

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 <div style="background-color: #cccccc; padding: 2px;">(b)(6)</div>
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	
Shi	Zhengli		Female	Professor	Chinese Academy of Sciences, Wuhan Institute of Virology	China	(b)(6)		Passport	China	(b)(6)						DMID/RDB	Erik Stemmy	
Zhou	Peng		Male	Associate Professor	Chinese Academy of Sciences, Wuhan Institute of Virology	China			Passport	China								DMID/RDB	Erik Stemmy
Li	Hongying		Female	China Programs Coordinator	EcoHealth Alliance	China			Passport	China								DMID/RDB	Erik Stemmy

HHS FOREIGN VISITOR MANAGEMENT PROGRAM
National Institute of Allergy and Infectious Diseases

Sponsor/Escort Phone #
(b)(6)

From: Morens, David (NIH/NIAID) [E]
Sent: Fri, 12 Mar 2021 11:37:21 -0500
To: Morens, David (NIH/NIAID) [E]
Subject: Fwd: Fwd: Your article has been published at <http://www.bmj.com>

----- Forwarded Message -----

Subject: Re: Fwd: Your article has been published at <http://www.bmj.com>

Date: Fri, 12 Mar 2021 11:14:49 -0500

From: Dennis Carroll (b)(6)

To: Morens, David (NIH/NIAID) [E] (b)(6)

BRIAND, Sylvie (b)(6) Christine Kreuder Johnson

(b)(6), Oyewale Tomori (b)(6) S

Wacharapluesadee (b)(6), Subhash Morzaria

CC: (b)(6) Sumption, Keith (CJW) (b)(6)

Thanks David. I've talked with Jono Quick at RF about this, not sure with much success. I've also been able to connect with Francis Desouza, Illumina CEO as part of a Milken Institute effort to promote such a system. Much support there. Also in discussion with Victor Dzou at NAM about co-hosting a series of workshop (with Illumina) to drill down into some of the details required for such a network (ie. what specific genetic and metadata needs to be collected). David Cameron is also using his position to get this topic on the G7 agenda. So agree, lots of movement- now for meaningful action

d

On Fri, Mar 12, 2021 at 10:12 AM Morens, David (NIH/NIAID) [E] (b)(6) wrote:

Dennis, great news, thanks for doing this!!! Over the past couple weeks many others have been weighing in with similar ideas. Among those I have been in touch with are the academicians Jim Musser at Houston and Scott Layne at UCLA, and a small think tank team put together by former Sec of State Madeleine Albright with Dr. Tedros, the WHO Director -General, and top folks from BMGF, Rockefeller, including Raj Shah and Rick Bright (who just joined RU), several health ministers of major countries, the CEO of Illumina, who has recently been writing and speaking about this, and separately there is also also Nickie Lurie and Jerry Keusch, who you know, also of course Peter and the EcoHealth folks. Thus these sorts of ideas are in play, but without an obvious mechanism to establish it. It might need some simultaneous networking both from the bottom and from the top down. david

On 3/12/2021 8:07 AM, Dennis Carroll wrote:

All, see link below for our article in the BMJ. Thanks for all your patience. Now, we need to make a global viral surveillance network a reality

Best to all and stay safe

d

On Fri, Mar 12, 2021 at 7:40 AM <bmj-mailer@highwire.stanford.edu> wrote:

We are delighted to tell you that your article

Preventing the next pandemic: the power of a global viral surveillance network

has now been published online by BMJ.

Access your article at: <http://bmj.com/cgi/content/full/bmj.n485>

Toll-free link:

<http://bmj.com/cgi/content/full/bmj.n485?ijkey=ZIx099cFiAbmNno&keytype=ref>

Promoting your article

Remember to tell your co-authors and colleagues about your article via email, social media or other collaboration tools. This will help to generate interest in and usage of your article. There are sharing options on the article's webpage to facilitate this. For further guidance please visit <http://authors.bmj.com/promote-your-paper/how-to-promote/>.

Article metrics and alerts

Online usage statistics and other metrics are available via the article's webpage. You can also register to receive alerts related to your specific article, or contents alerts for the journal.

Responses

Readers may submit an online response to your article which (if accepted for publication) will be linked to the paper online. If we receive any responses, the Editor will usually forward them to you for information and offer you the option to publish a reply.

Rights and permissions

The rights that you retain as the author of the work depend on the terms of the specific publication licence to which you have agreed. Please visit <http://authors.bmj.com/policies/copyright-and-authors-rights/> and <https://www.bmj.com/company/products-services/rights-and-licensing/author-self-archiving-and-permissions/> for further information. Permission to use the article in ways not covered under the terms of the selected licence can be requested online via the article's webpage.

Corrections

If you become aware of any errors in your article after publication, please contact the journal's Production Editor to discuss the publication of a correction. More information can be found at <http://authors.bmj.com/policies/correction-retraction-policies/>.

Feedback

We are always looking for ways to improve our services for authors and value your feedback. [Please take a few minutes to complete this short survey and tell us about your experience.](#)

--

Dr Dennis Carroll
Chair, Leadership Board, Global Virome Project

Senior Advisor, Global Health Security, URC

Senior Fellow, Scowcroft Institute of International Affairs at the Bush School of Government
and Public Service, Texas A&M University

mobile: (b)(6)

email: (b)(6)

--

David M Morens (b)(6) (b)(6)

--

Dr Dennis Carroll
Chair, Leadership Board, Global Virome Project

Senior Advisor, Global Health Security, URC

Senior Fellow, Scowcroft Institute of International Affairs at the Bush School of Government
and Public Service, Texas A&M University

mobile: (b)(6)

email: (b)(6)

From: Morens, David (NIH/NIAID) [E]
Sent: Fri, 16 Jul 2021 12:27:25 -0400
To: Morens, David (NIH/NIAID) [E]
Subject: Fwd: quote of the day.... i can't resist

----- Forwarded Message -----

Subject: Re: quote of the day.... i can't resist

Date: Wed, 30 Jun 2021 14:55:04 -0400

From: Karen Siatras (b)(6)

To: David Morens (b)(6)

I'd like to see any one of them TRY to pick their own fucking cotton.

On Wed, Jun 30, 2021 at 2:49 PM Karen Siatras (b)(6) wrote:
That's OK; it's not every day that my inbox is filled with notes from luminaries, after all.

I wish they would Build That Wall --- right around the middle of the country --- and let the crazies do their thing without bothering the rest of us.

On Tue, Jun 29, 2021 at 9:01 PM David Morens (b)(6) wrote:
Oops sorry for copying you on more than one of these emails with the science luminaries

You see, we are normal, depraved people, after all. d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

Begin forwarded message:

From: David Morens (b)(6)

Date: June 29, 2021 at 19:51:14 EDT

To: "Hotez, Peter Jay" (b)(6)

Cc: Siatras Siatras (b)(6), Peter Daszak (b)(6)

Gerald Keusch (b)(6)

Subject: Re: quote of the day.... i can't resist

Will do. And thanks from me for bitch-slapping (b)(6) He's the worst. d

Sent from my iPhone
David M Morens

OD, NIAID, NIH

On Jun 29, 2021, at 19:23, Hotez, Peter Jay (b)(6) wrote:

Many thanks, let (b)(6) know I'm defending him on Twitter today, that awful (b)(6)
(b)(6)

Peter Hotez, MD, PhD, FASTMH, FAAP

Dean, National School of Tropical Medicine

Professor, Departments of Pediatrics, Molecular Virology & Microbiology

Co-Head, Section of Pediatric Tropical Medicine

Health Policy Scholar

Baylor College of Medicine

Texas Children's Hospital Endowed Chair of Tropical Pediatrics

Co-Director, Texas Children's Hospital Center for Vaccine Development

University Professor

Department of Biology, Baylor University

Faculty Fellow, Hagler Institute for Advanced Study

Senior Fellow, Scowcroft Institute of International Affairs

Texas A&M University

Baker Institute Fellow in Disease & Poverty and Adjunct Professor of Bioengineering, Rice University

Adjunct Professor, University of Texas, School of Public Health

Founding Editor-in-Chief, PLoS Neglected Tropical Diseases

E-mail: (b)(6)

Twitter: @peterhotez

Skype: (b)(6)

Linkedin Peter Hotez

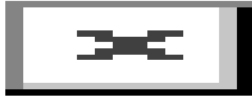
Amazon Author Center: <https://www.amazon.com/Peter-J.-Hotez/e/B001HPIC48>

Like us on Facebook <https://www.facebook.com/BCMNationalSchoolOfTropicalMedicine/>

Executive Assistant: Douglas Soriano

(b)(6)

Phone: (b)(6)



Sent from my iPhone

On Jun 29, 2021, at 6:16 PM, David Morens (b)(6) wrote:

Just on TV, a quotation from a White Trump-supporting woman who is angry about woke-ism, BLM, and Black progressives: “If we had known [you people would become who you are today] we would have picked our own fucking cotton”.

You gotta love the poetry of blind stupidity and evil. In this climate, we have to deal with a whole new field of demagoguery: Covid-originalism! One day someone will write a book about this. It will sit on a shelf next to Hannah Arendt and Theodore Adorno. And you may well be heroes! Congrats. d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

From: Edward Holmes
Sent: Sat, 18 Sep 2021 21:18:02 +0000
To: Peter Daszak
Cc: Garry, Robert F; Wang Linfa; Jason Gale; Stephen Goldstein; Kristian G. Andersen; Rasmussen, Angie; (b)(6) Morens, David (NIH/NIAID) [E]; Robert Kessler; David Morens; (b)(6)
Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

Yes, very good summary Peter.

As you note, despite a seemingly endless stream of papers, grants, genome sequences, theses, FOIAs and intelligence reports there is not a single piece of evidence that SC2 was in the lab. The work needed for a virus to escape a lab leaves a footprint, but there is none to be found.

We're in lockdown here in Sydney but as soon as I'm allowed out a fully English "cholesterol heaven" is on the cards.

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 (b)(6)

On 19 Sep 2021, at 2:05 am, Peter Daszak (b)(6) 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 (b)(6) wrote:

Of course, the momentum on the lab leak side will continue, with books by Sharri Markison, Alina Chan/Matt Ridley, Op Eds that criticize scientists, 70+ FoIAs by one organization alone, many other FoIAs on their way, 900 pages of FoIA'd grants and reports from EHA/NIAID showing zero evidence of lab leak.

Disclaimer

The information contained in this communication from the sender is confidential. It is intended solely for use by the recipient and others authorized to receive it. If you are not the recipient, you are hereby notified that any disclosure, copying, distribution or taking action in relation of the contents of this information is strictly prohibited and may be unlawful.

This email has been scanned for viruses and malware, and may have been automatically archived by Mimecast, a leader in email security and cyber resilience. Mimecast integrates email defenses with brand protection, security 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: Edward Holmes
Sent: Mon, 20 Sep 2021 06:16:06 +0000
To: Jason Gale
Cc: Peter Daszak; David Morens; Morens, David (NIH/NIAID) [E];
(b)(6) Kristian G. Andersen; Wang Linfa; Garry, Robert F; Taubenberger, Jeffery
(NIH/NIAID) [E];(b)(6)
Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

Yes, it's very odd. I just can't follow it.

What I did think was interesting - as you note - is that they talk a lot of the market which I thought was totally off the table in China. Also, it's from Nanshan Zhong and I last time I heard him speak he was strongly pushing the frozen food idea.

Perhaps a shift?

Or could just be a wet Wednesday afternoon's ramblings.

--

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 20 Sep 2021, at 4:06 pm, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

I thought the SARS-CoV-2 virus that emerged in Wuhan wasn't capable of infecting mice that weren't genetically engineered to express human ACE2? This paper, with its emphasis of meteorological factors, seems dodgy to me. But it's good to see research on the origins from researchers in China getting out, albeit it in an obscure journal.

From:

(b)(6)

At: 09/20/21 15:55:39 UTC+10:00

To:

Jason Gale (BLOOMBERG/ NEWSROOM:)

Cc:

(b)(6)

Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

Although I can't quite tell if it is sane.

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 20 Sep 2021, at 2:37 pm, Edward Holmes (b)(6) wrote:

Just found this in an obscure journal.

Interesting it is Nanshan Zhong and interesting that there's a lot about the market....

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 20 Sep 2021, at 10:52 am, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

I did this podcast episode on bats and zoonoses at the start of 2020 with the help of Hume Field, Trevor Drew, Mark Schipp and Linfa. Still seems relevant today.

<https://podcasts.apple.com/nz/podcast/how-it-all-started-rebroadcast/id1440051086?i=1000504072911>

From:

(b)(6)

At: 09/20/21 10:17:32 UTC+10:00

To:

Jason Gale (BLOOMBERG/ NEWSROOM:)

Cc:

(b)(6)

Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

100% agree.

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 20 Sep 2021, at 10:16 am, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

Suspect geopolitics is the biggest impediment to finding an animal source in China, and the best remedy for this is to rebuild/strengthen r'ships with scientists in China.

From:

(b)(6) At: 09/20/21 10:12:48 UTC+10:00

To: (b)(6)

Cc: Jason Gale (BLOOMBERG/ NEWSROOM:)

(b)(6)

Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

Just need to keep sampling, but that sampling ought to be broader.

We need something >99% similar across the whole genome.

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 20 Sep 2021, at 9:59 am, Morens, David (NIH/NIAID) [E] (b)(6) wrote:

Agree totally except your certainty that China is the ultimate source. Admittedly much data point in that direction but how can you be sure? d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Sep 19, 2021, at 19:28, Edward Holmes (b)(6) wrote:

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] (b)(6) wrote:

Eddie, please clarify, i don't « get » all the phylogenetic asumptions you guys understand, but can you put it in Isyman's terms? As you know, i have said repeatedly to look past Yunnan to all of SE Asia, as i have bennunconvinced 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 (b)(6) 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 (b)(6)
E

On 20 Sep 2021, at 7:52 am, Morens, David (NIH/NIAID) [E] (b)(6) 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:

(b)(6) At: 09/20/21 07:31:51 UTC+10:00

To: (b)(6)

Cc: Jason Gale (BLOOMBERG/ NEWSROOM:)

(b)(6)

Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

nPeter, as i am perennially swamped with work that has nothing to do with COVID issues of importance, i am always catching up on reading the important stuff

Just now i poured a martini and-read word for word your "A strategy..." paper with first author Sánchez. Also Kevin and Lin-fa were coauthors. Wow!!!

This is dynamite and also beautifully written. I mean, Hemingway, Conrad, Nin, couldn't have written it better. Beautiful job and so important.

I think you need to promote this work, and emphasize that the conclusions are far reaching and a sort of call to arms.

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 (b)(6) 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 (b)(6) wrote:

Of course, the momentum on the lab leak side will continue, with books by Sharri Markison, Alina Chan/Matt Ridley, Op Eds that criticize scientists, 70+ FoIAs by one organization alone, many other FoIAs on their way, 900 pages of FoIA'd grants and reports from EHA/NIAID showing zero evidence of lab leak.

Disclaimer

The information contained in this communication from the sender is confidential. It is intended solely for use by the recipient and others authorized to receive it. If you are not the recipient, you are hereby notified that any disclosure, copying, distribution or taking action in relation of the contents of this information is strictly prohibited and may be unlawful.

This email has been scanned for viruses and malware, and may have been automatically archived by Mimecast, a leader in email security and cyber resilience. Mimecast integrates email defenses with brand protection, security 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.

<Screenshot from 2021-09-19 17-04-25.png>

<healthcare-09-01132-v2.pdf>

From: Wang Linfa
Sent: Sat, 18 Sep 2021 15:56:48 +0000
To: Morens, David (NIH/NIAID) [E]; Peter Daszak; Edward Holmes; Jason Gale
Cc: Stephen Goldstein; (b)(6); Garry, Robert F;
(b)(6) Robert Kessler; David Morens;
Subject: RE: Study from 2007 shows SARS-infected civets on farms in Hubei

Game changer, dynamite and basically we (the international scientific community) have now found the natural/bat origin of "the functional core of SARS-CoV-2". As we all know, RBD is the key for sarbecovirus to infect human.

Just to put this in perspective: after 18 years of intensive searching, we still have NOT found the bat origin of "the functional core of SARS-CoV-1". The closest we had was WIV1 which has 10 aa difference from SARS-CoV-1 in the RBD region. Here we have a bat sarbecovirus RBD which has only 1 aa difference and that change has NO impact on its ability to bind human ACE2. I am completely amazed with the rapid progress of the research and it proved what we have been saying all along: pay more attention to SE Asia. There are more bats there, but with much less surveillance intensity than Southern China!

Case closed as far as I am concerned! Good night (morning) to all....

Linfa (Lin-Fa) WANG, PhD FTSE FAAM
Professor
Programme in Emerging Infectious Disease
Duke-NUS Medical School,
8 College Road, Singapore 169857
Tel: (b)(6)

From: Morens, David (NIH/NIAID) [E] (b)(6)
Sent: Saturday, 18 September 2021 11:44 PM
To: Peter Daszak (b)(6); Wang Linfa (b)(6); Edward Holmes (b)(6); Jason Gale <j.gale@bloomberg.net>
Cc: Stephen Goldstein (b)(6); Garry, Robert F (b)(6); Robert Kessler (b)(6); David Morens (b)(6)
Subject: RE: Study from 2007 shows SARS-infected civets on farms in Hubei

- External Email -

Yes, this is dynamite,. and all the more reason that more work needs to be done to characterize the bat sarbecovirus "universe" all over the region.

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

📞 (b)(6) (assistant: Whitney Robinson)

📠 301 496 4409

💻 (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.



From: Peter Daszak (b)(6)
Sent: Saturday, September 18, 2021 10:09 AM
To: Wang Linfa (b)(6); Edward Holmes (b)(6); Jason Gale (b)(6)
Cc: Stephen Goldstein (b)(6); Garry, Robert F (b)(6); Morens, David (NIH/NIAID) [E] (b)(6); Robert Kessler (b)(6); David Morens (b)(6)

Subject: RE: Study from 2007 shows SARS-infected civets on farms in Hubei
Importance: High

Yes – saw that paper Jason – really interesting

I looked through the paper and it's yet another game changer. So far, in the last few weeks/months, we've got the following new evidence supporting emergence via bat-to-intermediate host-to-human origin for COVID-19 (I've probably missed something):

Multiple new, SARS-CoV-2 related CoVs in SE Asia (Cambodia, Thailand, Japan, China etc.). I know of other work in review describing other related viruses in SE Asia also. We're also finding further novel SARS-CoV-2 related bat viruses in Malaysia, Thailand.

New evidence that live animals of the type that carry CoVs were present in the Wuhan markets (including Huanan).

Evidence from other bat SARS-CoVs that mutations occur where there FCS is found (eg.

RmYN02) <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7211627/>

a rat alpha-CoV with an FCS in wildlife farms, hotels and train stations in S. China, showing that FCS insertions are more common in nature than previously thought.

<https://journals.asm.org/doi/epdf/10.1128/JVI.01173-21>

Epidemiological analysis of early cases supporting early origin close to Huanan market, not WIV

[https://www.cell.com/cell/fulltext/S0092-8674\(21\)00991-0](https://www.cell.com/cell/fulltext/S0092-8674(21)00991-0)

Phylogenetic analyses suggesting there may have been multiple introductions into the human population, supporting presence of a virus circulating in animals rather than a lab leak (@virology paper)

Our work showing a very large interface for bat SARS-CoV spillover in a v. densely populated region, and potential for large numbers of missing cases each year

<https://www.medrxiv.org/content/10.1101/2021.09.09.21263359v1>

This paper showing ACE2 binding for bat SARS-CoV-2 related CoVs.

<https://www.researchsquare.com/article/rs-871965/v1>

On the lab leak side, we have convoluted accusations based on interpretations of intent about how Chinese scientists submitted genomes, wrote the papers, or how me and other scientists had collaborations with Chinese scientists. But, as far as new evidence goes, I could only find this:

- None

Of course, the momentum on the lab leak side will continue, with books by Sharri Markison, Alina Chan/Matt Ridley, Op Eds that criticize scientists, 70+ FoIAs by one organization alone, many other FoIAs on their way, 900 pages of FoIA'd grants and reports from EHA/NIAID showing zero evidence of lab leak.

This rate of research even in a pandemic is remarkable and suggests that we'll pretty quickly have such overwhelming evidence for the 'natural' origins that most people will move on from the lab leak.

(Off-the-record) However, the damage they leave behind is already horrific and will be worse by the time they decide to find another issue to focus on.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
520 Eighth Avenue, Suite 1200
New York, NY 10018-6507
USA

Tel.: (b)(6)
Website: www.ecohealthalliance.org
Twitter: [@PeterDaszak](https://twitter.com/PeterDaszak)

EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

From: Wang Linfa (b)(6)
Sent: Friday, September 17, 2021 10:56 PM
To: Edward Holmes (b)(6) Jason Gale <j.gale@bloomberg.net>
Cc: Stephen Goldstein (b)(6)
(b)(6) Peter Daszak (b)(6)
(b)(6)
Subject: RE: Study from 2007 shows SARS-infected civets on farms in Hubei

Almost identical SARS-CoV-2 RBD in several bat sarbecoviruses! This is as close as you can get for a natural RBD origin!

Also, the paper concluded that SARS-CoV-2 genome fragments are found in different sarbecoviruses, very similar to the PloS Path paper for SARS-CoV-1.

All we need is to find a sarbecovirus with a furin cleavage site and no more debate on the natural origin of SARS-CoV-2!

Linfa (Lin-Fa) WANG, PhD FTSE FAAM
Professor
Programme in Emerging Infectious Disease
Duke-NUS Medical School,
8 College Road, Singapore 169857
Tel: (b)(6)

From: Edward Holmes (b)(6)
Sent: Thursday, 16 September 2021 3:31 PM
To: Jason Gale <j.gale@bloomberg.net>
Cc: Stephen Goldstein (b)(6); (b)(6); Peter Daszak (b)(6); Wang Linfa (b)(6)
Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

- External Email -

Dismantles one key argument of the leakers - how could a virus get from Yunnan to Wuhan - in one simple move.

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 (b)(6)

On 16 Sep 2021, at 2:26 pm, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

And there's this:

"The discovery of civet-CoVs in the Hubei province should not be a surprise as SARS-CoV-like viruses were recently found in a bat species in the same province"

From: (b)(6) At: 09/16/21 14:24:33 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:)
Cc: (b)(6)

(b)(6)

Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

Just stumbled across it reading the discussion of another paper honestly. It's been cited since - there are certainly people who remembered it but I did not know of it and clearly had not penetrated the public origins discussion.

Stephen

Sent from my iPhone

On Sep 15, 2021, at 10:22 PM, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

Well done, Stephen for finding this:
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1900161/>

Jason Gale, MHlthSec
Senior editor & chief biosecurity correspondent | Bloomberg News
Level 30, 120 Collins St., Melbourne VIC 3000
Tel. (landline) +61-3-9228-8783 | Mobile (b)(6)
@jwgale | LinkedIn: <http://www.linkedin.com/pub/jason-gale/6/249/a56>

Disclaimer

The information contained in this communication from the sender is confidential. It is intended solely for use by the recipient and others authorized to receive it. If you are not the recipient, you are hereby notified that any disclosure, copying, distribution or taking action in relation of the contents of this information is strictly prohibited and may be unlawful.

This email has been scanned for viruses and malware, and may have been automatically archived by Mimecast, a leader in email security and cyber resilience. Mimecast integrates email defenses with brand protection, security 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: Sun, 19 Sep 2021 17:28:39 -0400
To: Peter Daszak
Cc: Garry, Robert F; Wang Linfa; Edward Holmes; Jason Gale; Stephen Goldstein; (b)(6) Robert Kessler; David Morens; (b)(6)
Bcc: Morens, David (NIH/NIAID) [E]
Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

Peter, as i am perennially swamped with work that has nothing to do with COVID issues of importance, i am always catching up on reading the important stuff

Just now i poured a martini and-read word for word your “A strategy...” paper with first author Sánchez. Also Kevin and Lin-fa were coauthors. Wow!!!

This is dynamite and also beautifully written. I mean, Hemingway, Conrad, Nin, couldn't have written it better. Beautiful job and so important.

I think you need to promote this work, and emphasize that the conclusions are far reaching and a sort of call to arms.

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 (b)(6) 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 (b)(6) wrote:

Of course, the momentum on the lab leak side will continue, with books by Sharri Markison, Alina Chan/Matt Ridley, Op Eds that criticize scientists, 70+ FoIAs by one organization alone, many other FoIAs on their way, 900 pages of FoIA'd grants and reports from EHA/NIAID showing zero evidence of lab leak.

Disclaimer

The information contained in this communication from the sender is confidential. It is intended solely for use by the recipient and others authorized to receive it. If you are not the recipient, you are hereby notified that any disclosure, copying, distribution or taking action in relation of the contents of this information is strictly prohibited and may be unlawful.

This email has been scanned for viruses and malware, and may have been automatically archived by Mimecast, a leader in email security and cyber resilience. Mimecast integrates email defenses with brand protection, security 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: Garry, Robert F
Sent: Sat, 18 Sep 2021 14:26:15 +0000
To: Peter Daszak
Cc: Wang Linfa; Edward Holmes; Jason Gale; Stephen Goldstein;
(b)(6) Morens, David (NIH/NIAID)
[E]; Robert Kessler; David Morens; (b)(6)
Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

Excellent summary - I'd add an intelligence community report despite some of the most biased news coverage I've ever seen. Reading the report it's clear that the IC including the top committee also leans heavily to natural - zip zero nada evidence for lab leak - all that's left for lab leakers is the Relman Special - that wiv had sc2 in a freezer and didn't know they had it - some lab person got infected and touched off transmission chains in multiple wet markets

Sent from my iPhone

On Sep 18, 2021, at 9:12 AM, Peter Daszak (b)(6) wrote:

External Sender. Be aware of links, attachments and requests.

Yes – saw that paper Jason – really interesting

I looked through the paper and it's yet another game changer. So far, in the last few weeks/months, we've got the following new evidence supporting emergence via bat-to-intermediate host-to-human origin for COVID-19 (I've probably missed something):

- Multiple new, SARS-CoV-2 related CoVs in SE Asia (Cambodia, Thailand, Japan, China etc.). I know of other work in review describing other related viruses in SE Asia also. We're also finding further novel SARS-CoV-2 related bat viruses in Malaysia, Thailand.
- New evidence that live animals of the type that carry CoVs were present in the Wuhan markets (including Huanan).
- Evidence from other bat SARSr-CoVs that mutations occur where there FCS is found (eg. RmYN02) <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7211627/>
- a rat alpha-CoV with an FCS in wildlife farms, hotels and train stations in S. China, showing that FCS insertions are more common in nature than previously thought. <https://journals.asm.org/doi/epdf/10.1128/JVI.01173-21>
- Epidemiological analysis of early cases supporting early origin close to Huanan market, not WIV [https://www.cell.com/cell/fulltext/S0092-8674\(21\)00991-0](https://www.cell.com/cell/fulltext/S0092-8674(21)00991-0)
- Phylogenetic analyses suggesting there may have been multiple introductions into the human population, supporting presence of a virus circulating in animals rather than a lab leak (@virology paper)
- Our work showing a very large interface for bat SARSr-CoV spillover in a v. densely populated region, and potential for large numbers of missing cases each year <https://www.medrxiv.org/content/10.1101/2021.09.09.21263359v1>

- This paper showing ACE2 binding for bat SARS-CoV-2 related CoVs.
<https://www.researchsquare.com/article/rs-871965/v1>

On the lab leak side, we have convoluted accusations based on interpretations of intent about how Chinese scientists submitted genomes, wrote the papers, or how me and other scientists had collaborations with Chinese scientists. But, as far as new evidence goes, I could only find this:

- None

Of course, the momentum on the lab leak side will continue, with books by Sharri Markison, Alina Chan/Matt Ridley, Op Eds that criticize scientists, 70+ FoIAs by one organization alone, many other FoIAs on their way, 900 pages of FoIA'd grants and reports from EHA/NIAID showing zero evidence of lab leak.

This rate of research even in a pandemic is remarkable and suggests that we'll pretty quickly have such overwhelming evidence for the 'natural' origins that most people will move on from the lab leak.

(Off-the-record) However, the damage they leave behind is already horrific and will be worse by the time they decide to find another issue to focus on.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
520 Eighth Avenue, Suite 1200
New York, NY 10018-6507
USA

Tel.: (b)(6)
Website: www.ecohealthalliance.org
Twitter: [@PeterDaszak](https://twitter.com/PeterDaszak)

EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

From: Wang Linfa (b)(6)
Sent: Friday, September 17, 2021 10:56 PM
To: Edward Holmes (b)(6) Jason Gale <j.gale@bloomberg.net>
Cc: Stephen Goldstein (b)(6)

(b)(6) Peter Daszak (b)(6)
(b)(6)

Subject: RE: Study from 2007 shows SARS-infected civets on farms in Hubei

Almost identical SARS-CoV-2 RBD in several bat sarbecoviruses! This is as close as you can get for a natural RBD origin!

Also, the paper concluded that SARS-CoV-2 genome fragments are found in different sarbecoviruses, very similar to the PLoS Path paper for SARS-CoV-1.

All we need is to find a sarbecovirus with a furin cleavage site and no more debate on the natural origin of SARS-CoV-2!

Linha (Lin-Fa) WANG, PhD FTSE FAAM
Professor
Programme in Emerging Infectious Disease
Duke-NUS Medical School,
8 College Road, Singapore 169857
Tel: (b)(6)

From: Edward Holmes (b)(6)
Sent: Thursday, 16 September 2021 3:31 PM
To: Jason Gale <j.gale@bloomberg.net>
Cc: Stephen Goldstein (b)(6)
(b)(6) Peter Daszak (b)(6)
(b)(6) Wang Linfa (b)(6)
Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

- External Email -

Dismantles one key argument of the leakers - how could a virus get from Yunnan to Wuhan - in one simple move.

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 (b)(6)

On 16 Sep 2021, at 2:26 pm, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

And there's this:

"The discovery of civet-CoVs in the Hubei province should not be a surprise as SARS-CoV-like viruses were recently found in a bat species in the same province"

From: (b)(6) At: 09/16/21 14:24:33 UTC+10:00

To: Jason Gale (BLOOMBERG/ NEWSROOM:)

Cc: (b)(6)

(b)(6)

Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

Just stumbled across it reading the discussion of another paper honestly. It's been cited since - there are certainly people who remembered it but I did not know of it and clearly had not penetrated the public origins discussion.

Stephen

Sent from my iPhone

On Sep 15, 2021, at 10:22 PM, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

Well done, Stephen for finding this:

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1900161/>

Jason Gale, MHlthSec

Senior editor & chief biosecurity correspondent | Bloomberg News

Level 30, 120 Collins St., Melbourne VIC 3000

Tel. (landline) +61-3-9228-8783 | Mobile (b)(6)

@jwgale | LinkedIn: <http://www.linkedin.com/pub/jason-gale/6/249/a56>

Disclaimer

The information contained in this communication from the sender is confidential. It is intended solely for use by the recipient and others authorized to receive it. If you are not the recipient, you are hereby notified that any disclosure, copying, distribution or taking action in relation of the contents of this information is strictly prohibited and may be unlawful.

This email has been scanned for viruses and malware, and may have been automatically archived by Mimecast, a leader in email security and cyber resilience. Mimecast integrates email defenses with brand protection, security 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: Edward Holmes
Sent: Mon, 20 Sep 2021 04:38:08 +0000
To: Jason Gale
Cc: Peter Daszak; (b)(6); Morens, David (NIH/NIAID) [E];
(b)(6); Wang Linfa; Garry, Robert F; Taubenberger, Jeffery
(NIH/NIAID) [E]; (b)(6)
Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei
Attachments: healthcare-09-01132-v2.pdf

Just found this in an obscure journal.

Interesting it is Nanshan Zhong and interesting that there's a lot about the market....

--

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 20 Sep 2021, at 10:52 am, Jason Gale (BLOOMBERG/ NEWSROOM:)
<j.gale@bloomberg.net> wrote:

I did this podcast episode on bats and zoonoses at the start of 2020 with the help of Hume Field, Trevor Drew, Mark Schipp and Linfa. Still seems relevant today.

<https://podcasts.apple.com/nz/podcast/how-it-all-started-rebroadcast/id1440051086?i=1000504072911>

From: (b)(6) At: 09/20/21 10:17:32 UTC+10:00

To: Jason Gale (BLOOMBERG/ NEWSROOM:)

Cc: (b)(6)

(b)(6)

Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

100% agree.

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 (b)(6)

On 20 Sep 2021, at 10:16 am, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

Suspect geopolitics is the biggest impediment to finding an animal source in China, and the best remedy for this is to rebuild/strengthen r'ships with scientists in China.

From: (b)(6) At: 09/20/21 10:12:48 UTC+10:00

To: (b)(6)

Cc: Jason Gale (BLOOMBERG/ NEWSROOM:) ,

(b)(6)

Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

Just need to keep sampling, but that sampling ought to be broader.

We need something >99% similar across the whole genome.

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 20 Sep 2021, at 9:59 am, Morens, David (NIH/NIAID) [E] (b)(6) wrote:

Agree totally except your certainty that China is the ultimate source. Admittedly much data point in that direction but how can you be sure? d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Sep 19, 2021, at 19:28, Edward Holmes (b)(6) wrote:

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] (b)(6) wrote:

Eddie, please clarify, i don't « get » all the phylogenetic assumptions you guys understand, but can you put it in Isyman's terms? As you know, i have said repeatedly to look past Yunnan to all of SE Asia, as i have bennunconconvined 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 (b)(6) 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 (b)(6)
E

On 20 Sep 2021, at 7:52 am, Morens, David (NIH/NIAID) [E] (b)(6) 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: (b)(6) At: 09/20/21 07:31:51 UTC+10:00

To: (b)(6)

Cc: Jason Gale (BLOOMBERG/ NEWSROOM:) ,

(b)(6)

Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

nPeter, as i am perennially swamped with work that has nothing to do with COVID issues of importance, i am always catching up on reading the important stuff

Just now i poured a martini and-read word for word your "A strategy..." paper with first author Sánchez. Also Kevin and Lin-fa were coauthors. Wow!!!

This is dynamite and also beautifully written. I mean, Hemingway, Conrad, Nin, couldn't have written it better. Beautiful job and so important.

I think you need to promote this work, and emphasize that the conclusions are far reaching and a sort of call to arms.

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 (b)(6) 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 (b)(6) wrote:

Of course, the momentum on the lab leak side will continue, with books by Sharri Markison, Alina Chan/Matt Ridley, Op Eds that criticize scientists, 70+ FoIAs by one organization alone, many other FoIAs on their way, 900 pages of FoIA'd grants and reports from EHA/NIAID showing zero evidence of lab leak.

Disclaimer


The information contained in this communication from the sender is confidential. It is intended solely for use by the recipient and others authorized to receive it. If you are not the recipient, you are hereby notified that any disclosure, copying, distribution or taking action in relation of the contents of this information is strictly prohibited and may be unlawful.

This email has been scanned for viruses and malware, and may have been automatically archived by Mimecast, a leader in email security and cyber resilience. Mimecast integrates email defenses with brand protection, security 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.

<Screenshot from 2021-09-19 17-04-25.png>

Article

SARS-CoV-2: Origin, Intermediate Host and Allergenicity Features and Hypotheses

Yuyi Huang¹, Junmou Xie¹, Yuhe Guo¹, Weimin Sun¹, Ying He¹, Kequn Liu^{2,*}, Jie Yan^{1,*}, Ailin Tao^{1,*}
and Nanshan Zhong^{3,*}

¹ The Second Affiliated Hospital, The State Key Laboratory of Respiratory Disease, Guangdong Provincial Key Laboratory of Allergy & Clinical Immunology, Guangzhou Medical University, Guangzhou 510260, China; huangyuyi@gzhmu.edu.cn (Y.H.); xiejunmou@126.com (J.X.); guoyuhe2013@126.com (Y.G.); sumyj1000@163.com (W.S.); heying0605@163.com (Y.H.)

² Wuhan Regional Climate Center, Wuhan 430074, China

³ The State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China

* Correspondence: kequnliu@126.com (K.L.); jieyan@gzhmu.edu.cn (J.Y.);

taoilin@gzhmu.edu.cn (A.T., Lead contact 2); nanshan@vip.163.com (N.Z., Lead contact 1)

Abstract: The goal of this study is to investigate the probable intermediate hosts and the allergenicity of the notorious virus SARS-CoV-2 to understand how this virus emerged. The phylogenetic analysis of the virus spike proteins indicates that SARS-CoV-2 falls into various small subclades that include a bat coronavirus RaTG13, suggesting bats as a likely natural origin. Refined alignment of the spike protein in NCBI found several fragments that are specific to SARS-CoV-2 and/or SARS-CoV are specific to *Rattus norvegicus* and/or *Mus musculus*, suggesting that rodents are the intermediate reservoir of SARS-CoV-2 and SARS-CoV. To evaluate the allergenicity values, the binding affinities of human leukocyte antigen (HLA) class I or II molecules with the spike proteins were calculated, and the results showed that both SARS-CoV-2 and SARS-CoV are predicted to bind to fourteen HLA class I and II molecules with super-high HLA allele-peptide affinities. The infection rate of individuals who have HLA alleles with very high binding affinities who might become infected and develop into refractory patients if there were no medical or non-medical interventions is about 7.36% and 4.78% of Chinese and Americans, respectively. Extremely high temperature and exceptionally low precipitation, the common climate factors between the outbreak sites of COVID-19 in Wuhan in 2019 and SARS in Guangdong in 2002, might have promoted coronavirus evolution into more virulent forms. Our hypothesis suggests that early immunization with an allergenically-engineered virus, in combination with continued surveillance of meteorological factors and viral mutations, may be one of the most powerful prophylactic modalities to fight this virus.

Keywords: SARS-CoV-2; spike protein; intermediary reservoir; allergenicity; MHC binding affinity; self-limitation; spontaneous mutation; early immunization



Citation: Huang, Y.; Xie, J.; Guo, Y.; Sun, W.; He, Y.; Liu, K.; Yan, J.; Tao, A.; Zhong, N. SARS-CoV-2: Origin, Intermediate Host and Allergenicity Features and Hypotheses. *Healthcare* **2021**, *9*, 1132. <https://doi.org/10.3390/healthcare9091132>

Academic Editors: Mariano Cingolani and Francesco Sessa

Received: 13 November 2020

Accepted: 27 August 2021

Published: 30 August 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The recent outbreak of COVID-19 across the whole world was caused by a novel beta coronavirus isoform which was designated as SARS-CoV-2 by the International Committee on Taxonomy of Viruses (ICTV) based on the sequence of the viral RNA genome. The World Health Organization (WHO) claimed an international public health emergency for the outbreak in January and, later, a global pandemic in March 2020. As of 22 March 2021, more than 25,794,100 infected cases were reported in over 200 countries and regions. This prompted scientists to identify how the epidemic affected such a substantial amount of people in the world. Coronaviruses are known to circulate in birds and mammals, including bats [1]. Several publications have recently explored the origin of SARS-CoV-2.

Based on genomic sequence analysis, Shi and colleagues demonstrated that the novel virus is 96% homologous to a Yunan bat coronavirus at the whole-genome level [2], while Wu et al. reported only 89.1% nucleotide similarity between the virus and other SARS-like coronaviruses (*Betacoronavirus sarbecovirus*) originally found in bats in China [3]. Guo et al. further demonstrated that bats and minks are two likely candidate reservoirs of the novel virus [4].

It is critical to identify the immediate reservoirs of SARS-CoV-2 and how the virus is passed onto humans. Recently reported results have been controversial. The results from Wei et al. suggested that snakes are the most probable wild animal reservoir for the virus, based on their relatively synonymous codon usage bias compared to other animal species [5]. Work from Guan and colleagues suggested that pangolins (*Manis javanica*) should be considered as a possible intermediate host for the novel coronavirus based on the 85.5% to 92.4% similarity of the viruses found in pangolins to a partial length (~86.3%) of the SARS-CoV-2 genome sequence [6]. Two other studies implied that cats may be involved in virus infection and transmission [7,8]. SARS-CoV-2 is a single-stranded RNA coronavirus bearing a high frequency of RNA recombination, and the stability of the virus would be affected by environmental conditions, such as temperature, humidity, atmospheric pressure, etc. [9–11]. How to keep codon-based evolution analysis of this virus accurate is therefore a perplexing and challenging question.

Allergenicity is referred to as the ability of an antigen to induce an aberrant or detrimental immune response in the host, which is an overreaction and different from a normal immune response in that it does not result in a protective/prophylactic effect but instead causes physiological dysfunction and/or tissue damage [12]. In the early phase of an allergic reaction, antigens are presented through major histocompatibility complex (MHC) in vertebrates and HLA (human leukocyte antigen) in humans to T cells to activate adaptive immunity [13–17]. Whether an antigen is able to be presented to T cells or not depends on the binding affinity of that antigen with MHC/HLA molecules of antigen-presenting cells (APCs). The stronger the binding affinity, the more likely the antigen would be presented outside of APCs, and thus, the higher the allergenicity. Such an antigen is more likely to trigger danger signals and activate downstream inflammatory pathways and cytokine storms [12,18]. Therefore, the binding affinity of antigens to HLA molecules is a key indicator of the allergenicity and the presentation potency of those antigens. Immunologically, proteins from SARS-CoV-2 should also be presented as antigens by APCs as they touch human bodies. High allergenicity of a viral antigen typically elicits a rapid elevation of various inflammatory factors, and often renders viral antigens liable to induce cytokine storms [19]. Profilin is a panallergen and exhibits a configuration of α - β - α layers, a similar structure component element shared by different allergens [20]. It can induce only mild symptoms like oral allergy syndrome in the allergic population [21,22]. Therefore, the highest values of profilin binding affinity to HLA I (≥ 0.9) and HLA II (≥ 0.8) molecules will be cited as the lowest cutoff values to discriminate the binding affinity of different HLA molecules to the spike protein of SARS-CoV-2 and/or SARS-CoV.

This study aims to: (1) Determine the potential virus intermediate reservoir by carrying out comprehensive amino acid sequence analysis and comparison of sliding sequence fragments of the novel virus with all sequences from mammals available in the NCBI database; (2) Analyze the allergenicity of the spike protein in SARS-CoV-2 and compare it with that of SARS-CoV to explain the mechanism of the COVID-19 pandemic from a new perspective. Furthermore, we systematically compare climate data in the past 50 years to predict any relationship between the meteorological conditions and the survival/development of the virus.

