments (10 minutes)**

2:00 – 2:05 International Issues (b)(5)

2:05 – 2:30 Final Perspectives and Action Items (George Korch)

From: Korch, George (HHS/ASPR/IO)
Sent: Tue, 10 Mar 2015 18:40:21 +0000
To: Korch, George (HHS/ASPR/IO); Swerdlow, David (CDC/OID/NCIRD); Uyeki, Timothy M. (CDC/OID/NCIRD); Gerber, Sue (CDC/CGH/DGHA); Haynes, Lia (CDC/OID/NCIRD); Erdman, Dean (CDC/OID/NCIRD); Pallansch, Mark A. (CDC/OID/NCIRD); (b)(6) (b)(6) ; Chaitram, Jasmine (CDC/OID/NCEZID); Carter, Wendy (FDA/CDER); Bavari, Sina; Miele, Peter (FDA/CDER); O'Rear, Julian (FDA/CDER); Deming, Damon (FDA/CDER); Merlin, Toby (CDC/OID/NCEZID); Feldmann, Heinrich (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Marinissen, Maria Julia (HHS/ASPR/OPP); Annelli, Joseph; DiEuliis, Diane (HHS/ASPR/OPP); Bright, Rick (HHS/ASPR); Donabedian, Armen (HHS/ASPR/BARDA); Robinson, Robin (HHS/ASPR/BARDA); Maher, Carmen (FDA/OC); Hensley, Lisa (NIH/NIAID) [E]; Kelley, Cynthia (FDA/CBER); Spiro, David (NIH/NIAID) [E]; Fisher, Robert (FDA/CBER); Hojvat, Sally A (FDA/CDRH); Beigel, John (NIH) [C]; Murray, Jeffrey S (FDA/CDER); Olinger, Gene (NIH/NIAID) [C];

(b)(6)

(b)(6) ; Thomas, Stephen J; Cho, David S (CBER) (FDA/CBER); (b)(6) ; Roberts, Rosemary (FDA/CDER); 'Cooper, Michael J CAPT USPHS USARMY MEDCOM AFHSC (US)'; (b)(6) (b)(6) Roberts, Rosemary (FDA/CDER); Gerber, Susan I. (CDC/OID/NCIRD);

(b)(6)

(b)(6) ; Zoon, Kathryn (NIH/NIAID) [E]; Bowen, Richard; (b)(6) Subbarao, Kanta (NIH/NIAID) [E]; (b)(6)

(b)(6)

(b)(6) Ferro, Philip (HHS/ASPR/IO); Wathen, Lynne (HHS/ASPR/BARDA);

(b)(6)

(b)(6) ; Gay, Cyril; Underwood, Lauren (HHS/ASPR/IO); Sutton, Troy (NIH/NIAID) [F]; Houser, Katherine (NIH/NIAID) [F]; Czako, Rita (NIH/NIAID) [F]; Gretebeck, Lisa (NIH/CC/OD) [F]; Vogel, Leatrice (NIH/NIAID) [E]; Lamirande, Elaine (NIH/NIAID) [E]; (b)(6)

(b)(6)

Cc: Aviles, Natalie (OS/ASPR) (CTR); Davis, Jon (OS/ASPR); 'Elliott Fineman'; 'Olinger, Gene G'; 'Dan Adams'; Erlandson, Karl (OS/ASPR); 'Baric, Toni C'; 'Sven Andreasson'; 'Jensen, Victoria M. (CTR)'; 'Zhu, Quan'; 'Tang, Xianchun,Phd'; (b)(5) ; 'Cheryl Kofford'; OHara, Michael (HHS/ASPR/BARDA) (CTR); Sciarretta, Kimberly (OS/BARDA)
Subject: PLEASE READ - FOR ACTUAL REGISTRATION - SAVE THE DATE - MERS - CoV Preparedness Workshop

This message is being sent on behalf of Dr. George Korch

Dear Colleagues,

This is a follow on message with the actual URL that links to the Workshop Registration Site. The earlier message below was only a placeholder, **so by clicking on the link below, you WILL BE REGISTERING** for the meeting.

Meeting Registration
Please go to: https://www.medicalcountermeasures.gov/federal-initiatives/public-meetings-and-conferences/mers-cov-preparedness-workshop/ to register for the workshop. **We kindly request non-US citizens who want to attend the workshop in person register by March 25, 2015 due to building access requirements.**

Location
Willow Conference Room
The Thomas P. O'Neill Federal Building
200 C Street, SW
Washington, DC 20024

Please proceed to the security desk to get signed in if you do not already have access to the HHS building.

Meeting POC
Dr. George Korch (HHS/ASPR)

Call/WebEx Information
For those unable to attend in person, please use the following teleconference number:

Toll free number: (b)(6)
Participant passcode: (b)(6)

Meeting Date: 3 April 2015
Meeting Time: 10:00 AM -1:00PM

Instant Net Conference Details:

Meeting Number: (b)(6)
Meeting Passcode: (none)
Meeting Host: (b)(5)

Instructions for Joining Instant Net Conference:

1. Join the meeting: (b)(6)
(b)(6)
2. Enter the required fields
3. Indicates that you have read the Privacy Policy
4. Click on Proceed

Please make sure that you have the proper plug-ins for WebEx installed on your computer. Click on the following link to test and see if you have the proper plug-ins: (b)(6). Be sure to select the WebEx plug-ins "setup" and follow the prompts.

I am writing on behalf of Dr. Nicole Lurie, Assistant Secretary for Preparedness and Response (ASPR) at the Department of Health and Human Services (HHS), to request your participation in an HHS sponsored stakeholder workshop to discuss MERS-CoV on April 3, 2015. This workshop will bring together senior members of the US Government and senior experts from academia and industry to discuss the current state of MERS-CoV clinical and non-clinical knowledge. The goal of the workshop is to identify current activities that have been conducted on a response plan to confront MERS-CoV and coordinate resources to achieve the desired public health goals.

We want to ensure a robust discussion among government leaders and senior representatives of academia and industry so seating will be limited. Please save this date on your calendar and an email with workshop registration details will be sent to you shortly. We hope to establish a WebEx for those unable to attend in person. WebEx site details and the teleconference line number will be provided closer to the workshop date.

If you are not able to participate, but can recommend another individual from your organization who could serve in this important capacity, please let us know. We very much appreciate your time and contributions.

With Regards,

George Korch Jr., Ph.D.
Senior Science Advisor
Assistant Secretary for Preparedness and Response
Department of Health and Human Services
Washington, D.C.
(b)(6)
202-690-7412 (FAX)

From: Peter Daszak
Sent: Sat, 5 Jul 2014 06:51:24 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura
Subject: FW: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER
Attachments: COV Additional Budget Request.xlsx
Importance: High

Dear Eric,

Thanks for the quick reply! I understand the strategy within NIAID, but want to make a plea to you for an increase to the award to cover the cut.

The reason is that because salary allocations won't increase in our budget, this effectively reduces the time originally requested for each of our funded personnel to allocate to this project work in years 2 through 5. Despite the NIH cap, the actual salary of EcoHealth Alliance personnel increases ~5% per year and our fringe rate increases by 1% per year both as per our negotiated DHHS fringe rate. In order to compensate for this, we would like to request permission to add an Assistant Research Scientist (tbd) at a fixed salary of (b)(4); (b)(6) only in years 4 and 5. This would not modify year 1 budgeted funding as per our Notice of Award nor increase us beyond the total award cap for direct costs. The Assistant Research Scientist would support the work done by the Research Scientist and Modeler/Statistician.

The attached budget outlines this request as per our proposal as well as increases the salary allocation for our Modeler/Statistician (Dr. Hosseini) from (b)(4); (b)(6) in our proposal budget to (b)(4); (b)(6) as per our email exchange on 24th January. If you approve this total increase of (b)(4); (b)(6) (b)(4); (b)(6) to our budget, then our AOR Aleksei Chmura (cc'ed here) will follow up with you or Laura to provide the budget line item details and update the budget narrative accordingly. I am available any time to discuss this.

Thanks for all your support and I look forward to hearing back from you

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

(b)(6) (direct)
+1.212.380.4465 (fax)
www.ecohealthalliance.org

EcoHealth Alliance builds innovative science-based solutions and partnerships that increase our global capacity to achieve two interrelated goals: protecting global health by preventing pandemics; and safeguarding ecosystems by promoting conservation.

From: Stemmy, Erik (NIH/NIAID) [E](b)(6)
Sent: Wednesday, June 25, 2014 8:06 AM
To: Peter Daszak
Cc: Aleksei Chmura
Subject: RE: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hi Peter,
Sincere apologies for my delayed response! I'd checked with our grant folks, but neglected to forward you their response. Thank you for reminding me. This policy took effect in FY2012, and NIAID Grants Management can't waive it. The NIH guide notice describing the policy can be found at the link below. Sorry I can't be more helpful!

Best,
Erik

NIH Guide Notice: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-12-036.html

Please note my updated contact information below:
Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-7630
Phone:(b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Peter Daszak (b)(6)
Sent: Tuesday, June 24, 2014 3:35 PM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura
Subject: RE: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER

Dear Erik,

I just wanted to check in with you and see if you were able to speak to Laura Pone about this issue.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

(b)(6) (direct)
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EcoHealth Alliance builds innovative science-based solutions and partnerships that increase our global capacity to achieve two interrelated goals: protecting global health by preventing pandemics; and safeguarding ecosystems by promoting conservation.

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Friday, June 6, 2014 1:05 PM
To: Peter Daszak
Subject: RE: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hi Peter,
Thanks for your message. I'll check in to the issue and get back to you as soon as possible.

Best,
Erik

Please note my updated contact information below:

Erik J. Stemmy, Ph.D.

Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Peter Daszak (b)(6)
Sent: Friday, June 06, 2014 12:56 PM
To: Stemmy, Erik (NIH/NIAID) [E]; Aleksei Chmura
Subject: Budget cut on 1R01AI110964 - 01 PI Name: DASZAK, PETER
Importance: High

Dear Eric,

We are very pleased and excited to receive our Coronavirus award (R01 AI110964-01) and have spent the last few months preparing so that we are now hitting the ground rolling on this!

I want to check in with you on one issue. Our proposal budget was cut by $275,604.30 (8%) over the five years (see attached excel file comparing the NoA budget and the proposal budget). My AOR (Mr Chmura, cc'd here) has communicated with Laura Pone who is handling the grant. Laura said this is due to an NIH/NIAID policy of not permitting annual cost-of-living escalation of salaries and fringe rates. Laura has stated that there now are no exceptions for cost-of-living increase despite our institutional policy on salary escalation and prior NIH escalation factors for recurring costs.
At the same time, our Grants and Contracting Office claim that NIH guidelines permit 3% escalation plus federally negotiated institutional annual fringe increases. Can you please confirm whether this is correct or not and direct us to a website with the corresponding language, so I can use it as guidance for this and all future NIH proposals.

This cut does affect us, and I'd like, if possible, either to reinstate the increase, or submit a revised budget with funds for extra support during the 'out years' so we can still maintain this work.

Any advice you can give on this would be greatly appreciated!

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

(b)(6) (direct)
+1.212.380.4465 (fax)
www.ecohealthalliance.org

EcoHealth Alliance builds innovative science-based solutions and partnerships that increase our global capacity to achieve two interrelated goals: protecting global health by preventing pandemics; and safeguarding ecosystems by promoting conservation.

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, February 11, 2014 10:09 AM
To: Peter Daszak; Aleksei Chmura
Cc: Pone, Laura (NIH/NIAID) [E]
Subject: RE: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

Hi Peter and Aleksei,
One other question about your application. There were a couple of human subjects concerns noted by the study section. I know your IRB approval is still pending, but were you able to address the other human subjects questions? Unless I missed it I didn't see anything in the JIT documents you uploaded.

Thanks,
Erik

Erik J. Stemmy, Ph.D.
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
6610 Rockledge Drive, Room 3210
Bethesda, MD 20892-7630
Phone: (b)(6)
Fax: 301-496-8030
Email: (b)(6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Pone, Laura (NIH/NIAID) [E]
Sent: Monday, February 10, 2014 3:56 PM
To: (b)(6) Stemmy, Erik (NIH/NIAID) [E]; (b)(6)
(b)(6)
Subject: Just-in-Time Request for Grant Number: 1R01AI110964 - 01 PI Name: DASZAK, PETER

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Institute of Allergy
and Infectious Diseases
Bethesda, Maryland 20892

Dear Mr. Chmura,

For applications well received by study section during peer review, we attempt to obtain documentation that must be submitted to the National Institute of Allergy and Infectious Diseases should an application subsequently be identified for funding. Since your application is among those favorably received, we request that you submit the information listed below:
Please submit this information by close of business **Thursday, February 13th.**

- Human Subjects Assurance documentation. **Include grant specific IRB approval date**. Grant specific IRB approvals must include either the project title or grant number.
- Documentation of the Required Education in the Protection of Human Subject Research Participants for all personnel involved.
- IACUC verification statement/letter with approval date.
- Response to Summary Statement Concern Regarding:
  - Protection of Human Subjects
  - Overlap
- Copy of EcoHealth Alliance's most recent F&A rate agreement.

Timely submission of the above information will enable us to expedite the issuance of an award should an application be identified for funding. Please submit this information by 02/13/14.

JIT information should be submitted using the Just-In-Time feature of the eRA Commons found in the Commons Status section. Submit **all** information at one time. For information on the Commons, go to the Commons Web site: https://commons.era.nih.gov/commons/index.jsp. If not submitting through the Commons ***or*** for information unable to be submitted through the Commons, please email the requested information signed by an authorized institutional business official. **Emailed documents not endorsed by an Institution Business Official will not be accepted as valid.**

Please feel free to contact me with any questions or concerns.

Thanks and have a nice day!

Laura Pone
Grants Management Specialist
DHHS/NIH/NIAID/GMP
6700B Rockledge Drive, Room 2240
Bethesda, MD 20892-7614 (Fed Ex zip 20817)
Phone: (b)(6)
e-Fax: 301-493-0597
Email: (b)(6)

please consider the environment before printing this e-mail.