2. Materials and Methods

2.1. Evolutionary Analysis

The amino acid sequences of the SARS-CoV spike glycoprotein derived from humans, civets and bats were downloaded from NCBI. Sequence alignment was performed

to identify three functional subunits: receptor binding domain, N-terminal domain and coronavirus S2 glycoprotein. A phylogenetic tree was constructed using the maximum likelihood method with best protein models for different sequence groups using the MEGA7.0 program [23,24].

2.2. Key Sites Analysis

First, protein sequences of SARS-CoV-2 and SARS-CoV derived from humans, civets and bats were analyzed through multiple sequence alignment to locate all potential homologous sites. Then, a homology site screening program was constructed to classify these sites and to screen out the homologous key sites in accord with consistent sites among human SARS-CoV-2, SARS-CoV, and civet SARS-CoV, but 50–100% different from bat SARS-CoV.

2.3. Mouse Derived Peptide Analysis

The fixed-length sliding window method was used to split the spike glycoprotein sequence into equal length, non-overlapping peptide segments. Based on the latest version of the NCBI Reference protein library of whole organisms, a peptide source scanner was constructed to analyze the source of each peptide of the spike glycoprotein. The proportion of mouse-derived peptide was calculated, and the possible mouse-derived peptides were retained. By scanning in the NCBI Reference protein library and the non-redundant protein sequence library, Protein BLAST was performed to verify the exclusive origin of the peptides retained by the scanner.

2.4. Allergenicity Assessment and Infection Population Estimating

The allergenicity of SARS-CoV-2 and SARS-CoV was assessed by using the software NetMHC-4.0 [25,26] and NetMHCII-2.3 [27] to predict the binding affinity of human SARS-CoV-2 and SARS-CoV with HLA class I and class II molecules. Briefly, the sliding window approach was used to extract peptides from the full-length spike proteins of the viruses which were 20 and 9 amino acids in length for HLA Class II and Class I alleles, respectively. Fifty kinds of HLA class II alleles and 81 kinds of HLA class I alleles were selected for binding affinity prediction. The distributions of peptides of different binding affinities with different HLA class I and class II molecules were calculated. Based on allele frequencies in worldwide populations (<http://www.allelefreqencies.net/>, accessed on 27 August 2021) [28], the numbers of individuals that have alleles which tightly bind with the spike protein were predicted and the infection rates were calculated according to the Hardy–Weinberg equilibrium for the populations of China and the United States, respectively. Binding affinities of more than 0.9 to HLA class I molecules and more than 0.8 to HLA class II molecules were used as the cutoff values for superhigh allergenicity discrimination.

2.5. Meteorological Parameters Analysis

The monthly climate data of Wuhan and Guangdong from 1951 to 2019 were downloaded from the China Meteorological Network, including six climate characteristics, such as average temperature, average maximum temperature, average minimum temperature, precipitation, sunshine hours, and relative humidity (<http://www.weather.com.cn/>, accessed on 27 August 2021). Different meteorological factors, including 6 climate factors and 12 months from 1959 to 2019, were analyzed to obtain any clues regarding the outbreak of SARS-CoV-2. Using 12 months and 6 climatic features as primary data, 257,985 different combinations between month and climatic feature were produced. The correlation coefficients of different years under the combination of month and climatic features were calculated using R language. To construct a screening program aimed at 2019 and 2002, we screened out the climate combination features that exhibited a special strong correlation between the two years; that is, the corresponding climate combination features of Wuhan 2019 are very similar to those of Guangdong in 2002 (Pearson correlation coefficient > 0.8), but are less similar to those in most other years (Pearson correlation coefficient < 0.5).

3. Results

3.1. Phylogenetic Analysis of SARS-CoV-2

To trace the source of SARS-CoV-2 and its evolutionary path, we analyzed the evolutionary relationship of the spike glycoproteins of human SARS-CoV-2, SARS-CoV, and the coronaviruses reported in bat and civets (Figure 1A). The spike protein contains S1 and S2 domains. S1 contains the receptor-binding domain (RBD) and S2 mediates fusion with host membranes. Although there is a distinct evolutionary difference among human SARS-CoV-2, SARS-CoV and other coronaviruses, bat SARS-CoVs (including RaTK13, CoVZC45, CoVZXC21, etc.) have evolutionary proximity to human SARS-CoV-2. These bat SARS-CoVs are also located proximally to civet and human SARS-CoVs in the evolutionary tree (Figure 1, Clade I). These results suggest that the natural human SARS-CoV-2 most likely originated from bats.

To demonstrate the evolutionary origin of human SARS-CoV-2 and other SARS-CoVs, we further analyzed the evolution of functional domains in spike glycoproteins. Phylogenetic results showed that the closest evolutionary relationship between SARS-CoV-2 and SARS-CoV mainly lies in the N-terminal and receptor binding domains (Figure 1A–D). However, the sequence in the S2 glycoprotein region of human SARS-CoV-2 is related more closely to that of bat SARS-CoV than human SARS-CoV (Figure 1D, Clade II), suggesting the S2 glycoprotein region is the likely cause for a pathogenetic difference between human SARS-CoV-2 and SARS-CoV. Therefore, the human SARS-CoV-2 is an independent branch located at the bottom of the evolutionary tree.

3.2. Intermediate Host Analysis

To identify the intermediate hosts of SARS-CoV-2, we scanned and aligned the human SARS-CoV-2 spike glycoprotein in the entire biological database using the peptide sliding window approach. Seventeen mouse-derived peptide fragments exactly matched with peptides within the human SARS-CoV-2 spike glycoprotein, which contained a total of 118 amino acids of mouse origin (Table S1). We further verified these fragments in the NCBI BLAST reference protein library and the non-redundant protein sequences library, and the results showed that seven fragments are mouse-specific peptides and exist only in mouse databases (*Mus* or *Rattus*) but no other mammal databases (Figure 2), indicating that human SARS-CoV-2 harbors the peptide fragments common with those found in the mouse. Thus, the mouse could be the intermediate host of human SARS-CoV-2.

Fifteen mouse-derived peptide fragments were found in human SARS-CoV with an 100% match, which accounted for 105 amino acids of mouse origin (Table S2). Four of these fragments were verified as rat-specific peptides via NCBI BLAST (Table S2).

The fragments EAEVQID and NHTSPDV are common mouse-derived peptides that exist in both SARS-CoV-2 and SARS-CoV (Table S1). Compared with SARS-CoV, human SARS-CoV-2 possesses more mouse-derived and mouse-specific peptides, indicating higher likelihood of mouse origin than SARS-CoV. There are two mouse-specific heterotopia peptides, NCTEVPVA(E) and ELLHAPA(H), that are uniquely identified in human SARS-CoV-2, but not in other SARS-CoVs derived from humans, civets or bats. Importantly, the mouse derived peptide TQRNFY found in human SARS-CoV-2 is also found in *Klebsella pneumoniae*, which may be related to a potentially shared pathogenetic pathway.

Another fragment HAIHVSGT in the SARS-CoV-2 spike glycoprotein was found to be specifically identical in *Rattus norvegicus* and *Mus musculus* (Figure S1). In comparison with SARS-CoV spike glycoproteins derived from humans, civets and bats, this peptide is located in a specific insertion area of the N-terminal domain in the SARS-CoV-2 spike glycoprotein (Figure 3). We then extracted this specific HAIHVSGT-containing N-terminal region of human SARS-CoV-2 for evolutionary analysis. The results showed that there is a close genetic relationship between human SARS-CoV-2 and SARS-CoV, and some bat SARS-CoVs (Figure 4) on this region, except for the AIHVSGTNGTK fragment specifically in the SARS-CoV-2 spike glycoprotein.

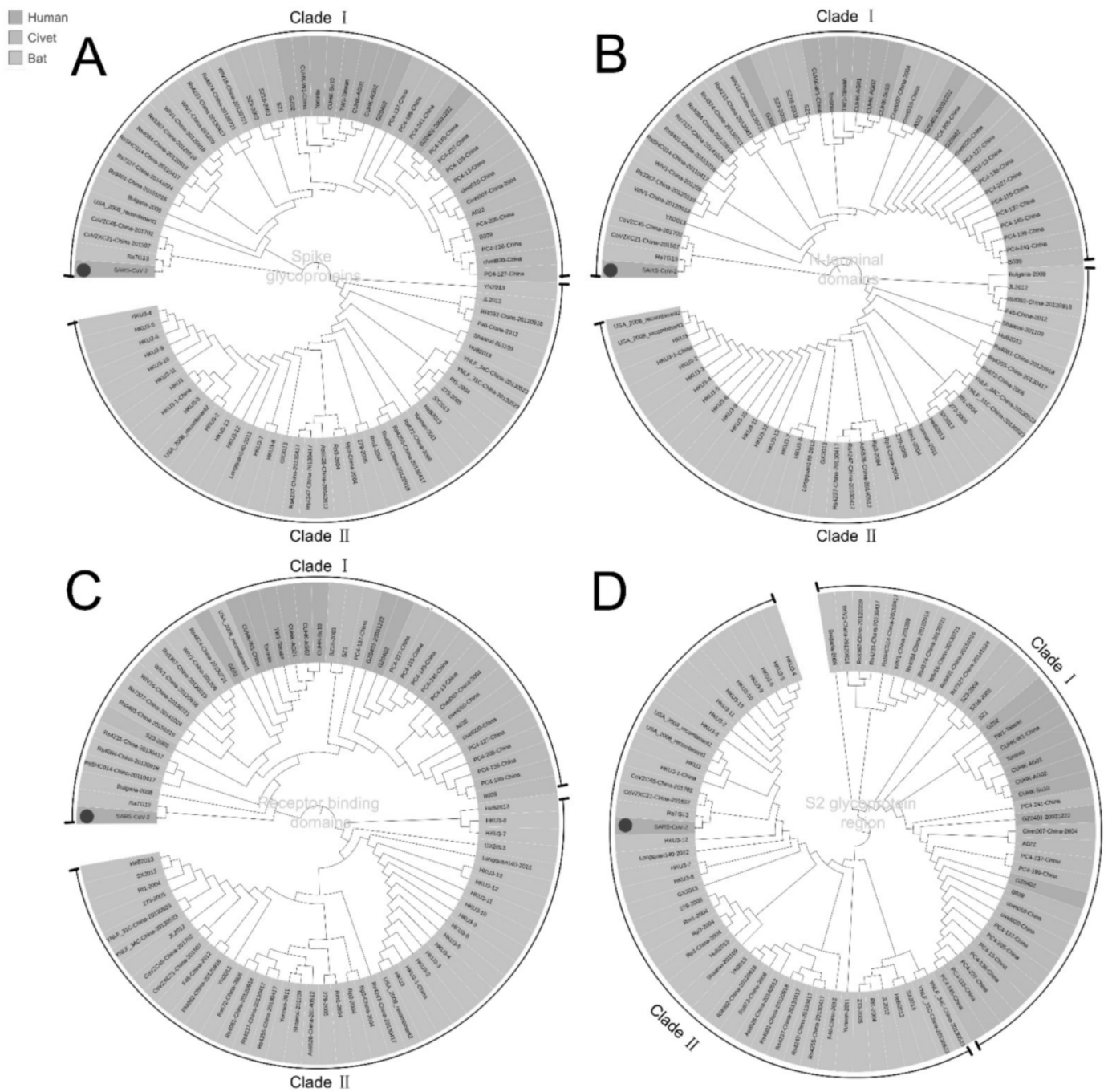


Figure 1. Phylogenetic analysis of different domains in the spike glycoproteins of SARS-CoV-2, SARS-CoVs, and other beta-coronavirus of bat and civet origin using the maximum likelihood method. (A). Full-length spike glycoproteins; (B). The N-terminal domains; (C). Receptor binding domains; (D). S2 glycoprotein region; two clades were divided in all phylogenetic trees. SARS-CoV-2, marked with a red dot, would fall into different subclades according to its different domains.

Protein	Peptide	Match	Nucleotides	Match
SARS-CoV-2	EAEVQID		GAG GCT GAA GTG CAA ATT GAT	
Mus musculus LAMA3	EAEVQID	7/7	GAG GCT GAA GTT CAG ATA GAC	17/21
Brandts bat LAMA3	EAEVQID	5/7	GAG GCC GAG CTG CAG GTG GAC	14/21
Homo sapiens LAMA3	EAEVQID	5/7	GAG GCT GAA CTC CAA GTG GAC	16/21
Felis catus LAMA3	ESELQVD	4/7	GAG TCT GAA CTC CAA GTG GAC	15/21
SARS-CoV-2	NCTEVPVA		AAC TGC ACA GAA GTC CCT GTT GCT	
Mus musculus Exph5	NCTEVPVA	8/8	AAC TGT ACG GAA GTT CCT GTG GCC	19/24
Brandts bat Exph5	SYTEVTVT	4/8	AGC TAC ACA GAA GTC ACT GTG ACC	18/24
Homo sapiens Exph5	SHTEVTVT	4/8	AGC CAT ACA GAA GTC ACT GTG ACC	16/24
Felis catus Exph5	SSTEVTVT	4/8	AGC TCT ACG GAA GTC ACT GTG ACC	16/24
SARS-CoV-2	TMSLGAE		ACT ATG TCA CTT GGT GCA GAA	
Mus musculus ADAMTS13	TMSLGAE	7/7	ACC ATG TCT CTG GGA GCG GAG	15/21
Brandts bat ADAMTS13	DPSLGAQ	4/7	GAC CCG TCC CTG GGC GCC CAG	10/21
Homo sapiens ADAMTS13	DPSLGAQ	4/7	GAC CCG TCC CTG GGG GCT CAG	10/21
Felis catus ADAMTS13	DPSLGTQ	3/7	GAC CCA TCC CTG GGG ACT CAG	8/21
SARS-CoV-2	HAIHVSGT		CAT GCT ATA CAT GTC TCT GGG ACC	
Mus musculus PDLIM7	HAIHVSGT	8/8	CAC GCT ATA CAC GTC TCC GGG ACT	20/24
Brandts bat PDLIM7	HATPASRT	4/8	CAC GCT ACA CCC GCC TCC AGG ACT	16/24
Homo sapiens PDLIM7	NAMAVTSR	2/8	AAT GCC ATG GCT GTG ACT TCA AGA	12/24
Felis catus PDLIM7	HATPASGT	5/8	CAC GCT ACA CCC GCC TCC GGG ACT	17/24
SARS-CoV-2	NHTSPDV		AAT CAT ACA TCA CCA GAT GTT	
Mus musculus Kcnb1	NHTSPDV	7/7	AAC CAC ACC TCC CCG GAC GTG	14/21
Brandts bat Kcnb1	NHISPDV	6/7	AAC CAT ATC TCC CCC GAC GTT	15/21
Homo sapiens Kcnb1	NHISPDV	6/7	AAC CAC ATC TCC CCT GAC GTC	13/21
Felis catus Kcnb1	HQRS AHV	2/8	CAC CAG CGC TCT GCC CAC GTG	9/21
SARS-CoV-2	DSFVIRGD		GAT TCA TTT GTA ATT AGA GGT GAT	
Mus musculus DSCC1	DSFVIRGD	8/8	GAC AGT TTT GTG ATT CGA GGA GAT	17/24
Brandts bat DSCC1	--MVIRGD	5/8	-- -- -- ATG GTG ATT CGT GGT GAT	13/24
Homo sapiens DSCC1	HSLVIRGD	6/8	CAC AGT CTT GTG ATT CGT GGT GAT	15/24
Felis catus DSCC1	HSLVIRGD	6/8	CAC AGT CTT GTG ATT CGT GGT GAT	15/24
SARS-CoV-2	ELLHAPA		GAA CTT CTA CAT GCA CCA GCA	
Mus musculus Wdr75	ELLHAPA	7/7	GAG CTT CTT CAC GCC CCA GCC	16/21
Brandts bat Wdr75	ELLHTPA	6/7	GAG CTG CTT CAC ACC CCC GCC	15/21
Homo sapiens Wdr75	ELLHTPA	6/7	GAG CTT CTT CAC ACT CCA GCC	13/21
Felis catus Wdr75	ELLHTPA	6/7	GAG CTT CTT CAT ACT CCA GCC	16/21

Figure 2. Alignments of the amino acid and nucleotide sequences from SARS-CoV-2, the rodent and other species.

Human	SARS-CoV-2	GVYYPDKVF	FRS	SVLHST	QDLFLPFF	SNVTG	FHAI	HVSGTNGTKR	FDN	47
Human	GZ0401-20031222	GVYYPDEI	FRSDTLYL	TQDLFLPFY	SNVTG	FHTIN	HTFDD		40
Bat	Rs4874-China-20130721	GVYYPDEI	FRSDTLYL	TQDLFLPFY	SNVTG	FHTIN	HREFDN		40
Bat	Rs4231-China-20130417	GVYYPDEI	FRSDTLYL	TQDLFLPFY	SNVTG	FHTIN	HREFDN		40
Bat	WIV16-China-20130721	GVYYPDEI	FRSDTLYL	TQDLFLPFY	SNVTG	FHTIN	HREFDN		40
Civet	Toronto	GVYYPDEI	FRSDTLYL	TQDLFLPFY	SNVTG	FHTIN	HTFGN		40
Civet	TW1-Taiwan	GVYYPDEI	FRSDTLYL	TQDLFLPFY	SNVTG	FHTIN	HTFGN		40
Civet	CUHK-AG01	GVYYPDEI	FRSDTLYL	TQDLFLPFY	SNVTG	FHTIN	HTFGN		40
Civet	CUHK-AG02	GVYYPDEI	FRSDTLYL	TQDLFLPFY	SNVTG	FHTIN	HTFGN		40
Civet	CUHK-Su10	GVYYPDEI	FRSDTLYL	TQDLFLPFY	SNVTG	FHTIN	HTFGN		40
	Consensus	gvyydpd	frs	l	tqdlflpf	snvtg	fh	i	f	

Figure 3. Alignment of the specific HAIHVSGT-containing region in the N-terminal domain of SARS-CoV-2 spike glycoprotein with that of human SARS-CoV and bat SARS-CoV.

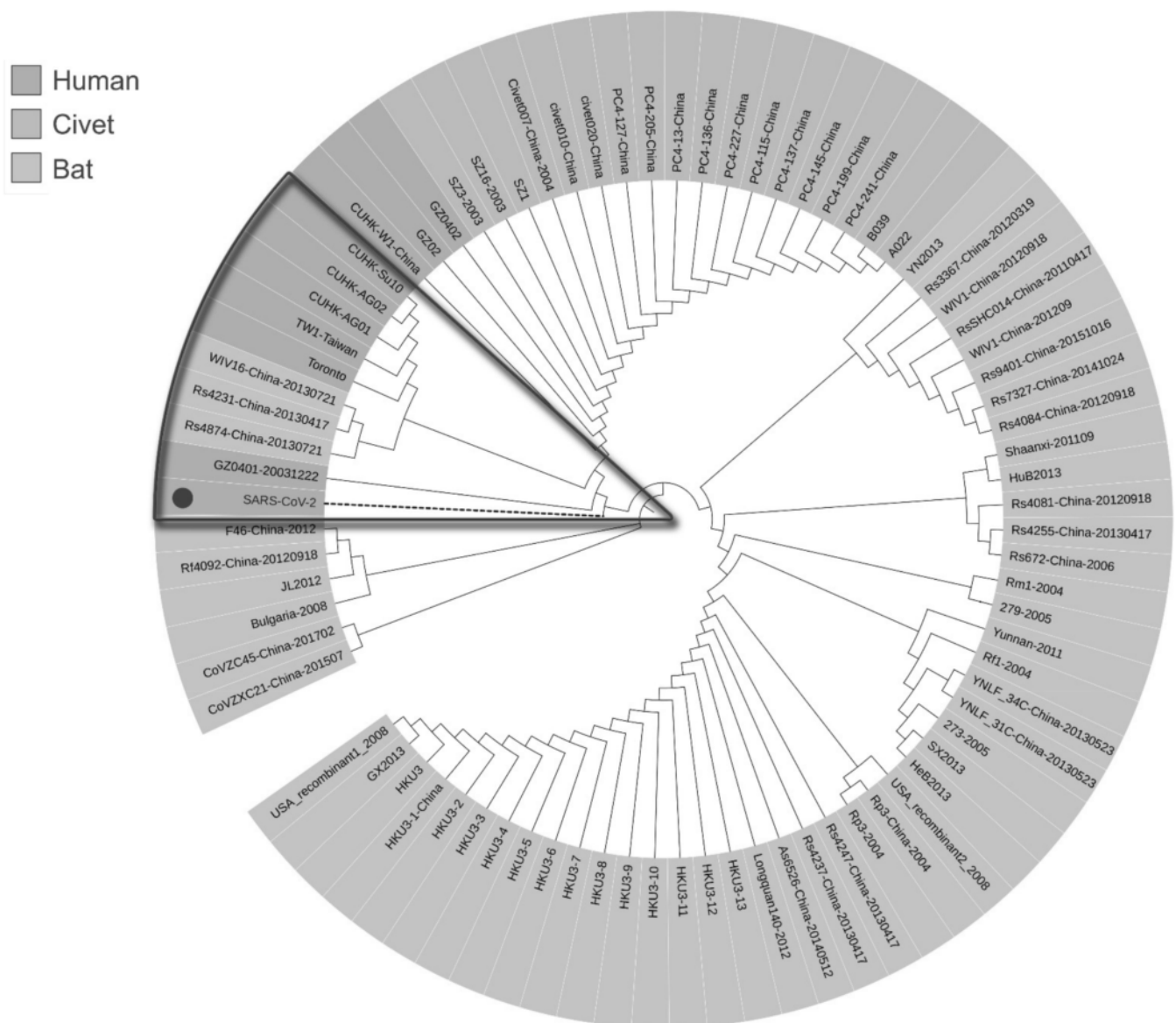


Figure 4. Phylogenetic analysis by the maximum likelihood method for the specific region in the N-Table 2. SARS-CoVs. Sequences that have a close evolutionary relationship with SARS-CoV-2 were highlighted in the blue box.

We further analyzed the homologous sites of the spike glycoproteins in human SARS-CoV-2 and SARS-CoV, as well as civet and bat SARS-CoVs. Among the homologous sites of the spike glycoprotein, only seven sites were found in both human SARS-CoV-2 and

human SARS-CoV, which were different from the highly conserved sites (over 80%) in bat SARS-CoV (Figure S2 and Table S3). The eight identical sites include four in the spike receptor-binding domains, one in the coronavirus S2 glycoprotein peptide and three in the N-terminal domains. Importantly, a locus in the N-terminal domain of SARS-CoV-2 is similar to the counterpart in the human SARS virus but different from the loci found in more than 90% of bat SARS-like viruses. These results suggest that a prerequisite for a coronavirus to have potential for human infection is that it contains at least eight key homologous sites to bat SARS-CoV in its genome. Thus, the accumulation of mutant sites on those specific fragments of bat SARS-like viruses may effectively predict the next virus outbreak.

3.3. Analysis of Virus-HLA Binding Affinity for Allergenicity Assessment

A variety of pathogenic viruses can cause hypersensitivity reactions and are threats to human health. Therefore, it is of interest to investigate the allergenicity between virus proteins and host immune defenses. To assess the allergenicity and the infection potential of the virus to humans, we analyzed and classified HLA molecules that can tightly bind to the spike proteins of SARS-CoV-2 and SARS-CoV. Through the binding affinity analysis of human SARS-CoV-2 and SARS-CoV with HLA class I and II molecules, we found those HLA alleles that are predicted to bind to the spike protein from SARS-CoV-2 are very similar to those from SARS-CoV. The spike proteins from SARS-CoV-2, as well as SARS-CoV, are predicted to bind with high affinity to five human HLA class I alleles (Figure S3) and seven HLA class II molecules (Figure S4). These results suggest that human SARS-CoV-2 and SARS-CoV could induce similar strong immune responses in populations with the same genetic background. However, it is worth noting that the number and location of the HLA alleles with strong affinities for the spike proteins are not exactly the same for human SARS-CoV-2 and SARS-CoV, suggesting that actual immune responses to them may differ.

As listed in Table 1, the HLA class II alleles with a high binding affinity to SARS-CoV-2 include DRB3*03:01, DRB1*10:01, DRB1*09:01, etc. Among them, DRB3*03:01 is the most frequent allele, to which 79.59% of the fragments in S protein of SARS-CoV-2 can bind tightly (Table 1), indicating that patients with this allele may have severe immune responses after SARS-CoV-2 infection. It is worth noting that SARS-CoV also has peptides with a very high binding affinity to this HLA allele (Table S6). Certain HLA alleles are generally prevalent in the population. For example, the proportion of the HLA allele DRB1*09:01 is 24.28% in a population of 103,259 Chinese. These results demonstrate that SARS-CoV-2 is capable of causing severe immune response in most people with the above HLA class II alleles.

$$N_i = \sum_{i=1}^n S_i * Q_i * (2 - Q_i)$$

On the other hand, most of the other HLA alleles exhibited low or no binding affinity to SARS-CoV-2 and SARS-CoV. The frequency of DQB1*03:02, for example, is about 10.54% in the Chinese population (<http://www.allelefrequencies.net>, accessed on 27 August 2021). This allele has a low binding affinity to SARS-CoV-2. In addition, SARS-CoV-2 harbors far fewer peptides with high binding affinity to HLA class I alleles than class II alleles (Tables 1 and S4). For example, the HLA I allele with the highest affinity to SARS-CoV-2, B*15:03, binds 97 peptides from SARS-CoV-2 with high affinity. Furthermore, the total proportion of high-affinity fragments for HLA I molecules in the total peptide fragments from SARS-CoV-2 is only 7.67%. Therefore, it would render SARS-Cov-2 more likely to induce the immune response through specific HLA class I/II molecules, resulting in distinct immune responses in different patients because of the genetic diversity of HLA genes.

Table 1. Specific HLA alleles and high affinity peptides from SARS-CoV-2 spike protein.

HLA Class.	Alleles	No. of High Affinity Peptides	% of High Affinity Peptides	No. of Ultrahigh Affinity Peptides	% of Individuals That Have the Specific HLA Allele in the Population of	
					Chinese	American
I	B*15:03	97	7.67	1	0.18	2.69
I	A*02:03	52	4.11	1	8.12	1.07
I	B*15:17	51	4.03	3	0.81	0.90
I	A*24:03	35	2.77	2	0.43	0.57
I	A*30:01	26	2.06	1	14.27	/
II	DRB3*03:01	998	79.59	64	/	12.72
II	DRB1*10:01	805	64.19	30	2.75	3.41
II	DRB1*09:01	555	44.26	9	24.28	4.94
II	DRB1*16:02	528	42.11	14	5.15	1.96
II	DRB1*13:02	481	38.36	38	7.58	9.56
II	DRB1*01:01	375	29.90	8	4.14	10.75
II	DRB1*11:01	175	13.96	2	8.49	/

Affinity over 0.5 was defined as “high affinity”, therefore, only peptides with affinity over 0.5 were accounted for high affinity peptides. The percentage of high affinity peptides is calculated as total high affinity peptides divided by total peptides that the spike protein harbors and the specific HLA molecule can bind, multiplied by 100. Affinity 0.9 (HLA Class I) and affinity 0.8 (HLA Class II), the top affinities of the panallergen profilin Q64LH0, were defined as the ultrahigh affinity cutoff values. All the original allele frequency and corresponding sample data were downloaded from the website (<http://www.allele-frequencies.net/>), where the frequency of DRB3*03:01 is unavailable for Chinese population, and those of A*30:01 and DRB1*11:01 are unavailable for American population, DRB5*01:01 and DRB3*02:02 (not shown) unavailable for both Chinese and American populations. The percentage of individuals that have the alleles in the sampled subpopulations was calculated based on sample sizes and allele frequencies. According to the Hardy-Weinberg Equilibrium, the number (N_i) of individuals that have the allele in the total of the sampled i subpopulation was roughly calculated based on the sample sizes (S_i) and allele frequencies (Q_i) through the following formula. The larger the subpopulation, the more accurate the N_i result.

3.4. Analysis of Meteorological Factors

Strong allergenicity could be a causative agent of the virus. In addition, the effect of meteorological factors on viral transmission and outbreak at the host population have not yet been determined. It was reported that extreme meteorological factors can accelerate the mutation of viruses [9,10,29–37]. In this study, in order to find out the key climatic features of two coronavirus outbreaks in Wuhan and Guangdong in 2019 and 2002, respectively, we analyzed the climate data (mean temperature, maximum temperature, minimum temperature, precipitation, hours of sunshine, relative humidity) of Wuhan and Guangdong from 1951 to 2019. Based on 12 months and 6 climatic features, we constructed 257,985 combinations with different months and climatic features. Among all possible combinations between the climate factors, 406 combinations exhibited strong correlation between Wuhan 2019 and Guangdong 2002 (no strong correlation existed among other years). The frequency of occurrence and percentage of total combination of corresponding climate feature combinations in the 406 selected combinations are listed in Table S5. For example, precipitation appeared 158 times, which is the highest of all climatic combination features, accounting for 32.31% of the total combinations. In addition, 27.81% of the 406 selected combinations contained relative humidity alone, while 22.29% of them contained both relative humidity and precipitation, indicating that these are the key features of strong correlation between the climates of Wuhan in 2019 and Guangdong in 2002 (Figure 5). These extreme climate factors may accelerate the viral mutation rate, which could be one of the factors causing the virus outbreak.

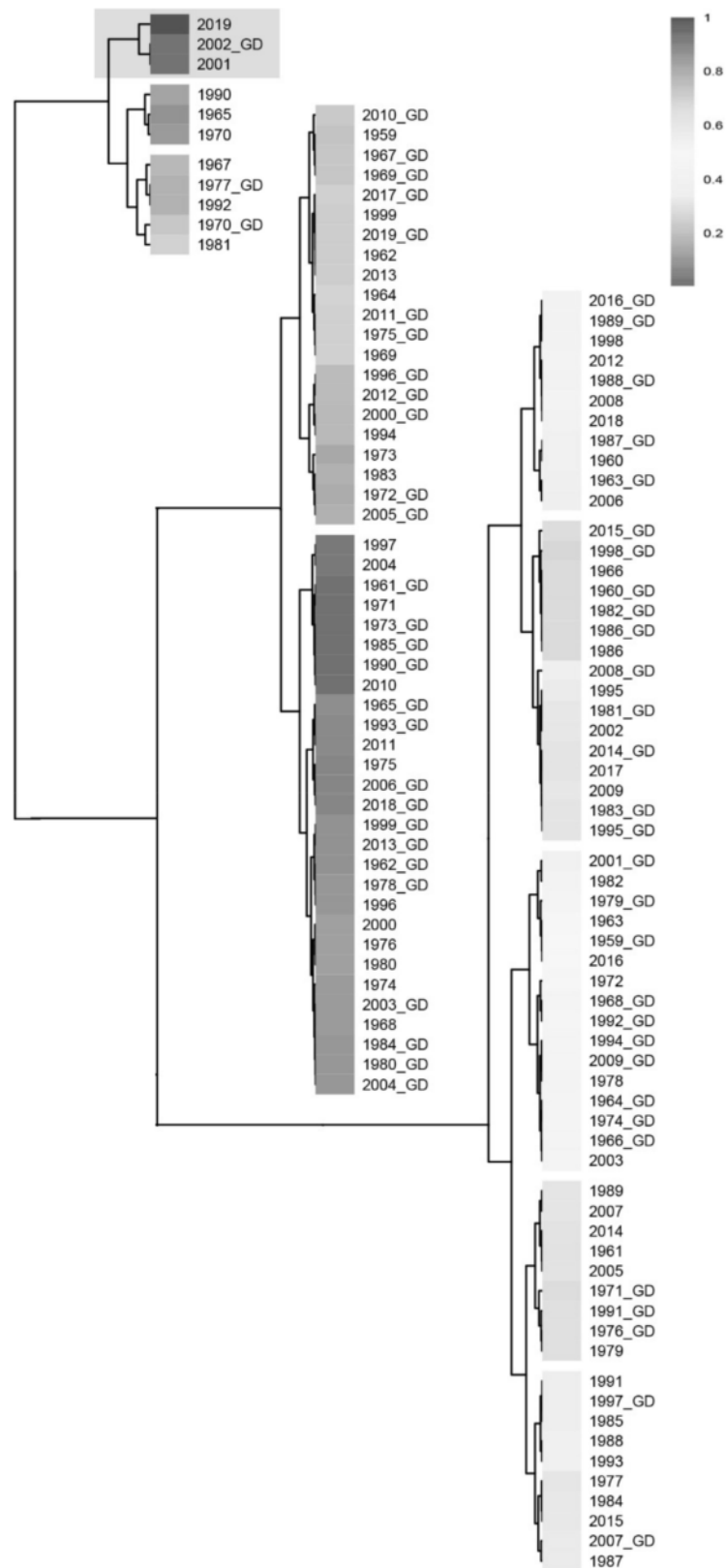


Figure 5. Correlation between Wuhan and Guangdong during 1951–2019 under the combined relative humidity characteristics. The correlation coefficient of each two years were calculated. G, the year of Guangdong. Cell color encodes correlation coefficients (red, positive correlation; blue, negative correlation). Color scale indicates the range of correlation coefficients. The correlation coefficient is assumed to be between 0 and 1, where 1 indicates the strongest possible association and 0 indicates the weakest possible association.

According to a report from the Hubei Meteorological Bureau, the most serious drought in Hubei Province in the past 69 years occurred in the summer and autumn of 2019. During this abnormally long drought, much less precipitation was accompanied by hot weather. In general, a large fluctuation in temperature in Wuhan City between August and October was observed. When the maximum temperature, the minimum temperature, and the precipitation were used as the meteorological parameters from the August to October periods in the past 69 years, the results show that Wuhan 2019 was an independent branch of clustering with the actual climate characteristics (Figure S5). When the mean diurnal ranges of temperature and precipitation were used as parameters, the curves showed the extreme conditions in Wuhan in 2019 in terms of high temperatures and low humidity, similar to but more extreme than the temperature-precipitation relationship in Guangdong 2002–2003 (Figure 6).

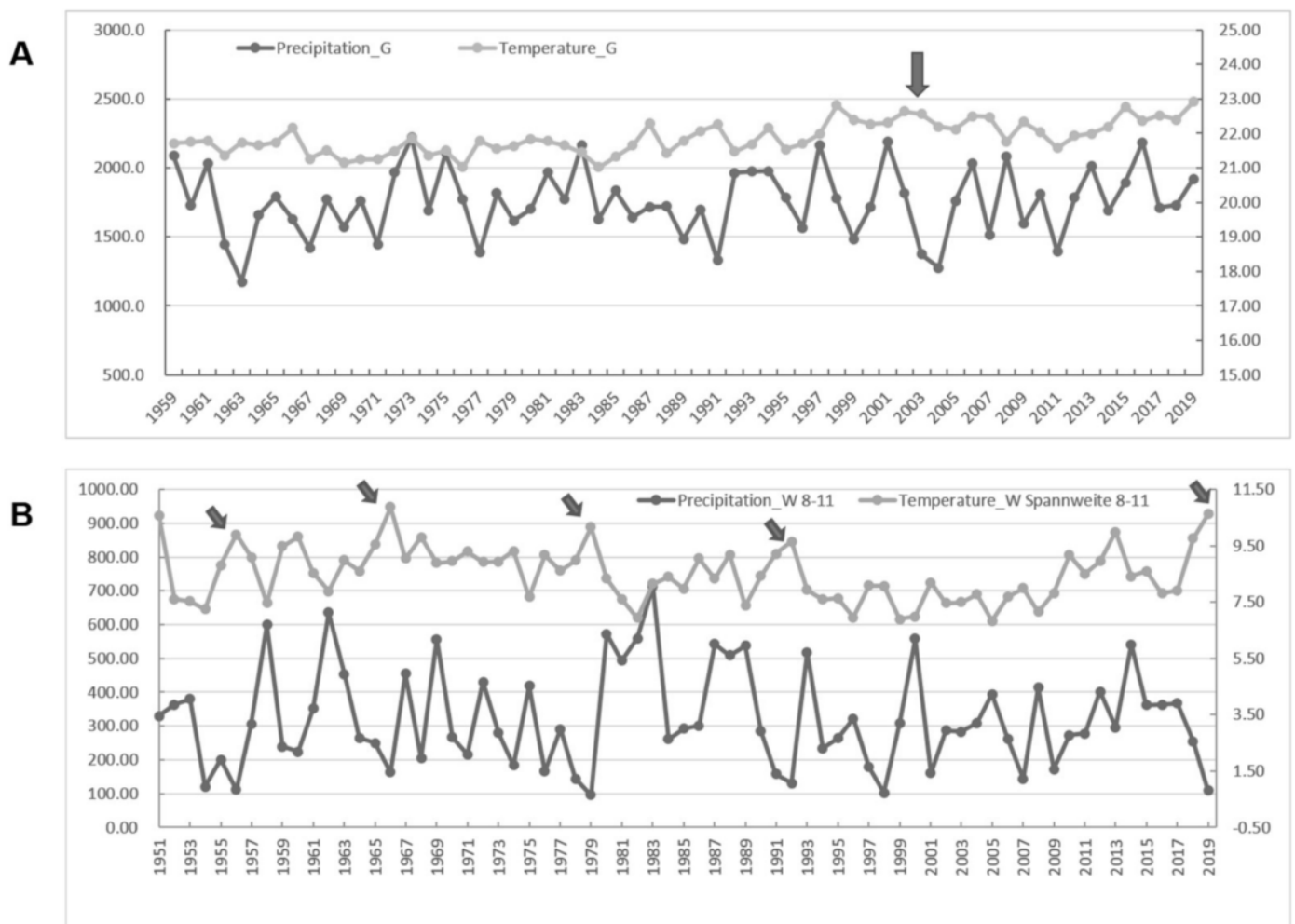


Figure 6. Analysis of meteorological factors in Guangdong and Wuhan in the years 1951 through 2019, where SARS and COVID-19 outbreaks occurred in 2002 and in 2019, respectively. (A). Annual precipitation and annual temperature. (B). Distributions of the mean diurnal range of temperature and precipitation from August through November, during which in 2019, Wuhan experienced a severe drought in the summer and autumn seasons. The arrows point to extremely high temperatures and lower precipitation in years 1955, 1966, 1979, 1992 and especially in 2019. The extreme weather may favor viral mutation to more virulent forms.

4. Discussion

The goal of this study is to investigate the probable intermediate hosts and the allergenicity of the notorious virus SARS-CoV-2 to understand how this virus emerged. The phylogenetic analysis of the virus spike proteins indicates bats as a likely natural origin

and rodents as the intermediate reservoir of SARS-CoV-2 and SARS-CoV. A variety of pathogenic viruses can cause hypersensitivity reactions and are threats to human health. Therefore, we evaluate the allergenicity between virus protein and host immune defenses. The results showed that both SARS-CoV-2 and SARS-CoV are predicted to bind to fourteen HLA class I and II molecules with super-high HLA allele-peptide affinities. Extreme climate might have promoted coronavirus to enable viral transmission and outbreak in the host population. Meteorological factors analysis shows that relative humidity and precipitation could be key factors causing the virus outbreak.

With the number of confirmed COVID-19 cases reaching 5,267,452 as of 24 May 2020, which is far more than the number of cases of severe acute respiratory syndrome (SARS), it is clear that the world is in the midst of a global pandemic. It is of the utmost importance to quickly discover the intermediate hosts of this virus and eradicate the source in order to prevent future outbreaks. Several research groups have recently attempted to address this issue [5–8]. Bats, minks, snakes, and pangolins, and many other creatures seemed to be possible candidates for the interspecies transfer of the novel virus from wildlife to humans, since these animals were sold as delicacies in this market. However, there are some challenging and unexplained facts. The first clinical cases published in *The Lancet* reported that >33% of the cases had no apparent link to the seafood market [2]. According to a report in the *NEJM*, although up to 84.5% of 1099 patients confirmed by laboratories had visited Wuhan city or had contact with Wuhan residents, only 1.9% of these patients had a history of direct contact with wildlife [38], which indicates a high potency of human-to-human transmission of this virus beyond the seafood market origin. Strikingly, of the 585 tested environmental samples, including 70 taken from the wildlife-trading shops and 515 collected from the COVID-19 patients served in shops and related blocks, 33 samples, 31 from the Western zone of the large market where wildlife was sold and 2 from other parts of the market, were positive for SARS-CoV-2 [39]. However, of the 31 positive samples, only 14 were derived from the wildlife-trading shops, whereas 19 positive samples were collected from other kinds of shops (<https://3w.huanqiu.com/a/24d596/9CaKrnKp4T3?agt=8>, accessed on 27 August 2021). Currently, it is unknown how the virus can be transferred directly from animal species to humans in the seafood market and how the virus could be spread among diverse foods in this market. This suggested that there may be animals that freely contact all kinds of foods and spread the virus everywhere in the market.

The house mouse (*Mus musculus*) and Norway rats (*Rattus norvegicus*, also known as brown rats) are the most widely distributed and most successful mammals, except for humans, on the planet and have been commensal with humans for thousands of years [40]. These rodents prefer habitats proximate to human populations and thus are likely to be the intermediate hosts of the virus SARS-CoV-2. In some blocks of the Huanan Seafood Wholesale Market, animals are actively traded as delicacies, with their carcasses and viscera littering away day and night (<https://tech.sina.com.cn/roll/2020-01-23/doc-iihnzhha4251798.shtml>, accessed on 27 August 2021), thereby providing a food source for rats and mice. (<https://tech.sina.com.cn/roll/2020-01-23/doc-iihnzhha4251798.shtml>, accessed on 27 August 2021). The viruses harbored by wild animals would be therefore taken away by the foodies, thus rendering the viruses scattered everywhere in or even outside the Market and then transferred to humans.

In this regard, it is interesting that both SARS-CoV and SARS-CoV-2 possess dozens of fragments derived from rodents (rats and/or mice), respectively. Two fragments (EAEVQID/NHTSPDV) shared by both viruses are more conserved than other proteins encoded by the viruses. This explains why the two viruses cross-react with the antibodies generated against the other [41]. It also strongly suggests that these rodents might be the intermediate hosts of both SARS-CoV and SARS-CoV-2 transferred to humans. This assertion is corroborated by the following facts. Among the samples from the rats and mice captured in Guangzhou hospitals in 2003, 12.5% were SARS-CoV positive by anus swab tests and in these positive samples, 90%–96% exhibited sequence homology with SARS-CoV [42]. Moreover, around the Amoy Gardens housing complex in Hong Kong in

2003, SARS-CoV remnants were detected in four of the eight samples of rat droppings and in the throat or rectal swabs from at least one rat [43].

With regard to SARS-CoV-2, even though the Huanan Seafood Wholesale Market was shut down on 1 January 2020, animal carcasses and viscera were observed and living rats and mice were still present through 17–19 January when the High-level Experts Group of the National Health Commission arrived to investigate the outbreak. Similar to the SARS-CoV outbreak 18 years ago, it is possible that rats and/or mice acquired the SARS-CoV-2 virus from the viscera of butchered animals, including, for example, bats, minks, pangolins that served as natural reservoirs for the virus, when these animals were traded as delicacies in the Market.

Two studies showed that SARS-CoV-2 has infected cat populations in Wuhan during the outbreak and argued that the virus was transmitted in cats [7,8]. This reinforces our proposal that mice and rats are the intermediate sources of SARS-CoV-2, since the rodents could eat many kinds of foods in the Market until they were eaten by cats. This conclusion could be strengthened if the SARS-CoV-2 viral sequence was found in rodents caught around that Market.

Mice and humans have large-scale synteny across over 90% of their genomes but have a much lower extent of sequence orthology covering less than half of the two genomes [44]. Therefore, there are significant differences between the two species, especially within each of their MHC (major histocompatibility complex) genomic regions [45]. In this study, we calculated the binding affinity of the two virus spike proteins with human/mouse MHCs to deduce the allergenicity of the viruses, based on the danger theory [18,46] and the MHC restriction phenomenon [15,16,47,48]. Our results show that five human HLA class I alleles and nine human HLA class II alleles can bind tightly with the S protein fragments of SARS-CoV-2, accounting for 0.18% to 24.28% of the sampled Chinese populations (Table 1). Provided that the locus recombination frequencies of 2%–3% are negligible [49], the proportion of infection-susceptible individuals would be about 7.36% of the Chinese population. These data suggest that people who have these HLA genotypes would be severely affected by COVID-19 and develop obvious pathological symptoms if there were no intervention. The rate of refractory patients calculated from the epidemic data from 14 February to 21 March 2020 in China was 24.7% of the hospitalized patients (about 0.5% of whole Wuhan population), obviously different from the estimated value of 7.36% of the whole population. The discrepancy may result from the powerful non-medical and medical interventions that were implemented to control COVID-19 in China. By contrast, the remaining 92.64% of the population would be healthy without severe symptoms even if they were infected by SARS-CoV-2. Under all kinds of intervention, the proportion of asymptomatic population and paucisymptomatic cases would be much higher than 92.64%, as estimated by different researchers at different times to be 39.9–50.5% [50], 59% [51], or 90% [52] in the Chinese population. Taken together, specific patients who have been infected by SARS-Cov-2 may not have obvious symptoms, making prevention of COVID-19 incredibly challenging. The phenotypic frequencies in different countries are expected to be somewhat different. For example, the rate of refractory virus-susceptible individuals was estimated to be 4.78% of the American population without any interventions (Table 1).

According to sequence alignment with other SARS-CoVs, SARS-CoV-2 has an unusual insert of 10 amino acids (HVSGTNGTKR) in the N-terminal domain (Figure 3). This insert is aligned specifically to RaTK15, a SARS-like coronavirus reported to originate from bats *Rhinolophus affinis* (but not *Rhinolophus sinicus*), with 96.2% identity at the whole-genome level to SARS-CoV-2 [2]. No other animals have been reported with a higher sequence identity with the SARS-CoV-2. Ge et al. strongly suggested that Chinese horseshoe bats were the natural reservoirs of SARS-CoV, and that intermediate hosts may not be necessary for direct human infection by some bat SL-CoVs [53]. It is notable that Chinese horseshoe bats, *R. sinicus* and *R. affinis*, have a similar appearance, and *R. affinis* is the main variety of bat in the Hubei Province. Nevertheless, no SARS-CoV-like virus has been identified from the Hubei *R. affinis* bats, but from the bats *R. macrotis* and *R. ferrumequinum*, in which no

viruses had been isolated by culture with Vero E6 cells from fecal swabs of the PCR-positive samples [54]. In addition, *R. pearsoni* bats are indigenous across the Yunnan province, China and Southeast Asia, and were suggested to harbor coronaviruses closely related to SARS that infected the human population [54]. The study [2] on viral infectivity into HeLa cells with or without the expressions of ACE2 proteins from human, Chinese horseshoe bats (*R. sinicus*, not *R. affinis*), civets, pigs, and mice concluded that SARS-CoV-2 could use all but mouse ACE2 as an entry receptor in the ACE2-expressing cells; that is to say, the mouse ACE2 would not facilitate SARS-CoV-2 entry to mouse cells. This conclusion may be doubtful based on the homology analysis performed on ACE2. The alignment result indicated that the identity of ACE2 amino acid sequences between human and mice (*Mus musculus*) or rats (*Rattus norvegicus*) are 81.05% to 82.49% (Figure 7 and Table 2), respectively, which exceeds the threshold of greater than 70% sequence identities usually required to trigger cross-reactivity between proteins [55]. This empirical law supports that mouse ACE2 is a receptor for SARS-CoV-2. In fact, a previous study reported that SARS-CoVs can proliferate in the mouse without severe symptoms [56]. Even if the above mouse ACE2 assays [2] were correct, an alternative route involving the CD147-spike protein would also help SARS-CoV-2 to invade host cells [57], further explaining the above conflicting results and supporting the rodents as a potential intermediate reservoir of SARS-CoV-2. That is to say, viral proliferation in rodents can be maintained without symptoms because MHC alleles in rodents have no ultrahigh binding affinity to proteins from SARS-CoV-2 or SARS-CoV (data not shown). It is therefore tempting to deduce that the mouse could be a long-term host of human SARS-CoV-2. Furthermore, according to our previous analysis, after a cross-species jump in 1991 and a human-adapted strain formed in 1998, SARS-CoV may still exist in humans (<https://arxiv.org/abs/1305.2659>, accessed on 27 August 2021). Therefore, the entry receptor ACE2 is not a problem for the coronavirus to attack humans from then on, no matter whether RaTK15 was isolated from *R. pearsoni*, *R. sinicus*, or *R. affinis*.

Table 2. Amino acid sequence comparison of human ACE2 with those from rat and mouse.

ACE2 Comparison	Max Score	Query Coverage	E Value	% of Identities
ACE2-Hs1 vs. ACE2-Hs2	1673	99%	0	99.01
ACE2-Hs1 vs. ACE2-mouse	1361	98%	0	81.05
ACE2-Hs1 vs. ACE2-rat	1353	96%	0	82.49
ACE2-Hs2 vs. ACE2-mouse	1369	98%	0	81.86
ACE2-Hs2 vs. ACE2-rat	1360	98%	0	82.37

ACE2 sequence information: **ACE2-Hs1**, ACE2_6M17_D from *Human sapien*. **ACE2-Hs2**, NP_068576.1 from *Human sapien*. **ACE2-mouse**, NP_081562.2 from *Mus musculus*. **ACE2-rat**, NP_001012006.1 from *Rattus norvegicus*.

```

***:*****;*
ACE2-Hs1 MRSSSWLLLSLVAVTAAWSHPQFEKQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNAGDKWSAFLEKQSTLAQMPLOEIQNLTVKLLQALQNGSSVLSBDKSKRLNTILNTMSTIY 136
ACE2-Hs2 -MSSSWLLLSLVAVTAA-----QSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNAGDKWSAFLEKQSTLAQMPLOEIQNLTVKLLQALQNGSSVLSBDKSKRLNTILNTMSTIY 136
ACE2-Mice -MSSSWLLLSLVAVTTA-----QSLTEENAKTFLNFNQEAEDLSYQSSLASWNYNTNITEENAKMSEAAAKWSAFYEEQSKTAQSFSLQEIQTPIIKRQLQALQNGSSSALSADKNKQLNTILNTMSTIY 136
ACE2-Rats -MSSSWLLLSLVAVATA-----QSLIEEKAESEFLNKNFQEAEDLSYQSSLASWNYNTNITEENAKMNEAAAKWSAFYEEQSKIAQNFSLQEIQNATIKRQLKALQNGSSSALSADKNKQLNTILNTMSTIY 136
1.....10.....20.....30.....40.....50.....60.....70.....80.....90.....100.....110.....120.....130.....

*****
ACE2-Hs1 STGKVCNPDNPQECLELLEPGLNEIMANSLDYNERLWAWESWRSEVGVKQLRPLRYEYVVLKNEIMARANHYEDYGDYWRGDEYVNGVDYDYSRGLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLP 272
ACE2-Hs2 STGKVCNPDNPQECLELLEPGLNEIMANSLDYNERLWAWESWRSEVGVKQLRPLRYEYVVLKNEIMARANHYEDYGDYWRGDEYVNGVDYDYSRGLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLP 272
ACE2-Mice STGKVCNPKNPQECLELLEPGLDEIMATSDYNSRLWAWEGWRAEVGVKQLRPLRYEYVVLKNEIMARANHYEDYGDYWRGDEYAEAGDGYNYNRNQLIEDVERTFAEIKPLYEHLHAYVRRKLMYPSYISPTGCLP 272
ACE2-Rats STGKVCNSMNPQECFLLEPGLDEIMATSDYNNRRLWAWEGWRAEVGVKQLRPLRYEYVVLKNEIMARANHYEDYGDYWRGDEYAEAGVEGYNYNRNQLIEDVENTFKEIKPLYEHLHAYVRRKLMVYPSYISPTGCLP 272
.140.....150.....160.....170.....180.....190.....200.....210.....220.....230.....240.....250.....260.....270..

*****
ACE2-Hs1 AHLLGDMWGRFWTNLYSLTVPFQKPNIDVTAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGFRIILMCTKVMTDDFLTAHHEMGIQYDMAYAAQPFLLRNGANEG 408
ACE2-Hs2 AHLLGDMWGRFWTNLYSLTVPFQKPNIDVTAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGFRIILMCTKVMTDDFLTAHHEMGIQYDMAYAAQPFLLRNGANEG 408
ACE2-Mice AHLLGDMWGRFWTNLYPLTVPFAKPNIDVTAMNQGWAERIFQEAKEKFFVSVGLPHMTQGFWANSMLTBPADGRKVVCHPTAWDLGHGDFRIKMCVKVMTDNFLLTAHHEMGIQYDMAYARQPFLLRNGANEG 408
ACE2-Rats AHLLGDMWGRFWTNLYPLTTPFLQKPNIDVTAMVNSQWDAERIFKEAEKFFVSVGLPMTQGFWANSMLTBPADGRKVVCHPTAWDLGHGDFRIKMCVKVMTDNFLLTAHHEMGIQYDMAYARQPFLLRNGANEG 408
....280.....290.....300.....310.....320.....330.....340.....350.....360.....370.....380.....390.....400.....4

*****
ACE2-Hs1 FHEAVGEIMSLSAATPKHLKSIIGLLSPDFQEDNETEINFLKQALITVGTLPFTYMLEKWRWVVFKEIPKQDMKKWWEKREIVGVVEVPVPHDETYCDPASLFHVSNDYSFIRYYTRTYQFQFQALCQAQAH 544
ACE2-Hs2 FHEAVGEIMSLSAATPKHLKSIIGLLSPDFQEDNETEINFLKQALITVGTLPFTYMLEKWRWVVFKEIPKQDMKKWWEKREIVGVVEVPVPHDETYCDPASLFHVSNDYSFIRYYTRTYQFQFQALCQAQAH 544
ACE2-Mice FHEAVGEIMSLSAATPKHLKSIIGLLSPDFQEDSETEINFLKQALITVGTLPFTYMLEKWRWVVFKEIPKQDMKKWWEKREIVGVVEPLPHDETYCDPASLFHVSNDYSFIRYYTRTYQFQFQALCQAQAH 544
ACE2-Rats FHEAVGEIMSLSAATPKHLKSIIGLLSPDFQEDNETEINFLKQALITVGTLPFTYMLEKWRWVVFQDKIPREQWTKKWWWEKREIVGVVEPLPHDETYCDPASLFHVSNDYSFIRYYTRTYQFQFQALCQAQAH 544
10.....420.....430.....440.....450.....460.....470.....480.....490.....500.....510.....520.....530.....540....

;*.*****;*
ACE2-Hs1 EGPLHKCDISNSTEAGQKLFNMLRGLKSEPWTLALENVVGAKNMNVRPLLNYFPLFTWLKDNKNSFVGVSTWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYAMROYFLKVNQMILFGEEDVR 680
ACE2-Hs2 EGPLHKCDISNSTEAGQKLFNMLRGLKSEPWTLALENVVGAKNMNVRPLLNYFPLFTWLKDNKNSFVGVSTWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYAMROYFLKVNQMILFGEEDVR 680
ACE2-Mice NGSLHKCDISNSTEAGQKLLKMLSLGNSPWTALALENVVGARNMDVPLNYFQPLFDWLKEQNRNSFVGVWNTWSPYADQSIKVRISLKSALGANAYEWNNDNEMFLFRSSVAYAMRKYFSIIKNQTVPFLEEDVR 680
ACE2-Rats DGPLHKCDISNSTEAGQKLLNMLSLGNSPWTALALENVVGSRNMDVPLNYFQPLFVWLKEQNRNSTVGVSTWSPYADQSIKVRISLKSALGANAYEWNNDNEMYLFRSSVAYAMREYFSREKNQTVPFLEEDVR 680
...550.....560.....570.....580.....590.....600.....610.....620.....630.....640.....650.....660.....670.....680

*::****;*
ACE2-Hs1 VANLKPRISENFVVTAPKNVSDIIPRTEVEKAIIRMSRSRINDAFRLNDNSLEFLGIQPTLGPNNQPPVSIWLIVFVGVVGVIVVGVILVIFPTGIRDRKKKKNKARSGENPYASIDI SKGENNPGFQNTDDVQTSF 814
ACE2-Hs2 VANLKPRISENFVVTAPKNVSDIIPRTEVEKAIIRMSRSRINDAFRLNDNSLEFLGIQPTLGPNNQPPVSIWLIVFVGVVGVIVVGVILVIFPTGIRDRKKKKNKARSGENPYASIDI SKGENNPGFQNTDDVQTSF 814
ACE2-Mice VSDLKPRVSYFFVVTSPQNVSDIIPRSEVEDAIRMSRGRINDVFLNDNSLEFLGIHPTLEPPYQPPVTIWLIIIFGVVGVVGVVGVVGVILVIVTGIRKGRKKKNETKREENPYDSMDIGKESNAGFQNSDDAQTSTF 814
ACE2-Rats VSDLKPRVSYFFVVTSPKNVSDIIPRSEVEEAIIRMSRGRINDIFGLNDNSLEFLGIYPTLKPYPPEPVTIWLIIIFGVVGVVGVVGVVGVILVIVTGIRKGRKKKNETKREENPYDSMDIGKESNAGFQNSDDAQTSTF 814
.....690.....700.....710.....720.....730.....740.....750.....760.....770.....780.....790.....800.....810....

ACE2 sequence information: ACE2-Hs1: 6M17_D; ACE2-Hs2: NP_068576.1; ACE2-Mice: NP_081562.2; ACE2-Rats: NP_001012006.1;

```

Figure 7. ACE2 Sequence alignment of humans, mice and rats.

SARS-CoV-2 is a positive-sense, single-stranded RNA coronavirus. It possesses a large RNA genome and undergoes RNA recombination, as in other coronaviruses, at a high frequency of nearly 25% for the entire genome [58], thus driving frequent species-transmission adaptation. Another report suggested that SARS-CoVs were likely caused by mutations and natural selection in addition to recombination [59]. Moreover, an average female rodent gives birth approximately seven times per year, which would lead to much a higher rate of mutations of the viruses maintained, compared to a deduced general mutation rate of $0.80\sim 2.38 \times 10^{-3}$ nucleotide substitutions per site per year for SARS-CoV [60]. Furthermore, during the August through November period in 2019, the most serious drought and highest temperatures in the summer and autumn time frame were experienced in Wuhan in the past 68 years (Figure 6). This climate could have provided favorable conditions for virus mutation from a mild form to the highly virulent SARS-CoV-2. Based on the causality triangle of viruses, hosts and environmental conditions, even if the nucleotide fragments of an intermediate host were integrated into the virus, those fragments could not easily be detected because of RNA recombination. Since cross-species transmissibility depends on protein functions, the amino acid sequences of the virus can provide compelling evidence to support identification of intermediate hosts.

Interestingly, both SARS-CoV and SARS-CoV-2 are inherently capable of reacting with different allelic forms of HLA molecules and tightly binding dozens of different HLA molecules. This means that these two viruses would have similar allergenicity and would trigger similar pathophysiological insults in humans. This is supported by autopsy and biopsies of cadavers of patients who died from SARS-CoV-2, because the pathological characteristics of COVID-19 strongly resemble those seen in SARS and Middle Eastern respiratory syndrome (MERS) coronavirus infections [61–63]. However, the cytokine-based endotypes of critically ill COVID-19 patients who are insensitive to treatment with steroids because of an increased concentration of the highly proinflammatory cytokine IL-17A produced by $CCR4^+CCR6^+$ Th17 in $CD4^+$ T cells [63,64] would be quite different from those of SARS patients for whom steroid treatment is beneficial because of the increased presence of type 2 cytokines [65,66]. Therefore, clinical treatments for SARS-CoV-2 patients will be different from those employed on SARS patients. On the other hand, unlike human HLA, mouse MHC does not bind strongly to the spike protein of SARS-CoV-2, suggesting that there would be no symptoms when rodents become infected with this virus. In fact, although SARS-CoV can replicate in the lungs of young mice following infection, such mice do not harbor replicated SARS-CoV in both lung and intestinal tissue and they do not show signs of illness. These mice present either subclinical infection or very mild disease after simultaneous inoculation intranasally and orally [56,67]. Therefore, it is likely that these rodents would not become ill in response to SARS-CoV-2 infection even if they harbored this virus. This situation provides conditions for the spread of the virus in humans and the rodents until herd immunity develops in the two populations. This is corroborated by our previous research data showing that SARS-CoV may still exist in humans (<https://arxiv.org/abs/1305.2659>, accessed on 27 August 2021).

It is thus likely that a SARS epidemic could recur when the meteorological conditions in the world are suitable for SARS-CoV-2 mutation. The virus would be maintained in general populations who have no high binding-affinity HLA alleles and be transferred between individuals. As described above, more than 92.64% of the population harbors the virus with no obvious symptoms, meaning that many people will be SARS-CoV-2 positive as detected by nucleic acid testing. We have demonstrated that weakly virulent SARS-CoVs might still exist in humans for years (<https://arxiv.org/abs/1305.2659>, accessed on 27 August 2021). These existing SARS-CoVs have significant potential to evolve into highly virulent strains when favorable meteorological conditions occur, highlighting the potential risk for reemergence of SARS as well. Based on the mutation rate of coronavirus and meteorological extremes occurring because of climate change, we speculate that SARS could re-emerge in the near future in a new form. A SARS vaccine is therefore urgently needed. However, a SARS-like chimeric virus experiment demonstrated that

both monoclonal antibody and vaccine approaches and prophylactic modalities failed to neutralize and protect from infection by those CoVs that possess a novel spike protein [68]. Advanced strategies and regimens will need to be developed. For example, a novel vaccine against a pool of the most highly virulent mutant strains could be prepared in advance.

Hypothesis 1. *Self-limitation and spontaneous mutation within the virus-infected population.*

With these results, we therein proposed a hypothesis for the future course of the coronaviruses. When a virus mutates into a novel one and severely infects one (or several) group(s) of individuals with specific MHC genotype(s), these susceptible individuals either die or heal with the development of immunity. The vast majority of individuals who range from being paucisymptomatic to asymptomatic or having recovered from the disease still harbor the novel virus while it is spread within the population with no epidemic. When conditions favor mutations, the virus becomes more virulent and targets individuals harboring other type(s) of MHC genotype(s), leading to soaring infection numbers and another epidemic. A new cycle would therefore start within the population. When the virus has acquired mutations favoring a cross-species jump, increasing infection numbers would lead to an epidemic followed by asymptomatic transmission within the new species into which the virus has jumped. This phenomenon will occur in many different mammals including humans, bats, rodents, etc. Furthermore, when the virus accumulates the requisite mutations enabling interspecies transmission and binding of all MHC alleles among another species population with high affinity, a much deadlier super virus could emerge to eradicate the species. The only way to defend against such a super virus is to employ prophylactic modalities, such as early immunization with a hypoallergenic virus that has been gradually attenuated from the super virus, as exemplified by the incidence of smallpox versus cowpox.

In summary, our results indicate that both SARS-CoV-2 and SARS-CoV are naturally originated from bats and might be transmitted to humans through rodents. This was demonstrated by carrying out comprehensive amino acid sequence analysis and comparison of sliding sequence fragments of the novel virus with all sequences from mammals available in the NCBI database. SARS-CoV-2 and SARS-CoV have similar binding affinities to the HLA antigen and would have similar potential to induce inflammation. Different populations have distinct allele distribution patterns and thus variable infection rates. It is predicted that the virus will severely infect 4.78% to 7.36% of the American and Chinese populations, respectively, and would make them suffer severe symptoms. Meteorological factor analysis indicates that Wuhan 2019 and Guangdong 2002–2003 have similar climate features, with extremely high temperatures and exceptionally low precipitation, which might imply some link between the climate environment and the survival and development of the coronaviruses. Early immunization with allergenically-engineered virus together with a continued surveillance of meteorological factors and viral mutations may serve as one of the most powerful prophylactic modalities to fight this virus.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/healthcare9091132/s1>, Figure S1. NCBI BLAST results (20200121) of HAIHVSGT, a murine specific peptide in the SARS-CoV-2 Spike glycoprotein using the NCBI reference protein library, Figure S2. The key sites for bat SARS-like virus infecting human. The key positions in the three functional regions of the spike glycoprotein (N-terminal domain, receptor binding domain, and Coronavirus S2 glycoprotein), and the amino acid distribution in human SARS-CoV and bat SARS-CoV are presented, Figure S3. Analysis of HLA class I molecular binding affinity with SARS-CoV-2 and SARS-CoV. HLA class I molecular were divided into two groups: high binding capacity and low binding capacity. For each molecular, the binding affinity with four different functional segments (marked with different colors) of spike glycoprotein were presented, Figure S4. Analysis of HLA class II molecular binding affinity with SARS-CoV-2 and SARS-CoV. HLA class II molecular were divided into two groups: high binding capacity and low binding capacity. For each molecular, the binding affinity with four different functional segments (marked with different colors) of Spike glycoprotein,

Figure S5. Correlation between specific climate characteristics including maximum temperature, minimum temperature and precipitation in Wuhan from 1951 to 2019. Cell color encodes correlation coefficients (Red, positive correlation; Blue, negative correlation). Color scale indicates the range of correlation coefficients. The correlation coefficient is assumed to be between 0 and 1, where 1 indicates the strongest possible associations and 0 indicates the weakest possible association. Table S1. Similarity screening of SARS-CoV-2 Spike glycoprotein in protein database were presented, Table S2. Similarity screening of SARS-CoV Spike glycoprotein peptides in protein database, Table S3. The pivotal loci for bat SARS-like viruses to infect humans, Table S4. Binding affinity of HLA Class I and II molecules with SARS-CoV spike protein, Table S5. The sample size and risk individuals of HLA Class I/II in Chinese and American, Table S6. Correlation analysis of climatic feature combinations between Wuhan 2019 and Guangzhou 2002.

Author Contributions: Data curation, Y.H. (Yuyi Huang), W.S. and Y.H. (Ying He); formal analysis, Y.H. (Yuyi Huang), J.X. and Y.G.; funding acquisition, A.T.; resources, K.L.; supervision, N.Z.; writing—original draft, J.Y.; writing—review & editing, A.T. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Natural Science Foundation of China (Grants No 81871266, 82100025).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: We are grateful for the academic help and wonderful research organization by Qifa Zhang, Professor of Huazhong Agricultural University. We thank Guojun Wang, Assistant Professor of the Harbor Branch Oceanographic Institute, Florida Atlantic University, Jongdae Lee and Weijie Guan at Guangzhou Medical University and Chiyu Zhang at the Pathogen Diagnostic Center, Institut Pasteur of Shanghai, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences for critical reading and the scientific discussion.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mišek, J.; Blicharz-Domańska, K. Coronaviruses in avian species—Review with focus on epidemiology and diagnosis in wild birds. *J. Vet. Res.* **2018**, *62*, 249–255. [CrossRef]
2. Zhou, P.; Yang, X.-L.; Wang, X.-G.; Hu, B.; Zhang, L.; Zhang, W.; Si, H.-R.; Zhu, Y.; Li, B.; Huang, C.-L.; et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **2020**, *579*, 270–273. [CrossRef]
3. Wu, F.; Zhao, S.; Yu, B.; Chen, Y.-M.; Wang, W.; Song, Z.-G.; Hu, Y.; Tao, Z.-W.; Tian, J.-H.; Pei, Y.-Y.; et al. A new coronavirus associated with human respiratory disease in China. *Nature* **2020**, *579*, 265–269. [CrossRef] [PubMed]
4. Guo, Q.; Li, M.; Wang, C.; Wang, P.; Fang, Z.; Tan, J.; Wu, S.; Xiao, Y.; Zhu, H. Host and infectivity prediction of Wuhan 2019 novel coronavirus using deep learning algorithm. *BioRxiv* **2020**. [CrossRef]
5. Ji, W.; Wang, W.; Zhao, X.; Zai, J.; Li, X. Homologous recombination within the spike glycoprotein of the newly identified coronavirus may boost cross-species transmission from snake to human. *J. Med. Virol.* **2020**, *92*, 433–440. [CrossRef]
6. Lam, T.T.-Y.; Jia, N.; Zhang, Y.-W.; Shum, M.H.-H.; Jiang, J.-F.; Zhu, H.-C.; Tong, Y.-G.; Shi, Y.-X.; Ni, X.-B.; Liao, Y.-S.; et al. Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. *Nature* **2020**, *583*, 282–285. [CrossRef]
7. Zhang, Q.; Zhang, H.; Gao, J.; Huang, K.; Yang, Y.; Hui, X.; He, X.; Li, C.; Gong, W.; Zhang, Y.; et al. A serological survey of SARS-CoV-2 in cat in Wuhan. *Emerg. Microbes Infect.* **2020**, *9*, 2013–2019. [CrossRef]
8. Shi, J.; Wen, Z.; Zhong, G.; Yang, H.; Wang, C.; Huang, B.; Liu, R.; He, X.; Shuai, L.; Sun, Z.; et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS–coronavirus 2. *Science* **2020**, *368*, 1016–1020. [CrossRef] [PubMed]
9. Streicker, D.G.; Lemey, P.; Velasco-Villa, A.; Rupperecht, C.E. Rates of Viral Evolution Are Linked to Host Geography in Bat Rabies. *PLoS Pathog.* **2012**, *8*, e1002720. [CrossRef]
10. Matson, M.J.; Yinda, C.K.; Seifert, S.N.; Bushmaker, T.; Fischer, R.J.; Van Doremalen, N.; Lloyd-Smith, J.O.; Munster, V.J. Effect of Environmental Conditions on SARS-CoV-2 Stability in Human Nasal Mucus and Sputum. *Emerg. Infect. Dis.* **2020**, *26*, 2276–2278. [CrossRef] [PubMed]
11. Li, J.; Rao, Y.; Sun, Q.; Wu, X.; Jin, J.; Bi, Y.; Chen, J.; Lei, F.; Liu, Q.; Duan, Z.; et al. Identification of climate factors related to human infection with avian influenza A H7N9 and H5N1 viruses in China. *Sci. Rep.* **2015**, *5*, 18094. [CrossRef] [PubMed]
12. Zhang, J.; Tao, A. Antigenicity, Immunogenicity, Allergenicity. In *Allergy Bioinformatics*; Tao, A., Raz, E., Eds.; Springer: Berlin/Heidelberg, Germany, 2015; Volume 8, pp. 175–186.
13. Knierman, M.D.; Lannan, M.B.; Spindler, L.J.; McMillian, C.L.; Konrad, R.J.; Siegel, R.W. The human leukocyte antigen class II immunopeptidome of the SARS-CoV-2 spike glycoprotein. *Cell Rep.* **2020**, *33*, 108454. [CrossRef] [PubMed]

14. Takagi, A.; Matsui, M. Identification of HLA-A* 02:01-Restricted Candidate Epitopes Derived from the Nonstructural Polyprotein 1a of SARS-CoV-2 That May Be Natural Targets of CD8 + T Cell Recognition In Vivo. *J. Virol.* **2021**, *95*. [CrossRef] [PubMed]
15. Sinigaglia, F.; Guttinger, M.; Romagnoli, P.; Takacs, B. Malaria antigens and MHC restriction. *Immunol. Lett.* **1990**, *25*, 265–270. [CrossRef]
16. Kazansky, D.B. MHC restriction and allogeneic immune responses. *J. Immunotoxicol.* **2008**, *5*, 369–384. [CrossRef]
17. Sakuraba, A.; Haider, H.; Sato, T. Population Difference in Allele Frequency of HLA-C * 05 and Its Correlation with COVID-19 Mortality. *Viruses* **2020**, *12*, 1333. [CrossRef]
18. Matzinger, P. The Danger Model: A Renewed Sense of Self. *Science* **2002**, *296*, 301–305. [CrossRef]
19. Jahrling, P.B.; Hensley, L.; Martinez, M.J.; LeDuc, J.W.; Rubins, K.H.; Relman, D.; Huggins, J.W. From The Cover: Exploring the potential of variola virus infection of cynomolgus macaques as a model for human smallpox. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15196–15200. [CrossRef]
20. He, Y.; Liu, X.; Huang, Y.; Zou, Z.; Chen, H.; Lai, H.; Zhang, L.; Wu, Q.; Zhang, J.; Wang, S.; et al. Reduction of the Number of Major Representative Allergens: From Clinical Testing to 3-Dimensional Structures. *Mediat. Inflamm.* **2014**, *2014*, 1–11. [CrossRef]
21. Asero, R.; Monsalve, R.; Barber, D. Profilin sensitization detected in the office by skin prick test: A study of prevalence and clinical relevance of profilin as a plant food allergen. *Clin. Exp. Allergy* **2008**, *38*, 1033–1037. [CrossRef]
22. Asero, R.; Tripodi, S.; Dondi, A.; Businco, A.D.R.; Sfika, I.; Bianchi, A.; Candelotti, P.; Caffarelli, C.; Dascola, C.P.; Ricci, G.; et al. Prevalence and Clinical Relevance of IgE Sensitization to Profilin in Childhood: A Multicenter Study. *Int. Arch. Allergy Immunol.* **2015**, *168*, 25–31. [CrossRef] [PubMed]
23. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874. [CrossRef]
24. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K.; Battistuzzi, F.U. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef] [PubMed]
25. Andreatta, M.; Nielsen, M. Gapped sequence alignment using artificial neural networks: Application to the MHC class I system. *Bioinformatics* **2015**, *32*, 511–517. [CrossRef] [PubMed]
26. Nielsen, M.; Lundegaard, C.; Worning, P.; Lauemøller, S.L.; Lamberth, K.; Buus, S.; Brunak, S.; Lund, O. Reliable prediction of T-cell epitopes using neural networks with novel sequence representations. *Protein Sci.* **2003**, *12*, 1007–1017. [CrossRef] [PubMed]
27. Jensen, K.K.; Andreatta, M.; Marcatili, P.; Buus, S.; Greenbaum, J.A.; Yan, Z.; Sette, A.; Peters, B.; Nielsen, M. Improved methods for predicting peptide binding affinity to MHC class II molecules. *Immunology* **2018**, *154*, 394–406. [CrossRef]
28. González-Galarza, F.F.; Takeshita, L.Y.; Santos, E.J.; Kempson, F.; Maia, M.H.T.; Da Silva, A.L.S.; Silva, A.L.T.; Ghataoraya, G.; Alfirevic, A.; Jones, A.; et al. Allele frequency net 2015 update: New features for HLA epitopes, KIR and disease and HLA adverse drug reaction associations. *Nucleic Acids Res.* **2014**, *43*, D784–D788. [CrossRef]
29. Chen, Z.; Zhu, Y.; Wang, Y.; Zhou, W.; Yan, Y.; Zhu, C.; Zhang, X.; Sun, H.; Ji, W. Association of meteorological factors with childhood viral acute respiratory infections in subtropical China: An analysis over 11 years. *Arch. Virol.* **2013**, *159*, 631–639. [CrossRef]
30. Morin, C.W.; Stoner-Duncan, B.; Winker, K.; Scotch, M.; Hess, J.J.; Meschke, J.S.; Ebi, K.L.; Rabinowitz, P.M. Avian influenza virus ecology and evolution through a climatic lens. *Environ. Int.* **2018**, *119*, 241–249. [CrossRef]
31. Mirsaedi, M.; Motahari, H.; Khamesi, M.T.; Sharifi, A.; Campos, M.; Schraufnagel, D.E. Climate Change and Respiratory Infections. *Ann. Am. Thorac. Soc.* **2016**, *13*, 1223–1230. [CrossRef] [PubMed]
32. Martin, G.A.; Yañez-Arenas, C.; Roberts, B.J.; Chen, C.; Plowright, R.K.; Webb, R.J.; Skerratt, L. Climatic suitability influences species specific abundance patterns of Australian flying foxes and risk of Hendra virus spillover. *One Health* **2016**, *2*, 115–121. [CrossRef]
33. Chen, Y.; Liu, Q.; Guo, D. Emerging coronaviruses: Genome structure, replication, and pathogenesis. *J. Med. Virol.* **2020**, *92*, 418–423. [CrossRef]
34. Tamerius, J.D.; Shaman, J.; Alonso, W.; Bloom-Feshbach, K.; Uejio, C.K.; Comrie, A.; Viboud, C. Environmental Predictors of Seasonal Influenza Epidemics across Temperate and Tropical Climates. *PLoS Pathog.* **2013**, *9*, e1003194. [CrossRef]
35. Sloan, C.; Moore, M.L.; Hartert, T. Impact of Pollution, Climate, and Sociodemographic Factors on Spatiotemporal Dynamics of Seasonal Respiratory Viruses. *Clin. Transl. Sci.* **2011**, *4*, 48–54. [CrossRef]
36. Tarek, F.; Hassou, N.; Benchekroun, M.N.; Boughribil, S.; Hafid, J.; Bessi, H.; Ennaji, M.M. Impact of rotavirus and hepatitis A virus by worldwide climatic changes during the period between 2000 and 2013. *Bioinformation* **2019**, *15*, 194–200. [CrossRef]
37. Kim, H.-C.; Gu, S.H.; Kim, W.-K.; Klein, T.A.; Lee, S.-H.; No, J.S.; Chong, S.-T.; Song, J.-W. Urban Rodent Surveillance, Climatic Association, and Genomic Characterization of Seoul Virus Collected at U.S. Army Garrison, Seoul, Republic of Korea, 2006–2010. *Am. J. Trop. Med. Hyg.* **2018**, *99*, 470–476. [CrossRef]
38. Guan, W.-J.; Ni, Z.-Y.; Hu, Y.; Liang, W.-H.; Ou, C.-Q.; He, J.-X.; Liu, L.; Shan, H.; Lei, C.-L.; Hui, D.S.; et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N. Engl. J. Med.* **2020**, *382*, 1708–1720. [CrossRef] [PubMed]
39. Cohen, J. Mining coronavirus genomes for clues to the outbreak's origins. *Science* **2020**, *31*. [CrossRef]
40. König, B. Chapter 2.11—The Behaviour of the House Mouse. In *The Laboratory Mouse*, 2nd ed.; Hedrich, H.J., Ed.; Elsevier: Amsterdam, The Netherlands, 2012; pp. 367–381. [CrossRef]

41. Lv, H.; Wu, N.C.; Tsang, O.T.-Y.; Yuan, M.; Perera, R.A.; Leung, W.S.; So, R.T.; Chan, J.M.C.; Yip, G.K.; Chik, T.S.H.; et al. Cross-reactive Antibody Response between SARS-CoV-2 and SARS-CoV Infections. *Cell Rep.* **2020**, *31*, 107725. [CrossRef] [PubMed]
42. Yi, J.-R.; Lin, L.-F.; Duan, J.-H.; Wu, J.; Pei, F.-Q.; Lu, W.-C.; Cai, S.-W.; Zheng, H.-Y.; Yin, W.-X. Analysis on murine-like animals carrying SARS coronavirus in the hygienic units related to SARS. *Chin. J. Vector Biol. Control.* **2004**, *15*, 7–9, (In Chinese with English Abstract).
43. Ng, S.K.C. Possible role of an animal vector in the SARS outbreak at Amoy Gardens. *Lancet* **2003**, *362*, 570–572. [CrossRef]
44. Mouse Genome Sequencing Consortium Initial sequencing and comparative analysis of the mouse genome. *Nature* **2002**, *420*, 520–562. [CrossRef]
45. Yuhki, N.; Beck, T.; Stephens, R.M.; Nishigaki, Y.; Newmann, K.; O'Brien, S.J. Comparative Genome Organization of Human, Murine, and Feline MHC Class II Region. *Genome Res.* **2003**, *13*, 1169–1179. [CrossRef] [PubMed]
46. Matzinger, P. The evolution of the danger theory. *Expert Rev. Clin. Immunol.* **2012**, *8*, 311–317. [CrossRef] [PubMed]
47. Braciale, T.J.; Morrison, L.A.; Sweetser, M.T.; Sambrook, J.; Gething, M.-J.; Braciale, V.L. Antigen Presentation Pathways to Class I and Class II MHC-Restricted T Lymphocytes. *Immunol. Rev.* **1987**, *98*, 95–114. [CrossRef]
48. Parham, P. Pictures of MHC restriction. *Nature* **1996**, *384*, 109–110. [CrossRef]
49. Hamed, C.T.; Meiloud, G.; Veten, F.; Hadrami, M.; Ghaber, S.M.; Boussaty, E.C.; Habti, N.; Houmeida, A. HLA class I (-A, -B, -C) and class II (-DR, -DQ) polymorphism in the Mauritanian population. *BMC Med. Genet.* **2018**, *19*, 1–10. [CrossRef]
50. Mizumoto, K.; Kagaya, K.; Zarebski, A.; Chowell, G. Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. *Eurosurveillance* **2020**, *25*, 2000180. [CrossRef]
51. Wang, C.; Liu, L.; Hao, X.; Guo, H.; Wang, Q.; Huang, J.; He, N.; Yu, H.; Lin, X.; Pan, A.; et al. Evolving epidemiology and impact of non-pharmaceutical interventions on the outbreak of coronavirus disease 2019 in Wuhan, China. *medRxiv* **2020**. [CrossRef]
52. Dong, Y.; Mo, X.; Hu, Y.; Qi, X.; Jiang, F.; Jiang, Z.; Tong, S. Epidemiology of COVID-19 Among Children in China. *Pediatrics* **2020**, *145*, e20200702. [CrossRef]
53. Ge, X.-Y.; Li, J.; Yang, X.-L.; Chmura, A.; Zhu, G.; Epstein, J.H.; Mazet, J.K.; Hu, B.; Zhang, W.; Peng, C.; et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **2013**, *503*, 535–538. [CrossRef]
54. Li, W.; Shi, Z.; Yu, M.; Ren, W.; Smith, C.; Epstein, J.H.; Wang, H.; Crameri, G.; Hu, Z.; Zhang, H.; et al. Bats Are Natural Reservoirs of SARS-Like Coronaviruses. *Science* **2005**, *310*, 676–679. [CrossRef]
55. Aalberse, R.C. Structural biology of allergens. *J. Allergy Clin. Immunol.* **2000**, *106*, 228–238. [CrossRef] [PubMed]
56. Wentworth, D.; Gillim-Ross, L.; Espina, N.; Bernard, K.A. Mice Susceptible to SARS Coronavirus. *Emerg. Infect. Dis.* **2004**, *10*, 1293–1296. [CrossRef]
57. Wang, K.; Chen, W.; Zhang, Z.; Deng, Y.; Lian, J.-Q.; Du, P.; Wei, D.; Zhang, Y.; Sun, X.-X.; Gong, L.; et al. CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. *Signal. Transduct. Target. Ther.* **2020**, *5*, 1–10. [CrossRef]
58. Lai, M.M. Recombination in large RNA viruses: Coronaviruses. *Semin. Virol.* **1996**, *7*, 381–388. [CrossRef]
59. Tang, X.; Wu, C.; Li, X.; Song, Y.; Yao, X.; Wu, X.; Duan, Y.; Zhang, H.; Wang, Y.; Qian, Z.; et al. On the origin and continuing evolution of SARS-CoV-2. *Natl. Sci. Rev.* **2020**, *7*, 1012–1023. [CrossRef]
60. Zhao, Z.; Li, H.; Wu, X.; Zhong, Y.; Zhang, K.; Zhang, Y.-P.; Boerwinkle, E.; Fu, Y.-X. Moderate mutation rate in the SARS coronavirus genome and its implications. *BMC Evol. Biol.* **2004**, *4*, 21. [CrossRef] [PubMed]
61. Li, X.; Wang, R.; Qu, G.; Wang, Y.; Liu, P.; Zhu, Y.; Fei, D.; Ren, L.; Zhou, Y.; Liu, L. Autopsy and general observation of the cadaver died from the novel coronavirus SARS-CoV-2. *J. Forensic Med.* **2020**, *36*, 1–3. (In Chinese)
62. Ding, Y.; Wang, H.; Shen, H.; Li, Z.; Geng, J.; Han, H.; Cai, J.; Li, X.; Kang, W.; Weng, D.; et al. The clinical pathology of severe acute respiratory syndrome (SARS): A report from China. *J. Pathol.* **2003**, *200*, 282–289. [CrossRef] [PubMed]
63. Xu, Z.; Shi, L.; Wang, Y.; Zhang, J.; Huang, L.; Zhang, C.; Liu, S.; Zhao, P.; Liu, H.; Zhu, L.; et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir. Med.* **2020**, *8*, 420–422. [CrossRef]
64. Russell, C.D.; Millar, J.E.; Baillie, J.K. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. *Lancet* **2020**, *395*, 473–475. [CrossRef]
65. Fujii, T.; Iwamoto, A.; Nakamura, T. Current concepts in SARS treatment. *J. Infect. Chemother.* **2004**, *10*, 1–7. [CrossRef] [PubMed]
66. Wang, Z.; Yang, B.; Li, Q.; Wen, L.; Zhang, R. Clinical Features of 69 Cases With Coronavirus Disease 2019 in Wuhan, China. *Clin. Infect. Dis.* **2020**, *71*, 769–777. [CrossRef] [PubMed]
67. Roberts, A.; Deming, D.; Paddock, C.D.; Cheng, A.; Yount, B.; Vogel, L.; Herman, B.D.; Sheahan, T.; Heise, M.; Genrich, G.L.; et al. A Mouse-Adapted SARS-Coronavirus Causes Disease and Mortality in BALB/c Mice. *PLoS Pathog.* **2007**, *3*, e5. [CrossRef]
68. Menachery, V.D.; Yount, B.L.; Debbink, K.; Agnihothram, S.; Gralinski, L.E.; Plante, J.A.; Graham, R.L.; Scobey, T.; Ge, X.-Y.; Donaldson, E.F.; et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat. Med.* **2015**, *21*, 1508–1513. [CrossRef] [PubMed]

From: Jason Gale (BLOOMBERG/ NEWSROOM:)
Sent: Sat, 18 Sep 2021 16:11:32 -0000
To: (b)(6)
(b)(6)
Cc: (b)(6); Garry, Robert F; (b)(6)
(b)(6); Morens, David (NIH/NIAID) [E];
(b)(6)
Subject: RE: Study from 2007 shows SARS-infected civets on farms in Hubei

Oh wow. Awesome summary! Thanks, David. Btw, we're all off the record here.