NOA BUDGET AMOUNTS

	Y1	Y2	Y3	Y4	Y5	Total
Salaries & Wages	$167,708	**$167,708**	**$167,708**	**$167,708**	**$167,708**	**$838,540**
Fringe Benefits	$54,168	**$54,168**	**$54,168**	**$54,168**	**$54,168**	**$270,840**
Salaries, Wages, & Fringe	$221,876	**$221,876**	**$221,876**	**$221,876**	**$221,876**	**$1,109,380**
Supplies	$21,400	$19,250	$7,250	$7,000	$3,500	$58,400
Travel Costs	$35,918	$35,918	$35,918	$35,918	$35,918	$179,590
Other Costs	$10,000	$13,550	$11,050	$9,800	$9,400	$53,800
Consortium/Contractual Costs	$227,663	$211,699	$213,239	$201,422	$191,576	$1,045,599
Direct Costs	$516,857	**$502,293**	**$489,333**	**$476,016**	**$462,270**	$2,446,769
F&A	$149,585	**$128,152**	**$121,757**	**$121,096**	**$119,376**	$639,966
Total Cost	$666,442	**$630,445**	**$611,090**	**$597,112**	**$581,646**	**$3,086,735**

PROPOSED ADDITIONAL BUDGET FOR YEARS 2 THROUGH 5

	Y1	Y2	Y3	Y4	Y5	Total
Salaries & Wages	$167,706	**$ 174,375**	**$ 174,375**	**$ 180,842**	**$ 180,842**	**$878,138**
Fringe Benefits	$54,169	**$ 56,323**	**$ 56,323**	**$ 58,412**	**$ 58,412**	**$283,639**
Salaries, Wages, & Fringe	$221,875	**$ 230,697**	**$230,697**	**$239,253**	**$239,253**	**$1,161,776**
Supplies	$21,400	$19,250	$7,250	$7,000	$3,500	$58,400
Travel Costs	$35,918	$35,918	$35,918	$35,918	$35,918	$179,590
Other Costs	$10,000	$13,550	$11,050	$9,800	$9,400	$53,800
Consortium/Contractual Costs	$227,663	$211,699	$213,239	$201,422	$191,576	$1,045,599
Direct Costs	$516,857	**$ 511,115**	**$498,154**	**$493,394**	**$479,647**	**$2,499,166**
F&A	$149,585	**$154,092**	**$147,698**	**$150,809**	**$149,089**	**$751,274**
Total Cost	$666,442	**$665,207**	**$645,852**	**$644,203**	**$628,737**	**$3,250,440**

From: Ellen Carlin
Sent: Fri, 23 Mar 2018 15:51:50 -0400
To: (b)(6); Barton Behravesh, Casey (CDC/DDID/NCEZID/OD); Brooks, Lance R CIV DTRA PARTNERSHIP AND INSP (US); Beth Cameron; Ellen Carlin; Cormier, Justin (CDC/OD/CDCWO); (b)(6); Jafari, Hamid (CDC/DDPHSIS/CGH/OD); Jones, Franca R CDR USN DHA HEALTH SURV (US); (b)(6); Morens, David (NIH/NIAID) [E]; Gerry Parker; (b)(6); Ron Waldman
Cc: Catherine Machalaba; William B. Karesh; Kanya Claudine Long; Franck Cesar Jean Berthe
Subject: Thank you
Attachments: Participant list.xlsx

Dear all,

Many thanks for your participation in Monday's Core Capacities for Global Health Security roundtable at the World Bank. We are incredibly grateful for your thoughtful and enthusiastic participation, and for traveling and taking time out of your busy schedules to provide it.

As promised, we have attached the list of participants and their email addresses. We have also pasted below highlights of some of the key messages that we heard at the meeting, and which we will incorporate into our analysis and findings. Thank you so much for bringing these ideas to our attention.

We may reach back to some of you over the coming months to assist on certain points, and will be sure to include each of you in our dissemination of the final deliverable.

Thanks again, and enjoy the weekend!

Ellen and Catherine

Preventing Pandemics:

Core Capacities for Global Health Security

Roundtable, 19 March 2018, IFC

Key Highlights

High-consequence pathogens warrant a different and more sustained approach than routine public health events. Preparedness for high-consequence pathogens requires both ensuring minimum public health capacities are attained and pursuing actions in other sectors that can reduce risks and impacts and assist with overall resilience.

Intentional, accidental and natural disease introduction should all be addressed under the *prevent-detect-respond* framework. Using the existing triad framework will enable easier uptake by policy-makers and implementers. Placing security concerns and security-sector skillsets into public health terms will help make synergies between public health and security sectors (law enforcement and military) more apparent. The security sector needs to be more involved with functions like risk mitigation.

Community engagement is a crucial, and under-incorporated, underpinning of local and global health security. The ultimate functioning of the core capacities is embedded within communities, including in workforce, detection and reporting, trust, and risk reduction opportunities. Pandemic preparedness approaches must be designed with the motivations of the end user in mind. Regional initiatives are highly relevant for successful implementation and should be incentivized.

The private sector has a particular incentive to operate in well-functioning societies and may be engaged in novel ways to mobilize resources and convene sectors. Private employers are an entry point for numerous interventions including workforce development, risk communication, and pandemic prevention. It may be useful to message the need for a common vocabulary for defining the role of the private sector in the global health security context. There remains a need to remove disincentives to reporting, and engagement of the private sector is critical on this point given reporting's impacts on trade and travel.

Some capacities should be viewed as cross-cutting. Community engagement, risk communication, and economic development/financing are among these. These cross-cutting elements should be added to those already included in the draft matrix (governance, information management, and research & development).

The nomenclature of outbreaks is important. Outbreaks, epidemics, and pandemics are not the same, and it is important to make that distinction, because the interventions are different. With respect to biosurveillance, it may be confusing to separate this term and function from situational awareness.

Independent accountability is needed. There would be value in recommending an independent accountability structure outside of the WHO.

Ellen P. Carlin, DVM
Senior Health and Policy Specialist

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
(mobile)
(b)(6)
www.ecohealthalliance.org

Research Associate, Smithsonian Conservation Biology Institute
Adjunct Research Scientist, Columbia University National Center for Disaster Preparedness
Courtesy Lecturer, Cornell University College of Veterinary Medicine

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

Core capacity	SME	Affiliation	Email address	Staff	Invitation sent	Yes/No
Threat/situational awareness	(b)(6)	HHS	(b)(6)			
		TAMU, Former DOD		Ellen	23-Feb	Yes
		Former DOD				
		Brookings, Prior State Department				
		White House NSC		Ellen	23-Feb	
Prevention		Gryphon Scientific				
		FBI				
		State Department International Health				
		NIEHS				
		Skoll Global Threats		Catherine	23-Feb	Yes
Protection		NIAID		Ellen	28-Feb	Yes
		NTI, Former WH		Catherine	23-Feb	Yes
		DOD, OSTP				
		CEPI		Ellen	23-Feb	Yes
		DOD DTRA		Ellen	23-Feb	Yes
		National Security Council				No
		HHS		Ellen	23-Feb	No
				Ellen		
Surveillance and detection		WHO (Geneva)		Catherine	23-Feb	Yes - webex +
		DOD GEIS		Ellen	23-Feb	Yes
		USAID		Catherine	23-Feb	Yes
		Finnish Defence Ministry				
		OIE - may send Billy; Bev; Cristobal Zapeda		Billy	23-Feb	No
		FAO		Catherine	23-Feb	Yes
Response		4SD		Catherine	23-Feb	No
		CDC Global Health Protection				
		World Bank		Franck		Someone
		NIAID				
		Principal Deputy Director for the Center for Global Health, CDC		Ellen	23-Feb	Yes
		Merial				
		Zoetis				
Risk reduction/mitigation		Georgetown University		Ellen	23-Feb	Yes
		Georgetown University				
		HHS Office of Global Affairs		Ellen	4-Mar	
		CDC Office of One Health		Catherine	23-Feb	Yes
		Chevron		Catherine	23-Feb	Probably
Recovery		Gates Foundation		Catherine	23-Feb	No
		In-Q-Tel				
		MSF				
		African Development Bank				
		SRF		Ellen	23-Feb	
				n/a		Yes
				n/a		Yes
				n/a		Yes
				n/a		Yes
				n/a		Yes
				n/a		Yes
				Franck		
				Ellen		No
				Hold		
				Billy will reach out		

(b)(6)

Environment and climate

Internal team

(b)(6)

(b)(6)

Final guest list

TAMU, Former DOD
Skoll Global Threats
NIAID
NTI, Former WH
CEPI
DOD DTRA
WHO (Geneva)
WHO (Geneva)
DOD GEIS
USAID
FAO
Principal Deputy Director for the Center for Global Health, CDC
Georgetown University
CDC Office of One Health
Chevron
OIE
World Bank

(b)(6)

(assistant Jessica Dickens)

17 participants
15 in the room

4 staff
1 Plus one

20 people that need to eat
Buffer for 22

(b)(6)

(b)(6)

Last	First	Affiliation	Email
(b)(6)		Chevron	(b)(6)
		CDC Office of One Health	
		OIE	
		DOD DTRA	
		NTI	
		EcoHealth Alliance	
		CDC	
		USAID	
		CDC Center for Global Health	
		DOD GEIS	
		EcoHealth Alliance	
		Ending Pandemics	
		World Bank	
		EcoHealth Alliance	
		NIAID	
		Texas A&M University	
		FAO	
		Georgetown University	

From: (b)(6)
Sent: Fri, 20 Aug 2021 20:13:30 -0400
To: Jason Gale
Cc: (b)(6)
(b)(6)
(b)(6) ; Garry, Robert F; (b)(6)
Bcc: Morens, David (NIH/NIAID) [E]
Subject: Re:

Good names. (b)(6) has a certain paranoid streak though…. I might add (b)(6) from (b)(6) (b)(6) the phylogeneticist (b)(6) (kinda hard assed but honest and detailed), (b)(6) (b)(6) (elder statesman with strong international experience including smallpox erad and ebola discovery, (b)(6) would be great!, (b)(6) the bat epidemiologist/epizootiologist, (b)(6) in Cambodia and many more!
d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Aug 20, 2021, at 20:02, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net>wrote:

I am big on diversity and think middle-aged white men (of which I am one) are usually over-represented, so I'd like to see a good array of very talented smart women considered...the likes of:
* (b)(6)
*
*
*
*
*

From: (b)(6) At: 08/21/21 09:54:52 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:)
Cc: (b)(6),
(b)(6),
Subject: Re:

Yes, i did see this, but assume it is all rigged. Can this group ID some folks who would be good candidates???? I can think of a few names…. d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Aug 20, 2021, at 19:30, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net>wrote:

Meantime, y'all saw this, right?
https://www.who.int/news-room/articles-detail/who-scientific-advisory-group-for-the-origins-of-novel-pathogens-(sago)

From: (b)(6) At: 08/21/21 09:27:14 UTC+10:00
To:
Cc: Jason Gale (BLOOMBERG/ NEWSROOM:) , (b)(6) ,
(b)(6) ,
Subject: Re:

Those Italian sequences are stone cold contamination David. Nothing nefarious, just a poorly done study.

The following Tweet threat by Michael Worobey explains it beautifully:

https://twitter.com/MichaelWorobey/status/1424483875384958981?s=20

Cheers,

Eddie

PROFESSOR EDWARD C. HOLMES FAA FRS
ARC Australian Laureate Fellow

THE UNIVERSITY OF SYDNEY
Marie Bashir Institute for Infectious Diseases &Biosecurity,
School of Life &Environmental Sciences and School of Medical Sciences,
The University of Sydney | Sydney | NSW | 2006 | Australia
T (b)(6)
E

On 21 Aug 2021, at 9:10 am, Morens, David (NIH/NIAID) [E] (b)(6) wrote:

Eddie, thanks so much, I had no idea that some of these conflicting data represented bullshit agendas. What has happened to scientific integrity that scientists would sell their souls over dishonest political agendas? I guess i am too naïve…. I have always believed or at least hoped that scientists had the utmost integrity….

If i may impose on you again, last week the Italian group published, finally, their data on viral sequences dating back to early-mid October 2019 and thereafter from Italy, suggesting, or so the data seem to say, that their sequences are upstream of the earliest Wuhan sequences two months later.

If true, this would suggest an earlier viral origin spread to Europe before being detected in Wuhan. The Italian sequences seemed to suggest that the Wuhan virus was a downstream offshoot?

Perhaps I misunderstand, either that or the authors are nuts? Surely you guys can figure this out? d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Aug 20, 2021, at 17:25, Edward Holmes (b)(6) wrote:

It's diabolical nonsense David. Irrespective of what they state in that 'paper', Linfa has found serological evidence for closely related viruses in pangolins dating back several years and the HKU team have similar data (see attachment). Plus the Guangdong pangolins have been my multiple groups in different ways and there is an independent lineage in Guangxi.

The attempt to undermine the pangolin data and the people that generated it one of the shameful examples of anti-science I have ever seen. The reality is that is because the RBD of the Guangdong pangolins is genetically similar to SARS-CoV-2 it becomes an inconvenient data point for those who believe the virus came from a lab in Wuhan hence their attempts to undermine it.

Cheers,

Eddie

PROFESSOR EDWARD C. HOLMES FAA FRS
ARC Australian Laureate Fellow

THE UNIVERSITY OF SYDNEY
Marie Bashir Institute for Infectious Diseases &Biosecurity,
School of Life &Environmental Sciences and School of Medical Sciences,
The University of Sydney | Sydney | NSW | 2006 | Australia
T (b)(6)
E <mailto:(b)(6)

On 21 Aug 2021, at 1:03 am, Morens, David (NIH/NIAID) [E]
(b)(6) <mailto:(b)(6) wrote:

Thanks to both you and Kristian. Very heelpful to know what the experts think, because 5o us mere mortals, phylogenetic and sequencing interpretation is a bit inscrutable.

Yes, although I don't know her personally, I know OF Alina Chan based on two papers of hers I came across, one of which was a screed against Eddie's recent review. It seemed biased, cherry-picked, and not the work of a scientist with integrity.

<image004.gif>

David M. Morens, M.D.
CAPT, United States Public Health Service
Senior Advisor to the Director
Office of the Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 31, Room 7A-03

31 Center Drive, MSC 2520
Bethesda, MD 20892-2520
• (b)(6) (assistant: Whitney Robinson)
• 301 496 4409
• (b)(6) <mailto:(b)(6)

From: Garry, Robert F (b)(6) <mailto:(b)(6)
Sent: Friday, August 20, 2021 10:38 AM
To: Morens, David (NIH/NIAID) [E] (b)(6) <mailto:(b)(6); Kristian G. Andersen (b)(6) <mailto:(b)(6)
Cc: Jason Gale <j.gale@bloomberg.net<mailto:j.gale@bloomberg.net>>; (b)(6) <mailto:(b)(6);
(b)(6) <mailto:(b)(6);
(b)(6) <mailto:(b)(6);
(b)(6) <mailto:(b)(6);
(b)(6) <mailto:(b)(6);
(b)(6) <mailto:(b)(6);
(b)(6) <mailto:(b)(6)
Subject: Re:

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“(a) the pangolin covs are actually from mice (b) actually, they were actually cloned artificial constructs, (c) actually, there were other viruses in the samples as well (oh no! who'd have thought), (d) actually, it's all contaminated with dog dna.”

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b

From: "Morens, David (NIH/NIAID) [E]" (b)(6) <mailto:(b)(6)
Date: Friday, August 20, 2021 at 8:56 AM
To: Kristian Andersen (b)(6) <mailto:(b)(6)
Cc: Jason Gale <j.gale@bloomberg.net<mailto:j.gale@bloomberg.net>>,
(b)(6) <mailto:(b)(6)
<mailto:(b)(6),
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(b)(6) <mailto:(b)(6) ,
(b)(6) mailto:(b)(6) "
<mailto:(b)(6) , Robert Garry
(b)(6) mailto:(b)(6) <mailto:(b)(6) "
<mailto:(b)(6) ,
(b)(6) <mailto:(b)(6)
(b)(6) <mailto:(b)(6)
Subject: <no subject>

External Sender. Be aware of links, attachments and requests.
Do you all know these data? see link below….
[2108.08163] Cloning vectors and contamination in metagenomic datasets raise concerns over pangolin CoV genome authenticity (arxiv.org)<https://protect-au.mimecast.com/s/s7cRCQnMBZfkxWRNQTxp1ID?domain=nam11.safelinks.protection.outlook.com>

<image006.gif>

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• (b)(6) (assistant: Whitney Robinson)
• 301 496 4409
• (b)(6) <mailto (b)(6)

<image007.jpg>

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Sent: Thursday, August 12, 2021 8:11 PM
To: Morens, David (NIH/NIAID) [E] (b)(6) <mailto:(b)(6)
Cc: Jason Gale <j.gale@bloomberg.net<mailto:j.gale@bloomberg.net>>;
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Subject: Re: The story behind the missing story about the story behind the missing raccoons

I hear La Jolla has some pretty nice beaches - just saying.