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

From: Peter Daszak (b)(6)
To: JASON GALE, (b)(6)
CC: (b)(6)
(b)(6)
At: 09/19/21 00:12:34 UTC+10:00

Yes – saw that paper Jason – really interesting

I looked through the paper and it's yet another game changer. So far, in the last few weeks/months, we've got the following new evidence supporting emergence via bat-to-intermediate host-to-human origin for COVID-19 (I've probably missed something):

- Multiple new, SARS-CoV-2 related CoVs in SE Asia (Cambodia, Thailand, Japan, China etc.). I know of other work in review describing other related viruses in SE Asia also. We're also finding further novel SARS-CoV-2 related bat viruses in Malaysia, Thailand.
- New evidence that live animals of the type that carry CoVs were present in the Wuhan markets (including Huanan).
- Evidence from other bat SARSr-CoVs that mutations occur where there FCS is found (eg. RmYN02) <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7211627/>
- a rat alpha-CoV with an FCS in wildlife farms, hotels and train stations in S. China, showing that FCS insertions are more common in nature than previously thought. <https://journals.asm.org/doi/epdf/10.1128/JVI.01173-21>
- Epidemiological analysis of early cases supporting early origin close to Huanan market, not WIV [https://www.cell.com/cell/fulltext/S0092-8674\(21\)00991-0](https://www.cell.com/cell/fulltext/S0092-8674(21)00991-0)
- Phylogenetic analyses suggesting there may have been multiple introductions into the human population, supporting presence of a virus circulating in animals rather than a lab leak (@virology paper)
- Our work showing a very large interface for bat SARSr-CoV spillover in a v. densely population region, and potential for large numbers of missing cases each year <https://www.medrxiv.org/content/10.1101/2021.09.09.21263359v1>

- This paper showing ACE2 binding for bat SARS-CoV-2 related CoVs.
<https://www.researchsquare.com/article/rs-871965/v1>

On the lab leak side, we have convoluted accusations based on interpretations of intent about how Chinese scientists submitted genomes, wrote the papers, or how me and other scientists had collaborations with Chinese scientists. But, as far as new evidence goes, I could only find this:

- None

Of course, the momentum on the lab leak side will continue, with books by Sharri Markison, Alina Chan/Matt Ridley, Op Eds that criticize scientists, 70+ FoIAs by one organization alone, many other FoIAs on their way, 900 pages of FoIA'd grants and reports from EHA/NIAID showing zero evidence of lab leak.

This rate of research even in a pandemic is remarkable and suggests that we'll pretty quickly have such overwhelming evidence for the 'natural' origins that most people will move on from the lab leak.

(Off-the-record) However, the damage they leave behind is already horrific and will be worse by the time they decide to find another issue to focus on.

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

520 Eighth Avenue, Suite 1200

New York, NY 10018-6507

USA

Tel.: (b)(6)

Website: www.ecohealthalliance.org

Twitter: [@PeterDaszak](https://twitter.com/PeterDaszak)

EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

From: Wang Linfa (b)(6)
Sent: Friday, September 17, 2021 10:56 PM
To: Edward Holmes (b)(6); Jason Gale <j.gale@bloomberg.net>
Cc: Stephen Goldstein (b)(6); Peter Daszak (b)(6); (b)(6); (b)(6)
Subject: RE: Study from 2007 shows SARS-infected civets on farms in Hubei

Almost identical SARS-CoV-2 RBD in several bat sarbecoviruses! This is as close as you can get for a natural RBD origin!

Also, the paper concluded that SARS-CoV-2 genome fragments are found in different sarbecoviruses, very similar to the PloS Path paper for SARS-CoV-1.

All we need is to find a sarbecovirus with a furin cleavage site and no more debate on the natural origin of SARS-CoV-2!

Linfa (Lin-Fa) WANG, PhD FTSE FAAM

Professor

Programme in Emerging Infectious Disease

Duke-NUS Medical School,

8 College Road, Singapore 169857

Tel: (b)(6)

From: Edward Holmes (b)(6)
Sent: Thursday, 16 September 2021 3:31 PM
To: Jason Gale <j.gale@bloomberg.net>
Cc: Stephen Goldstein (b)(6); (b)(6); Peter Daszak (b)(6); (b)(6); Wang Linfa (b)(6)
Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

- External Email -

Dismantles one key argument of the leakers - how could a virus get from Yunnan to Wuhan - in one simple move.

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 [redacted]

On 16 Sep 2021, at 2:26 pm, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

And there's this:

"The discovery of civet-CoVs in the Hubei province should not be a surprise as SARS-CoV-like viruses were recently found in a bat species in the same province"

From: (b)(6) At: 09/16/21 14:24:33 UTC+10:00

To: Jason Gale (BLOOMBERG/ NEWSROOM:)

Cc: (b)(6)

(b)(6)

Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

Just stumbled across it reading the discussion of another paper honestly. It's been cited since - there are certainly people who remembered it but I did not know of it and clearly had not penetrated the public origins discussion.

Stephen

Sent from my iPhone

On Sep 15, 2021, at 10:22 PM, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

Well done, Stephen for finding this:
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1900161/>

Jason Gale, MHlthSec
Senior editor & chief biosecurity correspondent | Bloomberg News
Level 30, 120 Collins St., Melbourne VIC 3000
Tel. (landline) +61-3-9228-8783 | Mobile (b)(6)
@jwgale | LinkedIn: <http://www.linkedin.com/pub/jason-gale/6/249/a56>

Disclaimer

The information contained in this communication from the sender is confidential. It is intended solely for use by the recipient and others authorized to receive it. If you are not the recipient, you are hereby notified that any disclosure, copying, distribution or taking action in relation of the contents of this information is strictly prohibited and may be unlawful.

This email has been scanned for viruses and malware, and may have been automatically archived by Mimecast, a leader in email security and cyber resilience. Mimecast integrates email defenses with brand protection, security 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: Morens, David (NIH/NIAID) [E]
Sent: Mon, 4 Oct 2021 13:27:50 -0400
To: Morens, David (NIH/NIAID) [E]
Subject: Fwd: Molnupiravir was tested against the bat-CoVs discovered by our NIAID collaboration with Wuhan Institute of Virology

----- Forwarded Message -----

SubjectRE: Molnupiravir was tested against the bat-CoVs discovered by our NIAID :collaboration with Wuhan Institute of Virology

Date: Mon, 4 Oct 2021 03:13:06 +0000

From: Keusch, Gerald T (b)(6)
Peter Daszak (b)(6) Hotez, Peter Jay (b)(6)
To: Roberts, Rich (b)(6) David Morens (b)(6)
CC: Robert Kessler (b)(6)

This is of real importance for all of us, because it commonly comes up when GOF is discussed that there is considerable risk without much benefit. This is a useful way to counter that impression – and incidentally show how ignorant the proponents are (but that is a bit of editorializing).

And David should go ahead and contact (b)(6). I could only talk off the record because of my commitment to (b)(6). I would stay out of the public press until we resolve what happens to the now terminated COVID Task Force on Origins etc. But I am itching to openly say what I want to.

Jerry

From: Peter Daszak (b)(6)
Sent: Sunday, October 3, 2021 7:48 PM
To: Hotez, Peter Jay (b)(6) Roberts, Rich (b)(6) Keusch, Gerald T (b)(6) David Morens (b)(6)
Cc: Robert Kessler (b)(6)
Subject: Molnupiravir was tested against the bat-CoVs discovered by our NIAID collaboration with Wuhan Institute of Virology
Importance: High

This is mainly for David Morens to let people know at NIAID, but may be of interest to you all.

The latest therapeutic to make headline news – Molnupiravir from Merck and Ridgeback Biotherapeutics (AKA EIDD-2801), was tested against the exact same bat-origin CoVs discovered in our terminated, now-suspended R01 from NIAID.

Here's an article on the drug: <https://www.reuters.com/business/healthcare-pharmaceuticals/mercks-covid-19-pill-cuts-risk-death-hospitalization-by-50-study-2021-10-01/>

..and here are a couple of papers that tested it against the bat-CoVs: <https://stm.sciencemag.org/content/12/541/eabb5883> “Here, we show that the ribonucleoside analog β -D-N⁴-hydroxycytidine (NHC; EIDD-1931) has broad-spectrum antiviral activity against SARS-CoV-2, MERS-CoV, SARS-CoV, and related zoonotic group 2b or 2c bat-CoVs, as well as increased potency against a CoV bearing resistance mutations to the nucleoside analog inhibitor remdesivir”.

And here: <https://www.nature.com/articles/s41586-021-03312-w> “Collectively, our results demonstrate the utility of LoM as a single in vivo platform to evaluate and compare the replication and pathogenesis of past, present and future pre-emergent, epidemic and pandemic coronaviruses, which will allow for accelerating the development and testing of therapeutic and pre-exposure prophylaxis agents such as EIDD-2801”.

Here's a full list from the information I have on the drugs/therapeutics that were tested against our viruses:

Vaccines, therapeutics tested against the bat-CoVs discovered by EHA/WIV in China

Remdesivir: Formerly known as GS-5734

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5567817/> Now in use via FDA EUA:

<https://www.cnn.com/2020/04/29/health/gilead-sciences-remdesivir-covid-19-treatment/index.html>

Molnupiravir: NHC; EIDD-1931 & prodrug EIDD-2801: tested against range of CoVs here <https://stm.sciencemag.org/content/12/541/eabb5883>. Shown to effectively block SARS-CoV-2 here <https://www.nature.com/articles/s41586-021-03312-w>. Currently in Phase II/III clinical trials, showing good efficacy to all variants in preliminary results (Oct 2021):

<https://www.reuters.com/business/healthcare-pharmaceuticals/mercks-covid-19-pill-cuts-risk-death-hospitalization-by-50-study-2021-10-01/>

Adagio ADG20 (refined from ADG2): <https://science.sciencemag.org/content/371/6531/823>. Now in Phase II/III clinical trials <https://www.clinicaltrialsarena.com/news/adagio-trial-covid-19-prevention/>

Broadly-neutralizing RBD-specific antibody DH1047: Martinez *et al.*

<https://www.biorxiv.org/content/10.1101/2021.04.27.441655v1>

Chimeric NTD/RBD spike mRNA vaccines: Martinez *et al.*

<https://www.biorxiv.org/content/10.1101/2021.03.11.434872v1>

Neutralizing Ab vaccine for pandemic and pre-emergent coronaviruses.

<https://www.nature.com/articles/s41586-021-03594-0> Cited as ‘proof-of-concept’ for Universal CoV vaccine initiative announced by Dr. Fauci on CNN 5/13/21

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance
520 Eighth Avenue, Suite 1200
New York, NY 10018-6507
USA

Tel.: (b)(6)
Website: www.ecohealthalliance.org
Twitter: [@PeterDaszak](https://twitter.com/PeterDaszak)

EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

From: Hotez, Peter Jay (b)(6)
Sent: Sunday, October 3, 2021 1:18 PM
To: Roberts, Rich (b)(6); Keusch, Gerald T (b)(6); Peter Daszak
(b)(6); David Morens (b)(6)
Cc: Robert Kessler (b)(6)
Subject: Re: Good LA Times article.

FYI (b)(6) contacted me and asked if we could speak, I gave him my cell

Peter Hotez, MD, PhD, DSc (hon), FASTMH, FAAP

Dean, National School of Tropical Medicine
Professor, Departments of Pediatrics, Molecular Virology & Microbiology
Health Policy Scholar

Baylor College of Medicine

Texas Children's Hospital Endowed Chair of Tropical Pediatrics
Co-Director, Texas Children's Hospital Center for Vaccine Development

University Professor, Baylor University

Faculty Fellow, Hagler Institute for Advanced Study

Senior Fellow, Scowcroft Institute of International Affairs

Texas A&M University

Baker Institute Fellow in Disease & Poverty and Adjunct Professor of Bioengineering, Rice University

Adjunct Professor, University of Texas, School of Public Health

Donate to our [COVID-19 Vaccine Development](#)

E-mail: (b)(6)

Twitter: [@peterhotez](#)

Skype: (b)(6)

Linkedin [Peter Hotez](#)

Amazon Author Center: <https://www.amazon.com/Peter-J.-Hotez/e/B001HPIC48>

Daily Beast Contributor <https://www.thedailybeast.com/author/peter-j-hotez>

Like us on Facebook <https://www.facebook.com/BCMNationalSchoolOfTropicalMedicine/>

Senior Coordinator / Executive Support: [Douglas Soriano](#)

(b)(6)

Phone: (b)(6)

Fax: 713-798-2299



From: Roberts, Rich (b)(6)
Sent: Sunday, October 3, 2021 11:09 AM
To: Keusch, Gerald T (b)(6); Peter Daszak (b)(6); David Morens (b)(6)
Cc: Robert Kessler (b)(6); Hotez, Peter Jay (b)(6)
Subject: RE: Good LA Times article.

CAUTION: This email is not from a BCM Source. Only click links or open attachments you know are safe.

Jerry:

I did get an answer from (b)(6) and anticipate a direct conversation soon. The contact I have was passed from (b)(6) in confidence so let me see if he would agree to converse with you directly after I have spoken with him.

I spoke with (b)(6) yesterday and found him to be quite reasonable. He did agree to pass any quotes he might use past me before publishing. I specifically mentioned that it would be greatly appreciated if he could write something in direct support of Peter within the article.

Rich

Richard J. Roberts
New England Biolabs
240 County Road
Ipswich, MA 01938-2723
USA
Tel: (b)(6)
Fax: (978) 412 9910
email: (b)(6)

From: Keusch, Gerald T (b)(6)
Sent: Saturday, October 2, 2021 6:20 PM
To: Roberts, Rich (b)(6); Peter Daszak (b)(6); David Morens (b)(6)
Cc: Robert Kessler (b)(6); peter hotez (b)(6)
Subject: RE: Good LA Times article.

EXTERNAL SENDER

I talk to him regularly, most recently about the attacks on (b)(6) and the task force we were on under the Lancet COVID Commission which (b)(6) has unilaterally terminated. I couldn't really allow him insights into where we are or to use anything I said (all off the record) because we are negotiating with Lancet for a different way to continue our work.

I think he is a decent guy. I know (b)(6) has concerns so if you do speak to (b)(6) be careful about what you say and how you say it, and require him to run any quotes he plans to use by you. That's his general modus operandi.

What we need is to get the amendment out of the appropriations bill, and while the senators are important it may be somebody like (b)(6) who is a cabinet member to get them to pay attention. The administration is mightily distracted.

Rich, if you are able to share your entre to (b)(6) I would happily follow up. I have contacted them, (b)(6), and my representation and all I get back is the auto thank you reply.

Jerry

From: Roberts, Rich (b)(6)
Sent: Saturday, October 2, 2021 3:47 PM
To: Peter Daszak (b)(6); Keusch, Gerald T (b)(6); David Morens (b)(6)
Cc: Robert Kessler (b)(6); peter hotez (b)(6)
Subject: RE: Good LA Times article.

Peter:

I just spoke with (b)(6) from (b)(6) I mentioned that you were being attacked completely unfairly and that it would be good if he could find some encouraging words to help you. I am wondering if one of you would also be prepared to talk to (b)(6)

Rich

Richard J. Roberts
New England Biolabs
240 County Road
Ipswich, MA 01938-2723
USA
Tel: (b)(6)
Fax: (978) 412 9910
email: (b)(6)

From: Peter Daszak (b)(6)
Sent: Tuesday, September 28, 2021 11:47 PM
To: Roberts, Rich (b)(6); Keusch, Gerald T (b)(6); David Morens (b)(6)
Cc: Robert Kessler (b)(6)
Subject: Good LA Times article.

EXTERNAL SENDER

Just saw a piece in the LA Times that sums up the state of the origins “debate” pretty clearly:

<https://www.latimes.com/business/story/2021-09-28/evidence-against-a-lab-leak-as-covid-source>

It’s firewalled so here’s the article:

Column: New evidence undermines the COVID lab-leak theory — but the press keeps pushing it

BY MICHAEL HILTZIK BUSINESS COLUMNIST

SEPT. 28, 2021 6 AM PT

When it comes to the pandemic, pseudoscience has outweighed real science at almost every turn. One of the best examples of that is the unsupported assertion that the virus causing COVID-19 escaped from a Chinese laboratory.

Despite mounting evidence that the virus reached humans through natural pathways — from infected animals such as bats — the lab-leak hypothesis recently jumped back into the news, thanks to [CNN](#), the investigative news site [the Intercept](#), and [the Atlantic](#).

All treat the idea that the virus escaped from a lab credulously. They downplay or entirely ignore the latest scientific findings that support the theory that the virus’ origin can be found in the animal kingdom — the view accepted by a preponderance of experts in virology.

It’s a likely probability that this one originated from animals as well. But the possibility also remains that the virus leaked from a lab.

CNN’S SANJAY GUPTA OVERSTATES THE LAB LEAK THEORY

This is known as the zoonotic theory, from the term for a disease that can be transmitted from animals to humans.

We’ve [reported](#) before on the [near absence of evidence](#) for a lab leak, whether or not it’s the product of a deliberate act.

Ever since the lab-leak claim first emerged during the Trump administration, where it was part of a White House information campaign demonizing China, one of the arguments in its favor has been that evidence for a zoonotic origin has also been spotty.

That argument has never been quite true — virologists know that animals have been the source of most of the viral diseases afflicting humanity — but it has become weaker than ever over the last year.

The question of the origin of COVID-19 isn't of merely academic interest. The answer could guide the world's preparation for future pandemics; if the virus emerged from a laboratory, then improving lab safety measures will be prioritized. If scientific opinion continues to coalesce around animal-to-human transmission, that will underscore the importance of regulating contact between humans and wildlife.

To put it another way, if we focus on the wrong answer, the right measures won't be taken. In a real sense, humankind's future depends on not being distracted by an unsupported, politically motivated claim about Chinese labs.

Before examining the flaws in the CNN, Intercept and Atlantic treatments, let's look at what's been published recently about the zoonotic path.

For context, keep in mind that the earliest cluster of COVID-19 cases, in late 2019, was identified in the environs of the Huanan seafood market in the Chinese metropolis of Wuhan. Lab-leak theorists find this significant, because it's 7.5 miles from the Wuhan Institute of Virology, which does research on bat viruses.

A paper posted online earlier this month chiefly by researchers at France's Institut Pasteur and under consideration for publication in a Nature journal, however, reports that three viruses were found in bats living in caves in northern Laos with features very similar to SARS-CoV-2, the virus responsible for COVID-19.

As Nature reported, those viruses are "more similar to SARS-CoV-2 than any known viruses."

Another paper, posted in late August by researchers from the Wuhan lab, reports on viruses found in rats also with features similar to those that make SARS-CoV-2 infectious in humans. Two other papers published on the discussion forum virological.org present evidence that the virus jumped from animals to humans at more than one animal market in Wuhan, not just the Huanan seafood market.

Given that these so-called wet markets have long been suspected as transmission points of viruses from animals to humans because they sell potentially infected animals, that makes the laboratory origin vastly less likely, according to a co-author of one of the papers.

"That a laboratory leak would find its way to the very place where you would expect to find a zoonotic transmission is quite unlikely," Joel Wertheim, an associate professor at UC San Diego's medical school, told me. "To have it find its way to multiple markets, the exact place where you would expect to see the introduction, is unbelievably unlikely."

As virologist Robert F. Garry of Tulane, one of Wertheim's co-authors, told Nature, the finding is "a dagger into the heart" of the lab-leak hypothesis.

Garry and Wertheim are among the 21 expert co-authors of a "critical review" of virological findings on the origins of COVID-19. The review concludes, "There is currently no evidence that SARS-CoV-2 has a laboratory origin."

Now let's look at the recent reporting in support of the lab-leak theory.

On Sept. 19, CNN aired an hourlong documentary entitled "The Origins of COVID-19: Searching for the Source." Hosted by the channel's star science anchor, Sanjay Gupta, the program carries the veneer of an evenhanded approach.

Proponents of the zoonotic origin theory are given airtime, including Kristian Andersen of the Scripps Research Institute in La Jolla and Peter Daszak, a prominent grant maker in the virology field.

But so are proponents of the lab-leak theory. They include Alina Chan, a researcher at the Broad Institute, a biomedical research center, and Josh Rogin, a Washington Post columnist. Neither has any experience in virology. Chan is co-writing a book about COVID's origins that is expected to feature the lab-leak theory prominently, a fact not mentioned by CNN.

Yet at the top of the hour, referring to the common pattern of viruses jumping from animals to humans, Gupta says, "It's a likely probability that this one originated from animals as well. But the possibility also remains that the virus leaked from a lab."

By posing these two theories as simply two equally plausible solutions to a mystery, CNN glosses over the fact that the virological community regards the animal origin as vastly more likely than a lab leak. In fact, the two hypotheses are miles apart in credibility.

One of the program's chief targets is a report by a World Health Organization team issued in early 2021 that found spillover from an animal host to be "likely to very likely" and a laboratory incident an "extremely unlikely pathway."

Gupta calls the WHO report "the only scientific study of COVID's origins to date." That's not remotely accurate. There have been countless scientific studies, both before the WHO report and since. Indeed, Gupta mentions one of them, a seminal paper by Andersen and colleagues, published in March 2020. That paper termed the lab-leak theory "a speculative incomplete hypothesis with no credible evidence."

Much of the rest of the CNN program is filled with speculation about the Wuhan Institute, typically presented with portentous music on the soundtrack, suggesting subliminally that something sinister is going on there. The absence of information from the institute or the Chinese government is generally taken as tantamount to an admission of guilt.

“Over the course of 2020,” Gupta declares, “more and more revelations emerged related to the Wuhan Institute of Virology.”

One of these revelations concerned three staff members who reportedly sought hospital treatment for a flu-like illness in November 2019, before the COVID pandemic emerged.

Nothing has ever transpired to suggest these workers had COVID — November is flu season, after all. That they sought treatment at a hospital is immaterial, since it is well-known that people in China often go to hospitals for primary care, which residents of other countries would tend to receive in a doctor’s office.

A CNN reporter appearing on air overstated the case, saying the patients were “hospitalized with an unknown illness.” There has been no evidence that they were admitted to the hospital or that their illness was “unknown.”

CNN doesn’t bring its audience up to date on any of the latest research supporting the zoonotic theory, though it was published well before the air date and superseded what Gupta described as “the only scientific study” of COVID origins.

More recently, the Intercept trumpeted a purported scoop based on a leaked document — a grant proposal submitted in 2018 by Daszak’s organization, the EcoHealth Alliance, to the Pentagon’s Defense Advanced Research Projects Agency, or DARPA.

The proposal, for a laboratory manipulation of a virus related to SARS, the viral disease that caused an outbreak of pulmonary disease in China in 2003. DARPA rejected the proposal, however, and there’s no evidence that it was submitted to, much less approved by, any other funding body.

“Many questions remain about the proposal, including whether any of the research described in it was completed,” the Intercept acknowledged.

Commentators on the Intercept’s disclosure have displayed, perhaps in spite of themselves, that they lack the courage of their own convictions. In an article published Sept. 24, the Atlantic, unable or unwilling to delve into what the Intercept’s document actually meant, if anything, settled for declaring that it made the lab-leak debate “even messier.”

The magazine's Daniel Engber and Adam Federman wrote: "Does the SARS-CoV-2 pandemic have an unnatural origin? The answer hasn't changed: probably not. But we have learned something quite disturbing in the past few days, simply from how and when this information came to light."

By pretending that the debate itself is important, as if both sides have something to offer, they manage to report on a claim that has no substance. The approach also protects journalists from their persistent fear of landing on the wrong side of things — the authors preserve an out in case the lab-leak hypothesis turns out to be true, as unlikely as that is. If that happens, they can point to their lily-livered observations and say, "See, we knew it all along."

In this debate, however, the zoonotic camp has evidence and the lab-leak camp nothing to offer but innuendo.

Here's the true state of the discussion. There is no evidence that the virus leaked from the Wuhan laboratory or any other lab. There is no evidence that the Wuhan lab was working with a bat virus that had anything but a very distant resemblance to SARS-CoV-2. Viruses that resemble it much more closely have been found in natural settings a thousand miles from Wuhan, as the crow, or bat, flies.

Evidence that artificial manipulation of a virus gave rise to SARS-CoV-2 has faded, as scientists find more evidence that features of SARS-CoV-2 thought to be unnatural occur in nature. Meanwhile, evidence for zoonotic transmission is constantly accumulating. No one who reports on the issue without acknowledging these two trends should be trusted.

Los Angeles Times columnist Michael Hiltzik writes [a daily blog](#) appearing on latimes.com. His seventh book, "[Iron Empires: Robber Barons, Railroads, and the Making of Modern America](#)," has just been published by Houghton Mifflin Harcourt. Follow him on Twitter at twitter.com/hiltzik and on Facebook at facebook.com/hiltzik.

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance
520 Eighth Avenue, Suite 1200
New York, NY 10018-6507
USA

Tel.: (b)(6)

Website: www.ecohealthalliance.org

Twitter: [@PeterDaszak](https://twitter.com/PeterDaszak)

EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

Disclaimer

The information contained in this communication from the sender is confidential. It is intended solely for use by the recipient and others authorized to receive it. If you are not the recipient, you are hereby notified that any disclosure, copying, distribution or taking action in relation of the contents of this information is strictly prohibited and may be unlawful.

This email has been scanned for viruses and malware, and may have been automatically archived by Mimecast, a leader in email security and cyber resilience. Mimecast integrates email defenses with brand protection, security 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: Baric, Ralph S
Sent: Tue, 18 Oct 2016 15:31:15 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: mers model

Hi Erik, just wanted you to know that the DPP4 paper is now in press in Nature Microbiology. ralph

From: Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]
Sent: Wednesday, October 12, 2016 4:03 PM
To: Cockrell, Adam; Feng Wang
Cc: Jeff Pouliot; Leyva-Grado, Victor; Umerah, Nina; Baric, Ralph S; Deborah Butler; Neil Pearson; Yount, Boyd L Jr
Subject: RE: GSK A57 Study control

Hi Adam,
That sounds like the best way to salvage information from the experiment.

Erik

From: Cockrell, Adam [mailto:(b)(6)]
Sent: Wednesday, October 12, 2016 3:49 PM
To: Feng Wang (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 (b)(6) Deborah Butler (b)(6) Neil Pearson
(b)(6) Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study control

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 [mailto:(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 [mailto:(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

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email: (b)(6)
Tel: (b)(6)

[gsk.com](#) | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image001.png>

From: Cockrell, Adam [mailto:(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 [mailto:(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

GSK

1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States

Email (b)(6)

Tel (b)(6)

gsk.com | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image001.png>

From: Cockrell, Adam [mailto:(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 [mailto:(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

GSK

1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States

Email (b)(6)

Tel (b)(6)

gsk.com | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image001.png>

From: Cockrell, Adam [mailto:(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

GSK

1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States

Email (b)(6)

Tel (b)(6)

[gsk.com](#) | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image001.png>

From: Cockrell, Adam [mailto:(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 [mailto:(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

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(6)
Tel (b)(6)

gsk.com | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image001.png>

From: Cockrell, Adam [mailto:(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 [mailto:(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

GSK

1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States

Email (b)(6)

Tel (b)(6)

gsk.com | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image001.png>

From: Cockrell, Adam [mailto:(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 [mailto:(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

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(6)
Tel (b)(6)

[gsk.com](#) | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image001.png>

From: Cockrell, Adam [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States

Email (b)(6)

Tel (b)(6)

gsk.com | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image002.png>

From: Cockrell, Adam [[mailto:\(b\)\(6\)](mailto:(b)(6)@gsk.com)]
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

(b)(6)

Best Regards,
Adam

From: Feng Wang [mailto:(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](#)

<image001.png>

From: Cockrell, Adam [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 dosing 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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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](#)

<image002.png>

From: Stemmy, Erik (NIH/NIAID) [E] [mailto:(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, (b)(6)
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.

NOTE: This material is intended for the individual or entity to which it is addressed. It may contain privileged, confidential information that is protected from disclosure under applicable laws. If you are not the addressee, or a person authorized to deliver the document to the addressee, please note that you are strictly prohibited from reviewing, copying, disclosing, disseminating or distributing this material 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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

From: Cockrell, Adam
Sent: Tue, 18 Oct 2016 15:27:55 +0000
To: Stemmy, Erik (NIH/NIAID) [E]; Umerah, Nina; Baric, Ralph; Heise, Mark T; Leyva-Grado, Victor
Subject: RE: HHSN272201000019I-HHSN27200003-Task A57 - Conference Call

Thanks Erik,

I have to be in meetings all Monday morning so would not be able to make it either. Once I have the titers done for the experiment I will assemble a summary of the GSK study, and circulate. Probably by mid-next week.

Best,
Adam

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, October 18, 2016 11:23 AM
To: Umerah, Nina (b)(6); Baric, Ralph S (b)(6); Heise, Mark T (b)(6); Leyva-Grado, Victor (b)(6); Cockrell, Adam (b)(6)
Subject: RE: HHSN272201000019I-HHSN27200003-Task A57 - Conference Call

Hi Everyone,
I've had a conflict come up for 11am on Monday 10/24. Do we need to reschedule the call or would you prefer to just update via email? Adam – not sure if you'll have any updates for the GSK study by next week... Any other topics to discuss?

Erik

-----Original Appointment-----

From: Umerah, Nina [mailto:(b)(6)]
Sent: Thursday, July 09, 2015 1:48 PM
To: 'Umerah, Nina'; Stemmy, Erik (NIH/NIAID) [E]; Baric, Ralph; (b)(6) PETERPALESE; 'Amy Sims'; Lim, Jean; Leyva-Grado, Victor; Moore, Victoria L
Subject: HHSN272201000019I-HHSN27200003-Task A57 - Conference Call
When: Monday, October 24, 2016 11:00 AM-12:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where:
Importance: High

Dear all,

The number for the conference call scheduled for the 4th Monday of the month at 11am EST is (b)(6). The participant passcode is (b)(6).

Thanks,

Nina

Nina Umerah
Grants and Contracts Manager
Department of Microbiology
Icahn School of Medicine at Mount Sinai
One Gustave L. Levy Place, Box 1124
New York, NY 10029
Tel.: (b)(6)
Fax: 212-534-1684

From: Baric, Ralph S
Sent: Fri, 14 Oct 2016 22:21:52 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: paper
Attachments: MERS Workshop Manuscript 8-25-2016-RSB.docx

Hi Erik, reads well. my comments. ralph

From: Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]
Sent: Wednesday, October 12, 2016 4:03 PM
To: Cockrell, Adam; Feng Wang
Cc: Jeff Pouliot; Leyva-Grado, Victor; Umerah, Nina; Baric, Ralph S; Deborah Butler; Neil Pearson; Yount, Boyd L Jr
Subject: RE: GSK A57 Study control

Hi Adam,
That sounds like the best way to salvage information from the experiment.

Erik

From: Cockrell, Adam [mailto:(b)(6)]
Sent: Wednesday, October 12, 2016 3:49 PM
To: Feng Wang (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 (b)(6); Deborah Butler (b)(6); Neil Pearson
(b)(6); Yount, Boyd L Jr (b)(6)
Subject: RE: GSK A57 Study control

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 [mailto:(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 [mailto:(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

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(6)
Tel (b)(6)

[gsk.com](#) | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image001.png>

From: Cockrell, Adam [mailto:(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 [mailto:(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

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(6)
Tel (b)(6)

gsk.com | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image001.png>

From: Cockrell, Adam [mailto:(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 [mailto:(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

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(6)
Tel (b)(6)

[gsk.com](#) | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image001.png>

From: Cockrell, Adam [mailto:(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

GSK

1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States

Email (b)(6)

Tel (b)(6)

[gsk.com](#) | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image001.png>

From: Cockrell, Adam [mailto:(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 [mailto:(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

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email: (b)(6)
Tel: (b)(6)

[gsk.com](#) | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image001.png>

From: Cockrell, Adam [mailto:(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 [mailto:(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

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(6)
Tel (b)(6)

[gsk.com](#) | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image001.png>

From: Cockrell, Adam [mailto:(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 [mailto:(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

GSK

1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States

Email (b)(6)

Tel (b)(6)

[gsk.com](#) | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image001.png>

From: Cockrell, Adam [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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

GSK
1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426-0989, United States
Email (b)(6)
Tel (b)(6)

gsk.com | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)

<image002.png>

From: Cockrell, Adam [mailto:(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

(b)(6)

Best Regards,
Adam

From: Feng Wang [mailto:(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](#)

<image001.png>

From: Cockrell, Adam [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 dosing 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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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](#)

<image002.png>

From: Stemmy, Erik (NIH/NIAID) [E] [mailto:(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, (b)(6)
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.

NOTE: This material is intended for the individual or entity to which it is addressed. It may contain privileged, confidential information that is protected from disclosure under applicable laws. If you are not the addressee, or a person authorized to deliver the document to the addressee, please note that you are strictly prohibited from reviewing, copying, disclosing, disseminating or distributing this material 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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

Page 049 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 050 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 051 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 052 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 053 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 054 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 055 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 056 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 057 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 058 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 059 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 060 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 061 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

From: Erlandson, Karl (OS/ASPR)
Sent: Thu, 13 Oct 2016 19:21:13 +0000
To: Stemmy, Erik (NIH/NIAID) [E]; Baric, Ralph; Hensley, Lisa (NIH/NIAID) [E]; Subbarao, Kanta (NIH/NIAID) [E]
Subject: RE: MERS Model Workshop Draft Manuscript
Attachments: MERS Workshop Manuscript 8-25-2016.docx

Hi Erik

Just a few tweaks and comments.

Karl

From: Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]
Sent: Friday, September 23, 2016 2:51 PM
To: Baric, Ralph; Erlandson, Karl (OS/ASPR); Hensley, Lisa (NIH/NIAID) [E]; Subbarao, Kanta (NIH/NIAID) [E]
Subject: RE: MERS Model Workshop Draft Manuscript

Hi Everyone,
Friendly reminder to please have a look at the attached draft manuscript from the MERS animal model workshop and to send me your comments.

Many thanks!
Erik

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Thursday, August 25, 2016 2:45 PM
To: Baric, Ralph (b)(6); Dreier, Thomas (OS/ASPR/BARDA) (b)(6); Erlandson, Karl (OS/ASPR) (b)(6); Hensley, Lisa (NIH/NIAID) [E]; (b)(6); Munster, Vincent (NIH/NIAID) [E]; (b)(6); Subbarao, Kanta (NIH/NIAID) [E]; (b)(6)
Cc: Spiro, David (NIH/NIAID) [E]; (b)(6)
Subject: MERS Model Workshop Draft Manuscript

Hi Everyone,
I know it's been a while since the MERS Model workshop, but David and I have put together a draft manuscript that we would like to submit to (b)(4) for publication. We've put this together based on the detailed summary provided by the science writer. Since you all were part of the organizing committee for the workshop, we thought it would be good to have you as co-authors writing on behalf of the entire group. We're asking for your comments and feedback first, and then we will circulate an updated draft to the larger presenter/panelist group. If possible, we would appreciate it if you could please send any comments back to us by September 12th. Please also let me know if you'd prefer not to be listed as an author, or are otherwise unable to participate in preparing the paper.

In addition to the draft, I've also attached two recent, related, EID papers. Our thought is that this paper would be a follow on to these two. Please let me know if you have any questions.

Thanks!
Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, (b)(6)
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.

NOTE: This material is intended for the individual or entity to which it is addressed. It may contain privileged, confidential information that is protected from disclosure under applicable laws. If you are not the addressee, or a person authorized to deliver the document to the addressee, please note that you are strictly prohibited from reviewing, copying, disclosing, disseminating or distributing this material 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.

Page 064 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 065 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 066 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 067 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 068 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 069 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 070 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 071 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 072 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 073 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 074 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

Page 075 of 260

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

From: Mathur, Punam (NIH/NIAID) [E]
Sent: Wed, 5 Oct 2016 14:48:37 -0400
To: Baric, Ralph; Graham, Rachel
Cc: Yao, Alison (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: latest paper drafts

Hi Ralph,

Thank you for sending us both the (b)(4) paper to be published in (b)(4). Do let us know when you submit the (b)(4) paper, which journal are you targeting?

All the best,
Punam

From: Baric, Ralph
Sent: Tuesday, October 04, 2016 4:14 PM
To: Mathur, Punam (NIH/NIAID) [E] (b)(6) Graham, Rachel (b)(6)
Cc: Yao, Alison (NIH/NIAID) [E] (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Subject: RE: latest paper drafts

Hi Punam, Alison and Erik, As promised, still some minor tweeking on the (b)(4) 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

From: Baric, Toni C
Sent: Mon, 3 Oct 2016 15:59:01 +0000
To: Baric, Ralph; Beisel, Christopher (NIH/NIAID) [E]; Damania, Blossom A; Spiro, David (NIH/FIC) [E]; Stemmy, Erik (NIH/NIAID) [E]; Graham, Rachel; Mathur, Punam (NIH/NIAID) [E]; Yao, Alison (NIH/NIAID) [E]
Subject: NIAID UNC Conference call

Hello

This is a reminder for the UNC-NIAID conference call scheduled for Tuesday Oct 4 at 12 noon. The calling instructions are:

Phone: (b)(6)
Passcode: (b)(6)

Best Regards

Toni Baric

Department of Microbiology and Immunology
9025 Burnett Womack
CB# 7292
Chapel Hill, NC 27599-7292
Office: (b)(6)
(b)(6)

From: Camping, Ryan
Sent: Wed, 28 Sep 2016 21:00:59 +0000
To: Uccellini, Melissa; Adolfo García-Sastre; Mena, Nacho; Randy Albrecht; PETERPALESE; Mullarkey, Caitlin; Ana Sesma; Irene Ramos; Florian Krammer; Shaw, Megan; Vausselin, Thibaut; Bouvier, Nicole (MSH); Harm Van Bakel; Ron Fouchier; (b)(6) Medina, Rafael; (b)(6) Jefferson Santos (b)(6) YOSHIHIROKAWAOKA; Neumann, Gabriele (NIH); (b)(6) (b)(6) (b)(6) Colin Parrish; Brian Wasik; Vincent, Amy; Nicola Lewis; Nelson, Martha (NIH/FIC) [V]; Wentworth, David E. (CDC/DDID/NCIRD/ID); Baric, Ralph; Baric, Toni C (b)(6) Neville, Amey B; Philip Dormitzer; Post, Diane (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]
Cc: Bragg, Jordan; Kitazono, Lori; Mckinney, Brigitte; Umerah, Nina; Cespedes, Marianela
Subject: RE: CRIP Scientific Advisory Board Meeting in NYC
Attachments: CRIP SAB Meeting Agenda 10-6-16.pdf

Dear all,

Attached please find the agenda for the CRIP SAB meeting next Thursday, October 6. Please review and let me know of any needed changes ASAP.

We will host a dinner the night before on Wednesday, October 5 from 7-9PM, location TBD. Please mark your name down here if you would like to attend: <http://doodle.com/poll/kd3mh8e3f78c3w4t>

We're looking forward to a productive meeting. See you soon!

Ryan

From: Camping, Ryan
Sent: Monday, July 11, 2016 4:12 PM
To: Uccellini, Melissa; Garcia-Sastre, Adolfo; Albrecht, Randy; Palese, Peter; Mullarkey, Caitlin; Sesma, Ana; Ramos-lopez, Irene; Krammer, Florian; Shaw, Megan; Vausselin, Thibaut; Bouvier, Nicole (MSH); Van bakel, Harm; (b)(6) Medina, Rafael; (b)(6) (b)(6) (b)(6) 'Colin Parrish'; 'Vincent, Amy'; 'Nicola Lewis'; 'Martha Nelson'; 'Wentworth, David E. (CDC/OID/NCIRD)'; (b)(6) Neville, Amey B; (b)(6) 'Philip Dormitzer'
Cc: 'Post, Diane (NIH/NIAID) [E]'; (b)(6) Degrace, Marciela (NIH/NIAID) [E] (b)(6) Markey, Courtney (NIH/NIAID) [E] (b)(6)
Subject: RE: CRIP Scientific Advisory Board Meeting in NYC

Dear all,

According to the availability poll, Thursday, October 6, 2016 is the best date for the CRIP Scientific Advisory Board meeting in New York, NY. The meeting will be a full-day (8AM-approx. 4PM) of

discussions and presentations. We will host a dinner the evening before for anyone who would like to attend. I will send details regarding travel reimbursement and honoraria in separate communications. Please mark your calendars. Thank you.

Ryan

Ryan Camping

Manager, Grants & Contracts
Center for Research on Influenza Pathogenesis
Department of Microbiology
Icahn School of Medicine at Mount Sinai
One Gustave L. Levy Place, Box 1124
New York, NY 10029
Office: (b)(6)
E-mail: (b)(6)
Web: <http://labs.icahn.mssm.edu/garcia-sastre/>

From: Camping, Ryan
Sent: Thursday, June 02, 2016 3:17 PM
To: Uccellini, Melissa; Garcia-Sastre, Adolfo; Albrecht, Randy; Palese, Peter; Mullarkey, Caitlin; Sesma, Ana; Ramos-lopez, Irene; Krammer, Florian; Shaw, Megan; Vausselin, Thibaut; Bouvier, Nicole (MSH); Van bakel, Harm; (b)(6) Medina, Rafael; (b)(6)
(b)(6)
(b)(6) 'Colin Parrish'; 'Vincent, Amy'; 'Nicola Lewis';
'Martha Nelson'; 'Wentworth, David E. (CDC/OID/NCIRD)'; (b)(6)
(b)(6) 'Philip Dormitzer'
Cc: 'Post, Diane (NIH/NIAID) [E]'; (b)(6) Degrace, Marciela (NIH/NIAID) [E]
(b)(6) Markey, Courtney (NIH/NIAID) [E]; (b)(6)
Subject: CRIP Scientific Advisory Board Meeting in NYC

Dear all,

We are planning a CRIP external Scientific Advisory Board (SAB) meeting in either August, September or October at Mount Sinai in New York. Outside advisors will provide CRIP with critical advice and help us with directions and implementation of new CRIP projects during a full one-day meeting. Program officials from NIH will also be present. CRIP co-investigators are invited to attend either in-person or by WebEx and contribute to the discussions and talks.