Oh wait, I live here - here's what's outside my office:

<image008.jpg>

Happy to save you a spot - you know, 'field' research.

K

On Thu, Aug 12, 2021 at 5:09 PM Morens, David (NIH/NIAID) [E]
(b)(6) <mailto:(b)(6) wrote:

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Sent from my iPhone
David M Morens
OD, NIAID, NIH

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<j.gale@bloomberg.net<mailto:j.gale@bloomberg.net>>wrote:

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From: (b)(6) <mailto: (b)(6) At: 08/13/21 09:05:19 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:) <mailto:j.gale@bloomberg.net>,
(b)(6) <mailto:(b)(6) <mailto:(b)(6)
(b)(6) <mailto:(b)(6)
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Kindest regards,
Jason

<Pangolin-Serology-Nido2021-Poster.pdf>

From: Morens, David (NIH/NIAID) [E]
Sent: Fri, 13 Aug 2021 23:34:36 +0000
To: Jason Gale; (b)(6)
(b)(6) Garry, Robert F; (b)(6)
Subject: RE: Guys, I have another round of questions that I need to answer

Peter is the perfec t guy to do that!

David

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301 496 4409
(b)(6)

From: Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net>
Sent: Friday, August 13, 2021 7:04 PM
To: (b)(6) ; Morens, David (NIH/NIAID) [E] (b)(6) Garry, Robert F (b)(6)
Subject: Guys, I have another round of questions that I need to answer

It mostly relates to why knowing the Xiao paper earlier would have made a difference in the origins research.
Does anyone have 10 mins to talk off-the-record to help, perchance?
Thanks.
Jason

From: Jason Gale (BLOOMBERG/ NEWSROOM:) At: 08/13/21 07:52:37
To: (b)(6), (b)(6)
Subject: The story behind the missing story about the story behind the missing raccoons

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From: Morens, David (NIH/NIAID) [E]
Sent: Fri, 20 Aug 2021 15:03:21 +0000
To: Garry, Robert F; Kristian G. Andersen
Cc: Jason Gale; (b)(6)
(b)(6)
Subject: RE:

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Yes, although I don't know her personally, I know OF Alina Chan based on two papers of hers I came across, one of which was a screed against Eddie's recent review. It seemed biased, cherry-picked, and not the work of a scientist with integrity.

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Cc: Jason Gale <j.gale@bloomberg.net>, (b)(6)
(b)(6) ,
(b)(6) , Robert Garry
(b)(6) ,

(b)(6)

Subject: <no subject>

External Sender. Be aware of links, attachments and requests.

Do you all know these data? see link below….

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David

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(b)(6)

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(b)(6)

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Sent: Thursday, August 12, 2021 5:53 PM
To: (b)(6);
(b)(6); Morens, David (NIH/NIAID) [E]
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From: Morens, David (NIH/NIAID) [E]
Sent: Wed, 21 Jul 2021 21:52:26 +0000
To: Keusch, Gerald T; Peter Daszak ((b)(6))
Subject: RE: Thank you

These are both greAt, I'll put them in Tony's (metaphorical) pile.
d

David M. Morens, M.D.
CAPT, United States Public Health Service
Senior Advisor to the Director
Office of the Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 31, Room 7A-03
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520
B (b)(6) (assistant: Whitney Robinson)
W 301 496 4409
3 (b)(6)

-----Original Message-----
From: Keusch, Gerald T (b)(6)
Sent: Wednesday, July 21, 2021 11:24 AM
To: Morens, David (NIH/NIAID) [E] (b)(6); Peter Daszak ((b)(6)) (b)(6)
Subject: RE: Thank you

That is sweet. I hope TF will see it. I'm loving the press coverage of his skirmish with an ophthalmologist who cannot see the difference between science and shit, and has no insight to guide how he thinks and what he says.

I was so proud of Tony's direct response - even his patience (limited though it was) when Paul bullied and interrupted him.

Stacey Knobler at Sabin Vaccine Institute sent me the attached two pieces on COVID vaccine development and its relevance for pandemic influenza preparedness in which I am quoted stemming from the work Nicki and I did for the Sabin-Aspen Institutes think tank on vaccine science and policy, and subsequent interviews with their PR team which produced these articles. They don't address the origins controversy but they are relevant to public perception of the value of vaccines and the science underlying their development.

Jerry

-----Original Message-----
From: Morens, David (NIH/NIAID) [E] (b)(6)

Sent: Wednesday, July 21, 2021 9:29 AM
To: Peter Daszak ([redacted]) [redacted]; Keusch, Gerald T [redacted]
Subject: FW: Thank you

David M. Morens, M.D.
CAPT, United States Public Health Service Senior Advisor to the Director Office of the Director National Institute of Allergy and Infectious Diseases National Institutes of Health Building 31, Room 7A-03
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520
B [redacted] (assistant: Whitney Robinson) W 301 496 4409
3 [redacted]

-----Original Message-----
From: Billet, Courtney (NIH/NIAID) [E] [redacted]
Sent: Tuesday, July 20, 2021 4:34 PM
To: NIAID OD AM <NIAIDODAM@niaid.nih.gov>
Subject: FW: Thank you

Another one, lol

-----Original Message-----
From: Carol Maloney Nelson [redacted]
Sent: Tuesday, July 20, 2021 4:19 PM
To: NIAID Ocpostoffice (NIH/NIAID) <OCPOSTOFFICE@niaid.nih.gov>
Subject: Thank you

Thank you Dr Fauci. For everything.
We listens d we understand because we are educated and at least somewhat intelligent.

We already knew Rand Paul is an ass and we thank you for speaking up for all of us.

PS [redacted]

From: Peter Daszak
Sent: Wed, 21 Jul 2021 11:32:45 -0400
To: Morens, David (NIH/NIAID) [E]
Cc: Keusch, Jerry ((b)(6))
Subject: Re: Thank you

Lovely but I'm now also getting tony Fauci's hate mail (they Cc me on some). I won't forward, it's pretty disgusting!!!

Cheers,

Peter

Peter Daszak
(Sent from my iPhone)

President
EcoHealth Alliance

460 West 34th Street, New York, NY10001, USA

www.EcoHealthAlliance.org

> On Jul 21, 2021, at 9:29 AM, Morens, David (NIH/NIAID) [E] (b)(6) wrote:
>
>
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> David M. Morens, M.D.
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> 31 Center Drive, MSC 2520
> Bethesda, MD 20892-2520
> B (b)(6) (assistant: Whitney Robinson)

> W 301 496 4409
> 3 (b)(6)
>
> Disclaimer: This message is intended for the exclusive use of the recipient(s) named above. It may contain information that is PROTECTED, PRIVILEGED, and/or CONFIDENTIAL, and it should not be disseminated, distributed, or copied to persons not authorized to receive such information. All sensitive documents must be properly labeled before dissemination via email. If you are not the intended recipient, any dissemination, distribution, or copying is strictly prohibited. If you have received this communication in error, please erase all copies of the message and its attachments and notify us immediately.
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> PS (b)(6)

(b)(6)

awareness training, web security, compliance and other essential capabilities. Mimecast helps protect large and small organizations from malicious activity, human error and technology failure; and to lead the movement toward building a more resilient world. To find out more, visit our website.

From: (b)(6)
Sent: Mon, 4 Oct 2021 19:01:35 -0400
To: Peter Daszak; Gerald Keusch
Bcc: Morens, David (NIH/NIAID) [E]
Subject: Fwd: Dr. Fauci request from the Senate Dems

Sent from my iPhone
David M Morens
OD, NIAID, NIH

Begin forwarded message:

From: "Conrad, Patricia (NIH/NIAID) [E]" (b)(6)
Date: October 4, 2021 at 18:54:32 EDT
To: NIAID OD AM <NIAIDODAM@niaid.nih.gov>
Cc: "Selgrade, Sara (NIH/NIAID) [E]" (b)(6)
Subject: Fwd: Dr. Fauci request from the Senate Dems

Sent from my iPhone

Begin forwarded message:

From: "Dodin, Reema B. EOP/WHO" (b)(6)
Date: October 4, 2021 at 6:43:36 PM EDT
To: "Conrad, Patricia (NIH/NIAID) [E]" (b)(6)
Cc: "Anderson, Charlie D. EOP/WHO" (b)(6)
Subject: Dr. Fauci request from the Senate Dems

Hello Patricia!

I hope this finds you well.

Senator Stabenow runs the weekly DPCC Dem caucus luncheon, and said that the February visit from Dr. Fauci was among everyone's favorite and they were wondering if he could come back – perhaps this Thursday? Or the Thursday of the week of the 18th?

Many thanks for considering!

rd

Reema Dodin


(b)(6)

From: Ellen Carlin [b6]
Sent: 3/23/2018 7:51:50 PM
To: b6; Barton Behravesh, Casey (CDC/DDID/NCEZID/OD) [b6 b6]; Brooks, Lance R CIV DTRA PARTNERSHIP AND INSP (US) [b6]; Beth Cameron [b6]; Ellen Carlin [b6]; Cormier, Justin (CDC/OD/CDCWO) [b6 b6]; b6; Jafari, Hamid ([b6 b6]); Jones, Franca R CDR USN DHA HEALTH SURV (US) [b6]; b6; Morens, David (NIH/NIAID) [E] [b6]; Gerry Parker [b6]; b6; Ron Waldman [b6]
CC: Catherine Machalaba [b6]; William B. Karesh [b6]; Kanya Claudine Long [b6]; Franck Cesar Jean Berthe [b6]
Subject: Thank you
Attachments: Participant list.xlsx

Dear all,

Many thanks for your participation in Monday's Core Capacities for Global Health Security roundtable at the World Bank. We are incredibly grateful for your thoughtful and enthusiastic participation, and for traveling and taking time out of your busy schedules to provide it.

As promised, we have attached the list of participants and their email addresses. We have also pasted below highlights of some of the key messages that we heard at the meeting, and which we will incorporate into our analysis and findings. Thank you so much for bringing these ideas to our attention.

We may reach back to some of you over the coming months to assist on certain points, and will be sure to include each of you in our dissemination of the final deliverable.

Thanks again, and enjoy the weekend!

Ellen and Catherine

Preventing Pandemics:

Core Capacities for Global Health Security

Roundtable, 19 March 2018, IFC

Key Highlights

High-consequence pathogens warrant a different and more sustained approach than routine public health events. Preparedness for high-consequence pathogens requires both ensuring minimum public health capacities are attained and pursuing actions in other sectors that can reduce risks and impacts and assist with overall resilience.

Intentional, accidental and natural disease introduction should all be addressed under the *prevent-detect-respond* framework. Using the existing triad framework will enable easier uptake by policy-makers and implementers. Placing security concerns and security-sector skillsets into public health terms will help make synergies between public health and security sectors (law enforcement and military) more apparent. The security sector needs to be more involved with functions like risk mitigation.

Community engagement is a crucial, and under-incorporated, underpinning of local and global health security. The ultimate functioning of the core capacities is embedded within communities, including in workforce, detection and reporting, trust, and risk reduction opportunities. Pandemic preparedness approaches must be designed with the motivations of the end user in mind. Regional initiatives are highly relevant for successful implementation and should be incentivized.

The private sector has a particular incentive to operate in well-functioning societies and may be engaged in novel ways to mobilize resources and convene sectors. Private employers are an entry point for numerous interventions including workforce development, risk communication, and pandemic prevention. It may be useful to message the need for a common vocabulary for defining the role of the private sector in the global health security context. There remains a need to remove disincentives to reporting, and engagement of the private sector is critical on this point given reporting's impacts on trade and travel.

Some capacities should be viewed as cross-cutting. Community engagement, risk communication, and economic development/financing are among these. These cross-cutting elements should be added to those already included in the draft matrix (governance, information management, and research & development).

The nomenclature of outbreaks is important. Outbreaks, epidemics, and pandemics are not the same, and it is important to make that distinction, because the interventions are different. With respect to biosurveillance, it may be confusing to separate this term and function from situational awareness.

Independent accountability is needed. There would be value in recommending an independent accountability structure outside of the WHO.

Ellen P. Carlin, DVM
Senior Health and Policy Specialist

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

b6 (direct)
(mobile)
b6
www.ecohealthalliance.org

Research Associate, Smithsonian Conservation Biology Institute
Adjunct Research Scientist, Columbia University National Center for Disaster Preparedness
Courtesy Lecturer, Cornell University College of Veterinary Medicine

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

Core capacity	SME	Affiliation	Email address	Staff	Invitation sent	Yes/No
Threat/situational awareness	b6	HHS	b6			
		TAMU, Former DOD		Ellen	23-Feb	Yes
		Former DOD				
		Brookings, Prior State Department				
		White House NSC		Ellen	23-Feb	
Prevention		Gryphon Scientific				
		FBI				
		State Department International Health				
		NIEHS				
		Skoll Global Threats		Catherine	23-Feb	Yes
Protection		NIAID		Ellen	28-Feb	Yes
		NTI, Former WH		Catherine	23-Feb	Yes
		DOD, OSTP				
		CEPI		Ellen	23-Feb	Yes
		DOD DTRA		Ellen	23-Feb	Yes
		National Security Council				No
		HHS		Ellen	23-Feb	No
				Ellen		
Surveillance and detection		WHO (Geneva)		Catherine	23-Feb	Yes - webex +1
		DOD GEIS		Ellen	23-Feb	Yes
		USAID		Catherine	23-Feb	Yes
		Finnish Defence Ministry				
		OIE - may send Billy; Bev; Cristobal Zapeda		Billy	23-Feb	No
		FAO		Catherine	23-Feb	Yes
Response		4SD		Catherine	23-Feb	No
		CDC Global Health Protection				
		World Bank		Franck		Someone
		NIAID				
		Principal Deputy Director for the Center for Global Health, CDC		Ellen	23-Feb	Yes
		Merial				
		Zoetis				
Risk reduction/mitigation		Georgetown University		Ellen	23-Feb	Yes
		Georgetown University				
		HHS Office of Global Affairs		Ellen	4-Mar	
		CDC Office of One Health		Catherine	23-Feb	Yes
		Chevron		Catherine	23-Feb	Probably
Recovery		Gates Foundation		Catherine	23-Feb	No
		In-Q-Tel				
		MSF				
		African Development Bank				
		SRF		Ellen	23-Feb	

b6

Internal team

b6

n/a — Yes
n/a — Yes
n/a — Yes
n/a — Yes
n/a — Yes
n/a — Yes — Environment and climate
Franck

Ellen — No
Hold
Billy will reach out

Final guest list

b6

TAMU, Former DOD
Skoll Global Threats
NIAID
NTI, Former WH
CEPI
DOD DTRA
WHO (Geneva)
WHO (Geneva)
DOD GEIS
USAID
FAO
Principal Deputy Director for the Center for Global Health, CDC
Georgetown University
CDC Office of One Health
Chevron
OIE
World Bank

b6

(assistant Jessica Dickens)

17 participants
15 in the room

4 staff
1 Plus one

20 people that need to eat
Buffer for 22

b6

EHA/World Bank Global Health Security Roundtable
March 19, 2018
Participant list

Last	First	Affiliation	Email
b6		Chevron	b6
		CDC Office of One Health	
		OIE	
		DOD DTRA	
		NTI	
		EcoHealth Alliance	
		CDC	
		USAID	
		CDC Center for Global Health	
		DOD GEIS	
		EcoHealth Alliance	
		Ending Pandemics	
		World Bank	
		EcoHealth Alliance	
		NIAID	
		Texas A&M University	
		FAO	
		Georgetown University	

From: Alison Andre [b6]
Sent: 2/20/2020 4:07:02 PM
To: Taubenberger, Jeffery (NIH/NIAID) [E] [b6]
b6
CC: Peter Daszak [b6]; Morens, David (NIH/NIAID) [E] [b6]
b6
Subject: Re: New England Journal of Medicine 20-02106
Attachments: NEJM CTA-2017.pdf; ICMJE Disclosure Form.pdf; Change of Authorship Form_daszak.doc

Hi Jeff,

Attached forms here. For the change in authorship form, I was unsure if the order mattered but I changed it to what you had in your previous email. Peter's delighted to be a co-author and the author order of Morens, Daszak, Taubenberger is great.