Please welcome our newly reformed SAB. Ralph Baric and Dave Wentworth have stayed on from the previous incarnation of the SAB, and we welcome Philip Dormitzer, Mary Estes, and Ian Wilson as new members.

Please fill out your availability to attend the one-day in-person meeting at Mount Sinai in New York: <http://doodle.com/poll/nywdty7h5547q4mh>

We look forward to productive discussions and review of the CRIP program. Please let me know if you have any questions. Thank you.

Ryan

Ryan Camping

Manager, Grants & Contracts

Center for Research on Influenza Pathogenesis

Department of Microbiology

Icahn School of Medicine at Mount Sinai

One Gustave L. Levy Place, Box 1124

New York, NY 10029

Office: (b)(6)

E-mail: (b)(6)

Web: <http://labs.icaohn.mssm.edu/garcia-sastre/>

ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI
NIAID Centers of Excellence for Influenza Research and Surveillance (CEIRS)
Center for Research on Influenza Pathogenesis (CRIP)
Thursday, October 6, 2016

Department of Microbiology
Annenberg Building, Room 16-02
1468 Madison Ave. @ 100th St.
New York, NY

CRIP SAB Meeting Agenda

Wednesday, October 5

7:00 – 9:00 PM **Dinner (Location TBD)**

Thursday, October 6

7:30 – 8:00 AM **Breakfast & Setup**

8:00 – 8:05 AM **Introduction**
Diane Post

8:05 – 8:25 AM **CRIP Overview**
Adolfo García-Sastre

8:30 – 9:00 AM **Antibody-dependent cell-mediated cytotoxicity (ADCC) of broadly protective hemagglutinin antibodies**
Peter Palese

9:05 – 9:35 AM **The NS1 of influenza A viruses**
Adolfo García-Sastre

9:40 – 10:10 AM **EMC wild bird surveillance and studies on pathogenesis and transmission of emerging influenza viruses**
Ron Fouchier (WebEx)

10:15 – 10:30 AM **Break**

10:35 – 11:05 AM **Research Project 5 and avian and swine influenza surveillance in Argentina and Guatemala**
Jefferson Santos

11:10 – 11:40 AM **Progress Reports**
Yoshihiro Kawaoka

11:45 – 12:45 PM **Lunch**
SAB in 16-90
Other Participants in 16-02

- 12:50 – 1:20 PM **Updates on surveillance research from the Pacific Rim and beyond**
Jon Runstadler
- 1:25 – 1:40 PM **Diversity of animal and human influenza virus in Chile**
Rafael Medina (WebEx)
- 1:45 – 2:00 PM **Pre-emptive vaccine updates, and immunity management**
Derek Smith
- 2:05 – 2:20 PM **Evaluation of host and virus factors associated with the pathogenicity
and transmission of influenza viruses in avian species**
David Suarez & Mary Pantin-Jackwood
- 2:25 – 2:40 PM **The evolutionary dynamics of IAVs in swine**
Amy Vincent (WebEx) & Martha Nelson
- 2:45 – 3:00 PM **CRIP surveillance and experimental data for influenza viruses**
Eric Bortz
- 3:05 – 3:20 PM **An improved workflow for high-throughput influenza genome
sequencing in the CEIRS network**
Harm van Bakel
- 3:25 – 4:25 PM **Discussions**
SAB, Diane Post, Erik Stemmy & Adolfo García-Sastre in 16-90
Other Participants in 16-02
- 4:30 – 5:00 PM **Wrap-up Discussion & Departures**

From: Cockrell, Adam
Sent: Mon, 26 Sep 2016 14:22:19 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Baric, Ralph; Leyva-Grado, Victor; Umerah, Nina
Subject: RE: AMC Call?

Hi Erik.

It looks like GSK will send the drug solubilized and I will initiate the testing October 10th.

Best,
Adam

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Monday, September 26, 2016 9:34 AM
To: Cockrell, Adam (b)(6)
Cc: Baric, Ralph S (b)(6); Leyva-Grado, Victor (b)(6); Umerah, Nina (b)(6)
Subject: RE: AMC Call?

Hi Adam,
We can cancel today. The only update I had on my list was the GSK study. Following along on the emails it looks like you're good to go when the BSL3 reopens. You solved all the solubility issues?

Erik

From: Cockrell, Adam (b)(6)
Sent: Monday, September 26, 2016 9:25 AM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Baric, Ralph (b)(6)
Subject: AMC Call?

Hi Erik,

Checking to make sure that we do not have a call today.

Best,

Adam Cockrell
Post-Doctoral Fellow
Department of Epidemiology
University of North Carolina at Chapel Hill
Chapel Hill, NC, 27599
Phone: (b)(6)

From: Cockrell, Adam
Sent: Thu, 8 Sep 2016 18:16:30 +0000
To: Leyva-Grado, Victor
Cc: Baric, Ralph; Umerah, Nina; Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: GSK A57 Study

Thanks Victor and Nina.

From: Leyva-Grado, Victor [mailto:(b)(6)]
Sent: Thursday, September 08, 2016 2:13 PM
To: Cockrell, Adam (b)(6)
Cc: Baric, Ralph S (b)(6); Umerah, Nina (b)(6); Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Subject: RE: GSK A57 Study

Hi Adam,

I talked to Nina and we think it's ok for UNC to sign, so please go ahead.

V

From: Cockrell, Adam [mailto:(b)(6)]
Sent: Thursday, September 08, 2016 1:34 PM
To: Leyva-Grado, Victor
Cc: Baric, Ralph S; Umerah, Nina; Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: GSK A57 Study

Hi Victor,

Are you alright with us signing/returning this document to them and moving forward with the study as planned?

Thanks,
Adam

From: Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]
Sent: Thursday, September 08, 2016 1:31 PM
To: Cockrell, Adam (b)(6)
Cc: Baric, Ralph S (b)(6); Umerah, Nina (b)(6); Leyva-Grado, Victor (b)(6)
Subject: RE: GSK A57 Study

It seems like it's just an acknowledgement of receipt of compound. If MSSM is ok with you signing I'd say go ahead and sign, and proceed as planned with the study.

Erik

From: Cockrell, Adam [mailto:(b)(6)]
Sent: Thursday, September 08, 2016 1:29 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Baric, Ralph (b)(6); Leyva-Grado, Victor (b)(6)
Umerah, Nina (b)(6)
Subject: RE: GSK A57 Study

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] [mailto:(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 [mailto:(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 [mailto:(b)(6)]

Sent: Thursday, September 08, 2016 10:52 AM

To: Cockrell, Adam (b)(6)

Cc: Yount, Boyd L Jr (b)(6)

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 vortex 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 [mailto:(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 [mailto:(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)

gsk.com | [Twitter](#) | [YouTube](#) | [Facebook](#) | [Flickr](#)



From: Cockrell, Adam [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 dosing 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 [mailto:(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 [mailto:(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 [mailto:(b)(6)]
Sent: Wednesday, August 03, 2016 5:20 PM
To: Jeff Poulit; 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 [mailto:(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] [mailto:(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, (b)(6)
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.

NOTE: This material is intended for the individual or entity to which it is addressed. It may contain privileged, confidential information that is protected from disclosure under applicable laws. If you are not the addressee, or a person authorized to deliver the document to the addressee, please note that you are strictly prohibited from reviewing, copying, disclosing, disseminating or distributing this material 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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

From: Umerah, Nina
Sent: Tue, 6 Sep 2016 14:01:27 +0000
To: Stemmy, Erik (NIH/NIAID) [E]; Cockrell, Adam; Leyva-Grado, Victor
Cc: Baric, Ralph; Adams, Miranda (NIH/NIAID) [E]
Subject: RE: A57 NCE?

Hi Erik,

I'm not sure what the holdup is, but I'll make sure it's sent out today.

Thanks,
Nina

Nina Umerah

(b)(6)

From: Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]
Sent: Tuesday, September 06, 2016 7:20 AM
To: Umerah, Nina; Cockrell, Adam; Leyva-Grado, Victor
Cc: Baric, Ralph; Adams, Miranda (NIH/NIAID) [E]
Subject: RE: A57 NCE?

Hi Nina,
Any update on the NCE? I still don't think we've received it.

Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, (b)(6)
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.

NOTE: This material is intended for the individual or entity to which it is addressed. It may contain privileged, confidential information that is protected from disclosure under applicable laws. If you are not the addressee, or a person authorized to deliver the document to the addressee, please note that you are strictly prohibited from reviewing, copying, disclosing, disseminating or distributing this material 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: Umerah, Nina [mailto:(b)(6)]
Sent: Wednesday, August 31, 2016 1:57 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Cockrell, Adam (b)(6)
Leyva-Grado, Victor (b)(6)
Cc: Baric, Ralph (b)(6) Adams, Miranda (NIH/NIAID) [E] (b)(6)
Subject: RE: A57 NCE?

Hi Erik,

The NCE request was sent directly to our finance office last week. I will follow up today.

Thanks,
Nina

Nina Umerah

(b)(6)

From: Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]
Sent: Wednesday, August 31, 2016 1:40 PM
To: Cockrell, Adam; Leyva-Grado, Victor
Cc: Baric, Ralph; Umerah, Nina; Adams, Miranda (NIH/NIAID) [E]
Subject: A57 NCE?
Importance: High

Hi Adam,

Any news on the NCE? Technically we need to process the request 30 days before the end of the performance period, which is the end of September. If we don't get it in soon, the contract will end next month.

Erik

From: Cockrell, Adam [mailto:(b)(6)]
Sent: Monday, August 22, 2016 11:14 AM
To: Leyva-Grado, Victor (b)(6) Stemmy, Erik (NIH/NIAID) [E]
(b)(6)
Cc: Baric, Ralph (b)(6) Umerah, Nina (b)(6)
Subject: RE: AMC call today?

Hi Victor,

I will speak with Ralph about putting together the NCE and get that over to you.

Best Regards,

Adam

From: Leyva-Grado, Victor [mailto:(b)(6)]

Sent: Monday, August 22, 2016 11:10 AM

To: Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)

Cc: Baric, Ralph S (b)(6) Umerah, Nina (b)(6)

Subject: RE: AMC call today?

Hi Adam,

Is Amy still helping you out with the administrative part of the contract? I talked to Nina last week and we haven't received the request from UNC (is this still correct Nina?). The only one we have is the previous NCE for the 5 months.

Cheers,

V

From: Jeff Pouliot
Sent: Tue, 30 Aug 2016 16:07:33 +0000
To: Cockrell, Adam; Feng Wang; Stemmy, Erik (NIH/NIAID) [E]; 'Leyva-Grado, Victor'; 'Umerah, Nina'; Baric, Ralph; 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

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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 [mailto:(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 dosing 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 [mailto:(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 [mailto:(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 [mailto:[\(b\)\(6\)](#)]
Sent: Wednesday, August 03, 2016 5:20 PM
To: Jeff Poulit; 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 [mailto:(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] [mailto:(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, (b)(6)
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.

NOTE: This material is intended for the individual or entity to which it is addressed. It may contain privileged, confidential information that is protected from disclosure under applicable laws. If you are not the addressee, or a person authorized to deliver the document to the addressee, please note that you are strictly prohibited from reviewing, copying, disclosing, disseminating or distributing this material 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.

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.

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.

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.

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.

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.

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.

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.

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.

From: Post, Diane (NIH/NIAID) [E]
Sent: Tue, 23 Aug 2016 15:40:48 -0400
To: Stemmy, Erik (NIH/NIAID) [E]; 'Walter Orenstein'; Richard Compans; Treanor, John; (b)(6) Stephan Bour; Richard Webby; Stacey Schultz-Cherry; 'Richard Rothman (b)(6) Andrew Pekosz; Katz, Jackie M. (CDC/DDID/NCIRD/ID); Bright, Rick (OS/ASPR/BARDA); (b)(6) Dennis Carroll'; 'David E Wentworth'; 'Frances E Lund'; Thomas Braciale; (b)(6) Gellin, Bruce (HHS/OASH); Degrace, Marciela (NIH/NIAID) [E]; 'Braciale, Thomas (b)(6) Wentworth, David E. (CDC/DDID/NCIRD/ID); Donis, Ruben (OS/ASPR/BARDA); 'McKenzie, Pamela'; 'Erin-Joi Collins-McNeal' (b)(6) Lauren Sauer (b)(6) 'Neu, Donna'; Ryan Camping; Uccellini, Melissa (b)(6) Andrew Burnham; Markey, Courtney (NIH/NIAID) [E]
Subject: CEIRS NEC Meeting
Attachments: CRIP Report 2016.pdf, Emory-UGA Report 2016.pdf, JHCEIRS Report 2016.pdf, NYICE Report 2016.pdf, SJCEIRS Report 2016.pdf, CEIRS NEC MEETING_2_2016.docx

Dear all,

I hope this email finds you well! Hard to believe that the summer is almost over. We are looking forward to our meeting scheduled for next Monday August 29th set to occur from 12:30 – 4:30. The goal of the meeting is to receive feedback from the external NEC members regarding the activities being supported under the CEIRS contracts.

Attached is an agenda for the meeting. There are some discussion questions on page 2 for your consideration. One of the topics that we'd like to discuss is the NECs ideas regarding measures of success. Some of the PI's felt that if you have some ideas on this topic it may help to hear them prior to the meeting so that they can try to make sure to address those points during their presentations. Please let us know if there are things you'd like to make sure that they address.

We do have time allotted on the agenda to have a general discussion of the network. This can be either a closed discussion with just the external NEC members participating or this could be an open discussion with everyone on-line. Please let me know which option you would prefer.

Information for joining the meeting is below. I will also update the meeting invite. In addition to the agenda – attached again here for your convenience are the executive summaries from each of the centers.

CEIRS NEC Meeting

Mon, Aug 29, 2016 12:30 PM - 4:30 PM Eastern Daylight Time
Please join my meeting from your computer, tablet or smartphone.
[https://global.gotomeeting.com/join/\(b\)\(6\)](https://global.gotomeeting.com/join/(b)(6))

You can also dial in using your phone.

United States (Toll-free): (b)(6)
United States : (b)(6)

Access Code: (b)(6)

Thank you very much and we look forward to speaking with you all soon.

Best wishes,
Diane

Diane J. Post, Ph.D.

Influenza Program Officer
Contracting Officer Representative (COR), CEIRS
Respiratory Diseases Branch
DMID/NIAID/NIH/DHHS
5601 Fishers Lane Room 8E16, MSC 9825
Bethesda, MD 20892

Office: (b)(6)

Cell: (b)(6)

Email: (b)(6)

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 original 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 Institute 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.

Contract: HHSN272201400008C
PI: Adolfo García-Sastre

Annual Progress Report

Title NIAID Centers of Excellence for Influenza Research and Surveillance (CEIRS)

Contract Number HHSN272201400008C

Period Covered April 1, 2015 – March 31, 2016

Type of Report Annual

Investigators Adolfo García-Sastre
Peter Palese
Florian Krammer
Ana Fernandez-Sesma
Megan Shaw
Nicole Bouvier
Harm van Bakel
Randy Albrecht
Ivan Marazzi

Performing Organization Name and Address Icahn School of Medicine at Mount Sinai
1468 Madison Ave, Box 1124
New York, NY 10029

Subcontracts & Lead Investigators

Erasmus MC – Ron Fouchier
University of Wisconsin-Madison – Yoshihiro Kawaoka
University of Georgia – Daniel Perez
University of California Davis – Walter Boyce
MIT – Jonathan Runstadler
Catholic University of Chile – Rafael Medina
INIA-Spain – Gustavo Real-Soldevilla
Cambridge University – Derek Smith
University of Alaska – Eric Bortz
USDA-SERPL – David Suarez, Mary Pantin-Jackwood, Erica Spackman
USDA-ARS – Amy Vincent
Cornell University – Colin Parrish
University of Cambridge – Nicola Lewis

Sponsoring Agency Name and Address Respiratory Diseases Branch
NIAID
National Institutes of Health
Bethesda, MD 30892

Executive Summary

General update

CRIP has maintained an active surveillance network including avian surveillance in the Netherlands, the Republic of Georgia, Indonesia, Vietnam, California, Alaska, Argentina, Guatemala, and Chile. Swine surveillance sites include Spain, Chile, Argentina, and Guatemala, and marine mammal surveillance sites include California and the Gulf of Maine. HPAI H5N8 was last detected in February of 2015 in The Netherlands, with clade-specific antibodies detected during and after the emergence of this virus. Enhanced avian surveillance in California revealed that HPAI H5N8 did not become established in waterfowl. Surveillance in Alaska revealed LPAI H5N2 circulating prior to the emergence of HPAI H5N8 with an internal gene cassette highly related to HPAI H5N1 and H5N2 suggesting a possible precursor. Studies of swine influenza viruses in Argentina and Guatemala demonstrated continuous circulation and reassortment representing multiple independent introductions from unknown sources. Viral isolates were not detected from marine mammals, however serological evidence indicates substantial exposure. Technical improvements including rocket net launchers for live capture of gulls and Bluetooth tagging for grey seals, have revealed a high seroprevalance of influenza virus in adult gulls despite the absence of viral shedding, and allowed for repeat sampling of seals. Animal surveillance in Chile identified two novel swine H1N2 virus clusters and a novel H5N5 LPAIV. Avian-swine interface studies in Spain have not found evidence for transmission of avian influenza viruses from wild birds to wild boars or pigs, suggesting that epizootic transmission differs for wild and industrial swine.

Research examining potentially pandemic viruses including H10N7 viruses that emerged in seals and H5N6 viruses that emerged in humans, revealed clinical disease in ferrets. H10N7 was airborne-transmissible, and H5N6 was very pathogenic. Efficient replication and transmission of H9 subtype viruses was demonstrated in pigs and quail. Work investigating the H5N2 and H5N8 viruses that infected US waterfowl and poultry revealed that 2014 viruses were highly adapted to mallards and domestic waterfowl but not to gallinaceous species, while 2015 viruses were more adapted to chickens. H7N8 LPAI and HPAI viruses that caused outbreaks in commercial turkey flocks in 2016 caused more disease in experimentally infected turkeys than chickens. H9N2 lineage viruses transmitted easily in chickens but H7N9 viruses with similar internal genes transmitted poorly. Swine viruses identified through the USDA swine surveillance system were characterized phylogenetically and a new spillover of human seasonal H3 continues to evolve and spread among swine. A pipeline for risk assessment of swine viruses was established and strain selection and anti-sera production has begun. To quantify the risk of interspecies transmission, the ability of non-swine viruses to infect swine was examined. Risk assessment of bat influenza-like viruses demonstrated their inability to reassort with conventional influenza A viruses.

CRIP made a number of developments in vaccine-related research during the last year. High-yield influenza A and B candidate vaccine viruses are under development. Substantial progress has been made in predicting the antigenic evolution of seasonal influenza viruses, with a clear path to developing improved seasonal influenza vaccines. cHA vaccines were tested in pigs and no association was found between anti-stalk antibodies and VAERD. Clinical trial samples demonstrated that baseline stalk-specific antibodies correlate with age. Research on the mechanism of action broadly-neutralizing HA-specific antibodies has revealed cooperation in polyclonal mixtures between

Contract: HHSN272201400008C

PI: Adolfo García-Sastre

neutralizing and non-neutralizing antibodies, and a requirement for alveolar macrophages for the function of non-neutralizing antibodies.

Research on host factors required for viral replication has identified cellular interaction partners for MxA and Mx1 and testing of diversity outbred mouse susceptibility to influenza has continued. siRNA screening for host factors has been completed and bioinformatics analyses are ongoing. Chemical screening identified novel roles for the helicase Sentaxin, and Topoisomerase 1 in antiviral gene expression. We have found that IRF7 deficiency in humans leads to severe influenza virus infection, and we have identified UBR4 as a host factor required for viral surface protein expression and viral budding. The role of CD43 and the contribution of NS1 dimerization to host responses in dendritic cells has been examined.

A number of reagents have been generated for the influenza community and NIAID, including reagents for H5NX, H6N1, H7N9, and H10N8 research, and reagents for assessing anti-stalk immunity. Resources for the guinea pig model of influenza virus transmission were developed, including cell lines for sialic acid profiling. A number of molecular probes for modified sialic acids were generated and used to test sialic acid expression on a variety of host tissues and flu-relevant cell lines. Two new sequencing protocols were deployed that allowed for the sequencing of a large number of full influenza-virus genomes.

Changes to the CEIRS projects, structure and organization

- New Concepts:
 - Concept 7: Option 11/12 (Boyce, Runstadler, García-Sastre, Enhanced H5 Surveillance)
 - Concept 8: Option 20A (Lewis, Vincent, García-Sastre, Swine pipeline)
 - Concept 9: Option 17 (van Bakel, Runstadler, Kawaoka, DIGS sequencing core, H5 vaccine)
- Continuations:
 - Concept 4: Option 22B/C (Krammer, Reagent Core)
 - Option 21B (Albrecht, Training program)
- Completed Concepts:
 - Concept 1: Option 15 (Parrish, Sialic acid probes)
 - Concept 2: Option 15 (Perez, Strep pneumo)
 - Concept 3: Option 17 (Albrecht, Ferret genomic signatures)
- Project Transferred to new lab:
 - Concept 3: Option 17 (Katz component, Ferret genomic signatures)

Project Progress Description and Highlights

Surveillance Project 1

- We have maintained an up-to-date sample collection from the Netherlands and the Republic of Georgia, and performed culture and subtyping of all PCR positive surveillance samples.
- The last detection of HPAI H5N8 virus in The Netherlands was on February 25, 2015 in a Eurasian widgeon. Clade-specific antibodies to HPAI H5 clade 2.3.4.4 have been detected by HI and VN assays in serum samples obtained during and after the emergence of this virus subtype in Europe.

Surveillance Project 2

- We detected influenza viruses in surveillance samples from Vietnam
- Some Vietnamese H5N1 viruses isolated in 2012-2013 have dual α 2,3/ α 2,6 sialic acid receptor-binding specificity, based on binding to erythrocytes expressing primarily human- and/or avian-type receptors.

Surveillance Project 3

- We demonstrated the continuous circulation and constant reassortment of swine influenza viruses in Argentina.
- Swine influenza viruses in Argentina and Guatemala represent multiple independent introductions from yet to be determined sources
- Avian Influenza viruses from wild birds have been obtained from Argentina and Guatemala.

Surveillance Project 4

- A total of 1332 swabs from 664 marine mammals in California were tested by RTPCR – all were negative for influenza virus. A total of 199 sera samples from 937 marine mammals were seropositive by ELISA.
- Analyses of waterfowl microbiota revealed that 39 bacterial OTUs serve as potential biomarkers on influenza infection.

Surveillance Project 5

- We collected and screened 5,024 avian and marine mammal viral swabs, and identified 344 as AI matrix positive.
- We established live captures of adult glaucous-winged gulls at Cordova, AK using a rocket net launcher. This allowed us to identify high seroprevalence (64.52%) despite an absence of viral shedding, suggesting that recently fledged juveniles are the primary source of virus in this population.
- We completed our 4th year of surveillance on live caught grey seals, established repeat sampling, piloted a Bluetooth tagging approach, and expanded collaborations to better address ecology of disease within this host.
- We demonstrated that bacterial lipopolysaccharide directly reduces the infectivity of avian H3N8 and human H1N1 influenza viruses.

Surveillance Project 6

- We identified two novel swine H1N2 influenza virus clusters endemic to the commercial swine population in Chile.
- We identified a novel H5N5 LPAIV in Antarctic penguins, and characterized franklin's gulls as an important reservoir for the introduction of AIV into South America.

Surveillance Project 7

- In spite of sharing habitat very closely, we have not found evidence of transmission of avian influenza viruses from resident or migratory birds to wild boars or Iberian pigs, suggesting that epizootiology of free-range *Suidae* appears to be different from the classical industrial swine.
- There is a significant difference of seroprevalence between pigs of pure Iberian breed and hybrid Iberian pigs: 13.8% vs 44.3% pointing out the potential involvement of breed-associated genetic traits. There is also significant difference of seroprevalence and virus subtype distribution between Iberian pigs and wild boars sharing the same habitat pointing out a limited transmission between them and a different natural evolution in both hosts.

Research Project 1

- We showed that non-neutralizing HA antibodies can cooperate with neutralizing antibodies to enhance protection *in vitro*, and that they require alveolar macrophages to mediate clearance *in vivo*.
- Using serum samples from a clinical trial where participants received a recombinant HA vaccine, we found that IgG and IgA antibodies recognizing the stalk domain of HA at baseline correlated with age.

Research Project 2

- We infected 600 Diversity Outbred mice with influenza virus and collected a variety of data.
- We identified cellular interaction partners of MxA and Mx1.
- We identified amino acid changes in the viral polymerase proteins that may affect virulence.

Research Project 3

- We completed a siRNA screen of 263 host factors, and used statistical and bioinformatics analyses to identify host factors and biological processes involved in the replication of both H1N1 and H5N1 viruses.
- We analyzed the effect of CD43 expression on H5N1 virus binding, replication, cytokine/chemokine production, and oxidative stress and apoptosis, and evaluated the ability of different influenza virus strains to cleave CD43 in THP1 cells.
- We analyzed the contribution of the dimerization domain of NS1 to IFN antagonism in human primary DCs using an NS1 dimerization mutant (W187R).
- We rescued infectious influenza viruses containing the 6 internal genes from bat influenza-like viruses and demonstrated inability to reassort with conventional influenza A viruses, and studied the role of bat NS1.
- We have found that IRF7 deficiency in humans leads to severe influenza virus infection, and we have identified UBR4 as a host factor required for viral surface protein expression and viral budding.

Research Project 4

- We continued studies on the emergence of potentially pandemic viruses including pandemic H2 and H3 viruses as well as H10N7 and H5N6 viruses that recently emerged in seals and humans respectively. The latter two viruses caused clinical infections in ferrets, with the H5N6 virus being very pathogenic and the H10N7 virus being airborne-transmissible between ferrets.
- We developed an experimental framework to predict the evolution of seasonal influenza viruses.
- We completed deep sequencing of the mRNA transcriptome of influenza-virus infected and uninfected Hartley stock guinea pigs, and developed epithelial and fibroblast cell lines for SA expression studies.

Research Project 5

- We demonstrated efficient replication and transmission in pigs and quail of H9 subtype virus carrying ferret-adaptive changes, and showed plasticity of amino acid 226 of H9 HA.

PP Project 1

- We have made substantial progress, in collaboration with the Kawaoka and Fouchier groups and US CDC, on predicting the antigenic evolution of seasonal influenza viruses. The evolution is sufficiently predicable that there is a clear path to developing an improved seasonal influenza vaccine.

PP Project 2

- We completed sequencing of more than 500 influenza A genomes.

PP Project 3

- The 2014 H5N2 and H5N8 HPAI viruses (clade 2.3.4.4) from the U.S. were determined to be highly adapted to mallards and domestic waterfowl but variably adapted to gallinaceous species. The 2015 H5N2 HPAI viruses causing outbreaks in poultry in the U.S. were more adapted to chickens than the 2014 H5N2 wild duck index virus. In mallards, these viruses were still highly infectious but differences in pathogenicity were observed.
- Experimentally infected turkeys were more susceptible than chickens to the H7N8 LPAI and HPAI viruses that caused the outbreaks in commercial turkey flocks in Indiana in January 2016.
- The H9N2 lineage of virus is highly adapted to poultry and transmits easily in chickens, but the H7N9 viruses with similar internal gene cassette is much less transmissible.

PP Project 4

- Two sets of QA/QC testing were completed in the past year: August-September 2015 and February-March 2016.

PP Project 5

- The USDA-ARS National Animal Disease Center (NADC) investigators analyzed ~1000 new viruses identified through the USDA IAV swine surveillance system at the genetic level and representative strains were obtained from the repository for antigenic or pathotype characterization. A new spillover of a human seasonal H3 (circa 2011) that is antigenically distinct from swine H3 and precursor human H3 continues to evolve and spread among swine.
- Non-swine lineage viruses were investigated for their ability to infect and transmit in the swine host to quantify risk of interspecies transmission, including 4 strains of HPAI H5Nx, the newly emerged canine H3N2, and a swine isolate of H4N6 of North American wild bird lineage.

Concept 1

- Molecular probes for modified sialic acids, including O-acetyl variants and linkage-types, have been generated and verified from recombinant expression of Nidovirus and Influenza glycoproteins
- Through survey of tissue sections, we find that modified sialic acids are present in the respiratory tissues of multiple natural and laboratory hosts of Influenza virus, in species, tissue, and cellular-specific patterns. They are also present in major cell culture lines (MDCK, A549, 293T) used for Influenza virus research.

Concept 2

- We established an *in vitro* tissue culture system to better study mechanisms underlying IAV/S. pneumoniae synergism.
- We determined that at least two different two-component systems in S. pneumoniae sense IAV-infected cells leading to increased survival of bacterial cells in A549 cells.

Concept 3

- Tissue samples collected from ferrets infected with an avian or human isolates of H7N9 were analyzed by plaque assay for virus titers and tissues were sent to University of Washington (Katze lab) for genomics analysis.

Concept 4

- We tested cHA vaccine in pigs and found no association between anti-stalk antibodies and VAERD. VAERD was associated with PBMCs secreting IFN-gamma without stimulation.

Concept 5

- We identified a novel role for Sentaxin, a helicase with unknown function in immunity, in controlling premature termination of antiviral genes and showed that knock out mice and cells derived from people deficient in Senataxin over-respond to infection.
- We identified that depletion and chemical inhibition of Topoisomerase 1 suppresses antiviral gene expression *in vitro* and *in vivo*. This study also highlighted the usage of Top1 inhibitors for treatment of exacerbated response to infection.

Concept 6

- We produced reagents for H5NX, H6N1, H7N9 and H10N8 research, and batches of reagents for assessing anti-stalk immunity for NIAID.
- We characterized mechanisms of protection from H10N8.

Concept 7

- Enhanced avian surveillance in California revealed that while HPAI H5N8 was isolated in January 2015, the virus did not become established in waterfowl and no further detections were made. A total of 147 swabs from 1397 California waterfowl were positive for LPAI by RTPCR, and 84 sera samples from 312 birds were seropositive by ELISA. Sixty-three LPAI viruses and 1 HPAI virus were isolated (in collaboration with SERPL) and full genomes were sequenced.
- We identified LPAI H5N2 circulating in Alaskan mallards prior to emergence of HPAI H5N8 in poultry. A high degree of genetic relatedness between the LPAI H5N2 internal genes and the HPAI H5N1 and H5N2 that later emerged, suggests a possible precursor to reassortment.
- Potential vaccines against H5 viruses have been generated.

Concept 8

- The global analyses of swine and human influenza A viruses which forms the starting point for the swine characterization pipeline has been accepted for publication in eLIFE.
- Significant progress was made in selection of human seasonal strains and swine strains to prepare swine and ferret anti-sera for subsequent HI tests of swine viruses. SJCEIRS shared human seasonal vaccine strains and swine-antisera were generated against the entire starting panel.
- Mexican swine viruses have been isolated and sequenced, identifying a recent common ancestor to pandemic human H1N1 2009 viruses.

Contract: HHSN272201400008C

PI: Adolfo García-Sastre

Concept 9

- The Bakel lab has deployed two new sequencing protocols that have allowed us to scale our sequencing capacity and meet DIGS contract needs. The Perez lab established wet lab and in silico methods for NGS and sequenced 121 full genomes.
- We are currently generating high-yield influenza A candidate vaccine viruses for comparative studies.

Focus and goals for the next contract year

Surveillance Project 1

- H13/H16 evolution in black-headed gulls – epidemiological modelling, genetic and antigenic analysis
- Poultry-wild bird interface in cooperation with the Dutch Central Veterinary Institute (CVI Lelystad): comparison of subtypes and sequences

Surveillance Project 2

- Continue surveillance activities in Vietnam and Indonesia
- Identify potentially novel markers of H5Nx pathogenicity

Surveillance Project 3

- Continue with biological and molecular characterization of H9, H3, and H7 subtype viruses

Surveillance Project 4

- Focus on marine mammal surveillance along the California Pacific Coast, using PCR and serology (ELISA, VN) to identify virus shedding and exposure among Northern Elephant Seals, California Sea Lions, Pacific Harbor Seals, and Southern Sea Otters

Surveillance Project 5

- Continue surveillance efforts in Alaskan seabirds and waterfowl, furthering sequence and phylogenetic analyses to better understand precursors to reassortment and pathways of viral dissemination
- Continue surveillance efforts in marine mammals, focusing on expanded longitudinal analysis and measures of contact structure, as well as additional characterization of positive samples

Surveillance Project 6

- Publish our recent data on both swine and avian influenza viruses, to contribute with novel insights on the circulation and diversity of IAV in South America
- Continue to nurture strong collaborations with government and other academic institutions and the swine industry, to maintain a robust long-term animal surveillance program in Chile

Surveillance Project 7

- Identification and characterization of circulating Influenza viruses in wild boars and free-range Iberian pigs in habitats with abundant wild bird populations
- Identification of ecological, breeding system, genetic traits, and other drivers that affect the epidemiology of swine influenza.

Research Project 1

- Compare the ability of IgG and IgA monoclonal antibodies that display HAI activity, broadly neutralizing stalk-specific antibodies, or antibodies that lack *in vitro* neutralization to protect against lethal challenge in a murine challenge model
- Examine the ability of two broadly-neutralizing influenza B antibodies to neutralize *in vitro* and protect mice *in vivo* against lethal challenge

Research Project 2

- Perform SNP analysis for influenza virus-infected Diversity Outbred mice
- Perform siRNA knockdown studies for potential cellular interaction partners of Mx1 and MxA

Research Project 3

- Confirm the role of the identified host factors in the context of whole virus infection, and assess the role of the confirmed host factors in the adaptation of the avian polymerases to human cells
- Further characterize the cleavage of the sialic acids of CD43 and use a proteomics approach to identify proteins that are phosphorylated after interaction of H5N1 with CD43
- Continue testing selected NS1 mutants for their ability to induce pro-inflammatory cytokines and type I IFN production in human DCs and macrophages
- Characterize the phenotype of fully-infectious HL18NL11 bat influenza-like viruses in vitro and in vivo, and determine the cellular receptor used by the virus

Research Project 4

- Continue to determine the site of the respiratory tract from which influenza viruses are transmitted via the airborne route
- Continuation of research on subtype/clade-dependency of AA substitutions that affect airborne transmissibility and their associated phenotypes
- Generate and test influenza A virus H4 HA proteins possessing H3 HA-specific antigenic epitopes
- Generate high-yield influenza B candidate vaccine viruses
- Identification of species-specific immune regulatory signatures in the respiratory tracts of influenza virus-infected and uninfected guinea pigs and ferrets

Research Project 5

- Expand studies on flu B mechanisms of attenuation
- Restart preparation and characterization of monoclonal antibodies against various influenza virus proteins
- Reestablish ferret model of influenza pathogenesis and transmission

PP Project 1

- Continued progress on all fronts (landscapes, cartography, molecular basis, evolutionary processes, risk assessment) and their integration for being able to predict the evolution of influenza viruses, and how to protect against them with vaccines

PP Project 2

- Provide complete genome sequence for at least 750 influenza A isolates

PP Project 3

- Identify molecular markers of adaptation of AI viruses (H5 clade 2.3.4.4 and H7N9) in different avian species
- Conduct pathogenicity and transmission studies with emerging AI viruses in avian species

PP Project 4

- Continue QA/QC program

PP Project 5

- Continue genetic and antigenic characterization of USA swine IAV, including follow-up work on the recent human-to-swine seasonal H3 spillover viruses, H3 antigenic sites, and gamma H1 and H1N1pdm09

Concept 1

- Funding expired

Contract: HHSN272201400008C

PI: Adolfo García-Sastre

Concept 2

- Funding expired

Concept 3

- Coordinate with Ian Lipkin and Juliet Morrison to complete the transcriptional analysis of the tissue samples obtained from H7N9 infected ferrets

Concept 4

- Finalize cHA studies in the pig model

Concept 5

- Evaluation of the role of Topoisomerase enzyme during *in vivo* influenza infection, in both preventive and therapeutic settings

Concept 6

- Continue producing reagents for CEIRS, NIAID and the global influenza virus research community

Concept 7

- Pending funding, Conduct surveillance of 500-1000 waterfowl during the summer, fall and winter where HPAI viruses were identified in 2014 and 2015

Concept 8

- Determine antigenic relatedness between swine and human strains using swine and ferret antisera and antigenic cartography to identify highly divergent swine strains
- Finalize pipeline workflow with all global partners and commence genetic and antigenic real-time analyses
- *In vitro* and *in vivo* characterization of the genetic determinants of swine adaptation of the human seasonal H3 and reverse engineered reassortants, and newly emerging non-swine lineage IAV

Concept 9

- Further develop and test high-yield influenza A virus candidate vaccine viruses
- Optimize methodology for virus isolation of difficult to grow wild isolates.
- Provide complete genome sequence for at least 300 influenza A isolates

Specific concerns or delays

- Research Project 5
 - This project has been severely affected by the Stop Work Order on GOF studies. As a consequence we have developed alternative studies that preserve the overall goal of the original project (understanding interspecies transmission) but do not include GOF studies.
- Concept 4
 - The time/budget for finalizing all proposed work may not be sufficient.
- Concept 8
 - Although the concept was awarded in August 2015, no formal agreement was in place between the University of Cambridge and NIH until December 2015. Thus, the initial phase of the option was carried out at risk, and we were unable to advertise for the key post-doc position until the sub-contract was signed.
- Concept 9
 - A delay was caused by the GoF assessment for the testing of high-yield influenza A candidate vaccine viruses with the HA and NA genes of different H5N1 viruses. We have now obtained permission to carry out the proposed experiments.

Summary Tables

Full list of CEIRS Projects

Project Title	Funding Mechanism	PI	Status
Surveillance Project 1	Base	Fouchier	Ongoing
Surveillance Project 2	Base	Kawaoka	Ongoing
Surveillance Project 3	Base	Perez	Ongoing
Surveillance Project 4	Base	Boyce	Ongoing
Surveillance Project 5	Base	Runstadler	Ongoing
Surveillance Project 6	Base	Medina	Ongoing
Surveillance Project 7	Base	Real-Soldevilla	Ongoing
Research Project 1	Base	Palese	Ongoing
Research Project 2	Base	Kawaoka	Ongoing
Research Project 3	Base	García-Sastre, Fernandez-Sesma, Shaw	Ongoing
Research Project 4	Base	Fouchier, Bouvier	Ongoing
Research Project 5	Base	Perez	Ongoing
PP Project 1	Base	Smith	Ongoing
PP Project 2	Base	van Bakel	Ongoing
PP Project 3	Base	Suarez, Pantin- Jackwood	Ongoing
PP Project 4	Base	Spackman	Ongoing
PP Project 5	Base	Vincent	Ongoing
Concept 1	Option 15	Parrish, Perez	Completed 2/16
Concept 2	Option 15	Perez	Completed 11/15
Concept 3	Option 17	Albrecht	Completed 11/15
Concept 4	Option 18	Krammer	Ongoing
Concept 5	Option 18	Marazzi	Ongoing
Concept 6	Option 22	Krammer	Ongoing
Concept 7	Option 11 and 12	García-Sastre, Rundstadler, Boyce	Ongoing
Concept 8	Option 20	García-Sastre, Lewis, Vincent	Ongoing
Concept 9	Option 17	van Bakel, Runstadler, Kawaoka	Ongoing

Active Surveillance Sites

Country, State/Province	Surveillance Type				Project
	Avian	Swine	Human	Other	
Netherlands	X				Surveillance Project 1
Republic of Georgia	X				Surveillance Project 1
Indonesia	X		X (if available)		Surveillance Project 2
Vietnam	X		X (if available)		Surveillance Project 2
California	X			X (marine mammal)	Surveillance Project 4 Concept 7
Spain		X			Surveillance Project 7
Chile	X	X	X		Surveillance Project 6
USA, Alaska	X			X	Surveillance Project 5
USA, Gulf of Maine				X	Surveillance Project 5
Argentina	X	X			Surveillance Project 3
Guatemala	X	X			Surveillance Project 3

Publication Summary

Reporting period April 1, 2015-March 31, 2016

Current contract number: HHSN272201400008C	41
Previous contract number: HHSN266200700010C	15
USDA/SERPL IAA: AAI12004	1
USDA/ARS IAA: AAI14006	0
Current Emory-UGA contract number: HHSN272201400004C	1
Previous SJCEIRS contract number: HHSN266200700005C	1
No reference to CEIRS contract number	6
Total	65

Data Summary

	Samples collected	Samples submitted to DPCC	Influenza viruses identified	Full genomes sequenced	Reagents
HHSN272201400008C	34,532	33,079	829	677	295

Annual Progress Report

Title NIAID Centers of Excellence for Influenza Research and Surveillance (CEIRS)

Contract Number HHSN272201400004

Period Covered April 1, 2015 – March 31, 2016

Type of Report Annual

Investigators Walter A. Orenstein, MD., Richard W. Compans, Ph.D. Erin-Joi Collins, MS, MPH, David A. Steinhauer, Ph.D., Jens Wrammert, Ph.D., Anice Lowen, Ph.D., John Steel, Ph.D., Rafi Ahmed, Ph.D., Bali Pulendran, Ph.D., Aneesh Mehta, MD., Edmund K. Waller, MD, Ph.D, Saad Omer, MPH, PhD, Chinglai Yang, Ph.D., Ioanna Skountzou, M.D., Ph.D., Jacob Kohlmeier, Ph.D., Vicki Hertzberg, Ph.D., Andrea Plotsky, MSPH, Ramya Govindarajan, MS, MSPH

Performing Organization Name and Address Emory University
Atlanta, GA 30322

Subcontracts & Lead Investigators

- 1) University of Georgia, Athens, GA
Ralph A. Tripp, Ph.D.
S. Mark Tompkins, Ph.D.
- 2) Harbin Veterinary Research Institute, China
Hualan Chen, Ph.D.
- 3) University of Georgia, Atlanta, GA
Timothy Denning, Ph.D.
- 4) Beth Israel Deaconess Medical Center, Boston, MA
Richard Cummings, Ph.D.

Sponsoring Agency Name and Address Respiratory Diseases Branch
NIAID
National Institutes of Health
Bethesda, MD 30892

Project Officer: Diane J. Post, Ph.D.

Contact: Erin-Joi Collins, MS, MPH, CPH
Associate Director

(b)(6)

Executive Summary

General update

Research Project 1 is focused on gaining a better understanding of how influenza viruses bind to glycan receptors and the relationship between the hemagglutinin of influenza viruses and the neuraminidase. Significant progress has been made by Research Project 1 on the technology being developed for influenza receptor binding studies. Notably, the Cummings lab has made a breakthrough in the capacity to efficiently isolate glycans from cells and tissues, which will allow for broad applicability and availability of the shotgun microarray technology for identifying natural influenza receptors. A report detailing these approaches, termed Oxidative Release of Natural Glycans (ORNG), has recently been accepted for publication in Nature Methods (Song et al, in press). The techniques have already been applied to pig lung tissue, and demonstrate that the efficiency of glycan release can be increased by up to 100-fold relative to enzymatic methods. We have recently obtained several human lungs from the LifeLink Organ Donor Program of Georgia, and are currently expanding our studies to identify influenza receptors in the human respiratory tract. We have also made significant progress in assay development for influenza NA activity and specificity using three separate but complementary approaches; an ion exchange chromatography-based assay, a glycan reductive isotope labeling (GRIL) mass spectroscopy-based assay, and a glycan microarray substrate-based assay. Future studies will apply these assays in conjunction with HA binding assays on natural influenza substrates, and will be critical for our understanding of HA-NA functional balance. We have also analyzed a broad spectrum of HA subtypes for reactivity to anti-HA stem antibodies, and defined residues in the HA stem involved in the trigger for acid-induced conformational changes and HA stability. Significantly, we have identified a histidine residue at HA2 position 111 that plays a critical role for the triggering of Group-1 HAs. This residue is highly conserved in the Group-1 subtypes, and appears to serve the same purpose as the conserved histidine at HA1 position 17 of Group-2 HAs.

Research project 2 is focused on understanding factors which increase transmissibility of influenza viruses and influence reassortment. In Year 2, work carried out under Research Project 2 further solidified a role for the viral M1 protein in determining transmission phenotypes. An M segment from an avian adapted strain was found not to support transmission in a guinea pig model, while that from a human adapted strain does. A motif in M1 which appears to be important for transmissibility was also identified. Other efforts revealed that RNA packaging signals on influenza virus gene segments can be important in determining the outcome of reassortment between heterologous virus strains. In a publication in PLOS Pathogens, we reported that two classes of influenza A virus (IAV) particles that are not fully infectious impact the frequency of reassortment. Defective interfering particles were found to suppress reassortment, likely due to the suppression of infectious progeny production. Conversely, semi-infectious particles, which deliver fewer than eight segments to

the site of replication, augment reassortment by creating a requirement for co-infection to achieve productive infection and thereby increasing the proportion of infected cells that are co-infected. Recent data reveal that levels of semi-infectious particles are greatly increased in a mismatched virus/host system, when variants of an avian influenza virus are used to co-infect mammalian cells. This finding suggests that reassortment may be highly prevalent in the context of host species transfers.

The first arm of Project 4 focuses on gaining better understanding of the adaptive immune response to influenza vaccination. The second arm focuses on evaluating the innate immune response to influenza vaccination and infection. Research Project 4 has found that antibody secreting cells (ASCs) only can be transiently detected in peripheral blood after influenza vaccination, peaking at 7 day post-vaccination. However, activated B cells (ABCs) we described in our last report persist for up to two weeks post-vaccination. ASC and ABC clonal lineages most likely have originated from the same memory B cells and later diverged into distinct fates. The ABC clones in vaccinated individuals are enriched for vaccine-induced lineages at day 7, and a few members of these clonal lineages remain in a non-activated state in the blood at day 14 after vaccination and contribute to the resting memory B cell population. To achieve the goals of the second arm of the project, we used in vitro experiments with human innate cells, samples from influenza patients and also in vivo mouse models. We have established the in vitro susceptibility of different innate cells to influenza virus strains. In influenza patients, analysis of nasal wash cell samples revealed distinct temporal profiles of innate cell accumulation and their gene expression in the airways that could correlate with the clinical course of infection. Experiments in mice with DC-specific deletion of mTOR has revealed the critical role for CD103+ mucosal DCs in generation of virus-specific CD8+ T cell responses.

Surveillance project 1 is focused on understanding the characteristics of influenza infections in swine. During the reporting period, Surveillance Project 1 received and analyzed 3609 clinical specimens from 404 farms from the Southeastern US and the Midwest. We identified 256 positive samples and were able to isolate 71 viruses, while we conducted Next Generation sequencing (NGS) in 94 clinical specimens and 91 viruses and plaque isolates. NGS data are currently being analyzed. We have generated phylogenetic trees and we are comparing our isolated viruses to reference viruses and vaccine strains. We also began testing our viruses for resistance against antiviral drugs. Finally, we tested field samples by enhanced Raman spectroscopy (SERS) and compared these results with PCR to optimize the application of the technology.

Surveillance Project 2 is focused on studying the characteristics of influenza viruses circulating in domestic chickens, ducks, and pigs both in farms and live bird markets in southeastern China. Surveillance Project 2 collected samples from over 4000 animals (including 1060 chickens, 950 ducks, and 1800 pigs)

during this period as compared to 1500 animals (500 from each species) planned for the project. Whole virus sequencing has been completed for 21 virus isolates and mouse infectivity has been characterized in 9 viruses. Some AIVs of the H3 and H4 HA subtypes are able to infect and replicate in mammalian hosts at high levels without adaptation. Further, some of these viruses also exhibit affinity for human like sialic acid receptors and could potentially transmit in mammalian hosts.

After establishing a pregnancy model in BALB/c mice, Pilot Project 1 investigated the consequences of low infectious dose (0.5xLD₅₀) of pandemic H1N1 influenza A/California/07/09 during the mid to late gestational period of pregnancy and compared those consequences to infection with seasonal H1N1 influenza A/Brisbane/59/07. Severity of A/California/07/09 infection, completion of gestation, and health status of the fetus were all closely monitored and compared to non-infected pregnant mice. We found significantly increased viral replication within the lungs of pregnant mice infected with pandemic or seasonal influenza compared to non-pregnant infected controls indicating a less effective immune response in the pregnant mice. H1N1 infection significantly reduced the gestation length of pregnant mice and increased the likelihood of small for gestational age (SGA) and stillborn offspring. Increased morbidities and mortalities found in pregnant mice and their offspring may be explained by observed changes in the expression of hormones and cytokines. We expanded our study on the effect of seasonal influenza, A/Brisbane/59/07 on progesterone. Progesterone, which is necessary to sustain pregnancy, was reduced in sera, lung, and placenta tissues after infection. At the same time, we found increased levels of prostaglandin F_{2α}, an abortifacient known to induce labor, in the placentas of these pregnant infected mice compared to non-infected controls. Cytokine expression in the lungs and sera of pregnant and non-pregnant mice varied after infection, indicating compartmentalization of innate immune responses. The vast majority of cytokine levels were also suppressed in the placentas and fetuses of pregnant infected mice possibly due to feto-placental tolerance.

The overall objective of Pilot Project 2 is to define the role of individual mucosal antigen presenting cells (APC) subsets in the differentiation and protective immune functions of influenza-specific T and B cell responses in the upper respiratory tract and the lung. We have determined that monocytes are the primary antigen-presenting cell subset in the lung harboring influenza antigens at the late stages of the effector T cell response, which is when we observe the establishment of flu-specific lung-resident T cell memory (lung T_{RM}). Using genetic depletion in mice, we have found that defective monocyte recruitment to the lung significantly decreased the number of flu-specific lung T_{RM} following influenza infection. Furthermore, our data show that only the non-classical monocyte subset is capable of promoting the expression of tissue-resident markers on antigen-specific T cells *in vitro*, suggesting that this subset of monocytes is required for optimal lung T_{RM} following influenza infection or pulmonary vaccination.

Since receipt of the funds in late 2015, Cross Center Project 1, the Ferret Reagent Core, has expressed seven recombinant ferret proteins (see table below), including four ferret cytokines. All cDNAs are optimized for mammalian expression and contain 6x-His and Myc tags for purification and detection. We are currently confirming successful purification of the cytokines by nickel chromatography and expect to test for biological activity using primary ferret cells. The remaining three receptor proteins are on hold while we focus on the cytokines. We also have mice immunized with ferret IFN λ (received from Mount Sinai), however immune responses were not robust, so we are boosting as soon as we have additional recombinant protein and are preparing for fusion for mAb generation.

In the first 6 months of CEIRS- Distributed Influenza Genomic Sequencing (DIGS), Cross Center Project 2, the project leaders coordinated their work and exchanged protocols between the centers in an effort to homogenize the procedures and results that will be generated. Virus isolates and control viruses were tested by Next Generation Sequencing (NGS) and gene software analysis software were compared. With the participation of DPCC we expect to have better coordination for the distribution of samples from CEIRS partners to analyze.

Over the past year, our Data Management Team has continued collecting data from scientists and delivering as required per contract. We developed RedCap databases for the two Clinical studies, wrote documentation and trained the Research Coordinators and Quality Manager on their use. We also worked extensively with the School of Medicine IT Department, who manages our data warehouse, in their efforts to ensure our FISMA compliance. This is in the final steps of completion. We worked with new personnel to train them on the various Data Management tools that we use. We actively participated with the various DPCC Working Groups and attended almost 100% of the meetings.

Changes to the CEIRS projects, structure and organization

In the past year, we have added one Option to our contract, Option 17a, Cross Center Projects. Included in this option were the Ferret Reagent Core and CEIRS Distributed Influenza Genomic Sequencing Core (DIGS), and a subcontract to Beth Israel Deaconess Medical Center of Harvard University to continue our work in glycomics research.

Project Progress Description and Highlights

Research Project 1:

- Developed the Oxidative Release of Natural Glycans (ORNG) technique for efficient generation and expanded use of natural glycan microarray technology
- Development of NA specificity assays using three alternative approaches; a) ion exchange chromatography, b) glycan reductive

PI(s): Walter A. Orenstein, Richard W. Compans

isotope labeling (GRIL) and mass spectroscopy, c) glycan microarrays as substrates

- Identified HA group-specific trigger residues involved in acid-induced conformational changes, membrane fusion, and HA stability.

Research Project 2:

- Influenza virus reassortment is enhanced by semi-infectious particles but suppressed by defective interfering particles (Fonville et al., PLOS Pathogens, 2015)
- Preliminary results suggest that semi-infectious particle levels and therefore reassortment frequencies are dependent on host adaptation
- A motif in M1 which appears to be important for transmissibility has been identified.
- Reassortment of the HA segment between Pan/99 (H3N2) and NL/602 (H1N1) viruses is limited by divergence in the RNA packaging signals.

Research Project 4:

- Activated B cell (ABC) clonal lineages originate from the same memory B cells as ASC and later diverge into distinct fates.
- A few of the ABCs clonal lineages remain in a non-activated state in the blood for 14 days post-vaccination and contribute to the resting memory B cell population.
- Established the in vitro susceptibility of human innate cells to influenza virus infection.
- Developed flow cytometry panels for staining nasal wash immune cells.
- Measured temporal profiles of immune cells in airways following influenza infection.
- Analyzed the gene expression changes in immune cells of airways.
- Demonstrated the critical role for mTOR in development of lung CD103+ DC required for CD8+ T cell response to influenza vaccine Live Attenuated Influenza Vaccine (LAIV, Flumist)

Surveillance Project 1

- 3609 clinical specimens were collected and analyzed, 256 Swine Influenza Virus (SIV)-positive samples were identified; 71 viruses were isolated
- 94 clinical specimens and 91 virus and plaque isolates were sequenced by Next Generation Sequencing; genetic analysis was completed on 50% of the specimens and phylogenetic trees were generated and compared to relevant strains
- We have begun testing viruses for possible antiviral drug resistance
- All samples were analyzed by Surface Enhance Raman Spectroscopy (SERS) and PCR and analysis is ongoing.

Surveillance Project 2:

- Collected samples from over 4000 animals (including 1060 chickens, 950 ducks, and 1800 pigs) during this period as compared to 1500 animals (500 from each species) planned for the project.
- Completed whole genome sequencing of 21 virus isolates.
- Characterized 9 viruses for their infectivity in mice.

- Investigated H4 AIV infectivity and transmissibility in mammalian hosts.

Pilot Project 1:

- Lung virus titers in pregnant infected mice were significantly higher compared to non-pregnant controls, indicating reduced viral clearance and a less effective immune response.
- Sub-lethal H1N1 A/California/07/09 infection significantly reduced the gestation length of pregnant mice and increased the likelihood of small for gestational age (SGA) and/or stillborn pups.
- Exposure to influenza virus induced early pregnancy termination in mice via a significant reduction of progesterone levels and increase in prostaglandin F_{2α}. There was a 5-fold reduction of progesterone levels in sera and lung tissue as well as a 40% decrease found in the placentas of pregnant mice after infection. This reduction of progesterone in pregnant mice correlated with viral load found in the lungs. Prostaglandin F_{2α} levels in the placentas of infected mice were increased 5-fold.
- Differences were identified in cytokine expression in the sera and lungs of pregnant and non-pregnant cohorts before and after infection. Overall, cytokine expression was suppressed in the placental and fetal tissues of mothers who were infected by influenza virus, providing additional evidence for feto-maternal tolerance.

Pilot Project 2:

- We have identified the pulmonary APC subsets that harbor influenza antigens in the lung over the course of influenza infection and after viral clearance
- We have discovered that lung-resident monocytes are a predominant source of influenza antigens during the late stages of the immune response following influenza infection, which coincides with the initial development of flu-specific lung-resident memory T cells after viral clearance
- We have demonstrated that mice with defective recruitment of monocytes to the lung (CCR2 deficient mice) have a significant decrease in the number of influenza-specific, lung-resident memory CD8 T cells, despite normal numbers of systemic influenza-specific memory CD8 T cells
- We have found that the non-inflammatory monocyte subset is primarily responsible for the differentiation of flu-specific CD8 T cells into a tissue-resident memory T cell phenotype.

Cross Center Project 1:

- We have cloned and expressed the following recombinant ferret cytokines
 - Interferon lambda 1 & 3
 - Interferon beta
 - Interferon gamma
- Each cytokine has a 6xHis tag for detection and purification. We are currently optimizing for purification by nickel chromatography.
- We are beginning functional assays for the cytokines using primary ferret cell cultures.

PI(s): Walter A. Orenstein, Richard W. Compans

- We have cloned and expressed three ferret receptors for future reagent development.

Cross Center Project 2 (CEIRS-DIGS):

- NGS protocols were exchanged
- UGA has run clinical samples and control viruses with the protocols acquired from other centers to standardize methodology and succeeded in sequencing low-titer samples
- Sample analysis was confirmed with standard software
- We participated in surveys by DPCC and contributed towards the optimization of communication between the centers

Data Management:

- Completion of the data warehouse and documentation
- Reporting of all data received from scientists – including surveillance, genomic and reagents
- Development of RedCap databases for the Clinical studies
- Prepared for changes to data warehouse to correspond with DPCC template changes

Focus and goals for the next contract year

Research Project 1: We plan to extend the use of ORNG technology to begin generating glycans from human lung tissue for shotgun glycomics experiments to identify influenza receptors in the human respiratory tract. We will also continue development of the NA specificity assays in order to complement our HA binding assays. These will be critical for developing a broad understanding of HA-NA functional balance, and we plan to apply these techniques to studies on isolates from different hosts and human clinical isolates, as well as studies planned in collaboration with other CEIRS Centers.

(b)(4)



Research Project 4: The focus of the next year will be to examine the quality of the influenza HA-specific memory B cell responses; determine if the specificity or level of hypermutation of responding B cells clones play a role in deciding fate and longevity. The second focus will be to continue to evaluate responses to influenza vaccination and infection using a systems biology approach.

Surveillance Project 1: We plan to continue our surveillance, expand our sequencing and genetic analyses and focus on developing a model for the evolution of influenza in swine. Furthermore we will test our virus isolates against

antiviral drugs to determine possible resistance and deploy the SERS hand-held device in the field.

Surveillance Project 2: Surveillance of animal influenza virus activity requires accumulation of data for better understanding the dynamics of influenza virus activity in domestic animal species. We will continue our analysis of the genotypes of the influenza virus isolates we have obtained from our surveillance activity so far, to determine their gene lineage for understanding their prevalence and reassortment in domestic animals. We will put more emphasis on integrating the analysis of virus isolates obtained from our surveillance in Guangxi, China with viruses obtained from other regions of China through surveillance to gain better understanding of the overall animal influenza activities with respect to evolution and prevalence in domestic animals and to determine their potential for infecting and transmitting in mammalian hosts to improve our preparedness against outbreaks by these viruses in humans.

Pilot Project 1: We are planning to continue our studies on the impact of influenza virus infection on the fetoplacental interface. Since infection is associated with apoptosis and fibrin degradation by matrix metalloproteinases (MMPs), we will examine the upregulation of apoptosis-related caspases (caspase 3 and 7) and MMP 1, 2, 3, 7, 8 and 9 in the amniotic fluid and placenta of infected females. Specifically, circulating MMP-9 and MMP-2/TIMP-2 are associated with early termination of pregnancy.

Pilot Project 2: In the next contract period, we will complete the remaining tasks in Aim 1 to determine the importance of mucosal CD11b⁺ dendritic cells and CD103⁺ dendritic cells for the generation of flu-specific lung resident T cell memory. We will also continue our investigation into the role of non-classical monocytes in this process by depleting these cells during influenza infection and determining the impact of depletion on flu-specific lung resident T cell memory and cellular immunity to heterosubtypic influenza challenge.

Cross Center Project 1 (Ferret Reagent Core): During no cost extension (since funds arrived late) and if funds are renewed in fall 2016, we will focus on confirming biological activity of the four ferret cytokines and then generating mAbs. We will also test existing reagents from commercial sources for cross-reactivity. Once we have made progress with the cytokines, we will shift to the receptor proteins to purify for antibody generation.

Cross Center Project 2 (CEIRS-DIGS): In the next year of the project, we expect to receive viruses from CEIRS partners and provide NGS services to them. These will include generating consensus sequences and raw data. Sample distribution will be coordinated by DPCC and we expect to start in the next couple of months.

Data Management: For the coming contract year, we have three main projects outlined. The first is to bring our data warehouse into agreement with the changes made by DPCC to reporting templates during the past year. We will then be able to upload more data to our warehouse. The other is to participate with development of the new reporting requirements for experimental data, followed by reporting the data we have been collecting for our published studies over the past year.

Specific concerns or delays

Research Project 1: The relocation of the Cummings lab from Emory University to Harvard University in 2015 resulted in a temporary slowdown in several of the glycan-based studies due to the inevitable changes in equipment and laboratory settings, as well as continuity disruptions for personnel and adaptation to the new environment. The lab is now up and running, and we feel that the long-term benefits associated with the move will far outweigh the initial hurdles.

(b)(4)



Research Project 4: Delays in the initial approval of clinical projects have affected the start of the clinical arm of the project and enrollment of subjects. This has resulted in no enrollments in the base year of the contract. In Option 1, we were able to partially make up for the delay in the Healthy Volunteer Study by enrolling 16 instead of the potential 20. Additional delays in the approval of the Bone Marrow study has resulted in only 3 patients being enrolled in Option 1 with 1 lost to follow-up. Changes in Center personnel have contributed to the delays in the Bone Marrow Study and the still to be submitted Pregnancy Study.

Cross Center Project 1: Notice of funding for the concept was given in August 2015. Funding was to be effective September 4, 2015 – May 3, 2016. Unfortunately, the contract took some time to establish. UGA provided pre-award funds effective October 27, 2015 and the actual award arrived December 3, 2015. With funds arriving amidst the November and December holiday season, remarkable progress did not start until 2016. On a positive note, we have requested a no-cost extension to enable continued progress through the summer in the hope of receiving renewed funding in the fall of 2016. We hope with a renewal, we will be able to streamline the funding. On the scientific front, preliminary immunizations suggest ferret IFN λ is poorly immunogenic in BALB/c mice we are boosting for fusion, but may need to utilize NZB/W or similar autoimmune-prone mice.

Data Management: We have had two things which have slowed down our reporting progress over the past year – one is the required FISMA security checks – specifically, completion of the IT-SC&A Report. Because of this, our data warehouse has been unavailable for adding data for much of the year. Fortunately, this is nearly completed. The second is the lack of a method for reporting Experimental data, since ImmPort has been abandoned. Because of this, we have not been able to report much of our experimental data. However, we are hopeful that both of these problems will be eliminated in the coming few months. We look forward to the new, simpler reporting method for Experimental data.

Administrative Core: Over the past year, we have had significant personnel changes replacing all personnel in the administrative core with the exception of the PIs. It took time for training and to have everyone become familiar with the contract.

Summary Tables

Full list of CEIRS Projects

Project Title	Funding Mechanism	PI	Status
Project 1: Influenza glycoprotein functions as determinants of host range, transmission, pathogenicity, and pandemic potential	Base Period Option 1 Option 2	Steinhauer	Ongoing
Project 1: Beth Israel Medical Center Subcontract	Option 17A-1	Cummings	Ongoing
Project 2: Host adaptation, reassortment and transmission of influenza viruses at the animal-human interface	Base Period Option 1 Option 2	Steel/Lowen	Ongoing
Project 4: Human immune responses to influenza virus vaccination and infection	Base Period Option 1 Option 2	Ahmed	Ongoing
Surveillance Project 1: Multidisciplinary studies investigating the natural history of influenza in swine	Base Period Option 1 Option 2	Tripp	Ongoing
Surveillance Project 2: Animal influenza surveillance in Guangxi, China	Base Period Option 1 Option 2	Yang/Chen	Ongoing
Pilot Project 1: Effect of influenza pathogenesis in pregnant mothers and their fetuses in a mouse model	Option 18A	Skountzou	Ongoing
Pilot Project 2: Mucosal antigen presentation and the control of local immunity to influenza virus	Option 18B	Kohlmeier	Ongoing
Cross Center Project 1: Development of Novel Ferret Reagents	Option 17A-2	Tompkins	Ongoing
Cross Center Project 2: CEIRS Distributed Influenza Genomic Sequencing (DIGS)	Option 17A-3	Tripp	Ongoing
Data Management and Analysis Team	Base Period Option 1 Option 2	Hertzberg	Ongoing
Administrative & Management Core	Base Period Option 1 Option 2	Orenstein/ Compans	Ongoing

Active Surveillance Sites

Country, State/Province	Surveillance Type				Project
	Avian	Swine	Human	Other	
USA, NC, SC, IA, IL, MO, UT		X			Surveillance Project 1
China, Guangxi	X	X			Surveillance Project 2

Publication Summary

Reporting Period	HHSN272 201400004C	HHSN266 200700006C	No CEIRS contract number cited
Base Year	12	13	0
Option 1	6	7	1
Total	18	20	1

Data Summary

Cumulative numbers 04/01/2014- 03/31/2016	Samples collected	Samples submitted to DPCC	Influenza viruses identified	Full genomes sequenced	Reagents
HHSN272201400004C	10173	10173	171	14	206
HHSN266200700006C	19350	19350	49	39	291
TOTALS	29523	29523	220	53	491

Annual Progress Report

Title	JHCEIRS: A NIAID Center of Excellence for Influenza Research and Surveillance (CEIRS)
Contract Number	HHSN272201400007C
Period Covered	April 1, 2015 – March 31, 2016
Type of Report	Annual
Investigators	PIs: Richard Rothman and Andrew Pekosz Co-Investigators: Andrea Dugas, Joshua Epstein, Andrew Feldman, Charlotte Gaydos, Gabor Kelen, Sabra Klein
Performing Organization Name and Address	Johns Hopkins Department of Emergency Medicine 5801 Smith Avenue, Davis Building, Suite 220 Baltimore, MD, 21209
Subcontracts & Lead Investigators	Applied Physics Laboratory, PI: Peter Thielen Vanderbilt University, PI: James Crowe
Sponsoring Agency Name and Address	Respiratory Diseases Branch NIAID National Institutes of Health Bethesda, MD 30892

Executive Summary

General update

The overarching objective of the Johns Hopkins University Center for Excellence in Influenza Research and Surveillance (JHCEIRS) is to improve the ability of the medical and public health infrastructure to respond to influenza pandemics and seasonal influenza. We aim to achieve this by advancing innovative tools and algorithms for rapid molecular surveillance and viral fitness characterization, building new knowledge regarding host immune response, and applying robust global modeling methods to evaluate the effectiveness of various public health intervention strategies for pandemic planning response.

The second year of the JHCEIRS contract was focused on our first year of clinical surveillance, and secondary protocol development and project start up including development of the infrastructure required to initiate the pipeline of human clinical samples to virus characterization model. The JHCEIRS Management Core primed the entire team including all investigators, staff and students and in our first year, successfully ran all respective projects, meeting enrollment targets (and exceeding some) and initiating the virus selection pipeline. The role of the JHCEIRS the Management Core is to monitor all areas of the JHCEIRS and provide assurance of the effective implementation of the overarching goals of JHCEIRS to all areas and personnel who support JHCEIRS. The Management Core is responsible for coordination, supervision and oversight of all JHCEIRS research and administrative activities. In the second year of the JHCEIRS contract, the Management core has successfully submitted all appropriate regulatory documents, including documents for OMB emergency and routine review. In the beginning of this year, we obtained site-activation for all our clinical sites and received OMB protocol approval.

In addition, JHCEIRS leadership has recruited for and selected all necessary personnel, initiated protocol development for a new clinical study, participated in all data management aspects, submitted 11 monthly invoices and technical reports, initiated 2 subcontracts, submitted 7 potential option projects for EOY funding (two renewals, five EOY proposals) and continued three CEIRS network projects. JHCEIRS has also hosted their first external advisory committee meeting, with the goal of improving oversight and guidance of the Center's activities.

The makeup of the external advisory board is as follows:

Maryna Eichelberger, PhD

Senior Investigator
Division of Viral Products
Center for Biologics Evaluation and Research
Food and Drug Administration

(b)(6)

Scott E. Hensley, PhD
Assistant Professor
Member, Vaccine Center
The Wistar Institute

(b)(6)

W. Ian Lipkin, MD (JHCEIRS NEC Representative)

John Snow Professor of Epidemiology
Center for Infection and Immunity
Mailman School of Public Health
Columbia University

(b)(6)

Kathleen Neuzil, MD
Professor and Director
Center for Vaccine Development
Department of Medicine
University of Maryland
School of Medicine

(b)(6)

Gregory Storch MD
Professor and Director
Divisions of Pediatric Infectious Diseases and Pediatric Laboratory Medicine
Department of Pediatrics
Washington University
School of Medicine in St. Louis

(b)(6)

The feedback of the EAB was overwhelmingly positive. Their full feedback can be found below, along with JHCEIRS responses. Their concerns focused on three main areas – Addition of surveillance sites with critically ill and pediatric patient populations; Addition of influenza surveillance in Africa; and, expansion and/or continued reanalysis of our rapid diagnostics approach.

General Progress Description and Results:

- On target to meet all enrollment goals for all surveillance studies
- Initiation of Virus Selection Committee and clinical specimen pipeline
- Attended First Annual Network Meeting
- Finalized NIAID protocols including:
 - Clinical Concept Templates
 - Concept Templates (nonclinical studies)
 - Human Surveillance protocol and components (DMID and JHIRB)

- Generic OMB Review
- Shelf Protocol and components (DMID and JHIRB)
- Obtained COA for all subcontract sites
- Hosted first external advisory board meeting (see above for advisory board makeup)

CEIRS Network Engagement

- Participated in several CEIRS Network Events
 - 2nd Annual Meeting
 - Surveillance Meeting
 - Monthly Coordinator Calls
 - Monthly Data Management Meetings
 - Monthly PI Meetings
 - Gain of Function Activities
 - Presented at CEIRS Webinars
 - Andrew Pekosz
 - Sabra Klein
- Continued work on three CEIRS Cross-Network Projects
 - Determining early genomic signatures from ferret infection
 - Systems biology of innate immunity and vaccination
 - Immune responses to predict clinical outcomes
- Continued engagement with other CEIRS Centers on Pandemic Research Response Plan development
- JHCEIRS PI Co-Chairs human surveillance working group

Changes to the CEIRS projects, structure and organization

Studies starting this year:

Option 19:

- 1) Respiratory cell repository core
- 2) Ferret reagent core
- 3) Pandemic potential of swine influenza viruses

Studies ending this year:

Option 8A: Additional effort to base

Project Progress Description and Highlights

Human Influenza Surveillance in the United States and Taiwan

- In our first year, we have met original passive surveillance enrollment target; increased enrollment targets
- We have also met original active surveillance flu positive enrollment target; increased enrollment targets
- We are on track to meet all enrollment targets during this year's influenza season.

Genotype Determination with RT-PCR/ESI-MS (PLEX-ID)

- Utilizing RT-PCR/ESI-MS, we genotyped Influenza positive samples (N=54) collected from Johns Hopkins affiliate hospitals. Of the 54 samples tested, 87% (47/54) tested positive.
 - As expected, all positives were Influenza A H3N2, with the predominant genotype being A/Singapore/H2013.718b/2013 at 32% (15/47) samples tested, followed by A/Helsinki/951/2013 at 26% (12/47).
- For Influenza positive samples from the 2015-2016 Influenza season, we have completely analyzed samples passively collected from Johns Hopkins affiliate hospitals (N=40), actively collected surveillance samples from Chang Gung Memorial Hospital (N=48) and passively collected samples from Wright Patterson Air Force base (N=30).
 - Of the samples analyzed, 87% (103/118) tested positive.
 - 81.4% (96/118) were Influenza A H1N1, although a small percentage, 7% (13/118) were Influenza A H3N2 or Influenza B virus at 7.6% (9/118).

Determining the Disease Potential of Influenza A Virus Isolates

- In our first year of active surveillance, we were able to coordinate receipt of clinical samples, culture on human nasal epithelial cells and sequencing of clinical sample and hNEC amplified virus
- We demonstrated the synergy in using PCR/MS, whole genome sequencing, gene specific sequencing and virus isolation in characterizing the genotypes and phenotypes of various influenza A virus strains
- A panel of recombinant H1N1 viruses containing HA mutations accumulated between 2009 and 2015 were generated and shown to vary in virus fitness in MDCK cells and human nasal epithelial cell cultures.
- A panel of recombinant H3N2 viruses was generated representing viruses present in the 2014-15 influenza season and the stability of antigenic drift mutations was shown to be quite variable, with several viruses losing the mutations associated with antigenic drift during culture in hNECs

Influenza A virus Interactions with Epithelial Cells

- We have determined that hNEC cultures kept at physiologically relevant temperatures (33C versus 37C) have different innate immune responses to influenza virus infection. This has important implications for our understanding of the kind of innate immune responses induced by infection
- H3N2 strains from 2014-15 appear to have varied viral fitness on hNEC cultures due to the presence of the mutations associated with antigenic drift. We are initiating detailed studies of these viruses to understand the relationship between virus fitness and antigenic drift.
- A manuscript describing the effect of estrogens on the transcriptional responses of IAV-infected hNECs from female donors was published. This publication received significant citations in national and international news forums and was highlighted by the DPPC on the CEIRS website

Pandemic Public Health Research Response Plan and Risk Assessment

- The utility of our pipeline analysis was validated as it revealed a novel escape route for an H1N1 virus under Tamiflu drug pressure through a mutation in the HA protein that has not been previously published. We hypothesize selection of this HA variant is due to its restoration of the required HA-NA activity balance during the viral replication cycle (Tamiflu inhibits NA activity).
- We performed microfluidic passaging to analyze differences in projected changes in the quasi-species fitness distributions for naturally circulating pandemic H1N1 variants using 2 variants with an observed 30% change in fitness in primary nasal epithelial cells.
- As part of developing a multi-scale model to perform risk assessments at the epidemiological scale based on laboratory measurements of specific viral parameters, an initial formulation of a mathematical dose-response relationship for viral transmission was completed and then implemented in software.
- In response to EAB comments, we are currently investigating methods to increase throughput of analysis of HA proteins using microfluidics tools to identify antigenic drift variants in evolutionary neighborhoods of circulating viruses.

Pandemic Public Health Research Response Plan and Risk Assessment (Sub Contract)

- We have now fully integrated clinical influenza genomic surveillance into JH-CEIRS, providing descriptive and quantitative characterization of samples collected from contributing hospitals. These data are being used across clinical, research, and modeling efforts throughout our network,

- and sample handling and bioinformatics methods have been transitioned from previous JHU/APL viral genomics projects for use within CEIRS. These include methods for graphical visualization via our web-based genomics data manipulation interface, as well as newly developed tools for expediting data analysis and dissemination.
- A total of 92 influenza samples were processed for sequencing at JHU/APL during this funding year. These included primary nasal isolates, as well as matched isolates that had been passaged in human nasal epithelial cell (hNEC) culture or MDCK cell lines. Optimized methods for minimally biased sequencing, which do not require genomic amplification by multi-segment PCR, were compared to standard CDC approaches for multi-segment PCR (ms-PCR) based sample preparation. Minimally-biased methods have helped define antigenic drift that occurs upon passaging in primary hNEC cells vs. MDCK cells for Area 2 of JH-CEIRS.
 - In collaboration with the United States Department of Agriculture (USDA), we recently shared JHU/APL bioinformatics tools and USDA historical data for benchmarking rapid, direct classification of influenza sequencing data prior to genomic assembly.

Immune responses in the elderly during influenza surveillance

- Completed mouse experiments for validation of microneutralization, IgG, and IgA responses in serum and BAL;
- Developed an ELISPOT to measure influenza-specific antibody secreting cells (ASCs); currently conducting animal experiments to evaluate sex differences in numbers of ASCs in the lungs;
- Engineered a drift variant of the mouse-adaptive 2009 H1N1 for challenge studies; we have sequenced and validated the drift variant in vitro and in vivo;
- Following receipt of a fellowship from CEIRS to learn stalk antibody assays at Mt. Sinai, these assays are currently being set up in our JH-CEIRS Serology Core.

The Human Influenza Immunome Project

- In collaboration with the NIH VRC, we have completed next-generation sequencing of a large panel of samples and are continuing to process the raw data through our in-house proprietary pipeline for analyzing, storing, and querying these sequences for repertoire studies.
- We have significantly advanced the analysis of large subsets antibody variable-gene sequences targeting H3N2 and H5N1 virus HAs.

- In addition to the H5N1 study, we are examining the genetic and molecular basis of the anti-H3 influenza B cell response in 4 recipients of a H7N9 vaccine. We have completed next-generation sequencing of a panel of samples and have also completed processing the raw data through our next-generation sequencing bioinformatics pipeline.

Focus and goals for the next contract year

Our focus for year 3 of JHCEIRS will be to streamline our approach to human surveillance and our virus pipeline. We have made significant progress this past year and will continue to work to make most efficient and effective use of our contract funds and efforts to ensure maximum scientific discovery and specimen collection.

Specific concerns or delays

The only current concerns are tied to option renewals during EOY funding.

Our surveillance program requires more study coordinators than the effort of the contract allows. Last year, through an EOY mechanism, we were able to expand the effort of option 1. This year, this is not available.

Both of our option 16 projects will expire in the upcoming year. We have submitted EOY request for renewals

Additionally, our EAB was strongly in favor of expanding our surveillance to both critically ill patients, and to at least one African site. We submitted two EOY proposals to be responsive to this concern.

Summary Tables

Full list of CEIRS Projects

Project Title	Funding Mechanism	PI	Status
Project 1: Administrative Core	Option 2	Rothman	Ongoing
Project 2: Human Surveillance in the US and Taiwan	Option 2	Rothman	Ongoing
Project 3: Laboratory A	Option 2	Gaydos	Ongoing
Project 3: Laboratory B	Option 2	Pekosz	Ongoing
Project 4: Pathogenesis	Option 2	Pekosz	Ongoing
Project 5: Pandemic Influenza	Option 2	Epstein/Kelen	Ongoing
Project 5: Subcontract	Option 2	Feldman	Ongoing
Project 6: Immune Responses in the Elderly	Option 16	Klein	Ongoing
Project 7: Human Influenza Immunome Project	Option 16	Crowe	Ongoing
Project 8: Ferret and Respiratory Cell Cores	Option 19	Pekosz	Start Up

Active Surveillance Sites

Country, State/Province	Surveillance Type				Project
	Avian	Swine	Human	Other	
USA, National Capitol Region			X		Project 2
Taiwan			X		Project 2

Publication Summary

From DPCC-Table

Reporting Period	HHSN2722014...	No. CEIRS contract number cited
Base Year	2	HHSN272201400007C
Option 1	6	HHSN272201400007C
Total	8	HHSN272201400007C

Data Summary

 # Total Samples Submitted to
 DPCC
 # Center: JH-CEIRS
 # 09/01/2015
 # File Generated: 05/10/2016
 # -----

Submission Type	Reagents Created
Virus Reagent	30
Antibody Reagent	0
Plasmid Reagent	69
Protein Reagent	0
Total	99

Contract
PI

Annual Progress Report

Title NIAID Centers of Excellence for Influenza Research
and Surveillance (CEIRS)

Contract Number HHSN272201400005C

Period Covered April 1, 2015 – March 31, 2016

Type of Report Annual

Investigators John Treanor, MD
David Topham, PhD
Andrea Sant, PhD
Jeanne Holden-Wiltse, MPH

Performing Organization Name and Address University of Rochester
601 Elmwood Avenue
Rochester, NY 14642

Subcontracts & Lead Investigators

University of Washington
PI: Michael Katze, PhD
University of Chicago
PI: Patrick Wilson, PhD
University of Massachusetts
PI: Masanori Terajima, PhD
Cornell University
PI: Gary Whittaker, PhD

Sponsoring Agency Name and Address Respiratory Diseases Branch
NIAID
National Institutes of Health
Bethesda, MD 30892

Contract
PI

Executive Summary

This report constitutes the semi-annual progress report for contract HHSN272201400005C, entitled "NIAID Centers of Excellence for Influenza Research and Surveillance" covering the project period from April 1, 2015 to September 30, 2015.

The primary focus of NYICE is on the pathogenesis of influenza and the host response in humans. Research programs within NYICE evaluate the effects of antigenic variation on vaccine protection, the specificity of the B cell response, the specificity and function of CD4 T cells in the response to influenza vaccination and infection, the biology of innate immune responses to infection and determination of pathways involved in disease severity, and evaluation of viral factors that contribute to pathogenesis. This report describes work done in these areas in the first half of the second contract year (Option 1 and associated projects)

The structure of the CEIRS centers includes both projects that are considered part of the base contract, as well as optional projects that fall into specific categories defined by program as being of high priority. The base component of NYICE was renewed in April of 2015 for an additional year of funding. With the base component are 4 individual projects (described below), a pandemic response plan, and the administrative, clinical, and data management cores. The size of the base was expanded during the first contract year through an equitable adjustment which was also awarded during the option 1 period. In addition to the base, there are several optional projects, including cross-CEIRS collaborations, and a pathogenesis option to evaluate the role of the HA cleavage sequence in pathogenesis. Additional optional projects are also possible, if they align with program goals and if adequate funds are available. It is currently anticipated that applications for additional non-severable optional projects may be funded throughout the duration of the contract period.

General update

Investigations conducted by NYICE, often in collaboration with other CEIRS centers, have focused on understanding the relationship between vaccine induced responses and infecting viruses, how the immune response is regulated in response to vaccine and infection, and what avenues might be available to improve vaccine protection by stimulating a more broadly cross protective and durable immune response. We have detected significant antigenic variation in influenza viruses isolated from humans in our surveillance studies, and have found that some of this variability could be predicted from assessment of serial passage of virus in the presence of post vaccination human sera. These results are being used in our recently developed computational algorithms to predict antigenic changes in future influenza viruses. We found, using recently developed assays for memory B cells, the initial B cell response to vaccination in humans to be directed towards influenza variants that were likely to represent initial influenza exposures based on subject age. We are also continuing our efforts to understand the factors that impact the development of more broadly cross protective antibody responses to infection and vaccination, taking advantage of the opportunity to evaluate samples from human subjects undergoing pandemic vaccination with avian influenza vaccines. B cell responses from these subjects are enriched for broadly protective antibodies, some of which are protective in murine models despite not having detectable neutralizing activity in vitro. In conjunction with these studies evaluating B cell responses, we are assessing how the specificity and function of CD4 cells impacts the ability to provide help. We have determined that in conventional influenza vaccine preparations, influenza M and NA proteins, but not NP, are physically associated with HA. We are now evaluating the relative contributions of M, NA, HA, and NP specific CD4 cells to antibody responses, in a unique clinical study comparing rIV-3, ccIV-3 and IIV-3 formulations in healthy adults. Finally, we are evaluating the innate immune response to infection and

Contract
PI

vaccination in humans and in a variety of in vitro and animal models. These studies have indicated several potential medical targets for repurposed drugs, and a surprising finding that live attenuated vaccine elicits an enhanced interferon response in the nasal epithelial cell culture model than does wild-type infection. These findings have implications for vaccine development. We are using these techniques to evaluate how the different formulations of influenza vaccine impact innate immunity, and how this then influences the cellular and antibody responses to vaccine.

We are also evaluating viral factors that contribute to replication and transmission, in a continued effort to contribute to pandemic risk assessment. In the previous project period, we evaluated population-wide immunity to H2 viruses, and found substantial pre-existing immunity to human H2 viruses in persons born before 1968. However, these antibodies were less cross reactive to current avian H2 viruses. We are now using similar techniques to evaluation population immunity to H3N2v. We have now created a panel of single cycle influenza viruses that are non infectious and can be used to assess HAI and neutralizing antibody levels against any HA or NA subtype under BSL-2 conditions. We have also identified specific residues in the PB2 protein of H9N2 virus that appear to be responsible for the robust polymerase activity in mammalian cells of the A/Quail/Hong Kong/G1/1997 virus compared to other avian influenza A viruses. These findings may explain the frequency with which H9 reassortment take place in the generation of other avian influenza viruses associated with human infection

Through our option 13 project, we are continuing to assess the role of the host microbiome in facilitating HA cleavage and enhancing the replication and transmission potential of both influenza A and B viruses, including recent North American lineage H5Nx viruses. These studies have shown an important role of the *S. aureus* SspB protease, and are now evaluation proteases from other normal respiratory tract bacteria such as *P. gingivalis*. We have found that for H5N8 viruses, transport of HA to the cell surface is highly dependent on expression of the NA. We have also developed a novel and highly accurate single-particle fusion assay that can be used to assess the phenotype of novel HA proteins in a way that can provide more accurate results than routine fusion pH-threshold stability assays currently used to assess the transmission potential of novel influenza viruses.