Happy to send these straight to Maria at NEJM if easier – just let me know.

Thanks,
Alison

Alison Andre
Executive Assistant to the President

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

b6 (direct)
1.212.380.4465 (fax)
www.ecohealthalliance.org

EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation.

From: "Taubenberger, Jeffery (NIH/NIAID) [E]" b6
Date: Thursday, February 20, 2020 at 10:44 AM
To: Alison Andre b6, "Morens, David (NIH/NIAID) [E]" b6
Cc: Peter Daszak
Subject: FW: New England Journal of Medicine 20-02106

Hi Alison,

I was given your name by Peter about this upcoming NEJM perspectives article for which Peter will be a coauthor. Cc'ing you in hopes that you can coordinate with Peter to get all the needed author information in their system as soon as possible.

Thanks

Jeff

Jeffery K. Taubenberger, M.D., Ph.D.,
Chief, Viral Pathogenesis and Evolution Section

Deputy Chief, Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
33 North Drive, Room 3E19A.2 MSC 3203
Bethesda, MD 20892-3203 USA

Tel. b6 ; Fax. 1-301-480-1696
email: b6

==========

From: Morens, David (NIH/NIAID) [E] b6
Sent: Thursday, February 20, 2020 10:34 AM
To: Peter Daszak b6 ; Peter Daszak b6
Cc: Taubenberger, Jeffery (NIH/NIAID) [E] b6
Subject: Fwd: New England Journal of Medicine 20-02106

Sent from my iPhone
David M Morens
OD, NIAID, NIH

Begin forwarded message:

> **From:** "Sedjo, Maria" b6
> **Date:** February 20, 2020 at 10:17:52 EST
> **To:** "Taubenberger, Jeffery (NIH/NIAID) [E]" b6
> **Cc:** "Morens, David (NIH/NIAID) [E]" b6
> **Subject: RE: New England Journal of Medicine 20-02106**
>
> Dear Dr. Taubenberger,
>
> The editors have approved your request to add Dr. Daszak as a co-author. As we have already accepted your piece, and it is flying through our production process to go online quickly, time is of the essence. I will need the following ASAP to make this a possibility:
>
> -an email address for Daszak
> -his completed CTA and ICMJE forms
> -a change of authorship form - each of you please sign a form and return to me
> -an updated title page with his information
>
> Forms are attached. All forms can be returned to me at b6 .
>
> Thanks!

Maria Sedjo
Executive Assistant to the Editor-in Chief

New England Journal of Medicine
10 Shattuck Street
Boston, MA 02115
b6
http://www.nejm.org

-----Original Message-----
From: Taubenberger, Jeffery (NIH/NIAID) [E] b6
Sent: Wednesday, February 19, 2020 10:12 AM
To: NEJM Editorial <editorial@nejm.org>
Cc: Morrissey, Stephen b6; Morens, David (NIH/NIAID) [E]
b6; Taubenberger, Jeffery (NIH/NIAID) [E] b6
Subject: RE: New England Journal of Medicine 20-02106

Dear Debbie,

Thank you for the opportunity to submit a revision to our coronavirus perspectives manuscript. I have submitted a revision online this morning. I am attaching a tracked version here.

As you consider this revision, We would like to ask you and Dr. Baden, who emailed with co-author David Morens about this manuscript before we submitted it, if we can get a special dispensation to add a third co-author, Dr. Peter Daszak. The three of us had originally planned to write this, not recalling that you had a two author limit, but when we were asked to submit quickly we could not reach Dr. Daszak to approve the final draft (it turned out he was at WHO Geneva dealing with the coronavirus epidemic), so we submitted without his name, but assuming he would approve it when we reached him. If it is not possible to add him, then we would at least like to acknowledge him for helpful discussion.

With best wishes,

Jeff Taubenberger

Jeffery K. Taubenberger, M.D., Ph.D.,
Chief, Viral Pathogenesis and Evolution Section Deputy Chief, Laboratory of Infectious Diseases National Institute of Allergy and Infectious Diseases National Institutes of Health
33 North Drive, Room 3E19A.2 MSC 3203
Bethesda, MD 20892-3203 USA

Tel. b6; Fax. 1-301-480-1696
email: b6

==========

statements made that are sender's own and not expressly made on behalf of NIAID.

-----Original Message-----
From: New England Journal of Medicine <onbehalfof@manuscriptcentral.com>
Sent: Tuesday, February 18, 2020 8:17 PM
To: Taubenberger, Jeffery (NIH/NIAID) [E] b6
Subject: New England Journal of Medicine 20-02106

Dear Jeff,

Thank you for submitting your Perspective manuscript about the new coronavirus. We plan to move forward with it quickly (always pending acceptance of a final version by the editor-in-chief), so I am attaching an edited version for your review, revisions, and responses. Although I've saved a copy with all the changes tracked, this version has the edits tentatively accepted and a number of queries embedded in it as "comments"; if you can't locate these, please let me know.

Feel free to e-mail me directly (b6) if you have questions or concerns. Otherwise, please go ahead and work on this version, tracking your changes, and upload a revision when you're done.

To upload your revision, log into https://nam12.safelinks.protection.outlook.com/?url=http%3A%2F%2Fmc05.manuscriptcentral.com%2Fnejm&data=02%7C01%7Cmsedjo%40nejm.org%7Ccaccc42e9c2748740c4008d7b5849df9%7C458a53272e354039ab37680f1f49c047%7C0%7C0%7C637177453505730137&sdata=e59AaPyFEEjr7yzTGFmCO%2FRpktyou%2BV%2BW4fhwbPSENA%3D&reserved=0 and enter For Authors, where you will find your title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision. So that it will convert properly, please select "Manuscript text - clean" from the menu.

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Please send completed forms to Maria Sedjo at b6 (preferred), or upload them to your Author Dashboard of ScholarOne Manuscripts along with your revision.

Thank you.

Best,

Debbie Malina

Debra Malina, Ph.D.
Perspective Edigtor
New England Journal of Medicine
10 Shattuck Street
Boston, MA 02115
b6
Fax: (617) 739-9864
http://www.nejm.org

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10 SHATTUCK STREET, BOSTON, MA 02115 U.S.A.
(781) 207-6529 FAX

Contribution/Manuscript Number (required): NEJM 20-02106

Short Title or description of Contribution: Another Novel Coronavirus Escapes Pandora's Box

Corresponding Author: Jeffrey Taubenberger

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If author was a U.S. Government employee at the time the Work was written, please check below.

[___]

PRINTED NAME Peter Daszak

SIGNATURE b6

DATE SIGNED: February 20, 2020

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Rev. 7/2017

ICMJE INTERNATIONAL COMMITTEE of MEDICAL JOURNAL EDITORS

ICMJE Form for Disclosure of Potential Conflicts of Interest

Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. It contains programming that allows appropriate data display. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in six parts.

1. Identifying information.

2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes".

3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. You should disclose interactions with ANY entity that could be considered broadly relevant to the work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work's sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. Intellectual Property.

This section asks about patents and copyrights, whether pending, issued, licensed and/or receiving royalties.

5. Relationships not covered above.

Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.

Definitions.

Entity: government agency, foundation, commercial sponsor, academic institution, etc.
Grant: A grant from an entity, generally [but not always] paid to your organization
Personal Fees: Monies paid to you for services rendered, generally honoraria, royalties, or fees for consulting , lectures, speakers bureaus, expert testimony, employment, or other affiliations
Non-Financial Support: Examples include drugs/equipment supplied by the entity, travel paid by the entity, writing assistance, administrative support, etc.
Other: Anything not covered under the previous three boxes
Pending: The patent has been filed but not issued
Issued: The patent has been issued by the agency
Licensed: The patent has been licensed to an entity, whether earning royalties or not
Royalties: Funds are coming in to you or your institution due to your patent

ICMJE INTERNATIONAL COMMITTEE of MEDICAL JOURNAL EDITORS

ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 1. Identifying Information

1. Given Name (First Name): Peter
2. Surname (Last Name): Daszak
3. Date: 20-February-2020

4. Are you the corresponding author? ☐ Yes ☑ No
Corresponding Author's Name: Jeffrey Taubenberger

5. Manuscript Title: Another Novel Coronavirus Escapes Pandora's Box

6. Manuscript Identifying Number (if you know it): NEJM 20-02106

Section 2. The Work Under Consideration for Publication

Did you or your institution **at any time** receive payment or services from a third party (government, commercial, private foundation, etc.) for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Are there any relevant conflicts of interest? ☐ Yes ☑ No

Section 3. Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the "Add +" box. You should report relationships that were **present during the 36 months prior to publication**.

Are there any relevant conflicts of interest? ☐ Yes ☑ No

Section 4. Intellectual Property – Patents & Copyrights

Do you have any patents, whether planned, pending or issued, broadly relevant to the work? ☐ Yes ☑ No

ICMJE INTERNATIONAL COMMITTEE of MEDICAL JOURNAL EDITORS

ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 5. Relationships not covered above

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

☐ Yes, the following relationships/conditions/circumstances are present (explain below):

☑ No other relationships/conditions/circumstances that present a potential conflict of interest

At the time of manuscript acceptance, journals will ask authors to confirm and, if necessary, update their disclosure statements. On occasion, journals may ask authors to disclose further information about reported relationships.

Section 6. Disclosure Statement

Based on the above disclosures, this form will automatically generate a disclosure statement, which will appear in the box below.

Dr. Daszak has nothing to disclose.

Evaluation and Feedback

Please visit http://www.icmje.org/cgi-bin/feedback to provide feedback on your experience with completing this form.

RE: NEJM 20-02106

STATEMENT OF AUTHORSHIP CHANGE

Eric J. Rubin, MD, PhD
Editor-in-Chief
New England Journal of Medicine
10 Shattuck Street
Boston, MA 02115
USA

We hereby allow a change in authorship for "" from:

David Morens, Jeffery Taubenberger

To:

David Morens, Peter Daszak, Jeffery Taubenberger

Sincerely,

Date: **February 20, 2020**

Printed Name: **Peter Daszak**

Signature: **b6**

From: Morens, David (NIH/NIAID) [E] [b6 b6]
Sent: 8/6/2021 4:50:29 PM
To: Wang Linfa [b6]; Stephen Goldstein [b6]; Jason Gale [j.gale@bloomberg.net]
CC: b6; Garry, Robert F [b6 b6]; b6 b6
Subject: RE: Chris Newman interview

Lin-Fa, not sure what this test is, but such a test, if it really correlates with Nt, it could be helpful in figuring out what caused the positive EIAs in the Cambodian populations our colleagues here have studied, these sera being strongly positive in spike and RBD EIA, but negative in Nt with an early SARS-CoV-2.

Stephen, you I think asked me a couple weeks ago whether they planned to publish this and I said I thought not, but now they have changed their minds, and are doing additional tests so they can publish. I will talk to the PI on a zoom call at 2 today.

David

David M. Morens, M.D.
CAPT, United States Public Health Service
Senior Advisor to the Director
Office of the Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 31, Room 7A-03
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520
☎ b6 (assistants: Kimberly Barasch; Whitney Robinson)
301 496 4409
b6

From: Wang Linfa b6
Sent: Thursday, August 5, 2021 11:57 PM
To: Stephen Goldstein b6 ; Jason Gale <j.gale@bloomberg.net>
Cc: b6 ; Garry, Robert F b6 ;
b6 ; Morens, David (NIH/NIAID) [E] b6
Subject: RE: Chris Newman interview

Hi all,

We have developed a multiplex surrogate virus neutralization test platform which can detect specific neutralizing antibodies to different sarbecoviruses. The paper is coming out on 18 Aug and happy to discuss how we can use this novel approach to test different sera. The test is species independent and we have used it for human and more than 10 animal species.

Cheers,

LF

Linfa (Lin-Fa) WANG, PhD FTSE FAAM
Professor
Programme in Emerging Infectious Disease
Duke-NUS Medical School,
8 College Road, Singapore 169857
Tel: b6

From: Stephen Goldstein b6
Sent: Friday, 6 August 2021 10:13 AM
To: Jason Gale <j.gale@bloomberg.net>
Cc: b6 ;
b6 ; Wang Linfa b6
Subject: Re: Chris Newman interview

- External Email -

In terms of conclusions, I think if seropositivity among workers in the wildlife trade is higher than background it would be strongly suggestive of occupational, not just community, exposure.

Sent from my iPhone

On Aug 5, 2021, at 8:11 PM, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

I suspect that Xiao Xiao is connected to many of the vendors he was visiting and hanging out with via WeChat, so he would've been critical for contact-tracing and reaching these folks. I guess there's no upside for the vendors to voluntarily give blood to check for neutralizing antibodies etc. Plus is would be too long ago now to draw any conclusions, right?

From: b6 At: 08/06/21 12:04:52 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:)
Cc: b6,
b6
Subject: Re: Chris Newman interview

I'll let you know.

PROFESSOR EDWARD C. HOLMES FAA FRS
ARC Australian Laureate Fellow

THE UNIVERSITY OF SYDNEY
Marie Bashir Institute for Infectious Diseases & Biosecurity,
School of Life & Environmental Sciences and School of Medical Sciences,
The University of Sydney | Sydney | NSW | 2006 | Australia
T
E b6

On 6 Aug 2021, at 12:03 pm, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

Very interested to see that when it's ready, Eddie. The animals Xiao observed were clearly not in great shape, so no doubt stressed and shedding loads of whatever pathogens they were infected with. Plus, their fecal matter was dropping on animals stacked below them.

----- Original Message -----
From: Edward Holmes b6
To: b6
CC: JASON GALE, b6,
b6
At: 08/06/21 12:00:45 UTC+10:00

Hard to interpret this. Could of course mean that the animals didn't have the virus BUT I'm involved in another project looking at market animals and I can tell you that these animals carry *a lot* of viruses. Not just coronas. Accident waiting to happen.

Cheers,

Eddie

PROFESSOR EDWARD C. HOLMES FAA FRS
ARC Australian Laureate Fellow

THE UNIVERSITY OF SYDNEY
Marie Bashir Institute for Infectious Diseases & Biosecurity,
School of Life & Environmental Sciences and School of Medical Sciences,
The University of Sydney | Sydney | NSW | 2006 | Australia
T
E b6

On 6 Aug 2021, at 11:57 am, Stephen Goldstein b6 wrote:

Yes serosurveys back then showed >50% seroprevalence in traders specializing in civets. Clearly occupational exposure, and probably outside of just the SARS epidemic period. Serosurveys of everyone in the wildlife chain from farm to market are my number one dream study to crack this nut.

Sent from my iPhone

On Aug 5, 2021, at 7:50 PM, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

If I eventually become the 1000th journalist to write a book about SARS-CoV-2, this has got to make it in:

Chris Newman: *[00:29:10]* One thing he interestingly did tell us, and it was in our original paper but didn't make it into the scientific reports sort of sanitized version is that he (Xiao) knew these vendors very well. He would go and see them weekly. He was on first-name terms. They'd chat, have a cigarette and a drink together and so forth. None of them got sick. Not one of them got sick from coronavirus. So they were selling these animals, but they themselves didn't get it.

Am I right in thinking that a serosurvey of workers in the two wet markets in Guangdong implicated in the SARS outbreak found 30% had cross-reactive antibodies? Would be fasssssssscinating to know whether Wuhan's wildlife vendors had some level of immune protection from prior exposure to SARS-related coronaviruses.

JG

From: b6 At: 08/06/21 09:57:55 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:) , b6
Cc: b6 ,
b6

b6
Subject: Re: Chris Newman interview

Agree with Eddie. They tests to do with those blood samples depending on quantity, storage, and availability would be to look for antibodies to SARS-CoV-2 rather than looking for evidence of the virus itself. But yes, I can imagine that being difficult or impossible in the current climate.

Stephen

From: Edward Holmes b6
Sent: Thursday, August 5, 2021 5:55:20 PM
To: Jason Gale
Cc: b6; Peter Daszak; b6; Wang Linfa; b6; Stephen Goldstein
Subject: Re: Chris Newman interview

That's interesting Jason.

The blood samples could be very useful (depending on how they are stored) but they would to find a lab that is willing and able to look at them. Again, the politics could be tricky.

PROFESSOR EDWARD C. HOLMES FAA FRS
ARC Australian Laureate Fellow

THE UNIVERSITY OF SYDNEY
Marie Bashir Institute for Infectious Diseases & Biosecurity,
School of Life & Environmental Sciences and School of Medical Sciences,
The University of Sydney | Sydney | NSW | 2006 | Australia
T
E b6

On 6 Aug 2021, at 9:27 am, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

Howdy,
I had a very interesting convo just now over Zoom with Chris Newman, the wildlife ecologist who worked on the Xiao paper in Scientific Reports. The publication's history is even more interesting than I thought. Couple of interesting things: the corresponding author Zhou was part of China's wildlife police/border control efforts (so knows a LOT!) and Xiao collected ticks from the wildlife he was surveying, so should have blood samples from infested animals from May 2017 until the market closure stopped data collection in Nov. 2019.
Jason

From: Morens, David (NIH/NIAID) [E] [b6 b6]
Sent: 8/6/2021 2:02:25 AM
To: Jason Gale [j.gale@bloomberg.net]
CC: b6 [b6; Garry, Robert F [b6 b6]]
BCC: Morens, David (NIH/NIAID) [E] [b6 b6]]
Subject: Re: Chris Newman interview

Yes, and let's remember it is possible, maybe likely, to be infected but never sick. d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Aug 5, 2021, at 21:50, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

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b6

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Jason

From: Morens, David (NIH/NIAID) [E] [b6 b6]
Sent: 10/4/2021 11:01:35 PM
To: Peter Daszak [b6]; Gerald Keusch [b6]
BCC: Morens, David [b6 b6]
Subject: Fwd: Dr. Fauci request from the Senate Dems

Sent from my iPhone
David M Morens
OD, NIAID, NIH

Begin forwarded message:

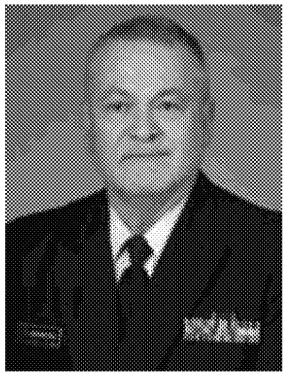
> **From:** "Conrad, Patricia (NIH/NIAID) [E]" b6
> **Date:** October 4, 2021 at 18:54:32 EDT
> **To:** NIAID OD AM <NIAIDODAM@niaid.nih.gov>
> **Cc:** "Selgrade, Sara (NIH/NIAID) [E]" b6
> **Subject: Fwd: Dr. Fauci request from the Senate Dems**
>
> Sent from my iPhone
>
> Begin forwarded message:
>
> > **From:** "Dodin, Reema B. EOP/WHO" b6
> > **Date:** October 4, 2021 at 6:43:36 PM EDT
> > **To:** "Conrad, Patricia (NIH/NIAID) [E]" b6
> > **Cc:** "Anderson, Charlie D. EOP/WHO" b6
> > **Subject: Dr. Fauci request from the Senate Dems**
> >
> > Hello Patricia!
> >
> > I hope this finds you well.
> >
> > Senator Stabenow runs the weekly DPCC Dem caucus luncheon, and said that the February visit from Dr. Fauci was among everyone's favorite and they were wondering if he could come back – perhaps this Thursday? Or the Thursday of the week of the 18th?
> >
> > Many thanks for considering!
> >
> > rd
> >
> > Reema Dodin
> > b6

From: Morens, David (NIH/NIAID) [E] [b6 b6]
Sent: 8/5/2021 9:25:18 PM
To: Peter Daszak ([b6]) [b6]; Keusch, Jerry ([b6]) [[b6]]; Kessler, Robert [b6]) [b6]; Rich Roberts ([b6]) [b6]; Eddie Holmes ([b6]) [b6]
Subject: FW: CNN: Exclusive: Intel agencies scour reams of genetic data from Wuhan lab in Covid origins hunt https://cnn.it/3fzBbsp

David

David M. Morens, M.D.
CAPT, United States Public Health Service
Senior Advisor to the Director
Office of the Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 31, Room 7A-03
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520

b6 (assistant: Whitney Robinson)
301 496 4409
b6



From: Folkers, Greg (NIH/NIAID) [E] b6
Sent: Thursday, August 5, 2021 4:18 PM
Subject: CNN: Exclusive: Intel agencies scour reams of genetic data from Wuhan lab in Covid origins hunt
https://cnn.it/3fzBbsp

Exclusive: Intel agencies scour reams of genetic data from Wuhan lab in Covid origins hunt

By Katie Bo Williams, Zachary Cohen and Natasha Bertrand, CNN

Updated 9:02 AM ET, Thu August 5, 2021

*Washington (CNN)*US intelligence agencies are digging through a treasure trove of genetic data that could be key to uncovering the origins of the coronavirus -- as soon as they can decipher it.

This giant catalog of information contains genetic blueprints drawn from virus samples studied at the lab in Wuhan, China which some officials believe may have been the source of the Covid-19 outbreak, multiple people familiar with the matter tell CNN.

It's unclear exactly how or when US intelligence agencies gained access to the information, but the machines involved in creating and processing this kind of genetic data from viruses are typically connected to external cloud-based servers -- leaving open the possibility they were hacked, sources said.

Still, translating this mountain of raw data into usable information -- which is only one part of the intelligence community's 90-day push to uncover the pandemic's origins -- presents a range of challenges, including harnessing enough computing power to process it all. To do that, intelligence agencies are relying on supercomputers at the Department of Energy's National Labs, a collection of 17 elite government research institutions.

There's also a manpower issue. Not only do intelligence agencies need government scientists skilled enough to interpret complex genetic sequencing data and who have the proper security clearance, they also need to speak Mandarin, since the information is written in Chinese with a specialized vocabulary.

"Obviously there are scientists who are (security) cleared," one source familiar with the intelligence told CNN. "But Mandarin-speaking ones who are cleared? That's a very small pool. And not just any scientists, but ones who specialize in bio? So you can see how this quickly becomes difficult."

Officials conducting the 90-day review hope this information will help answer the question of how the virus jumped from animals to humans. Unlocking that mystery is essential to ultimately determining whether Covid-19 leaked from the lab or was transmitted to humans from animals in the wild, multiple sources told CNN.

Investigators both inside and outside the government have long sought genetic data from 22,000 virus samples that were being studied at the Wuhan Institute of Virology. That data was removed from the internet by Chinese officials in September 2019, and China has since refused to turn over this and other raw data on early coronavirus cases to the World Health Organization and the US.

The question for investigators is whether the WIV or other labs in China possessed virus samples or other contextual information that could help them trace the coronavirus' evolutionary history.

Two scientists who study coronaviruses told CNN they are skeptical that there is any genetic data either in the tranche of 22,000 samples or any other database from the WIV that scientists don't already know about.

"Basically in [a 2020 research paper published in Nature], the WIV talked about all the sequences they had up until a certain point in time -- it's what most scientists virologists believe, that's pretty much what they had," said Dr. Robert Garry, a virologist at the Tulane University School of Medicine.

A source familiar with the US investigation would neither confirm nor deny that any of the data pertaining to those 22,000 samples is among what US intelligence agencies are currently analyzing.

No 'smoking gun'

Sources familiar with the effort say filling in that missing genetic link won't be enough to definitively prove whether the virus originated in the lab at Wuhan or first emerged naturally. Officials will still need to piece together other contextual clues to determine the true origins of the pandemic.

But it is a critical puzzle piece that the Biden administration has been prioritizing.
"The most prized technical data in this context are genetic sequences, database entries and contextual information about the provenance of the samples and the time and context in which they were acquired -- information people would use to place them in a narrative of the origins of SARS, Covid," one source familiar with the investigation told CNN.

For now, senior intelligence officials still say that they are genuinely split between the two prevailing theories on the pandemic's origins, or some combination of both scenarios. CNN reported last month that senior Biden administration officials overseeing the 90-day review now believe the theory that the virus accidentally escaped from a lab in Wuhan is at least as credible as the possibility that it emerged naturally in the wild -- a dramatic shift from a year ago, when Democrats publicly downplayed the so-called lab leak theory.

Multiple sources told CNN that absent an unexpected windfall of new information, officials don't expect to uncover a "smoking gun" -- like intercepted communications, for example -- that would offer definitive proof for either theory. The Biden administration's 90-day push is predicated on the expectation that science, not intelligence will be the key.

Intelligence officials are tasked with addressing several "scientific knowledge gaps" about the virus' evolution, according to the collection guidance governing the 90-day push, distributed to more than a dozen agencies on June 11 by the Office of the Director of National Intelligence and obtained by CNN.

The memo instructs the intelligence community to "expand its collection" and consider data already in its possession to identify both the initial host of the coronavirus and any species that it may have passed through as it adapted to humans -- or to find as "any progenitor virus and/or virus that could serve as backbone for genetic engineering purposes."

But former Director of National Intelligence John Ratcliffe told CNN that the US intelligence community already had sufficient collection on the topic of Covid origins.

"Obviously the more, the better. But we've had extraordinary insight into this topic for many months, much more than has been declassified. Pretending we didn't is political theater and a classic example of a politician trying to buy time by using the IC as a scapegoat," he told CNN in a statement.

Digging into the science

That's where the genomic data from the Wuhan lab could come in.

The genetic code of a given virus is the signature that allows scientists to tell the difference between the Delta and Beta variants of the coronavirus, for example. It can also offer clues as to how the virus has adapted or mutated over time, including whether it shows signs of human manipulation -- a kind of genetic history.

Many scientists continue to believe that the most likely scenario is that the virus jumped from animals to humans naturally. But despite testing thousands of animals, researchers still haven't identified the intermediate host through which the virus passed as it adapted to humans.

But some researchers, intelligence officials and Republican lawmakers believe that researchers at the WIV might have genetically altered a virus in the lab, using a controversial kind of research known as "gain of function" that could have infected researchers who then spread it in their community.

It's also plausible that the initial infection took place naturally outside of the lab, perhaps while a scientist was collecting a sample from an animal in the wild, and that scientist then spread the virus unknowingly when he returned to the lab with the samples, multiple sources familiar with the intelligence explained.

"If it was the latter, it was likely brought into a lab to study because someone got sick ... which means there were an unknowable number of other people who were already sick," the source familiar with the probe said.
Understanding exactly which viruses researchers at the WIV were working on could provide important evidence for any one of these theories. It's one of the reasons that investigators on Capitol Hill and elsewhere have been keenly focused on the database that was taken offline in 2019.

But it might not prove anything definitively, sources familiar with the intelligence say. Even if scientists in the intelligence community are able to use the data from the lab to stitch together a complete genetic history that shows how the virus mutated, they might not have enough information about how it was handled by the Chinese lab to determine with a high level of confidence that it leaked.

"Despite having that complete history of variants, [officials might] lack the contextual information to make sense of it in a narrative way," the source familiar with the investigation explained.
"Even a complete sequence history is difficult to obtain. And doesn't really tell us anything about the origins of the pandemic itself without the context," this person added.

Some Republicans on Capitol Hill have jumped into the uncertainty with their own report claiming that "the preponderance of evidence suggests" the coronavirus was "accidentally" released from a lab in Wuhan in 2019 -- an assertion that goes far beyond the intelligence community's current view of the matter.

90 days -- and then what?

It's possible that at the end of Biden's 90-day push, the intelligence community won't have reached what's known as a "high-confidence" assessment as to the pandemic's origins. Administration officials have previously suggested to CNN that it's possible a second review could be ordered at the end of the 90 days.

A bipartisan group of lawmakers on the Senate Intelligence and Foreign Relations Committees earlier this week sent a letter urging the administration to continue to prioritize the hunt until such a judgment can be made in order to prevent future pandemics.

But the lawmakers also zeroed in on a related focus for intelligence officials probing the pandemic's origins: China's "efforts to conceal the severity and scope of the outbreak of the SARS-CoV-2 virus that caused the COVID-19 pandemic."
"We also believe that the investigation should address PRC efforts to prevent international inquiries into the origins of SARS-CoV-2, and other actions PRC authorities have taken to obscure the nature of the virus and its transmission," the lawmakers said.

Republican lawmakers in the House, meanwhile, have latched onto the theory that the virus escaped from a lab. GOP lawmakers in a report released Monday by Rep. Michael McCaul of Texas have claimed that "the preponderance of evidence suggests" the coronavirus was "accidentally" released from a lab in Wuhan in 2019.

Intelligence officials say it's still far too soon to say.

From: Peter Daszak [b6]
Sent: 7/21/2021 3:32:45 PM
To: Morens, David ([b6]
([b6]
CC: Keusch, Jerry ([b6]) [[b6]]
Subject: Re: Thank you

Lovely but I'm now also getting tony Fauci's hate mail (they Cc me on some). I won't forward, it's pretty disgusting!!!

Cheers,

Peter

Peter Daszak
(Sent from my iPhone)

President
EcoHealth Alliance

460 West 34th Street, New York, NY10001, USA

www.EcoHealthAlliance.org

> On Jul 21, 2021, at 9:29 AM, Morens, David (NIH/NIAID) [E] [b6] wrote:
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>
> David M. Morens, M.D.
> CAPT, United States Public Health Service
> Senior Advisor to the Director
> Office of the Director
> National Institute of Allergy and Infectious Diseases
> National Institutes of Health
> Building 31, Room 7A-03
> 31 Center Drive, MSC 2520
> Bethesda, MD 20892-2520
> B [b6] (assistant: Whitney Robinson)
> W [b6]
> 3 [b6]
>
> Disclaimer: This message is intended for the exclusive use of the recipient(s) named above. It may contain information that is PROTECTED, PRIVILEGED, and/or CONFIDENTIAL, and it should not be disseminated,

distributed, or copied to persons not authorized to receive such information. All sensitive documents must be properly labeled before dissemination via email. If you are not the intended recipient, any dissemination, distribution, or copying is strictly prohibited. If you have received this communication in error, please erase all copies of the message and its attachments and notify us immediately.
>
>
>
>
> -----Original Message-----
> From: Billet, Courtney (NIH/NIAID) [E] b6
> Sent: Tuesday, July 20, 2021 4:34 PM
> To: NIAID OD AM <NIAIDODAM@niaid.nih.gov>
> Subject: FW: Thank you
>
> Another one, lol
>
>
> -----Original Message-----
> From: Carol Maloney Nelson b6
> Sent: Tuesday, July 20, 2021 4:19 PM
> To: NIAID Ocpostoffice (NIH/NIAID) <OCPOSTOFFICE@niaid.nih.gov>
> Subject: Thank you
>
> Thank you Dr Fauci. For everything.
> We listens d we understand because we are educated and at least somewhat intelligent.
>
> We already knew Rand Paul is an ass and we thank you for speaking up for all of us.
>
> PS [redacted] b6
b6

From: Morens, David (NIH/NIAID) [E] [b6]
[b6]
Sent: 8/13/2021 11:34:36 PM
To: Jason Gale [b6]; [b6]; [b6]; Garry, Robert F [b6]
[b6]
Subject: RE: Guys, I have another round of questions that I need to answer

Peter is the perfec t guy to do that!