Changes to the CEIRS projects, structure and organization

Dr. Katze, the lead investigator for project 4, Systems biology of innate immunity and vaccination, is moving his laboratory from the University of Washington to Columbia University to facilitate closer interaction on additional research projects in which he collaborates with Dr. Ian Lipkin. Members of his team are accompanying him to Columbia. We are currently in the process of transferring this subcontract from UW to Columbia and hope to have that in place within the next month.

There were no new project options awarded during the current contract year, although option 13 "Viral markers of pathogenesis" was renewed for an additional year.

Project Progress Description and Highlights

Highlights of the individual projects within the base are briefly described below:

Project 1: Antigenic evolution and immunity to influenza.

- Showed that propagation of viruses in the presence of human post-vaccination sera selected the same mutations as seen in viruses circulating during the subsequent influenza season.
- Developed improved methodology for detection of memory B cells and assessment of antibody production, these studies have suggested that mBc obtained early after infection are often specific for the priming variant of influenza virus.
- We developed a computational model of antigenic distance, and in collaboration with investigators from the St. Jude CEIRS, have used this model to assess antigenic variation among recent North American H5 viruses
- We have observed significant sequence variability in the NS1 gene of recent influenza A viruses, with effects on the ability to antagonize type 1 interferon. The significance of these findings is under investigation.
- Highlights (1-4 bullets for each project)

Project 2: Targeting B cell responses to provide broad protection against influenza:

- We characterized the binding and functional properties of twelve human H7-reactive antibodies induced by a candidate A/Anhui/1/2013 (H7N9) vaccine. Both neutralizing and non-neutralizing antibodies protected mice in vivo during passive transfer challenge experiments. In collaboration with the CRIP CEIRS, we also generated viral escape mutants which identified unique epitopes on the head and stalk domains
- We collaborated with investigators at the intramural laboratory of infectious diseases to show a good correlation between different methods of assessing antibody dependent cellular cytotoxicity, and helped to validate a novel flow cytometry based assay.
- We have successfully isolated plasmablasts from subjects in the vaccine comparison study, demonstrating that conditions for assessing plasmablast responses are favorable. We are in the process of determining the specificity of these antibodies
- We made progress towards the generation of immunoglobulin knock-in mice

Project 3: Links between specificity and function of influenza specific CD4 T cells

- We generated a panel of T cell hybridomas that can be used to determine the epitope display on HA-specific B cells
- We determined that in commercially available subunit influenza vaccine, M and NA, but not NP, are physically bound to HA. This will have implications for later studies determining the relative roles of CD4 cells specific for these proteins in providing help for antibody responses
- We developed and validated novel flow cytometry panels to differentiate functional subsets of human CD4 cells.
- We documented a broad array of epitope specificities of human CD4 cells and have begun to associate those with functional specificity

Project 4: Systems Biology of Innate Immunity and Vaccination

- In collaboration with Andy Pekosz at the JHU CEIRS, we evaluated gene expression signatures in human nasal epithelial cell culture infected with antigenically matched wild-type of cold adapted H3N2 viruses, and found a greater induction of IFN- λ , pro-inflammatory and chemotactic responses in LAIV- versus WT
- We performed transcriptional analysis of the response of nasal epithelial cells of human subjects following natural infection with H3N2 viruses and found very similar patterns to those seen after WT H3N2 infection of hNEC
- We have performed a complete analysis of historical microarray data from animal models infected with a wide variety of pathogenic influenza viruses, and used a digital, cell quantification (DCQ) algorithm to estimate the relative proportions of different immune cell subsets induced by these viruses.

Pandemic Research Plan

- We have shown that the influenza A/quail/Hong Kong/G1/97 H9N2 polymerase has robust activity in mammalian cells as compared to other avian origin influenza A viruses, and that the elevated activity is mediated by the PB2 and PA polymerase subunits
- We generated a battery of MDCK cell lines constitutively expressing influenza A H1-H16 hemagglutinin (HA) proteins that will be used to HA-pseudotype our single-cycle infectious, reporter expressing influenza A viruses to assess the levels of pre-pandemic immunity in the general population

Option 13 Pathogenesis Option: Viral Markers of Pathogenesis

- We have performed a preliminary analysis of a variety of bacterial proteases from common bacteria in the upper respiratory tract, including the *S. aureus* protease SspB, novel protease activities from *H. influenza* and *S. aureus*, and a novel HA-activating proteases from *P. gingivalis*, for HA activating properties
- We are extending studies of protease activation to influenza B viruses, which have not previously been evaluated.
- We are obtaining human samples from a variety of sources to evaluate the role of human proteases, and proteases in influenza and bacteria coinfecting subjects, in the cleavage of seasonal influenza HA

Option 15 Cross CEIRS Collaborative Project: Systems biology of ferrets

- In collaboration with the Kawaoka lab, we carried out an extensive comparison of the systems biology of ferret infection with either the recent A/California/04/2009 or the Spanish influenza A/BrevigMission/1/1918. Integrative analysis of high-throughput omics data with virologic and histopathologic data showed an unexpected correlation of proinflammatory lipid precursors in the trachea following 1918 infection with severe tracheal lesions human pandemic H1N1 influenza viruses.

Contract
PI

Option Ferret Reagent Development (Team Ferret).

- We identified a set of antibodies that selectively and reliably detects CD8 (T cells), CD11b (myeloid cells), CD79 and CD20 (B cells), CD74 (invariant chain, class II positive cells), MHC class II (antigen presenting cells), CXCR5. Highlights (1-4 bullets for each project)
- Using the CD8 and CD4 specific monoclonal antibodies and monoclonal antibodies specific for ferret IFN-g, we have developed methods to separate CD4 and CD8 T cells and assay them for influenza reactivity

Focus and goals for the next contract year

In the coming project period, we plan to follow up on several interesting new findings outlined above. We will finish the analysis of MBC and ASC responses to seasonal H3N2 infection using previously collected samples, and then assess whether the same findings are true in the carefully characterized H1N1 cases monitored in the current Acute Flu study. This will also allow us to extend our observations on the potential significance of NS1 variability to H1N1 influenza viruses. We will also extend these studies by assessing the specificity and function of monoclonal antibodies derived from these infected cohorts, and compare these to antibodies derived from subjects receiving pandemic vaccination. The most interesting of these antibodies will be fully characterized by crystallography, as well as epitope mapping and functional assessments.

We are also intensively evaluating the CD4 response to infection and vaccination, and in particular the effects of antigen formulation on the specificity of the CD4 cell response, and subsequent provision of help for B cells, concentrating on the samples from our ongoing randomized study comparing standard, cell culture, and recombinant influenza vaccines. We have developed new CRISPR/Cas9 reagents using the higher fidelity Nickase Cas9, and expect to be able to produce the immunoglobulin knock-in mice that will be used in future studies of the relationship between CD4 and B cells. We will also produce and characterize influenza specific CD4 T cell hybridomas and assess them by probing epitope display by these HA-specific cells. These studies can then be extended to influenza B viruses, about which much less is known.

We are also intensively characterizing the systemic biology of infection and vaccination by transcriptional and lipidomic approaches, and expect to derive important new information about the relationship between specific vaccine formulation, innate and inflammatory responses, and the antibody and cellular response to infection and vaccination from these samples. These human studies will provide a unique opportunity to integrate both sets of data in a real world setting.

Human samples will also be used to identify and characterize influenza-bacteria co-infected patients and sequence HAs from these samples, with a goal of performing deep-sequence analysis. We will continue to characterize novel proteolytic activity for *H. influenza*, *S. aureus* and *P. gingivalis* with the goal of identification of protease by mass spectrometry. These studies will also incorporate assessment of the effects on novel influenza

Contract
PI

viruses by continuing the study of H10N8 as well as variant H1 and H3 human influenza viruses, with a focus on cleavage activation and the use of human respiratory proteases. Ultimately, we will attempt to confirm these findings by establishing a mouse model for influenza co-infection with *H. influenza*, *S. aureus* and the role of *P. gingivalis* as a risk-factor for influenza infection

Specific concerns or delays

None

Summary Tables

Full list of NYICE Projects

This section will be provided under separate cover as a report supplement.

Project Title	Funding Mechanism	PI	Status

Active Surveillance Sites

Country, State/Province	Surveillance Type				Project
	Avian	Swine	Human	Other	
USA, New York			X		Project 1 Project 2 Project 3 Project 4

Publication Summary

Reporting Period	HHSN272201400005C	HHSN266200700008C	No. CEIRS contract number cited
Base Year	3	12	43
Option 1	6	6	36
Total	9	18	69

Data Summary

	Samples collected	Samples submitted to DPCC	Influenza viruses identified	Full genomes sequenced	Reagents
HHSN272201400005C	78 (human)	0 (will sent when QC completed)	37 H1N1+ isolates	0 (Sanger sequencing done and submitted on contract 1 isolates)	282
TOTALS					

HHSN272201400006C
Richard Webby & Stacey Schultz-Cherry

Annual Progress Report

Title	NIAID Centers of Excellence for Influenza Research and Surveillance (CEIRS)
Contract Number	HHSN272201400006C
Period Covered	April 1, 2015 – March 31, 2016
Type of Report	Annual
Investigators	Richard Webby, PhD, Stacey Schultz-Cherry, PhD, Elena Govorkova, PhD, Charles Russell, PhD, Paul Thomas, PhD, Scott Krauss, MS and Robert G. Webster, PhD, FRS.
Performing Organization Name and Address	St. Jude Children's Research Hospital Department of Infectious Diseases 262 Danny Thomas Place Memphis, TN 38105-3678
Subcontracts & Lead Investigators	The University of Hong Kong PIs: Yi Guan, Malik Peirs, Leo Poon, Zhu Huachen, Hui-Ling Yen, Denis Ip, Michael Chan National Research Centre PI: Mohamed Ahmed Ali Kansas State University PIs: Juergen Richt, Wenjun Ma National University of Singapore Duke-NUS Graduate Medical School PIs: Gavin Smith, Vijay Dhanasekaran The University of Texas Health Science Center at Houston PI: Justin Bahl University of Wisconsin-Madison PI: Jorge Osorio University of Michigan PI: Aubree Gordon

HHSN272201400006C
Richard Webby & Stacey Schultz-Cherry

University of Georgia Research Foundation, Inc.
PI: Dave Stallknecht

Ohio State University
PI: Andrew Bowman

University of Minnesota
PI: Michael Osterholm

Sponsoring Agency
Name and Address

Respiratory Diseases Branch
NIAID
National Institutes of Health
Bethesda, MD 30892

Executive Summary

General update

Year 2 of the CEIRS award has seen a continuation of established projects and initiation of new ventures within SJCEIRS. Based on recommendations of our SAB there have been no major changes in emphasis and our focus remains understanding the human-animal interface with surveillance and laboratory-based research targeting animals, humans, and the interface itself. The Center has continued its productivity with its investigators authoring over 90 CEIRS-funded manuscripts during the past 12 months. SJCEIRS publications have appeared in PNAS, PLoS Pathogens, Lancet Infectious Diseases, Nature Genetics, Nature Communications, Cell Host and Microbes, and Clinical Infectious Diseases.

Our surveillance-based research projects are of two types, continuations of long term active samplings and shorter term projects aimed at answering specific scientific questions. Our studies in wild birds feature continued detection of influenza A viruses in Alberta and Delaware Bay, studies which were initiated in 1976 and 1985, respectively. This season's isolation rates were 9.3% for Delaware and 5.3% for Alberta; studies further down the Mississippi and Atlantic flyways revealed a 2.77% isolation rate. This year we have also initiated studies in sea birds in North America to examine their roles as influenza virus hosts. Our studies in South America have matured and avian influenza viruses were isolated in Chile and Colombia. A major highlight of our wild bird studies was the recovery of multiple geolocators in Brazil from ruddy turnstones that were tagged in Delaware Bay. The data is being collected and we anticipate a wealth of information on the exact routes of shorebird movement. Also of note is that no trace of the clade 2.3.4.4 H5 HPAI viruses was found.

Our swine-based surveillance research has a wider geographic spread. In the US where we have multiple surveillance streams we have initiated a 12-month study to follow individual animals through the production chain. Initial results show a spike (10% positive) in influenza prevalence as animals enter the weaning barns. All endemic viruses were detected including the H1N2 variant viruses which we were able to show transmit more efficiently than other viruses in swine. Of note, sampling of 104 agricultural exhibitions occurring in six states showed a reduction in virus prevalence (4.7%) as compared to previous years. In Hong Kong and Southern China we noted a reduction in diversity of swine influenza viruses in recent years suggesting a changing viral ecology. Our studies in South America continue to show dominance of pandemic H1N1 viruses in Colombia and have contributed to the characterization of a unique lineage of swine virus related to H1N1 viruses circulating in humans some 30 years ago. Using our established swine challenge models we demonstrated that HA stalk-based vaccines are protective in pigs even in the presence of maternal antibodies.

In domestic bird species our focus has remained in areas of H5 and H9 endemicity. In Egypt, detection of H5N1 viruses was rarer than in recent years but overall 3% of sampled birds were either H5 or H9 positive. Little recent antigenic or genetic change was noted in the viruses isolated although detection of these subtypes in wild birds suggest that the poultry viruses do spill over into wild birds as we have detected previously in Asia. In Bangladesh we trialed a new sampling site and found H7N1, H7N9, H10N1 and H15N9 influenza viruses from domestic ducks at Tangua Haor nature reserve in Bangladesh with potential links to the gene pool in China. In China (including Taiwan) itself our studies showed that local lineages of H3 and H6 viruses were establishing in domestic ducks with an overall increasing nucleotide substitution rate of viruses in this host in recent years. Of note, the clade 2.3.4.4 H5 viruses in Taiwan have reasserted generated multiple genotypes, monitoring of these viruses will tell us if all will be maintained. In Mainland China we were able to show that the H7N9 virus was generated through sequential reassortments in ducks and chickens, and has become persistent in chickens. This virus originated from eastern China and has spread to over 20 provinces. Repeated introductions of viruses from Zhejiang to other provinces were documented and multiple regionally distinct lineages have been established with different reassortant genotypes. Viruses present at live poultry markets fueled the recurrence of human infections. Animal experiments showed that early H7N9 isolates and the precursor H7N7 viruses had less transmissibility in ferrets than the H7N9 viruses from the established lineages. A new study in Guangzhou was initiated to look at impact of seasonality and environmental factors on viral loads in live bird markets.

As designed, viruses from our (and other) surveillance streams fed into other projects within our program. A new H9N2 candidate vaccine virus was produced in collaboration with the World Health Organization and we showed that the mutational landscape of HPAI H5N1 virus populations under oseltamivir, T-705 or their combination is different but did not lead to the emergence of drug-resistant variants in a mouse model. We also had a focus on influenza B viruses in humans examining their global evolution and ability to maintain drug resistance markers. Through the integration of evolution and demography we have identified differences in age distribution among seasonal influenza and we are developing models to identify the underlying factors. Key findings from our examination of factors influencing viral transmission were that the 2009 H1N1 virus required an acid-stable HA protein for its human pandemic potential and that swine tolerate a broad range of HA activation pH values and, thus, can serve as a bridging host between avian influenza viruses. We have constructed and validated transmission chambers with selective cut-off sizes and tested the transmission potential of seasonal H3N2, pandemic H1N1, and TRIG-lineage swine influenza viruses via virus laden particles from the exhaled breath of inoculated ferrets.

During this period we also continued to provide seminal data aiding our understanding and characterization of MERS CoV. Surveillance continued in animal populations in the Middle East and Africa showing endemicity of the virus

in camels in multiple countries in the region; no virus was detected in camels in Australia, Kazakhstan, or Mongolia. Of particular note, we described a long term MERS outbreak in hospital workers in Riyadh and detected asymptomatic infection in camel handlers and other hospital workers.

Our studies of influenza viruses in human populations have continued in Nicaragua and enrollment has started in our poultry exposed cohort in Egypt and in swine exposed workers in an abattoir in Hong Kong. Samples from these and other studies were used to show that viral variants, including antigenic variants, are often co-transmitted between humans. We also identified a novel regulatory SNP in the IFTIM3 promoter correlating with severe outcomes in multiple cohorts; studies are underway to determine the mechanisms involved. Following up on our earlier studies modeling disease in obesity in a mouse model we showed that viruses isolated from obese mice have increased replication and pathogenicity, potentially due to changes in the PB2 subunit. The more severe disease seen in the obese host is modulated by ALI and ARDS development. In the context of these disease states we were able to demonstrate that the alveolar epithelium's protein permeability and fluid clearance are dysregulated by soluble immune mediators released upon infection with highly pathogenic avian influenza H5N1 virus but not the low pathogenic seasonal influenza H1N1 virus and that mesenchymal stromal cells significantly reduce the impairment of alveolar fluid clearance induced by H5N1 infection.

Finally, we have continued a number of studies that provide reagents or guidance to the CEIRS network and wider community. These include provisions of updated web learning material, primary cell lines, ferret reagents, antigens and antiserum to specific viruses, and the development of algorithms to improve next generation sequencing. During this funding period we have completed a series of key-informant interviews with NIAID leadership regarding priorities and expectations for the CEIRS network's pandemic research response. To facilitate development of this plan, we established the Pandemic Planning Advisory Committee (PPAC), including senior investigators from each CEIRS center; convened a kickoff meeting during the 8th Annual CEIRS Meeting in Rochester and a follow up meeting by teleconference to review project documents that will lay the foundation moving forward.

In summary, the past 12 months have seen a continued productivity of the SJCEIRS team. No major changes in direction are anticipated in the next period with small tweaks to existing projects expected.

Changes to the CEIRS projects, structure and organization

No major changes to Center structure and organization have occurred. The following studies have been initiated. No studies have ended.

Option 14B- Pediatric Cohort in Nicaragua

Option 18B- Seasonality study in markets in China

Option 22B- Reagents

Equitable Adjustment- Sequencing Core

IAA- Influenza in sea ducks

Project Progress Description and Highlights

Project & Title:	PI:	Highlights:
Project 1: Risk Assessment	Webby, Schultz-Cherry	<ul style="list-style-type: none"> • Samples have arrived at St Jude and have been screened in a timely fashion.
Project 2: Wild Birds in the Americas	Webby, Krauss	<ul style="list-style-type: none"> • Isolated 54 AIV from surveillance in shorebirds at Delaware Bay: 9.3% isolation rate; subtypes – H1N1, H1N2, H1N3, H6N8, H7N1, H7N3, H11N2, H16N3 • Isolated 16 AIV from surveillance in migratory ducks in Alberta, Canada: 5.3% isolation rate (300 birds sampled); subtypes – H1N1, H1N8, H3N8, H4N6, H8N4, H10N7, • Larry Niles, LLC re-sighted and recovered geolocators in Brazil that had been attached to ruddy turnstones at Delaware Bay – data analysis pending. • Wild bird surveillance in South America has been expanded and 8 AIV isolates identified in Chile and Colombia: Subtypes – H4N2, H5N2, H5N3, H9N2, and H11N2. (See Projects 6 & 26)
Project 3: Swine in the Americas	Webby	<ul style="list-style-type: none"> • This project continues to progress well with rapid screening of incoming samples. • In final stages of 12 month active surveillance study to follow (virologically and serologically) the same animals through the US production system (4 independent systems are being followed). A high level of PCR positivity (>10%) has been observed. • Analysis of sequence data from our active and USDA's contemporary passive surveillance shows that similar viruses were identified in both. The prevalence of different genotypes did differ, however. • Attended and provided support for the OFFLU swine influenza technical working group meeting.
Project 4: Swine in the Americas- Syndromic Surveillance	Richt, Ma	<ul style="list-style-type: none"> • We collected and screened more than 800 swine samples and isolated 29 swine influenza viruses. • Three subtypes of influenza viruses including H3N2, H1N2 and H1N1 are circulating in US swine herds, and most of them contain the Eurasian swine influenza M gene. • The H1N2 variant swine influenza virus is more transmissible and more easily maintained in pigs than other tested H1N1, H3N2, and endemic H1N2 viruses based on our pig experiment to study molecular evolution of swine influenza viruses in pigs mimicking field situations.

Project 5: Animal Surveillance in Asia	Peiris, Guan	<ul style="list-style-type: none"> • Sampling of swine and avian specimens was carried out in Vietnam, Hong Kong and mainland China, as planned. • Over 50 genotypes and a few newly established viral lineages have been identified in the swine influenza viruses isolated in Hong Kong and mainland China. Preliminary results showed that most genotypes are transient, and virus replacement of different genotypes was commonly observed. From 2013-2015, the overall number of genotypes decreased when compared with that of 2010-2012. • Some H3 and H6 subtypes of influenza viruses in domestic ducks from southern China and South Korea diverged from the influenza gene pool, and developed into local persistence. • Nucleotide substitution rates of recent virus lineages in domestic ducks were mostly higher than those from early years or those in wild birds, but this is not driven by positive selection. • The highly pathogenic avian influenza (HPAI) H5 viruses causing recent outbreaks in Taiwan belonged to the Asian HPAI H5 lineage, clade 2.3.4.4 viruses, and were apparently introduced by migratory birds. These viruses reassorted with Eurasian influenza gene pool viruses and formed five genotypic variants. As Taiwan has a similar influenza ecosystem to southern China, the HPAI H5 lineage could become established and enzootic in the island.
Project 6: Human Animal Interface in Colombia	Schultz-Cherry	<ul style="list-style-type: none"> • Identification of an H11 “outbreak” in a live animal market in Medellin. • Risk characterization of viruses associated with the outbreak. • Endemic spread of pdmH1N1 viruses throughout swine herds. • Buy in from the Colombian government including the Ministry of Environment, ICA (Colombian USDA), Ministry of Health, National Parks and swine and poultry producers. This has enormous implications for the continued success of the project.
Project 7: Administrative Core- Asia	Guan, Peiris	<ul style="list-style-type: none"> • Provide the administrative support and infrastructure for the activities pertaining to surveillance and the animal human interface as well as the immunology, pathogenesis and transmission components which the HKU center participates. • Visits to mainland China and other surveillance sites were made to monitor the progress.
Project 8: Human-Animal Interface in Egypt	Ali, Kayali	<ul style="list-style-type: none"> • More than 4300 poultry samples collected. Overall positivity rate at 3% and detected subtypes were H5N1, H9N2, and co-infections of these subtypes • Genetic analysis indicated little variation in poultry viruses from the previous year. • Around 1400 samples obtained from wild birds, 20 samples tested positive.

		<ul style="list-style-type: none"> • Subtypes in wild birds included H5N1 and H9N2 in addition to H7 and H10.
Project 9: Human-Animal Interface in Bangladesh	Webby, Webster	<ul style="list-style-type: none"> • The year round co-circulation of highly pathogenic H5N1 and low pathogenic H9N2 in live bird markets in Dhaka, Bangladesh with continuing acquisition of genetic changes fostering mammalian transmissibility indicates that there is a continuing risk of the genesis of a human transmissible influenza virus. • The detection of H7N1, H7N9, H10N1 and H15N9 influenza viruses from domestic ducks at Tangua Haor nature reserve in Bangladesh with potential links to the gene pool in China provides a unique opportunity to study the gene flow into the Indian subcontinent and into live bird markets. • Recent surveillance and sequencing data has shown that HPAI H5N1 viruses that are currently circulating in LBMs are reassortant viruses with internal genes from low pathogenic viruses, which further stresses the need for continued surveillance at the human-animal interface. • Novel H7N5 influenza viruses from shorebirds (black tailed godwits) and H15N9 from domestic ducks are unique subtypes not previously detected at our center and merit complete characterization.
Project 10: Evolutionary Dynamics	Smith, Dhanasekaran, Bahl	<ul style="list-style-type: none"> • Human influenza B viruses. Evolutionary analysis of influenza B viruses resulting in publications on influenza B in Australia and Malaysia during 2015-2016. • Pandemic H1N1 virus. Published first comprehensive study on long-term evolution of pandemic H1N1 virus in humans. • Avian influenza virus. Performed surveillance of wild birds in Australia and Antarctica to detect diverse influenza A viruses. • Human and other mammalian surveillance. Completed pilot study of influenza surveillance in swine in Cambodia and continued global surveillance on influenza A & B viruses. • <i>Examined prevalence of neuraminidase inhibitor resistance in global swine populations.</i> • <i>Investigated spatial and host preference signals of wild bird viruses isolated in Eurasia.</i> • <i>Examined how genetic diversity transmitted between species can modulate pandemic potential.</i>
Project 11: Antivirals	Govorkova	<ul style="list-style-type: none"> • Demonstrated that HA stem-binding monoclonal antibody VIS410 controls the development of acute respiratory distress syndrome in BALB/c mice after infection with influenza A(H7N9) viruses. • Identified that mutational landscape of HPAI A(H5N1) virus populations under oseltamivir, T-705 or their combination is different but did not lead to the emergence of drug-resistant variants in a mouse model.

		<ul style="list-style-type: none"> • Determined that influenza B viruses carrying NA inhibitor-resistant substitutions (E119A or H274Y) did not possess replication fitness advantage over - susceptible virus in direct competition experiments in ferrets without antiviral drug pressure.
Project 12: Vaccine Seed	Webby	<ul style="list-style-type: none"> • Produced a GMP-grade H9N2 WHO candidate vaccine virus (CVV).
Project 13: Human Immunology	Thomas	<ul style="list-style-type: none"> • Identified a novel regulatory SNP in IFTIM3 promoter • Correlated SNP carriage with severe outcomes across three cohorts • Identified unique immunomodulatory signatures in humans of herpesvirus coinfection with influenza • Viral variants, including antigenic variants, are often co-transmitted between humans • Bacterially expressed HA “mini-stem” can cause heterosubtypic protection by inducing cross-reactive antibodies
Project 14: Risk Factors	Schultz-Cherry, Chan	<ul style="list-style-type: none"> • Obese mice have more severe ALI and develop ARDS, which we can inhibit by “knocking-out” the beta 6 integrin • Viruses isolated from obese mice have increased replication and pathogenicity, potentially due to changes in the PB2 subunit • The alveolar epithelium's protein permeability and fluid clearance were dysregulated by soluble immune mediators released upon infection with highly pathogenic avian influenza (H5N1) virus but not the low pathogenic seasonal influenza (H1N1) virus. • Mesenchymal stromal cells significantly reduce the impairment of alveolar fluid clearance induced by A/H5N1 infection in vitro and prevent or reduce A/H5N1-associated acute lung injury in vivo, suggested the possible therapeutic options • Measurement of alveolar fluid clearance and protein permeability by the in vitro lung injury model can be a useful risk assessment parameters for the influenza viruses
Project 15: Transmission	Russell, Yen	<ul style="list-style-type: none"> • Discovered that the 2009 H1N1 virus required an acid-stable HA protein for its human pandemic potential. This is important because it has identified HA acid stability as a marker for pandemic potential. • Determined that swine tolerate a broad range of HA activation pH values and, thus, can serve as a bridging host between avian influenza viruses (HA activation pH values > 5.5) and humans (HA activation pH values < 5.5). This helps define the role of pigs in the evolution of pandemic influenza viruses. • Determined the sequence, synthesized the genome, and rescued an avian-like precursor Eurasian swine influenza virus by reverse genetics. The precursor Eurasian swine influenza virus that showed efficient growth in both embryonic eggs and in MDCK cells

		<p>will be evaluated in swine cell line and ex vivo cultures along with Eurasian swine influenza viruses isolated between 1979 to 2009. This is important because it will help to understand the critical functionality associated with avian-to-mammalian adaptation.</p> <ul style="list-style-type: none"> • Constructed and validated transmission chambers with selective cut-off sizes at 10, 5, and 2.5 μm for airborne particles. Tested the transmission potential of seasonal H3N2, pandemic H1N1, and TRIG-lineage swine influenza viruses via virus laden particles from the exhaled breath of inoculated ferrets at sizes <10, <5.3, or <2.5 μm. Experiments investigating the role of HA, NA, M in facilitating influenza airborne transmission potential are ongoing. This is important because it help to understand droplet (particles >5 μm) and airborne (particles \leq5 μm that may remain suspended in the air) transmission efficiency among ferrets.
Project 16: Administrative Core- All sites	Webby, McKenzie	<ul style="list-style-type: none"> • Requested and received State Dept. Approvals and COAs for all Subcontract agreements and Fee for Service Agreements between St Jude Children's Research Hospital and foreign collaborative institutions as well as domestic sites. • Increased communication via teleconferences with all 10 subcontract collaborative and many FFSA sites. • SAB Review of the SJCEIRS program conducted during this contract period and SAB completed review of >20 concept proposals for potential EOY funding. • Data Management Team continues to work with DPCC to meet the needs of the CEIRS Contract. • Continue to address and meet administrative needs for 6 St Jude CEIRS Clinical Protocols.
Project 17: Logistics	Webby, Krauss	<ul style="list-style-type: none"> • USDA and CDC Import permits were renewed and virus shipment continued without interruption. • Processed USDA Form 2 for Select Agent Transfers and USDA Form 4 for reporting detection of select agents throughout the year. • Processed documentation for USDA exclusion from select agent status of attenuated high pathogenic strains. • Processed approximately 200 shipments of viruses, reagents and supplies. • Provided funds for all CEIRS related/approved travel.
Project 10 Expanded: Evolutionary Dynamics	Dhanasekaran	<ul style="list-style-type: none"> • Human influenza B viruses. Evolutionary analysis of influenza B viruses resulting in publications on influenza B in Australia and Hong Kong during 2015-2016 • Avian influenza virus. Performed surveillance of wild birds in Australia and Antarctica to detect diverse influenza A viruses. Performed surveillance, sequencing and analysis of AIV in poultry in India.

		<ul style="list-style-type: none"> • Pandemic influenza susceptibility. Estimating the susceptibility of pandemic H2N2 viruses based on CD8+ T cell responses. • Integrating evolution and demography. Identified differences in age distribution among seasonal influenza viruses. Developing models to identify factors affecting differential age distribution.
Project 18: Pandemic Planning	Osterholm	<ul style="list-style-type: none"> • Completed a series of key-informant interviews with NIAID leadership regarding priorities and expectations for the CEIRS network's pandemic research response. • Established the Pandemic Planning Advisory Committee (PPAC), including senior investigators from each CEIRS center; convened a kickoff meeting during the 8th Annual CEIRS Meeting in Rochester and a follow up meeting by teleconference to review project documents. • Drafted, circulated for review among PPAC members and CEIRS leadership, and revised two key documents: (1) questionnaire for documenting centers' capabilities and expertise relevant to pandemic research; and (2) comprehensive outline of the CEIRS Pandemic Research Response Plan. • Maintained, updated, and posted e-learning modules (including lessons and knowledge checks) on topics related to avian influenza, general influenza, and 2009 pH1N1 on the CEIRS Online Influenza Training Portal.
Project 19: Swine at Agricultural Fairs	Bowman	<ul style="list-style-type: none"> • Collected 2961 nasal swabs from swine at 104 agricultural exhibitions occurring in six states. Resulting in the recovery of 140 (4.7%) IAV isolates. • Observed markedly lower IAV prevalence in exhibition swine during the 2015 show season, as compared to previous years. • Identified weighing and tagging activities at fairs as major control points to limit IAV spread during fairs.
Project 20: Wild bird: risks & reservoirs	Stallknecht	<ul style="list-style-type: none"> • Tested over 6,500 birds and isolated 433 IAV including all HA and NA North American subtypes • Coordinated a large-scale collaborative study to detect antibodies to clade 2.3.4.4 H5Nx in North America wild birds. • Finalized three experimental studies relating to the effects of hetero-specific immunity in mallards. • Completed and collaborated in two studies to link wild birds with two recent introductions of H7 IAV in North American poultry.
Project 21: Risk Assessment- expanded	Webby, Schultz-Cherry, Krauss	<ul style="list-style-type: none"> • Contributed data, expertise, viruses, and other reagents to multiple cross-CEIRS projects. • Built on national and international partnerships to collect and analyze large panel of clade 2.3.4.4 H5 viruses. • Gained NIH approval to mouse adapt US H5N2 and H5N8 viruses. • Showed that chickens are more infectious than ferrets in terms of transmission to naïve ferrets. This

		is consistent with the epidemiologic observations of limited human-to-human H5N1 transmission, but more frequent bird to human infection.
Project 22A: Egyptian Poultry Growers Cohort	Ali, Kayali	<ul style="list-style-type: none"> • Study was activated and opened to enrollment. • More than 1500 subjects were enrolled. • Study ongoing.
Project 22B: MERS Surveillance in ME	Ali, Kayali	<ul style="list-style-type: none"> • Camel surveillance conducted in Egypt, Jordan, and Tunisia. • Bat surveillance conducted in Egypt, Tunisia, Algeria, and Lebanon. • MERS-CoV antibody and PCR detection levels varied by country and was highest in Egypt as most camels sampled were imported from Sudan. • Beta-coronaviruses were detected in bats from Lebanon and Egypt. • Alpha-coronavirus was detected in bats from Tunisia.
Project 23: Predicting Clinical Outcomes	Thomas	<ul style="list-style-type: none"> • Coordinated protocol development for respiratory sample capture • Acquired samples from NIH-funded La Red cohort and prepped for targeted re-sequencing
Project 24: Methods & applications w/MERS	Peiris	<ul style="list-style-type: none"> • MERS-CoV is endemic in dromedaries in North (Morocco, Tunisia), West (Burkina Faso), Central (Nigeria), East (Egypt, Ethiopia) Africa, as well as Jordan, but not in Central Asia (Kazakhstan), Australia or in Bactrian camels in Mongolia. • Full genome sequence and virus isolates have been obtained from MERS-CoV from dromedaries in Egypt, Morocco, Burkina Faso, Nigeria, as well as Saudi Arabia. • Passive immunotherapy with immune camel serum is effective prophylactically and therapeutically in a DPP4 transgenic mouse model of MERS-CoV infection • Performance characteristics and cross-reactivity of serological (pseudotype neutralization, microneutralization and PRNT) assays for MERS-CoV have been characterized with animal and human sera. • Molecular epidemiology of MERS-CoV within a hospital in Riyadh in 2014 showed that this outbreak was in fact part of a larger outbreak involving multiple hospitals which had been ongoing (with human-human transmission) for around 5 months. • Asymptomatic human infection with MERS (by RT-PCR) was documented in camel handlers and hospital workers.
Project 25: Wild Bird Surveillance- MISS. Flyway	Bowman	<ul style="list-style-type: none"> • A total of 4,977 samples were collected from wild birds across 10 states in the Mississippi and Atlantic flyways. A total of 138 (2.77%) isolates were recovered. • No HPAI H5 or H7 isolates were detected; although LP H5 and H7 were detected.

		<ul style="list-style-type: none"> • IAV isolates were not recovered from the testing of 381 samples collected from Passerine species through banding efforts. A total of 9 samples were positive for IAV matrix via rRT-PCR. • rRT-PCR was negative for 283 samples collected from birds housed at avian rehab centers in Ohio from April – October 2015.
Project 26: Surveillance in Chile	Schultz-Cherry, Hamilton-West	<ul style="list-style-type: none"> • Isolated 16 viruses and have ~40 sequences from wild birds. All appear to be unique North-South American lineage reassortants • Completing risk assessment studies on a novel swine H1N2 virus • 2 manuscripts submitted several more in preparation • Hope to expand sites
Primary Cell Core Project	Schultz-Cherry, Thomas	<ul style="list-style-type: none"> • SOPs for nasal and tracheobronchial cells established for a variety of species • Repository growing and QC underway • Had our first “customers”. Received swine nasal and tracheal cells; ferret nasal and tracheal cells; and human NHBEs
Project 27: Avian Influenza ecology in sea ducks	Hall	<ul style="list-style-type: none"> • Conducted AI wild bird surveillance in Iceland- 380 samples; 6 isolates; 245 sera (181 AI positive) • Conducted surveillance in Maine sea ducks- 483 samples; 7 isolates; 105 sera (54 AI positive) • Established collaborative agreements with researchers for upcoming sampling efforts in Alaska sea birds • Published manuscript on AI in sea ducks (Hall et al. PLoS One. December 2015; DOI: 10.1371/journal.pone.0144524.
NS-13A: High Risk Populations in Colombia	Schultz-Cherry, Osorio	<ul style="list-style-type: none"> • Training completed • 14-0084 IRB approvals obtained in Colombia, Wisconsin, and almost finalized at St Jude. • 14-0083 IRB approvals obtained in Colombia. Wisconsin and St Jude underway. • Enrollment to begin in May
NS-14A: Household Transmission in Nicaragua	Thomas, Gordon	<ul style="list-style-type: none"> • Enrolled 349 participants • Collected over 2000 samples • Determined duration of viral shedding in adults and children • Completed transcriptional analysis of initial sample set
NS-14B: Pediatric Cohort in Nicaragua	Thomas, Gordon	<ul style="list-style-type: none"> • Completed site activation • Enrolled 1533 participants • Collected over 1000 samples
NS-15A: Role of TRPM8	Webby	<ul style="list-style-type: none"> • Confirmed the phenotype of reduced H5N1 pathogenicity in mice after deletion of TRPM8 in a second knockout mouse line.
NS-16A: Autoreactive potential universal vaccines	McGargill	<ul style="list-style-type: none"> • We found that mice containing higher levels of cross-reactive influenza antibodies also have increased levels of autoreactive antibodies. • Preliminary data show that mice with higher levels of influenza cross-reactive antibodies may be more susceptible to autoimmune disease.

NS-18A: Protection by stalk-reactive antibodies	Richt, Krammer	<ul style="list-style-type: none"> • Effects of HA stalk-based immune responses for influenza was evaluated in a pig model with maternal antibodies. • The stalk-based universal vaccine has protective effects in pigs even in the presence of maternal antibodies, evidenced by reduced virus replication in the lung.
NS-18B: Seasonality of viral loads-China	Yen	<ul style="list-style-type: none"> • Determined and compared viral loads (M gene copy numbers and TCID50) from poultry swabs (oropharyngeal and cloacal) and environmental samples (fecal dropping, water, and air) collected at live poultry markets in Guangzhou since Nov. 2015. • Determined the M gene positive rates and the prevalence of H5, H7, H9, and non-H5/H7/H9 subtypes from poultry swabs and environmental samples. H9 is dominantly detected in all months. • Higher viral loads detected in the oropharyngeal samples than the cloacal or environmental samples. Seasonality will be analyzed with more data available in spring and summer months. • Assessed the quantity and viability of influenza virus on dressed poultry. Low copies of M genes can be detected from dressed poultry and viable H5 virus was detected in 1/19 dressed poultry samples.
NS-19A: Risk Factors in Swine Abattoir Workers	Poon, Ip, Peiris	<ul style="list-style-type: none"> • Surveillance at the swine-human interface is of major public health importance and swine abattoir workers may represent a valuable sentinel population for epidemiologists to evaluate potential transmission of emerging virus. • We recently conducted a longitudinal systematic surveillance of swine abattoir workers in Hong Kong (since Oct 2015) for evidence of influenza infection, with the aim to identify possible risk factors for zoonotic transmission from swine to human. The study was carried out in a large abattoir in Hong Kong which receives pigs from around 10 provinces in southeastern China and slaughters approximately 3000 swine daily. Current surveillance of the swine is ongoing in the same abattoir as part of the CEIRS project. • Since Oct 2015, 465 sets of nasal and throat swabs were collected from 46 abattoir workers. Results pending.
NS-19B: H7, H9, & H10 in Southern China	Guan, Zhu	<ul style="list-style-type: none"> • H10 viruses were regularly introduced by migratory ducks to domestic ducks on Poyang Lake, a major aggregative site of migratory birds in Asia. This subtype of viruses were maintained and amplified in domestic ducks, then transmitted to chickens and reassorted with enzootic H9N2 viruses, leading to an outbreak and human infections at live poultry markets. The emergence of the H10N8 virus, following a similar pathway to the recent H7N9 virus, highlights the role of domestic ducks and the current influenza ecosystem in China that facilitates influenza viruses moving from their reservoir hosts

		<p>through the live poultry system to cause severe consequences for public health.</p> <ul style="list-style-type: none"> • H7N9 virus was generated through sequential reassortments in ducks and chickens, and has become persistent in chickens. This virus originated from eastern China and has spread to over 20 provinces. Repeated introductions of viruses from Zhejiang to other provinces were documented. Multiple regionally distinct lineages have been established with different reassortant genotypes. Viruses present at live poultry markets fueled the recurrence of human infections. Chickens served as the source of human infections in each outbreak wave. H7N9 viruses have become enzootic in China and may spread beyond the region, following the pattern previously observed with H5N1 and H9N2 influenza viruses. • Animal experiments showed that early H7N9 isolates and the precursor H7N7 viruses had less transmissibility in ferrets than the H7N9 viruses from the established lineages, thus the adaptation and evolution of H7N9 virus continuously pose an increasing threat to public health.
NS-22A/B/C.1: Influenza Reagent Production	Govorkova	<ul style="list-style-type: none"> • Recombinant H1, H3 and H11 HA proteins were produced and deposited to BEI Research Resources Repository. • Polyclonal anti-H2, anti-H4 and anti-H11 reference goat antisera were produced and deposited to BEI Research Resources Repository. • A panel of anti-H10 monoclonal antibodies [derived from A/Chicken/Jiangxi/34609/2013 (H10N8) influenza virus] was produced.
NS-22A/B.2: Ferret Reagent Network Project	Thomas, Schultz-Cherry	<ul style="list-style-type: none"> • Established a global network of collaborators working on developing ferret reagents • Established a pipeline for generating recombinant proteins and antibodies • Hired a technician dedicated to the ferret reagent and primary cell core projects • Developed a repository of ferret products that will be made available to the CEIRS network through DPCC • Meeting our milestones • Recombinant TNF-α and IFN-γ purified. CD8 antibodies being generated
NS-22C.3 (formally under Option 9a EA): UTHSC CEIRS-DIG Sequencing Core	Bahl	<ul style="list-style-type: none"> • Developed a novel machine learning approach that uses iterative refinement to improve variant calling and data assembly for use with next generation sequencing technologies optimized for highly variable genomes such as influenza. • Developing (alpha version) a web-based interactive phylogenetic tree explorer for use with newly sequenced data (projects with >20 sequences). • Optimizing sequencing protocols for Influenza and MERS. Currently sequencing 100 MERS samples and 250 Influenza Genomes

		<ul style="list-style-type: none"> Sequencing services are now available for the CEIRS research community with the new data submission pipeline.
--	--	-----------------------------------------------------------------------------------------------------------------------------------------------------------------

Focus and goals for the next contract year

Due to the progress and productivity of current activities, no changes to the Center goals are expected for the next contract year. Focus and goals remain the same.

Specific concerns or delays

Delays to date relate to gain fo function issues and to setup of human study documents in Colombia. Resolution to the former is expected in the near future and protocols are now in palce in Colombia with enrollment initiation expected in coming weeks.