David

David M. Morens, M.D.
CAPT, United States Public Health Service
Senior Advisor to the Director
Office of the Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 31, Room 7A-03
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520

☎ [b6] (assistant: Whitney Robinson)
301 496 4409
[b6]

From: Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net>
Sent: Friday, August 13, 2021 7:04 PM

To: b6; Morens, David (NIH/NIAID) [E] b6; b6; Garry, Robert F b6;

Subject: Guys, I have another round of questions that I need to answer

It mostly relates to why knowing the Xiao paper earlier would have made a difference in the origins research.
Does anyone have 10 mins to talk off-the-record to help, perchance?
Thanks.
Jason

From: Jason Gale (BLOOMBERG/ NEWSROOM:) At: 08/13/21 07:52:37
To: b6, b6,
Subject: The story behind the missing story about the story behind the missing raccoons

Hi everyone,
Just letting you know that my story has been turned into a sh!tshow internally. My long awaited feature on why the raccoon dogs were there in Wuhan one minute, gone the next and why we waited 18 months to find out for sure that they were there in the first place, has taken more twists and turns than any Olympic diver, thanks to some egomaniac editors. (Please keep that bit to yourselves).
I have even more sympathy for Xiao et al. I'm told now Tuesday for publication, but I wouldn't be surprised if some a-hole higher up the food chain spikes it. To say I am exasperated (and a tad emotional after working 13 days straight) is an understatement.
Kindest regards,
Jason

From: Morens, David (NIH/NIAID) [E] [b6] ([b6])
Sent: 7/21/2021 9:52:26 PM
To: Keusch, Gerald T [b6]; Peter Daszak ([b6]) [b6]
Subject: RE: Thank you

These are both greAt, I'll put them in Tony's (metaphorical) pile.
d

David M. Morens, M.D.
CAPT, United States Public Health Service
Senior Advisor to the Director
Office of the Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 31, Room 7A-03
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520
B [b6] (assistant: Whitney Robinson)
W
3 [b6]

-----Original Message-----
From: Keusch, Gerald T [b6]
Sent: Wednesday, July 21, 2021 11:24 AM
To: Morens, David (NIH/NIAID) [E] [b6]; Peter Daszak ([b6])
[b6]
Subject: RE: Thank you

That is sweet. I hope TF will see it. I'm loving the press coverage of his skirmish with an ophthalmologist who cannot see the difference between science and shit, and has no insight to guide how he thinks and what he says.

I was so proud of Tony's direct response - even his patience (limited though it was) when Paul bullied and interrupted him.

Stacey Knobler at Sabin Vaccine Institute sent me the attached two pieces on COVID vaccine development and its relevance for pandemic influenza preparedness in which I am quoted stemming from the work Nicki and I did for the Sabin-Aspen Institutes think tank on vaccine science and policy, and subsequent interviews with their PR team which produced these articles. They don't address the origins controversy but they are relevant to public perception of the value of vaccines and the science underlying their development.

Jerry

-----Original Message-----
From: Morens, David (NIH/NIAID) [E] [b6]
Sent: Wednesday, July 21, 2021 9:29 AM
To: Peter Daszak ([b6]) [b6]; Keusch, Gerald T
[b6]
Subject: FW: Thank you

David M. Morens, M.D.

CAPT, United States Public Health Service Senior Advisor to the Director Office of the Director National Institute of Allergy and Infectious Diseases National Institutes of Health Building 31, Room 7A-03
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520
B b6 (assistant: Whitney Robinson) W b6
3 b6

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From: Billet, Courtney (NIH/NIAID) [E] b6
Sent: Tuesday, July 20, 2021 4:34 PM
To: NIAID OD AM <NIAIDODAM@niaid.nih.gov>
Subject: FW: Thank you

Another one, lol

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To: NIAID Ocpostoffice (NIH/NIAID) <OCPOSTOFFICE@niaid.nih.gov>
Subject: Thank you

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We already knew Rand Paul is an ass and we thank you for speaking up for all of us.

PS b6

b6

From: Edward Holmes [b6]
Sent: 9/19/2021 11:27:24 PM
To: Morens, David (NIH/NIAID) [E] [b6]
[b6]
CC: Jason Gale [j.gale@bloomberg.net]; Peter Daszak [b6]; [b6]; [b6]; Wang Linfa [b6]; Garry, Robert F [b6]; [b6]; Taubenberger, Jeffery (NIH/NIAID) [E] [b6]; [b6]]
Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

It's not phylogenetics.

One thing is ascertainment bias which could be huge.

Second thing is to distinguish the long-term ecology of these viruses from the short-term emergence of the virus. These Laos viruses are the former. Clearly these viruses are commonplace in SE Asia. And I don't just think that bats and pangolins will be the only animals with SC2-like viruses. Virus ecology does not work like that. But this is not the same as determining the events that happened in Wuhan. To me, China still looks like the most likely source.

Third, I'm pretty certain that groups in China are sitting on more SC2-like viruses. If you sample bats you find them. It is striking to me that CCDC have published so little on this yet have supposedly sampled so many animals. That doesn't add up. Never discount the politics.

Professor Edward C. Holmes FAA FRS
The University of Sydney

On 20 Sep 2021, at 9:00 am, Morens, David (NIH/NIAID) [E] [b6] wrote:

Eddie, please clarify, i don't « get » all the phylogenetic asumptions you guys understand, but can you put it in lsyman's terms? As you know, i have said repeatedly to look past Yunnan to all of SE Asia, as i have bennunconconvinced of the Yunnan centrality of all this, suspecting thAt the universe of these viruses crosses borders to include not only SW and S China but all of SEA.

If that is so, the implications ate huge: this is annintetnational problem demanding international cooperation. d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Sep 19, 2021, at 18:33, Edward Holmes [b6] wrote:

Yes, good idea.

The receptor binding domain of some of these Laotian bats is so close to that of SARS-CoV-2 even some of the die-hard leakers are beginning to see the light…

This also effectively excludes that virus-receptor relationship was generated through lab passage, that the pangolin sequences were faked, and that this outbreak had anything to do with the Mojiang mine as a virus from a different country is now closer. That mine will go down in history as the reddest of herrings.

That said, I am a little worried about confirmation bias for the origin being bats from Yunnan/Laos/Cambodia. The more they find there, the more they sequence. But no doubt these Laotian samples are of huge significance. As are the Hubei civets.

<Screenshot from 2021-09-19 17-04-25.png>

PROFESSOR EDWARD C. HOLMES FAA FRS
ARC Australian Laureate Fellow

THE UNIVERSITY OF SYDNEY
Marie Bashir Institute for Infectious Diseases & Biosecurity,
School of Life & Environmental Sciences and School of Medical Sciences,
The University of Sydney | Sydney | NSW | 2006 | Australia
T
E b6

On 20 Sep 2021, at 7:52 am, Morens, David (NIH/NIAID) [E] b6 wrote:

Yes, do it! This is important and i say modestly, game changing. The whole « origin » controversy needs to be rethought from the ground up

We have been too micro-focusing (as i have long said to hard push back) but the sarobecovirus and merbecovirus problems are geographically and virologically complex and require us to drop back and study the viral-host universe. Thst universe is huge, complicated , and holds surprises, in my view. d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Sep 19, 2021, at 17:36, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

I'm planning to pull the threads Peter has so eloquently laid out into a story. Bob, Stephen, Joel (and Kristian), if you have time/interest to get on Zoom today, let me know. Thanks a lot. Jason

From: b6 At: 09/20/21 07:31:51 UTC+10:00
To: b6
Cc: Jason Gale (BLOOMBERG/ NEWSROOM:) , b6 ,
b6

b6

Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

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Let us all keep pushing,
and keep our eyes on the
prize of getting to the bottom of it all
david

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Sep 18, 2021, at 12:05, Peter Daszak b6 wrote:

I put it all in a twitter thread while drinking coffee in my local diner (Saturday is “full English breakfast” day for me).

https://twitter.com/peterdaszak/status/1439236376776658945?s=21

No doubt ill be attacked by multiple lab leak aficionados but so be it - at least eddie, Garry and Kristian won’t see. The horrors of that…

Cheers,

Peter

Peter Daszak
(Sent from my iPhone)

President
EcoHealth Alliance

460 West 34th Street, New York, NY10001, USA

www.EcoHealthAlliance.org

On Sep 18, 2021, at 10:26 AM, Garry, Robert F [b6] wrote:

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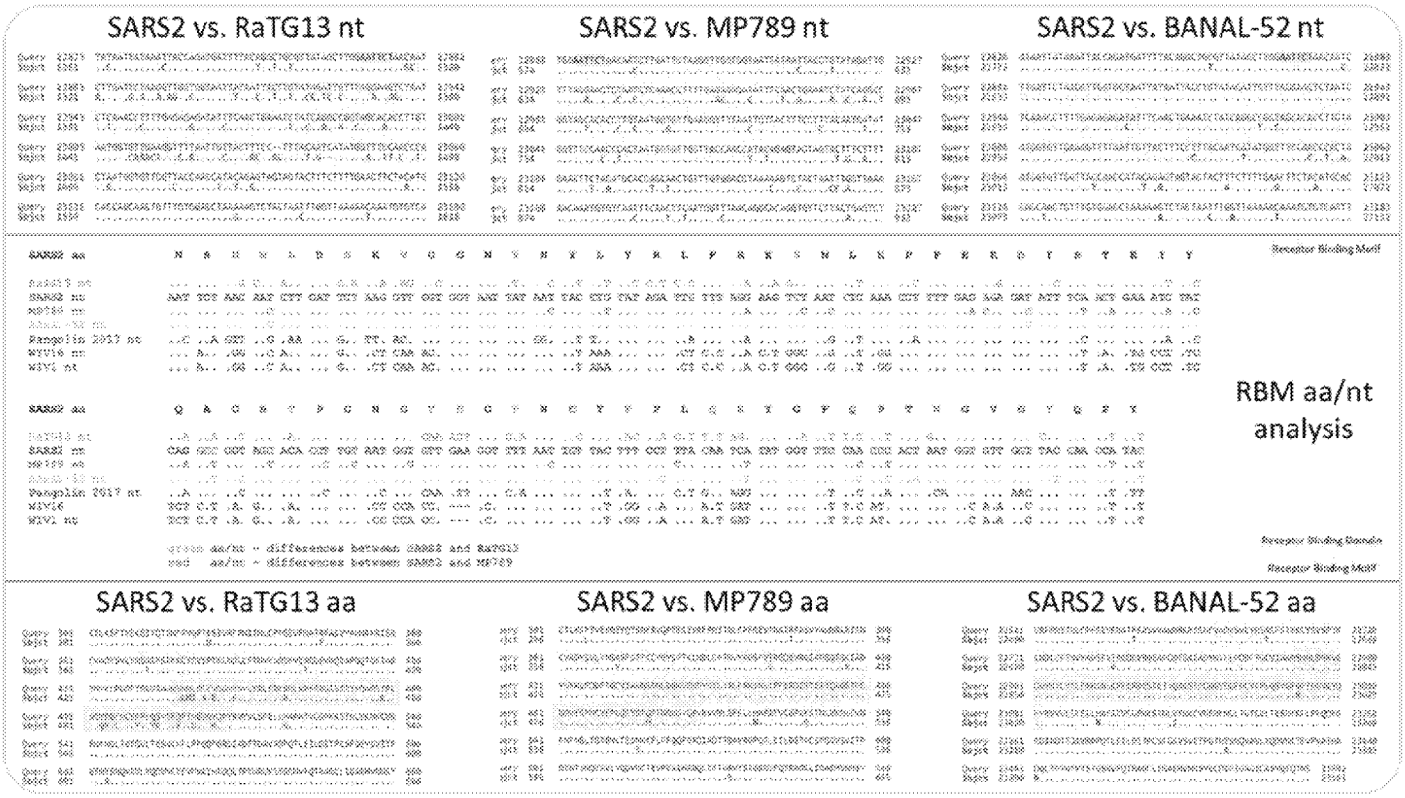
Yuri Deigin
@ydeigin

Wow. On a nucleotide level the new Laotian RBD/RBM is MUCH closer to SARS2 than that of the pangolin CoV. A VERY important piece of the puzzle that essentially proves the RBM *itself* was not engineered but actually came from a bat CoV. Doesn't rule out a lab leak/passaging etc.



2:26 PM · Sep 19, 2021 · Twitter Web App

11 Retweets **5** Quote Tweets **59** Likes

From: Morens, David (NIH/NIAID) [E] [b6 b6]
Sent: 9/19/2021 10:59:51 PM
To: Edward Holmes [b6]
CC: Jason Gale [j.gale@bloomberg.net]; Peter Daszak [b6]; b6; b6; Wang Linfa [b6]; Garry, Robert F [b6]; b6; Taubenberger, Jeffery (NIH/NIAID) [E] [b6 b6]
Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

Eddie, please clarify, i don't « get » all the phylogenetic asumptions you guys understand, but can you put it in lsyman's terms? As you know, i have said repeatedly to look past Yunnan to all of SE Asia, as i have bennunconconvinced of the Yunnan centrality of all this, suspecting thAt the universe of these viruses crosses borders to include not only SW and S China but all of SEA.

If that is so, the implications ate huge: this is annintetnational problem demanding international cooperation. d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Sep 19, 2021, at 18:33, Edward Holmes [b6] wrote:

Yes, good idea.

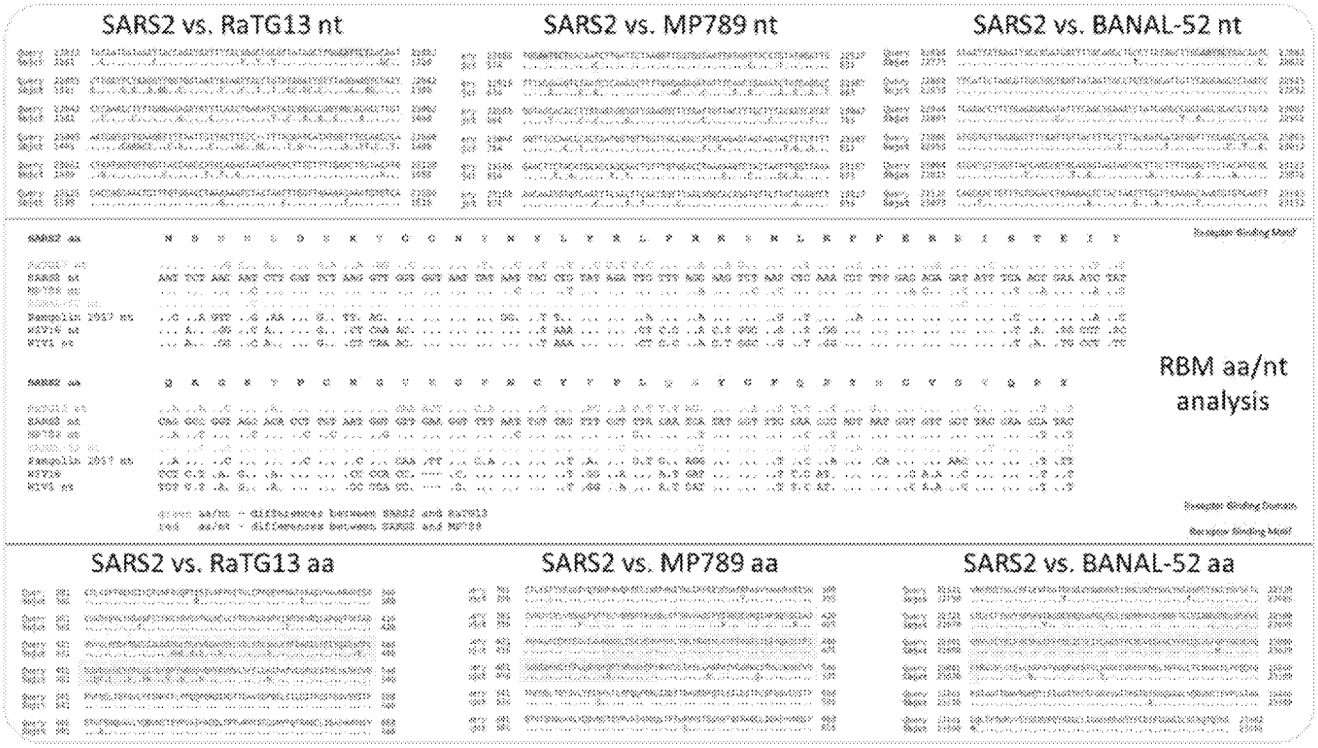
The receptor binding domain of some of these Laotian bats is so close to that of SARS-CoV-2 even some of the die-hard leakers are beginning to see the light…

This also effectively excludes that virus-receptor relationship was generated through lab passage, that the pangolin sequences were faked, and that this outbreak had anything to do with the Mojiang mine as a virus from a different country is now closer. That mine will go down in history as the reddest of herrings.

That said, I am a little worried about confirmation bias for the origin being bats from Yunnan/Laos/Cambodia. The more they find there, the more they sequence. But no doubt these Laotian samples are of huge significance. As are the Hubei civets.

Yuri Deigin
@ydeigin

Wow. On a nucleotide level the new Laotian RBD/RBM is MUCH closer to SARS2 than that of the pangolin CoV. A VERY important piece of the puzzle that essentially proves the RBM *itself* was not engineered but actually came from a bat CoV. Doesn't rule out a lab leak/passaging etc.



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--

PROFESSOR EDWARD C. HOLMES FAA FRS
ARC Australian Laureate Fellow

THE UNIVERSITY OF SYDNEY
Marie Bashir Institute for Infectious Diseases & Biosecurity,

School of Life & Environmental Sciences and School of Medical Sciences,
The University of Sydney | Sydney | NSW | 2006 | Australia
T
E b6

On 20 Sep 2021, at 7:52 am, Morens, David (NIH/NIAID) [E]
b6 wrote:

Yes, do it! This is important and i say modestly, game changing. The whole « origin » controversy needs to be rethought from the ground up

We have been too micro-focusing (as i have long said to hard push back) but the sarobecovirus and merbecovirus problems are geographically and virologically complex and require us to drop back and study the viral-host universe. Thst universe is huge, complicated , and holds surprises, in my view. d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Sep 19, 2021, at 17:36, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

I'm planning to pull the threads Peter has so eloquently laid out into a story. Bob, Stephen, Joel (and Kristian), if you have time/interest to get on Zoom today, let me know. Thanks a lot. Jason

From: b6 At: 09/20/21 07:31:51 UTC+10:00
To: b6
Cc: Jason Gale (BLOOMBERG/ NEWSROOM:) ,

b6

Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

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From: Taubenberger, Jeffery (NIH/NIAID) [E] [b6 b6]
Sent: 3/19/2020 5:32:23 PM
To: Morens, David (NIH/NIAID) [E] [b6 b6]
CC: Peter Daszak [b6]; Howard Markel [b6]
Subject: Re: Pandemic history manuscript, tracked
Attachments: HMPANDEMIC COVID draft 03 19 20 PD comments JKT clean.docx

David, et al.,

Here is a 'clean' version with tracked changes accepted for easier reading.

Best regards,

Jeff

From: Howard Markel [b6]
Date: Thursday, March 19, 2020 at 1:10 PM
To: "Taubenberger, Jeffrey (NIH/NIAID) [E]" [b6]
Cc: Peter Daszak [b6], "Morens, David (NIH/NIAID) [E]" [b6]
Subject: Re: Pandemic history manuscript, tracked

Hi Jeff!!!

Howard Markel, MD, PhD
George E. Wantz Distinguished Professor of the History of Medicine and Director
Center for the History of Medicine
The University of Michigan
[b6]

Sent from my iPhone

On Mar 19, 2020, at 1:06 PM, Taubenberger, Jeffery (NIH/NIAID) [E] [b6] wrote:

> Hi guys,
>
> I did a read through with some additional tracked changes and saved a new version here. With tracked comments from Peter, Howard, and me, it is looking a bit messy. David, do you want to have the next go at it? It might be easiest to make an accepted version for the next round of edits.
>
> We have all suggested references which are great. It will be easy to add those with endnote when we get to a closer to final draft. I am working from home today and do not have access to my endnote library.
>
> Thanks all,
>
> Jeff

PANDEMIC COVID-19 JOINS HISTORY'S PANDEMIC PANTHEON

Since December 2019, the world has watched the slow-motion birth of a new pandemic disease, Covid-19. As in Albert Camus' *The Plague*, the familiar rhythms of our very real lives have been shaken by an unfamiliar existential threat. Rising death and case numbers have changed every aspect of our work, school, recreation, travel, economic well-being, and interactions with friends and family.

Yet, ours is hardly the only era to face such tribulations. Deadly pandemics and large-scale epidemics have challenged human existence throughout history (Table).

YEAR	NAME	DEATHS	
430 BCE	"Plague of Athens"	~ 100,000	First identified trans-regional pandemic
541	Justinian plague (*Yersinia pestis*)	30-50 million	Pandemic; killed half of world population
1340s	"Black Death" (*Yersinia pestis*)	~ 50 million (25%)	Pandemic; killed at least a quarter of world population
1494	Syphilis (*Treponema pallidum*)	>50,000	
c. 1500	Tuberculosis	High millions	Ancient disease; became pandemic in Middle Ages
1520	*Hueyzahuatl* (*Variola major*)	3.5 million (50%)	Pandemic brought to New World by Europeans
1793-1798	"The American plague"	~ 25,000	Yellow fever terrorized colonial America
1832	2nd cholera pandemic (Paris)	18,402	Spread from India to Europe/Western Hemisphere
1918	"Spanish" influenza	~ 50 million	Led to additional pandemics in 1957, 1968, 2009
1976-2020	Ebola	15,258	First recognized in 1976; 29 regional epidemics to 2020
1981	Acute hemorrhagic conjunctivitis	rare deaths	First recognized in 1969; pandemic in 1981
1981	HIV/AIDS	~ 32 million	First recognized 1981; ongoing pandemic
2002	SARS	774	Near-pandemic
2009	H1N1 "swine flu"	284,000	5th influenza pandemic of the century
2014	Chikungunya	uncommon	Pandemic, mosquito-borne
2015	Zika	~ 1,000?*	Pandemic, mosquito-borne

Table. Some notable pandemic and epidemic diseases. For most historical pandemics, estimated deaths have varied widely, and figures cannot be considered accurate.

*Zika deaths occur mostly *in utero* or in newborns; death in older children and adults is extremely rare.

While these crises were once separated by centuries, or at least many decades, they are now becoming much more common. Since 2003 we have experienced SARS, an influenza pandemic (H1N1pdm in 2009), a chikungunya pandemic

(2014), a Zika pandemic (2015), and widespread pandemic-like extension of Ebola over five African countries, with cases exported globally (2014-2015). We now live in an era of pandemics, newly emerging infectious diseases and the return of old contagious foes.

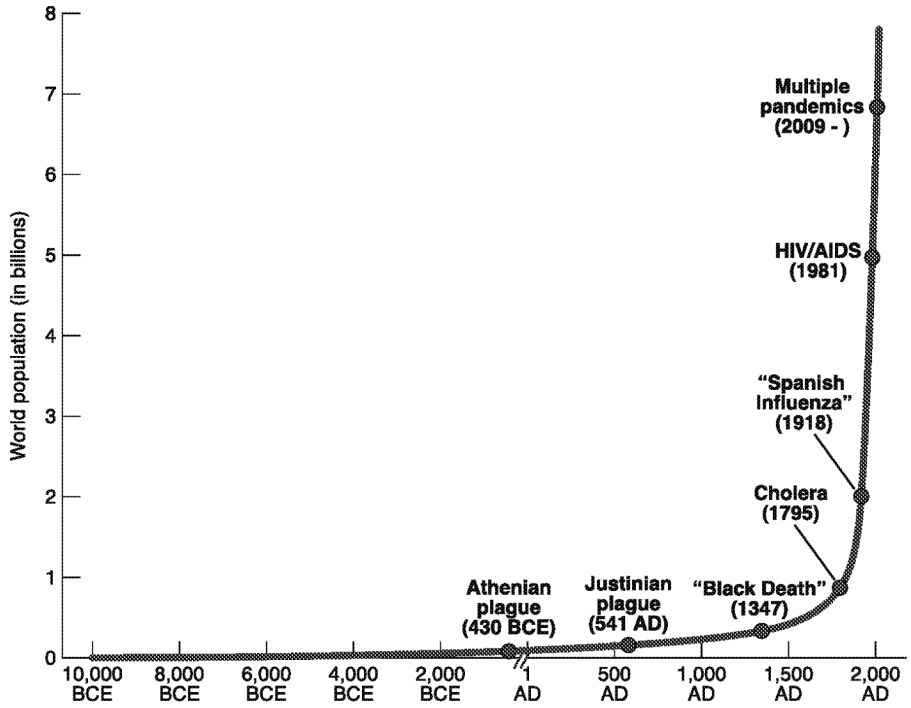

Figure 1. Estimated world population and selected known pandemics/widespread disease emergences, from 10,000 BCE – 2020 AD.

Early Pandemic History. Around 12,000 years ago, small family/clan groups of humans abandoned nomadic hunting-gathering to settle down in stable locations,

cultivating crops and raising domestic animals for food, labor, and clothing (the neolithic revolution). For the first time, humans and newly domesticated animals were living together in complicated ecosystems of villages, towns and cities. Under conditions of intense human-animal proximity and environmental alterations, enzootic and zoonotic diseases appeared. The agents of measles, smallpox, tuberculosis, gastric cancer-causing *Helicobacter pylori*, and many other future pandemic diseases evolved from animal pathogens that host-switched to become human infectious agents. As human populations continued to expand, these agents were able to initiate epidemics and pandemics (Figure 1). Some of the biblical plagues were probably emerging infectious diseases. The preserved mummy of Pharaoh Usermaatre Sekheperenre Ramesses V clearly shows smallpox lesions (Figure 2), indicating that fatal smallpox epidemics prevailed more than 3,000 years ago [2]. At some point, smallpox spread

Commented [PD1]: I'd insert a reference to a paper that goes into some detail on this: Dobson, A. P. & Carper, E. R. Infectious diseases and human population history. Biosci. 46: 115-126 (1996)

A BETTER SOURCE WOULD BE MCNEILL'S PLAGUES AND PEOPLE [HM]

Commented [TJ([2]: Replaced here with new version

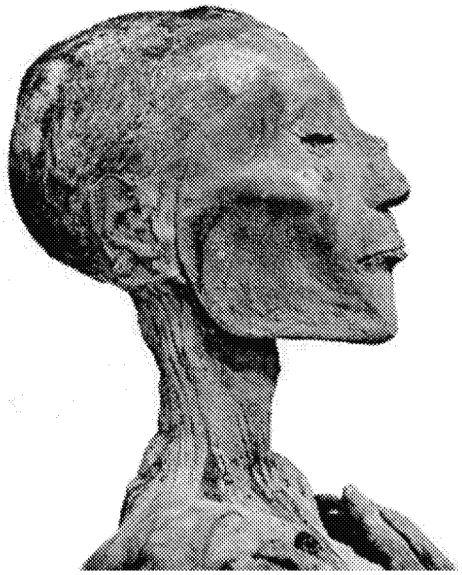

Figure 2. Mummy of Pharaoh Usermaatre Sekheperenre Ramesses V (c. 1196-1145 BCE), showing smallpox lesions, e.g., on the bridge of the nose.

pandemically over most of the world, sparing the Western Hemisphere for millennia, up to the 16th century with the first known outbreak there in 1520.

Heralding the end of Greece's "Golden Age", the explosive "Plague of Athens" (430-425 BCE) was perhaps the first recorded pandemic: it spread over much of the world known to the Greeks, including the Mediterranean and Africa [3]. Although the cause of the Athenian plague has not been identified (anthrax, bubonic/ pneumonic plague, smallpox, and typhus are leading candidates), it was the first disease investigated and described using clinical and epidemiological approaches. It remains today a benchmark for pandemic comparisons.

Since the Athenian plague, there has been a steady stream of new pandemics of even greater mortality (Table; reference 1). Confronting them, only to quickly forget the lessons they left behind, has become a recurring theme in human existence. The repetitive nature of our struggles to combat these diseases is illustrated in countless history books and plague tractates with sometimes striking similarities in strategies across the centuries (Figure 3).

Commented [PD3]: may be semantics, but 'deadliness' could be interpreted by the reader to mean that pandemic pathogens have become more lethal over time, but what you're really saying is that pandemics have caused greater mortality over time.

Commented [PD4]: Added a comment in the first para to this effect

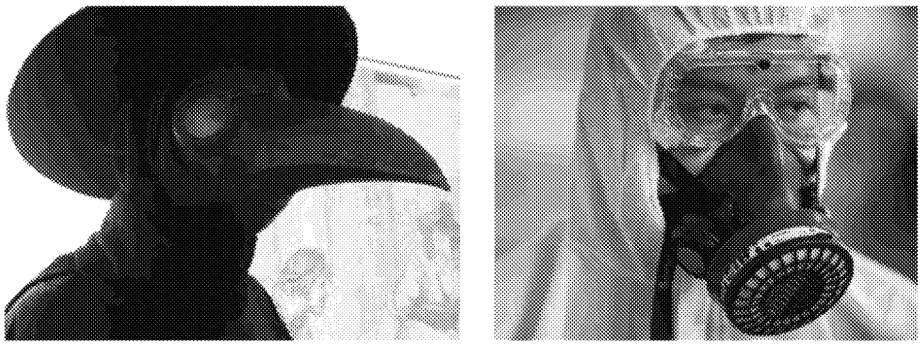

Figure 3. Fighting "plagues" in 1665 (caused by bubonic/pneumonic *Yersinia pestis*) and in 2020 (caused by SARS-CoV-2).

What is a pandemic? "Pandemic" has never been a scientific term, but rather a subjective popular term. In usage since the mid-1600s, the word "pandemic" (or "pandemick") was at first so imprecise that it could mean different, even

contradictory things in different contexts [4]. At its most specific, it conveyed the vague notion of an impressively large epidemic and its Greek roots, "pan"—all—and "demos"—people, reflect their widespread nature. "Epidemic" is often translated from the Greek as "that which is upon the people", i.e., a highly incident or widely prevalent condition, and usually one that has a rapid temporal and geographical spread. Following the sudden emergence of global influenza in 1889, the term "pandemic" acquired, and as of today retains, the narrower meaning of a disease "…occurring widely throughout a region, country, continent, or globally" [4]. "Pandemic" has also been sub-categorized into trans-regional (widespread within a continent or other large region), inter-regional (involving two or more regions), and global [5]. In practice, "pandemic" and "epidemic" are most often applied to infectious diseases, largely replacing such historical terms for emerging infections as *loimos*, *peste*, pestilence, and plague (in situations where "plague" is used generically, rather than in specific reference to bubonic/pneumonic plague caused by *Yersinia pestis*). [CITE Hippocrates, Epidemics, I, II, III]

Commented [PD5]: Isn't there a notion of the rapid temporal nature of the rise in cases – i.e. the percentage of people infected increases rapidly so that it becomes widely prevalent.

What lessons have we learned from this long history of pandemics, and how do they relate to the current situation with COVID-19?

1. **Human beings are the ultimate causes of pandemics**. Pandemics are caused by specific organisms, but these same organisms, or their ancestors, have almost always been around us for millennia without causing pandemic harm. As noted above, it was the historical congregation of humans and domestic animals in villages and cities that provided the opportunity for ancestral organisms to host-switch to humans and cause human smallpox, measles, and other diseases. While these originated in wild animals that we then domesticated, our growing ecological footprint is currently leading to an exponential rise in the spillover of other microbes directly from wildlife to people. Deforestation, agricultural intensification, urbanization and ecosystem disruption bring people into contact with wildlife and their potentially zoonotic pathogens. These activities have led to emerging diseases as diverse as hemorrhagic fevers, Nipah infection, and Zika [6-8]. Since 1999, China's numerous live animal markets have led to three important epidemics and now one pandemic: the emergence of deadly "bird flu" associated with poultry-adapted influenza A viruses known as

Commented [PD6]: I inserted 'almost' because some are truly novel and caused directly by our influence, e.g. some drug-resistant microbes (even though many already circulate in wildlife and other animals).

Commented [PD7]: Best ref probably Jones, K. E., Patel, N., Levy, M., Storeygard, A., Balk, D., Gittleman, J. L. & Daszak, P. Global trends in emerging infectious diseases. Nature 451: 990-993 (2008)

H5N1 and H7N9 have killed over a thousand people; SARS killed 774, and came close to causing a global pandemic in 2003; and now in 2019-2020 the SARS-like SARS-CoV-2 is causing our newest pandemic, COVID-19. One seemingly simple human behavior – establishment of multiple large live animal markets in a populous region – has within two decades caused the emergence of four fatal zoonotic diseases, including one barely-prevented near-pandemic, and one we have clearly failed to prevent.

Commented [PD8]: I think that sounds a bit too focused on China, when the truth is the wildlife trade is just as intense and diverse in countries like Laos, Vietnam, Cambodia, and others.

Commented [PD9]: That's redundant because pandemics by nature has spread 'globally'

2. **When people travel, germs travel [9]; when germs travel pandemics become possible.** Beginning around 1320, the "Black Death" followed trade routes from what is now Mongolia and China, across Asia, and into Europe (1347-1348). Likewise, cholera spread along travel routes from India to Europe in 1831-33, 1845, 1866 and 1892; its approach was reported in the media in "real time", forcing the realization, even without a concept of microbial infection, that cholera advanced exactly as fast as human travel. HIV is believed to have emerged at some time between 1880 and1920, but it only became pandemic in 1981 when population size had expanded, human movement had become more geographically extensive, and complex facilitative human behaviors had been more fully developed, e.g., trans-national road building and truck routes, leading to travel-related prostitution, and affordable international air travel. The 1889-1893 influenza pandemic was the first to have its progression 'tracked' in real time, spreading east to west from Asia and quickly reaching almost every region of the globe. The 1918-1919 influenza pandemic, which stands as the most fatal single event in human history, killed an estimated 50 million. The place of origin of the 1918 virus is obscure and there is little evidence of directionality of spread other than chaotic global multi-directionality. The 1957-1958 and 1968-1969 influenza pandemics followed the pattern of appearance in Southeast Asia with subsequent global spread, while the 2009 influenza pandemic originated in Mexico before spreading globally. The 1957, 1968, and 2009 pandemics were all genetic descendants of the 1918 influenza, such that we are still in the 1918 'pandemic era' today.

Commented [TJ([10]: Refs:
Pandemic influenza--including a risk assessment of **H5N1**.
Taubenberger JK, **Morens** DM.
Rev Sci Tech. 2009 Apr;28(1):187-202.

The persistent legacy of the 1918 influenza virus.
Morens DM, **Taubenberger** JK, **Fauci** AS.
N Engl J Med. 2009 Jul 16;361(3):225-9. doi: 10.1056/NEJMp0904819. Epub 2009 Jun 29. No abstract available. Erratum in: N Engl J Med. 2009 Sep 10;361(11):1123.

The *Aedes aegypti*-borne diseases (yellow fever, dengue, chikungunya and Zika) are all associated with human crowding/imperfect sanitation, peri-domestic water storage, exportation of vector mosquitoes, and human development of novel mosquito breeding sites such as discarded rubber tires. These four arboviral diseases have all exploded in recent decades, the delayed result of emergence and adaptation of a single mosquito species in response to water storage behaviors of humans beginning more than 5,000 years ago, and which are being greatly amplified today [10]. The unwitting spread of microbes by humans, a process termed "pathogen pollution", accelerates the geographic spread of emerging diseases and their impact on morbidity and mortality. In a world now dominated by a globalized economy that depends on international travel and trade, it has led to significant economic losses, e.g., $30-50 billion for SARS, and multiple hundreds of billions of dollars for COVID-19.

Commented [PD11]: I've got refs if you need them.

Commented [PD12]: Ref. to Jones et al. 2008 (above)

The reality that humans are the ultimate cause of pandemics is demonstrated most tragically by what historian Alfred Crosby has referred to as the "Columbian Exchange" [11]. After the first voyage of Columbus to the Americas in 1492, syphilis was apparently brought back to Europe; far more devastating consequences quickly followed. Europeans soon brought smallpox, measles, and other unknown diseases to the New World, wiping out millions of native peoples, e.g., the infamous *hueyzahuatl* pandemic of 1520, which killed 3.5 million. During the next several hundred years, all over the Americas, countless millions of native people died from these and other imported diseases. Beginning in the 1700s, the tragedy was extended to the Pacific islands and nations. The near-extinction of native peoples over half of the globe occurred on a scale so massive that it could not be adequately measured. The age of exploration might more appropriately be called the age of global microbial devastation.

3. **Expect the unexpected**. The exact time and place of the origin of a pandemic has never been anticipated; each appears unexpectedly with respect to time, place, and clinical-epidemiologic features. No explosive sexually-transmitted disease had ever been seen in Europe when the syphilis pandemic appeared suddenly in the late 15th century. The horrifying gummatous deformities (Figure 4) and tragic deaths characteristic of the first decades of the pandemic were likewise unprecedented [12]. Four centuries later, the HIV/AIDS pandemic was just

Commented [PD13]: I don't think that's completely correct – many of us repeatedly stated that Clade 2b bat-origin SARS-related coronaviruses in China are a likely source of the next pandemic, for example (exactly the nature of SARS-CoV-2)

Commented [PD14]: But we've shown that pandemics emerge largely from distinct geographic hotspots.

Commented [PD15]: Suggest this sentence instead: "It has not, so far, been possible to predict the exact timing, place of origin, or clinical-epidemiologic features of any of the recent pandemics." This sentence says what you intended, but leaves room for the section I've added on new strategies to predict the geography, host origin, and microbial nature of future pandemics.

as shocking in its ability to cause high fatality and tragic deaths, but this time in association with multiple modes of transmission (e.g., sexual, needle sharing, blood product transfusion, maternal transmission) significantly complicating control efforts. More than a millennium of at least 20 pandemic influenza recurrences (at least one every 57 years, and since 1700 AD,

Figure 4. 1665 Portrait of renowned painter, poet, and public intellectual Gérard de Lairesse (1641-1711), by Rembrandt Harmenszoon van Rijn (1606-1669). Lairesse's facial deformities, causing him to be shunned be some contemporaries, are now thought to have resulted from congenital syphilis.

one every 32 years [5]) has surprised us in each instance, in some cases, e.g., 1918, with extraordinary mortality and inexplicable epidemiologic features. Such reactions to the unexpected and frightening have characterized almost all pandemics, including reactions to highly fatal Ebola and the tragic deformities of babies during the Zika pandemic, and our fear and shock at the overwhelming of hospitals by COVID-19. However, science is beginning to provide hope that we can predict some aspects of pandemic emergence, and begin to lower the risk. Tracking past pandemic origins allows us to identify the underlying causes of

emerging diseases, and the hotspots where they are most likely to originate, albeit that these are large regions. Analyzing host-virus relationships allows us to identify the wildlife species that carry the highest risk of as-yet undiscovered viruses, and to estimate how many of these there are in wildlife. Analyses of air travel pathways provide real-time data to anticipate the likely spread of novel diseases once they have gained a foothold in the human population. Much remains to be done, but these efforts provide the first approaches to what may become a preventative approach to pandemic emergence. If land use change and agricultural intensification drive their emergence, future programs to reduce human-wildlife contact around these activities may reduce the risk of future pandemics.

Commented [PD16]: Ref to Jones et al. 2008

Commented [PD17]: Ref: Olival, K. J., Hosseini, P. R., Zambrana-Torrelio, C., Ross, N., Bogich, T. L. & Daszak, P. Host and viral traits predict zoonotic spillover from mammals. Nature 546: 646-650 (2017)

Commented [PD18]: Ref: Carroll, D., Daszak, P., Wolfe, N. D., Gao, G. F., Morel, C. M., Morzaria, S., Pablos-Méndez, A., Tomori, O. & Mazet, J. A. The global virome project. Science 359: 872-874 (2018)

Public health and civil organizational management are critical to pandemic control. Even in our modern era of drugs and vaccines, the most important first steps in pandemic control are preventive and educational. Infection-specific drugs and vaccines are rarely available at the outset and may not be available for years. When they become available, stockpiles may be insufficient, especially in the developing world. Moreover, diagnostics may be unavailable or non-specific, and there may be too few medical providers and facilities. An influenza or a COVID-19 pandemic as fatal as the 1918 influenza pandemic, even before adjustment for the significantly older US population age structure [DMM: make this calculation], might require, over a period of 2-3 months, as many as 2-4 million fully staffed ICUs with ventilators, drugs, and supplies. The current US surge capacity is estimated to be about 45,000. Public health efforts — including those organized by local and State health departments, and those provided by government, industry, and NGOs [13] — are by far the most critical components of early pandemic responses. These must be greatly strengthened.

Commented [PD19]: To distinguish from the earlier para about prevention.

Commented [TJ([20]: Perhaps qualify this a bit as estimated at the low end as 45000 ventilators.

Commented [PD21]: Ouch!

4. **What does pandemic history tell us about confronting COVID-19?** Every pandemic is different. Roughly 14 weeks into the COVID-19 pandemic (19 March 2020) we remain unsure of what lies ahead. Controlling a pandemic can be compared to dancing with an unpredictable leading partner. Neither where the dance is going, nor the direction of the next leading step, can be known. The trick is to remain alert, flexible, and capable of changing strategy at any moment as the situation itself changes. To complicate

Commented [PD22]: Redundant phrase

Commented [PD23]: Calculating from the first known case Dec 18th

matters, the changing situation requires not only good management of uncertainty, but good communication about uncertainty to a confused public.

That China has been able to achieve at least short-term regional control reminds us of the often-unused potential of public health police power. Yet other countries with sophisticated public health capacity, e.g., Italy, have not had early success at controlling viral spread, and there is growing realization that, as is true for many other respiratory viral diseases, "silent spreading" of SARS-CoV-2 by people who are either pre-symptomatic (incubating), asymptomatic, or with mild or atypical symptoms, may be driving the COVID-19 pandemic [14]. Confronting these dynamics will be of critical importance. Ever since the late 19th century, U.S. and most Western public health experts have recognized that there is usually far more to be gained by fostering public trust than by threatening public health police power, e.g., by forcibly isolating, quarantining, or preventing travel and movement. [CITE: Markel H, Lipman HB, Navarro JA, et al. Nonpharmaceutical Interventions Implemented by US Cities During the 1918-1919 Influenza Pandemic. *JAMA*. 2007;298(6):644–654 AND Markel, H. Quarantine! East European Jewish Immigrants and the New York City Epidemics of 1892 (Baltimore: Johns Hopkins University Press, 1997) Even so, public health control options lie on a continuum from informative/suggestive to coercive; the right balance must always be sought, and can be expected to change as the pandemic progresses. Already, in addition to formal public health efforts, businesses, schools, cultural entities, and government agencies are taking public health actions against COVID-19, including temporary or indefinite closures. So far, the US public seems to be moving in step with recommendations of public health, civic, and industry leaders. Personal, private and non-governmental efforts may be definitive. It is critical that such efforts be sustained as the pandemic worsens.

5. **"We must all hang together, or we will all hang separately".** How well we will succeed in mitigating the pandemic of COVID-19 cannot be predicted. But going forward, we must keep an eye on the abundant lessons left us by past pandemics. We must also take note of what is going on in nature all around us. Other species have not been as lucky as we have been so far. Species of bats, bees, and frogs are now being threatened with extinction by pan- and epizootic diseases; we should not imagine that humans will be

exempt from natural laws of microbial evolution [16]. The Justinian plague is said to have killed half of humanity. What assurance do we have that something as deadly will not soon appear?

When the COVID-19 pandemic has run its course, whatever the level of devastation it has left in its wake, it will be time to take stock and rethink how we can fix inadequate pandemic defenses. This must be a cooperative global undertaking, because we can expect to be facing pandemic challenges again and again, and global pandemic threats cannot be managed by national responses. In a densely interconnected world of nearly 8 billion humans, we have no choice but to follow Benjamin Franklin's revolutionary advice and hang together for the good of all.

Pandemics are nature's loud wake-up call that we humans are mismanaging our own existence in the complex ecosystem we have recklessly shaped, within which we live, and upon which our survival depends – planet earth. We must not only wake-up, we must now get up with energy and start building a safer future on a healthier planet.

2,293 words

Commented [PD24]: Two potential references that may be better:

Daszak, P. & Cunningham, A. Extinction by infection. Trends in Ecology & Evolution 14: 279 (1999).

Schloegel, L. M., Hero, J. M., Berger, L., Speare, R., McDonald, K. & Daszak, P. The decline of the sharp-snouted day frog (Taudactylus acutirostris): The first documented case of extinction by infection in a free-ranging wildlife species? Ecohealth 3: 35-40 (2006)

Commented [PD25]: This alludes to the growing 'Planetary Health' movement, which has some legs (e.g. the new Lancet Planetary Health journal.)

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