Summary Tables

Full list of CEIRS Projects:

Project Title	Funding Mechanism	PI	Status
Project 1: Risk Assessment	Option 1	Webby, Schultz-Cherry	Ongoing
Project 2: Wild Birds in the Americas	Option 1	Webby, Krauss	Ongoing
Project 3: Swine in the Americas	Option 1	Webby	Ongoing
Project 4: Swine in the Americas- Syndromic Surveillance	Option 1	Richt, Ma	Ongoing
Project 5: Animal Surveillance in Asia	Option 1	Peiris, Guan	Ongoing
Project 6: Human Animal Interface in Colombia	Option 1	Schultz-Cherry	Ongoing
Project 7: Administrative Core- Asia	Option 1	Guan, Peiris	Ongoing
Project 8: Human-Animal Interface in Egypt	Option 1	Ali, Kayali	Ongoing
Project 9: Human-Animal Interface in Bangladesh	Option 1	Webby, Webster	Ongoing
Project 10: Evolutionary Dynamics	Option 1	Smith, Dhanasekaran	Ongoing
Project 11: Antivirals	Option 1	Govorkova	Ongoing
Project 12: Vaccine Seed	Option 1	Webby	Ongoing
Project 13: Human Immunology	Option 1	Thomas	Ongoing
Project 14: Risk Factors	Option 1	Schultz-Cherry, Chan	Ongoing
Project 15: Transmission	Option 1	Russell, Yen	Ongoing
Project 16: Administrative Core- All	Option 1	Webby, McKenzie	Ongoing
Project 17: Logistics	Option 1	Webby, Krauss	Ongoing
Project 18: Pandemic Planning	Option 9a	Osterholm	Ongoing
Project 19: Swine at Agricultural Fairs	Option 9a	Bowman	Ongoing
Project 20: Wild bird: risks & reservoirs	Option 9a	Stallknecht	Ongoing
Project 21: Risk Assessment- expanded	Option 9a	Webby, Schultz-Cherry, Krauss	Ongoing
Project 22A: Egyptian Poultry Growers Cohort	Option 9a	Ali, Kayali	Ongoing

Project 22B: MERS Surveillance in ME	Option 9a	Ali, Kayali	Ongoing
Project 23: Predicting Clinical Outcomes	Option 9a	Thomas	Ongoing
Project 24: Methods & applications w/MERS	Option 9a	Peiris	Ongoing
Project 25: Wild Bird Surveillance- MISS. Flyway	Option 9a	Bowman	Ongoing
Project 26: Surveillance in Chile	Option 9a	Schultz-Cherry, Hamilton-West	Ongoing
Project: Primary Cell Core Project	Option 9a	Schultz-Cherry, Thomas	Ongoing
Project: CEIRS Genomic Sequencing Core	Option 9a	Bahl	Ongoing
Project 27: Influenza in Sea Ducks & N Atlantic	IAA	Hall	Ongoing
NS-13A: High Risk Populations in Colombia	Option 13A	Schultz-Cherry, Osorio	Ongoing
NS-14A: Household Transmission in Nicaragua	Option 14A	Thomas, Gordon	Ongoing
NS-14B: Pediatric Cohort in Nicaragua	Option 14B	Thomas, Gordon	Ongoing
NS-15A: Role of TRPM8	Option 15A	Webby	Ongoing
NS-16A: Autoreactive potential universal vaccines	Option 16A	McGargill	Ongoing
NS-18A: Protection by stalk-reactive antibodies	Option 18A	Richt, Krammer	Ongoing
NS-18B: Seasonality of viral loads-China	Option 18B	Yen	Ongoing
NS-19A: Risk Factors in Swine Abattoir Workers	Option 19A	Poon, Ip, Peiris	Ongoing
NS-19B: H7, H9, & H10 in Southern China	Option 19B	Guan, Zhu	Ongoing
NS-22A/B/C.1: Influenza Reagent Production	Option 22A/B.1	Govorkova	Ongoing
NS-22A/B.2: Ferret Reagent Network Project	Option 22A/B.2	Thomas, Schultz-Cherry	Ongoing

Active Surveillance Sites:

Country, State/Province	Surveillance Type				Project
	Avian	Swine	Human	Other	
USA (New Jersey); Colombia; Canada, Alberta	X				Project 2
USA (Illinois, Georgia, Oklahoma, Nebraska)		X			Project 3
USA (Kansas, Nebraska, Missouri, Colorado, Oklahoma, Iowa, South Dakota, Indiana, and North Dakota)		X			Project 4

China, Hong Kong, Vietnam	X	X			Project 5
Colombia	X	X			Project 6
Egypt	X				Project 8
Bangladesh, Dhaka	X				Project 9
USA (Ohio, Iowa, Kentucky, Indiana, West Virginia and Michigan)		X			Project 19
USA (New Jersey, Minnesota, Alaska, Texas, Louisiana, other east & gulf coast areas)	X				Project 20
Egypt			X		Project 22A
Egypt; Lebanon; Jordan; Tunisia; Algeria; Uganda				X (Camels, Bats, etc.)	Project 22B
Saudi Arabia & East, North, and West Africa*				X (Camels, etc.)	Project 24
USA, Mississippi Flyway	X				Project 25
Chile	X				Project 26
North America (USA and Canada) including Atlantic & Pacific coasts	X				Project 27
Colombia			X		NS 13A
Nicaragua			X		NS 14A
Nicaragua			X (Pediatric)		NS 14B
China, Guangzhou	X				NS 18B
China, Hong Kong			X		NS 19A
Southern China	X				NS 19B

Publication Summary:

- From DPCC Literature Report

Contract Publications	Base	Option 1	TOTAL
HHSN272201400006C	25	63	88
HHSN266200700005C	49	16	65
HHSN266200700007C	0	7	7
AAI16005	0	0	0
HHSN266200700006C	0	1	1
w/o reference to contract #	0	7	7
TOTALS	74	94	168

Data Summary:

	Samples collected	Samples submitted to DPCC	Influenza viruses identified	Full genomes sequenced	Reagents
HHSN272201400006C	97,514	66,870*	2,029	273*	2,820 [^]
HHSN266200700005C	0 [#]	0 [#]	0 [#]	243 [#]	0 ⁺
TOTALS	97,514	66,870*	2,029	273*	2,820[^]

*Based on DPCC report

#DPCC requested that we do not submit data from previous contract.

+No reagents made from the last contract have been submitted during this contract period.

[^]See Reagent Report for update.



NIAID/DMID Centers of Excellence for Influenza Research and Surveillance (CEIRS) Program Review

August 29th, 2016

12:30-4:30 pm

Web based meeting

12:30 – 12:45 PM: Welcome and Objectives for the Meeting

*NECs ideas regarding measures of success

Diane Post, National Institute of Allergy and Infectious Diseases, NIH

12:45 – 1:05 PM: Update from Emory University

Walt Orenstein, Emory University

1:05 – 1:25 PM: Update from NYICE

John Treanor, University of Rochester

1:25 – 1:45 PM: Update from SJCEIRS

Richard Webby, St. Jude Children's Research Hospital

1:45 – 2:05 PM: Update from JHCEIRS

Rich Rothman and Andrew Pekosz, Johns Hopkins University

2:05 – 2:25 PM: Update from CRIP

Adolfo Garcia-Sastre, Icahn School of Medicine at Mt. Sinai

2:25 – 2:45 PM: BREAK

2:45 – 3:05 PM: Update from DPCC

Stephan Bour, Digital Influzion

3:05 – 3:25 PM: Update on Cross Collaborations and Working Groups

Adolfo Garcia-Sastre, Icahn School of Medicine at Mt. Sinai

3:25 – 4:20 PM: General Discussion of Network

Option 1: Closed discussions

Option 2: Open session discussions

4:20 – 4:30 PM: Presentation of recommendations from NEC

4:30 PM Adjourn



Discussion Structure

1. Assess the type of activities being supported under these contracts and provide feedback regarding the network's accomplishments and their contributions to the advancement of research on influenza.
 - Are the centers meeting the metrics for success?
 - Are there activities that should be altered?
 - Are there activities that we are missing (ex reagent preparations)?
 - Are there any issues or concerns Centers currently face?

2. Identify scientific gaps and needs for influenza research and surveillance related research and opportunities for bringing additional value to the program utilizing current funding
 - Are there scientific gaps in the current program?

3. Make recommendations to DMID staff about opportunities to improve the program's effectiveness
 - Are there ideas on how to integrate surveillance with basic research?
 - Are there opportunities that could be implemented now (small steps)?
 - Are there opportunities that could be implemented over the next year?
 - What would you recommend be done differently in the future?

4. Define areas of collaboration among the Centers and with other virology/influenza networks
 - Are there areas for collaborations between Centers and other agencies that need to be further explored at this time?

From: Leyva-Grado, Victor
Sent: Mon, 22 Aug 2016 15:49:40 +0000
To: 'Cockrell, Adam'; Stemmy, Erik (NIH/NIAID) [E]
Cc: Baric, Ralph; Umerah, Nina
Subject: RE: AMC call today?

Thanks a lot Adam.

As soon as we get it from our finance office we can send it on Erik's way.

V

From: Cockrell, Adam [mailto:(b)(6)]
Sent: Monday, August 22, 2016 11:14 AM
To: Leyva-Grado, Victor; Stemmy, Erik (NIH/NIAID) [E]
Cc: Baric, Ralph S; Umerah, Nina
Subject: RE: AMC call today?

Hi Victor,

I will speak with Ralph about putting together the NCE and get that over to you.

Best Regards,

Adam

From: Leyva-Grado, Victor [mailto:(b)(6)]
Sent: Monday, August 22, 2016 11:10 AM
To: Cockrell, Adam (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Baric, Ralph S (b)(6) Umerah, Nina (b)(6)
Subject: RE: AMC call today?

Hi Adam,

Is Amy still helping you out with the administrative part of the contract? I talked to Nina last week and we haven't received the request from UNC (is this still correct Nina?). The only one we have is the previous NCE for the 5 months.

Cheers,

V

From: Cockrell, Adam [mailto:(b)(6)]
Sent: Monday, August 22, 2016 11:01 AM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Baric, Ralph S; Umerah, Nina; Leyva-Grado, Victor
Subject: RE: AMC call today?

Hi Erik,

I have not received the compound yet to test. I think they are waiting on a patent to be filed.

I will speak with Ralph regarding the NCE, and how to go forward with this through Mt. Sinai.

Thanks,
Adam

From: Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]
Sent: Monday, August 22, 2016 10:34 AM
To: Cockrell, Adam (b)(6)
Cc: Baric, Ralph S (b)(6); 'Umerah, Nina' (b)(6); 'Leyva-Grado, Victor' (b)(6)
Subject: RE: AMC call today?

Hi Adam,

I was just planning on a brief call, but if it's easier we can do it by email. Just wanted to check in and see if things are on track for the GSK study. I know you're waiting to receive the test compound to try out the sonication process. Any updates there? The only other issue is the No-Cost extension. We'll need to get that in ASAP, and OA has said that the NCE can extend past the contract end date. Can you let me know when you'll be able to send the request through Mt Sinai?

Thanks!
Erik

From: Cockrell, Adam [mailto:(b)(6)]
Sent: Monday, August 22, 2016 10:29 AM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Baric, Ralph (b)(6)
Subject: AMC call today?

Hi Erik,

Just wanted to check in to see if there is a call today. Anything to discuss? We will not be receiving drug from GSK until mid-September, which means we probably will not have results to discuss until the October call.

Thanks,
Adam

Adam Cockrell
Post-Doctoral Fellow
Department of Epidemiology
University of North Carolina at Chapel Hill
Chapel Hill, NC, 27599
Phone: (b)(6)

From: Baric, Toni C
Sent: Tue, 2 Aug 2016 18:13:24 +0000
To: Yao, Alison (NIH/NIAID) [E]; Mathur, Punam (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]
Cc: Baric, Ralph; Graham, Rachel
Subject: Doodle: Link for poll "Orfeome Face to Face meeting"

Dear Alison, Punam and Erik

Ralph would like to schedule a face to face meeting before the annual group meeting. He would like to plan it for January or February. Below is a doodle poll so we can narrow down the dates. No one in this group has an institutional ban to travel to North Carolina, so I intend to have the meeting at the Carolina Inn.

<http://doodle.com/poll/qksdb9aixfzsfe8s>

Thank you,
Toni

From: Graham, Rachel
Sent: Tue, 2 Aug 2016 16:40:27 +0000
To: Mathur, Punam (NIH/NIAID) [E]; Yao, Alison (NIH/NIAID) [E]
Cc: Baric, Ralph; Stemmy, Erik (NIH/NIAID) [E]
Subject: mBio Zika paper (not ORFeome funds)

Here is the link to the mBio paper Ralph discussed.

Rachel

<http://mbio.asm.org/content/7/4/e01123-16.long>

~~~~~

**Rachel Graham, Ph.D.**

Research Assistant Professor

UNC-Chapel Hill

(b)(6)

**From:** Baric, Toni C  
**Sent:** Mon, 1 Aug 2016 15:00:19 +0000  
**To:** Baric, Ralph; Beisel, Christopher (NIH/NIAID) [E]; Damania, Blossom A; Spiro, David (NIH/FIC) [E]; Stemmy, Erik (NIH/NIAID) [E]; Graham, Rachel; Mathur, Punam (NIH/NIAID) [E]; Yao, Alison (NIH/NIAID) [E]  
**Subject:** UNC-NIH monthly call for U19-AI 107810

Hello everyone,  
This is a reminder that we will have our monthly UNC-NIH call tomorrow, Tues August 2 at 12 pm EDT.  
The calling instructions are below.

Phone: (b)(6)  
Passcode: (b)(6)

Thank you.

## Toni Baric

Department of Microbiology and Immunology  
9025 Burnett Womack  
CB# 7292  
Chapel Hill, NC 27599-7292

Office: (b)(6)  
(b)(6)

**From:** Baric, Toni C  
**Sent:** Fri, 22 Jul 2016 13:02:09 +0000  
**To:** Stemmy, Erik (NIH/NIAID) [E]; Leyva-Grado, Victor; Umerah, Nina; Baric, Ralph; Deborah Butler; Neil Pearson; Jeff Pouliot; Cockrell, Adam  
**Subject:** RE: NIAID A57 Call with GSK

Dear Erik,  
This is fine for Ralph.  
Toni

-----Original Appointment-----

**From:** Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]  
**Sent:** Friday, July 22, 2016 8:57 AM  
**To:** Leyva-Grado, Victor; Umerah, Nina; Baric, Toni C; Baric, Ralph S; Deborah Butler; Neil Pearson; Jeff Pouliot; Cockrell, Adam  
**Subject:** NIAID A57 Call with GSK  
**When:** Wednesday, August 03, 2016 1:00 PM-2:00 PM (UTC-05:00) Eastern Time (US & Canada).  
**Where:** Skype Meeting

Hi Everyone,  
Apologies, but I've had a conflict arise for 10am. Can we shift this call to 1pm on 8/3?  
Erik

\*\*\*\*\*

Hi Everyone,  
Please see below for dial in details. You may use either the Skype link or direct dial in. We will plan to discuss the next Study under A57. I will circulate an updated information sheet on the compound once I receive an updated version.  
Erik

---

### → Join Skype Meeting

This is an online meeting for Skype for Business, the professional meetings and communications app formerly known as Lync.

#### Join by phone

(b)(6) NIAID) English (United States)  
(b)(6) NIAID) English (United States)

[Find a local number](#)

Conference ID: (b)(6)

[Forgot your dial-in PIN?](#) | [Help](#)

[LOC({1033})!]

---

**From:** Leyva-Grado, Victor  
**Sent:** Tue, 19 Jul 2016 14:42:38 +0000  
**To:** 'Cockrell, Adam'; Stemmy, Erik (NIH/NIAID) [E]; Umerah, Nina; Baric, Ralph  
**Cc:** Baric, Toni C  
**Subject:** RE: Call to discuss A57 Study

Hi Erik,

Monday both times will work for me , Wednesday only after 10:00.

V

---

**From:** Cockrell, Adam [mailto:(b)(6)]  
**Sent:** Tuesday, July 19, 2016 8:59 AM  
**To:** Stemmy, Erik (NIH/NIAID) [E]; Leyva-Grado, Victor; Umerah, Nina; Baric, Ralph S  
**Cc:** Baric, Toni C  
**Subject:** RE: Call to discuss A57 Study

Hi Erik,

I'm good for any time on Wed, Aug 3<sup>rd</sup>.

Thanks,  
Adam

---

**From:** Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]  
**Sent:** Tuesday, July 19, 2016 8:56 AM  
**To:** Leyva-Grado, Victor (b)(6); Umerah, Nina (b)(6)  
Baric, Ralph S (b)(6); Cockrell, Adam (b)(6)  
**Cc:** Baric, Toni C (b)(6)  
**Subject:** Call to discuss A57 Study

Hi Everyone,

I've been speaking with a group at GSK about testing a small molecule under A57. They've provided the potential times below for a call. Can you please let me know if any of them work for you?

Thanks!  
Erik

Mon Aug 1<sup>st</sup>: 11-12:00pm; 1-1:30pm

Wed Aug 3<sup>rd</sup>; 9-11am; 1-2pm

Erik J. Stemmy, Ph.D.  
Program Officer

Respiratory Diseases Branch  
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS  
5601 Fishers Lane, (b)(6)  
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.

\*\*\*\*\*

NOTE: This material is intended for the individual or entity to which it is addressed. It may contain privileged, confidential information that is protected from disclosure under applicable laws. If you are not the addressee, or a person authorized to deliver the document to the addressee, please note that you are strictly prohibited from reviewing, copying, disclosing, disseminating or distributing this material 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:** Peter Daszak  
**Sent:** Mon, 11 Jul 2016 14:28:11 +0000  
**To:** Greer, Jenny (NIH/NIAID) [E]; Aleksei Chmura  
**Cc:** Stemmy, Erik (NIH/NIAID) [E]; Kirker, Mary (NIH/NIAID) [E]; Glowinski, Irene (NIH/NIAID) [E]; Ford, Andrew (NIH/NIAID) [E]; Joseph Riccardi  
**Subject:** Re: Grant Number: 5R01AI110964 - 03 PI Name: DASZAK, PETER

Dear Jenny,

This is terrific! We are very happy to hear that our Gain of Function research funding pause has been lifted.

Cheers,

Peter

**Peter Daszak**

*President*

EcoHealth Alliance  
460 West 34<sup>th</sup> Street – 17<sup>th</sup> Floor  
New York, NY 10001

(b)(6) (direct)  
+1.212.380.4465 (fax)  
[www.ecohealthalliance.org](http://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:** Greer, Jenny (NIH/NIAID) [E] (b)(6)  
**Sent:** Thursday, July 7, 2016 10:00 AM  
**To:** Aleksei Chmura; Peter Daszak  
**Cc:** Stemmy, Erik (NIH/NIAID) [E]; Kirker, Mary (NIH/NIAID) [E]; Glowinski, Irene (NIH/NIAID) [E]; Ford, Andrew (NIH/NIAID) [E]  
**Subject:** Grant Number: 5R01AI110964 - 03 PI Name: DASZAK, PETER

Aleksei and Peter,

Please find attached a determination regarding your grant.

As always, don't hesitate to contact us with any questions.

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)

Disclaimer: The information in this email and any of its attachments is confidential and may contain sensitive information. It should not be used by anyone who is not the originally intended recipient. If you have received this email in error, please inform the sender and delete it from your mailbox or any other storage devices. The National Institute of Allergy and Infectious Diseases shall not accept liability for any statements made that are the sender's own and not expressly made on behalf of NIAID by one of its representatives.

**From:** Peter Daszak  
**Sent:** Mon, 11 Jul 2016 14:15:45 +0000  
**To:** Stemmy, Erik (NIH/NIAID) [E]  
**Cc:** Aleksei Chmura; Chen, Ping (NIH/NIAID) [E]  
**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*

EcoHealth Alliance  
460 West 34<sup>th</sup> Street – 17<sup>th</sup> Floor  
New York, NY 10001

(b)(6) (direct)  
+1.212.380.4465 (fax)  
[www.ecohealthalliance.org](http://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:** Aleksei Chmura  
**Sent:** Fri, 8 Jul 2016 01:28:21 +0800  
**To:** Greer, Jenny (NIH/NIAID) [E]  
**Cc:** Stemmy, Erik (NIH/NIAID) [E]; Dr. Peter Daszak  
**Subject:** Re: Grant Number: 5R01AI110964 - 03 PI Name: DASZAK, PETER

Fantastic to hear!  
Many thanks,  
-Aleksei

On Jul 7, 2016, at 20:36, Greer, Jenny (NIH/NIAID) [E] (b)(6) wrote:

Dear Aleksei,  
Thanks for checking in. We did receive your updated letter and are working through our internal review processes. We'll let you know as soon as we have an update.

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)

Disclaimer: The information in this email and any of its attachments is confidential and may contain sensitive information. It should not be used by anyone who is not the originally intended recipient. If you have received this email in error, please inform the sender and delete it from your mailbox or any other storage devices. The National Institute of Allergy and Infectious Diseases shall not accept liability for any statements made that are the sender's own and not expressly made on behalf of NIAID by one of its representatives.

---

**From:** Aleksei Chmura [mailto:(b)(6)]

**Sent:** Thursday, July 07, 2016 12:37 AM

**To:** Greer, Jenny (NIH/NIAID) [E] (b)(6)

**Subject:** Re: Grant Number: 5R01AI110964 - 03 PI Name: DASZAK, PETER

Dear Jenny,

We received an out-of-office message from Eric last month and I just wanted to make sure that you both received my email with the updated letter from Dr. Daszak.

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

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)

[www.ecohealthalliance.org](http://www.ecohealthalliance.org)

Visit our blog: [www.ecohealthalliance.org/blog](http://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.*

On Jun 29, 2016, at 11:58, Aleksei Chmura (b)(6) wrote:

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*

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

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

[www.ecohealthalliance.org](http://www.ecohealthalliance.org)

Visit our blog: [www.ecohealthalliance.org/blog](http://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.*

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](http://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*

EcoHealth Alliance

460 West 34<sup>th</sup> Street – 17<sup>th</sup> Floor

New York, NY 10001

(b)(6) direct)

+1.212.380.4465 (fax)

[www.ecohealthalliance.org](http://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] [mailto:(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](http://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, (b)(6)

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.

\*\*\*\*\*

NOTE: This material is intended for the individual or entity to which it is addressed. It may contain privileged, confidential information that is protected from disclosure under applicable laws. If you are not the addressee, or a person authorized to deliver the document to the addressee, please note that you are strictly prohibited from reviewing, copying, disclosing, disseminating or distributing this material 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)

Disclaimer: The information in this email and any of its attachments is confidential and may contain sensitive information. It should not be used by anyone who is not the originally intended recipient. If you have received this email in error, please inform the sender and delete it from your mailbox or any other storage devices. The National Institute of Allergy and Infectious Diseases shall not accept liability for any statements made that are the sender's own and not expressly made on behalf of NIAID by one of its representatives.

---

**From:** Aleksei Chmura [mailto:(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

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

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

[www.ecohealthalliance.org](http://www.ecohealthalliance.org)

Visit our blog: [www.ecohealthalliance.org/blog](http://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.*

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)

Disclaimer: The information in this email and any of its attachments is confidential and may contain sensitive information. It should not be used by anyone who is not the originally intended recipient. If you have received this email in error, please inform the sender and delete it from your mailbox or any other storage devices. The National Institute of Allergy and Infectious Diseases shall not accept liability for any statements made that are the sender's own and not expressly made on behalf of NIAID by one of its representatives.

---

**From:** Peter Daszak [mailto:(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

**Peter Daszak**

*President*

EcoHealth Alliance

460 West 34<sup>th</sup> Street – 17<sup>th</sup> Floor

New York, NY 10001

(b)(6) (direct)

+1.212.380.4465 (fax)

[www.ecohealthalliance.org](http://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:** Greer, Jenny (NIH/NIAID) [E] [mailto:(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

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 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.”*

Disclaimer: The information in this email and any of its attachments is confidential and may contain sensitive information. It should not be used by anyone who is not the originally intended recipient. If you have received this email in error, please inform the sender and delete it from your mailbox or any other storage devices. The National Institute of Allergy and

Infectious Diseases shall not accept liability for any statements made that are the sender's own and not expressly made on behalf of NIAID by one of its representatives.

**From:** Baric, Toni C  
**Sent:** Fri, 1 Jul 2016 16:14:56 +0000  
**To:** Baric, Ralph; Beisel, Christopher (NIH/NIAID) [E]; Damania, Blossom A; Spiro, David (NIH/FIC) [E]; Stemmy, Erik (NIH/NIAID) [E]; Graham, Rachel; Mathur, Punam (NIH/NIAID) [E]; Sims, Amy C; Dugan, Vivien (NIH/NIAID) [E]; Yao, Alison (NIH/NIAID) [E]  
**Subject:** UNC-NIH called cancelled

Hi Everyone,  
The NIH-UNC call schedule for July will be cancelled. We are set for our August 2 call.

Have a happy and safe 4<sup>th</sup>.

## Toni Baric

Department of Microbiology and Immunology  
9025 Burnett Womack  
CB# 7292  
Chapel Hill, NC 27599-7292  
Office: (b)(6)  
(b)(6)

**From:** Baric, Ralph S  
**Sent:** Fri, 17 Jun 2016 19:58:26 +0000  
**To:** Stemmy, Erik (NIH/NIAID) [E]  
**Cc:** Sims, Amy C  
**Subject:** RE: MERS Isolate Question

Ok, I will gladly do that from here on out. ralph

---

**From:** Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]  
**Sent:** Friday, June 17, 2016 3:14 PM  
**To:** Baric, Ralph S  
**Subject:** RE: MERS Isolate Question

Thanks Ralph. The EMC strain is available in BEI so if it's too much of a hassle for you to work through Erasmus and Bart you can feel free to direct them to BEI. The MTA BEI had allowed them to share as long as requesters fill out an intended use form, I believe. I'll let you know if we want to pursue any of the other strains you have for BEI.

Erik

---

**From:** Baric, Ralph  
**Sent:** Friday, June 17, 2016 12:42 PM  
**To:** Stemmy, Erik (NIH/NIAID) [E] (b)(6)  
**Subject:** RE: MERS Isolate Question

Hi Erik, People are still acquiring the EMC strain-a trickle now of requests.....most of the requests come to me and I either ask for permission via Bart Haagman-Erasmus (or he contacts me and asks me to send it to someone). Its never a bad idea to have more than one isolate on file. The Jordan isolate is also free of charge without any of the Erasmus BS in regards to MTA agreement. We also have a camel MERS that could be banked at BEI---received it from Malik Peiris, so I would have to ask him if its okay to do this if your interested. We also synthesized the chinse Beijing 01 strain (linked to Korean outbreak), but in collaboration with George Gao (less sure of his interest to continue after zikv emerged. Would have to ask him, although we made it in collaboration. ralph

---

**From:** Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]  
**Sent:** Friday, June 17, 2016 9:39 AM  
**To:** Baric, Ralph S  
**Subject:** MERS Isolate Question

Hi Ralph,  
We are thinking of having BEI try to acquire a Jordan isolate of MERS (Jordan-N3/2012), and I wanted to ask you if you think that's something that might be useful for the larger community. I've seen lots of work published using the EMC isolate, and it looks like the IRF has published with this Jordan strain as well, but I don't have a great sense if other groups have it. No one has ordered the EMC strain from BEI, but I suspect that's because by the time they listed it everyone who wanted it already had it. Do you have any thoughts on whether this Jordan isolate would be more popular?

Thanks for your help!  
Erik

Erik J. Stemmy, Ph.D.  
Program Officer  
Respiratory Diseases Branch  
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS  
5601 Fishers Lane, (b)(6)  
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.

\*\*\*\*\*

NOTE: This material is intended for the individual or entity to which it is addressed. It may contain privileged, confidential information that is protected from disclosure under applicable laws. If you are not the addressee, or a person authorized to deliver the document to the addressee, please note that you are strictly prohibited from reviewing, copying, disclosing, disseminating or distributing this material 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:** Peter Daszak  
**Sent:** Thu, 16 Jun 2016 19:45:43 +0000  
**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

Great – afternoon would be best. I'll ask Hongying to send in all our passport details..

Cheers,

Peter

**Peter Daszak**  
*President*

EcoHealth Alliance  
460 West 34<sup>th</sup> Street – 17<sup>th</sup> Floor  
New York, NY 10001

(b)(6) (direct)  
+1.212.380.4465 (fax)  
[www.ecohealthalliance.org](http://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] [mailto:(b)(6)]  
**Sent:** Thursday, June 16, 2016 6:04 AM  
**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

Peter,

Just want you to know that I have a meeting on July 7 11 am - 12 pm. I can meet you 9-11 or in the afternoon.

Thanks

Ping

Sent from my iPhone

On Jun 12, 2016, at 11:49 AM, Peter Daszak (b)(6) wrote:

Hi Ping,

I'll be in Beijing on Thursday the 7<sup>th</sup> 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 either in the morning or afternoon of the 7<sup>th</sup> 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 7<sup>th</sup>, I can rearrange things and do either the 5<sup>th</sup> or 6<sup>th</sup>.

Cheers,

Peter

**Peter Daszak**  
*President*

EcoHealth Alliance  
460 West 34<sup>th</sup> Street – 17<sup>th</sup> Floor  
New York, NY 10001

(b)(6) (direct)  
+1.212.380.4465 (fax)  
[www.ecohealthalliance.org](http://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] [mailto:(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

**Peter Daszak**  
*President*

EcoHealth Alliance  
460 West 34<sup>th</sup> Street – 17<sup>th</sup> Floor  
New York, NY 10001

(b)(6) (direct)  
+1.212.380.4465 (fax)  
[www.ecohealthalliance.org](http://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:** 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)  
(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*

EcoHealth Alliance  
460 West 34<sup>th</sup> Street – 17<sup>th</sup> Floor  
New York, NY 10001

(b)(6) (direct)  
+1.212.380.4465 (fax)  
[www.ecohealthalliance.org](http://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] [mailto:(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, (b)(6)  
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.**

\*\*\*\*\*  
*NOTE: This material is intended for the individual or entity to which it is addressed. It may contain privileged, confidential information that is protected from disclosure under applicable laws. If you are not the addressee, or a person authorized to deliver the document to the addressee, please note that you are strictly prohibited from reviewing, copying, disclosing, disseminating or distributing this material 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:** Stu Greenberg  
**Sent:** Thu, 2 Jun 2016 13:13:16 -0400  
**To:** 'Cockrell, Adam'  
**Cc:** Baric, Ralph; Stemmy, Erik (NIH/NIAID) [E]  
**Subject:** RE: Progress

Hi Adam,

My colleague Koichi Kimura is bringing over fresh anti-MERS IgY this evening. I would like to ship them to you on Monday for Tuesday delivery in Chapel Hill. What delivery address would you prefer?

Professor Tsukamoto had some thoughts to share on dosage based on an H5N1 study he has conducted with chicks in a BSL3 lab in Indonesia. The chicks were injected intra-muscularly with the ostrich IgY (1, 10 and 100mg/bird) at 1 hr post inoculation with A/H5N1. Then all chicks were boarded in individual cages. At 5-days post viral-challenge, the number of dead chicks was counted in each experimental group (over five individuals in each group). In the case of 10 and 100mg IgY, all birds were alive and no histopathologic lesions were found in the lungs. The body weight of the chicks was about 100g~150g. Professor Tsukamoto recommends that the dosage of IgY to mice be at least 2 mg per animal, because the mice in your study were about 20~30g in body weight.

Rather than taking time and effort to perform a neutralization assay, let me suggest an alternative approach. Before initiating another formal study, why not do a preliminary test with a 2mg dosage for just a few mice. If they survive, as we believe they will, then you could re-launch the study at the 2mg dosage. If they don't survive, then the study would not make much sense. What do you think?

Regards,  
Stu Greenberg

---

**From:** Cockrell, Adam [mailto:(b)(6)]  
**Sent:** Friday, April 15, 2016 9:51 PM  
**To:** Stu Greenberg (b)(6)  
**Cc:** Baric, Ralph S (b)(6); 'Stemmy, Erik (NIH/NIAID) [E]' (b)(6)  
(b)(6)  
**Subject:** RE: Progress

Hi Stu,

I agree. Definitely disappointing. Testing the antibodies in a neutralization assay would provide the necessary information regarding degradation of the antibodies. However, due to time constraints I have over the next 1.5 months, with ongoing projects, it will be difficult for me to get to this right away. I will do my best to put it in the queue, but cannot make an immediate time commitment.

Best Regards,  
Adam

**From:** Stu Greenberg (b)(6)  
**Sent:** Thursday, April 14, 2016 11:24 AM  
**To:** Cockrell, Adam (b)(6)  
**Cc:** Baric, Ralph S (b)(6) 'Stemmy, Erik (NIH/NIAID) [E]' (b)(6)  
**Subject:** RE: Progress

Hi Adam,

We are obviously disappointed that the sample we provided does not confer protection. I have discussed this with Dr. Tsukamoto, and he believes that the sample antibodies have degraded.

I think there might be a straightforward way to test Dr. Tsukamoto's hypothesis. The table below was produced by USAMRIID.

| 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) |             |

The MERS antibody sample and the negative control we provided you correspond to Compound IDs "MERS IgY 2" and "Pre-im IgY NC," respectively. The relative neutralization power of the MERS antibodies to the control is shown to be about 13 to 1. If the MERS antibodies have indeed degraded, an in vitro neutralization test of the MERS antibodies and the control should show relative neutralization power nearer 1 to 1.

Does this make sense to you?

Regards,  
 Stu

**From:** Cockrell, Adam [mailto:(b)(6)]  
**Sent:** Tuesday, April 12, 2016 9:53 AM  
**To:** Stu Greenberg (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6)  
**Cc:** Baric, Ralph S (b)(6)  
**Subject:** RE: Progress

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 [mailto:(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)

**From:** Baric, Ralph S  
**Sent:** Tue, 24 May 2016 22:04:51 +0000  
**To:** Stemmy, Erik (NIH/NIAID) [E]  
**Cc:** Lim, Jean; Leyva-Grado, Victor; Cockrell, Adam  
**Subject:** RE: May 2016 A57 Animal Models Report

Interesting data. Couple of points. The ostrigen neut titers are about (b)(4) than control ostrige serum ec50 values and (b)(4) than the lanzavechia and marasco human antibodies (b)(4) (b)(4) Ralph

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

From: Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]  
Sent: Monday, May 23, 2016 11:24 AM  
To: Baric, Ralph S  
Cc: Lim, Jean; Leyva-Grado, Victor; Cockrell, Adam  
Subject: RE: May 2016 A57 Animal Models Report

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.  
Program Officer  
Respiratory Diseases Branch  
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS  
5601 Fishers Lane, (b)(6)  
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.

\*\*\*\*\*

NOTE: This material is intended for the individual or entity to which it is addressed. It may contain privileged, confidential information that is protected from disclosure under applicable laws. If you are not the addressee, or a person authorized to deliver the document to the addressee, please note that you are strictly prohibited from reviewing, copying, disclosing, disseminating or distributing this material 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.

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

From: Lim, Jean [mailto:(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

>



**From:** Leyva-Grado, Victor  
**Sent:** Fri, 20 May 2016 15:58:42 +0000  
**To:** Stemmy, Erik (NIH/NIAID) [E]; Umerah, Nina; Baric, Ralph; Cockrell, Adam; Heise, Mark T  
**Subject:** RE: A57 Option 2

Hi Erik,

Please let us know if you need anything from our end.

Cheers,

V

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

---

**From:** Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]  
**Sent:** Wednesday, May 18, 2016 12:04 PM  
**To:** Leyva-Grado, Victor; Umerah, Nina; Baric, Ralph; Cockrell, Adam; Heise, Mark T  
**Subject:** A57 Option 2  
**Importance:** High

Hi Everyone,

I'm in the process of exercising option 2 for A57. The performance period stated for it in the SOW is 4 months. I know in the past we decided that wasn't a reasonable amount of time for 4 studies so I was thinking of setting the performance period 9 months instead. Can you let me know ASAP if that would be reasonable? Our contracts office would also need you to confirm that no additional funds would be required for the longer performance period.

Let me know if you have any questions.

Thanks!

Erik

Erik J. Stemmy, Ph.D.  
Program Officer  
Respiratory Diseases Branch

Division of Microbiology and Infectious Diseases NIAID/NIH/HHS

5601 Fishers Lane, (b)(6)

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.

\*\*\*\*\*

NOTE: This material is intended for the individual or entity to which it is addressed. It may contain privileged, confidential information that is protected from disclosure under applicable laws. If you are not the addressee, or a person authorized to deliver the document to the addressee, please note that you are strictly prohibited from reviewing, copying, disclosing, disseminating or distributing this material 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:** Bumbray-Quarles, Devon (NIH/NIAID) [E]  
**Sent:** Wed, 18 May 2016 07:10:10 -0400  
**To:** 'Kevin McKoskey'  
**Cc:** (b)(6) Kirker, Mary (NIH/NIAID) [E]; Glowinski, Irene (NIH/NIAID) [E]; Ford, Andrew (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; (b)(6) Baric, Ralph; Bumbray-Quarles, Devon (NIH/NIAID) [E]  
**Subject:** Grant Number: 2 R01 AI 089728 - 06, Li (PI)  
**Attachments:** NIAID Response to GoF- 2R01AI089728-06.pdf

Dear Mr. McKoskey,

Please find attached NIAID's response for Gain of Function (GoF) on the above subject grant.

If you have any questions or concerns, please do not hesitate to ask.

Thank you.

**Sincerely,**  
**Ms. Devon Bumbray-Quarles**  
**Grants Management Specialist**  
**Grants Management Program**  
**DHHS, NIH, NIAID, GMP**  
**5601 Fishers Lane, Room 4E28, MSC 9824**  
**Bethesda, MD 20892-9824**  
**Overnight Mail Only: Use Zip Code 20852**  
**P:** (b)(6)  
**F:** 301.493.0597  
(b)(6)



*“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.”*

\*\*\*\*\*

**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 original 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 Institute 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.





DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health  
National Institute of Allergy  
and Infectious Diseases  
Bethesda, Maryland 20892

May 18, 2016

Mr. Kevin McKoskey  
Director, Sponsored Projects  
University of Minnesota  
450 McNamara Alumni Center  
200 Oak Street SE  
Minneapolis, MN 55455-2070

RE: 2 R01AI089728-06

Dear Mr. McKoskey:

Thank you for your correspondence of April 15, 2016, regarding the October 17, 2014 White House announcement of a U.S. Government-wide pause on certain gain-of-function (GoF) experiments and its potential impact on your research (<http://www.whitehouse.gov/blog/2014/10/17/doing-diligence-assess-risks-and-benefits-life-sciences-gain-function-research>). The research funding pause pertains to GoF research projects that may be reasonably anticipated to confer attributes to influenza, MERS, or SARS viruses such that the resulting virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route.

NIAID reviewed the original grant application, and the additional information provided by you, and made the following assessments regarding Subaim 2.1 of the above-referenced grant:

- NIAID is in agreement that the work you proposed under Experiments 1, 2, and 3 is not subject to the GoF research funding pause. This determination is based on the fact that the work will be carried out using either recombinant proteins or replication-deficient pseudoviruses, and will not involve the generation of a replicating SARS-CoV variant with enhanced pathogenicity and/or transmissibility via the respiratory route.
- NIAID's determination is that the work proposed under Experiment 4 to generate SARS-Like viruses with enhanced affinity for human receptors (via both reverse genetics and serial passaging) is subject to the GoF research funding pause, and therefore may not be conducted under this grant. Given the lack of empiric evidence that increased receptor binding alone is insufficient to increase pathogenicity, NIAID has determined that the proposed work is reasonably anticipated to result in a SARS-Like virus with enhanced pathogenicity. NIAID acknowledges that in lieu of generating SARS-Like viruses with enhanced affinity for human receptors you will pursue alternative strategies using *loss-of-function* mutations, or mutations targeting the ACE2 receptor. NIAID also reviewed your statement that if any mutant

viruses you generate demonstrate either enhanced virus growth >1 log compared to the wildtype parental backbone strain or more efficient growth in primary human airway epithelial cells, you will immediately stop all experiments with the mutant and notify NIAID and the IBCs at the University of Minnesota and the University of North Carolina at Chapel Hill of these results.

Please remember that the institution must comply in full with all terms and conditions placed on this grant. If your research evolves to include experiments that may be subject to the GoF research funding pause or you observe enhanced pathogenicity and/or transmissibility of SARS or SARS-Like viruses in mammals via the respiratory route at any time during the course of conducting these experiments, you must immediately stop these research activities and provide the NIAID Program Officer and Grants Management Specialist with the relevant data and information related to these unanticipated outcomes.

As indicated above, NIAID determinations are based on information from multiple sources, but primarily on our communication with you about the details of your proposed experiments and your research results. Should NIAID's determination change based on information obtained through the U.S. Government GoF deliberative process, described here <http://www.phe.gov/s3/dualuse/Documents/gain-of-function.pdf>, you will be notified; however, until such time, or until the GoF research funding pause is lifted, NIAID's determination, indicated above, is final.

Please let us know if you have any questions, or if you require additional information.

Sincerely,

(b)(6)

Devon Bumbrey-Quarles  
Grants Management Specialist  
NIAID/NIH/DHHS

(b)(6)

Erik J. Stemmy, Ph.D.  
Program Officer  
Division of Microbiology and Infectious Diseases  
NIAID/NIH/DHHS

CC: Dr. Fang Li  
Ms. Erin Knudsen  
Ms. Mary Kirker  
Dr. Irene Glowinski  
Dr. Andrew Ford  
Dr. Ralph Baric

**From:** Peter Daszak  
**Sent:** Fri, 13 May 2016 16:55:15 +0000  
**To:** Stemmy, Erik (NIH/NIAID) [E]  
**Cc:** Normil, Carine (NIH/NIAID) [C]; Pone, Laura (NIH/NIAID) [E]; Aleksei Chmura  
**Subject:** Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER  
**Attachments:** Year 2 NIAID CoV Report as submitted via eRA Commons.pdf  
**Importance:** High

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*

EcoHealth Alliance  
460 West 34<sup>th</sup> Street – 17<sup>th</sup> Floor  
New York, NY 10001

(b)(6) direct)  
+1.212.380.4465 (fax)  
[www.ecohealthalliance.org](http://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.*



## A. COVER PAGE

|                                                                                                                                                                  |                                                                                                                                                                                                                                       |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Project Title:</b> Understanding the Risk of Bat Coronavirus Emergence                                                                                        |                                                                                                                                                                                                                                       |
| <b>Grant Number:</b> 5R01AI110964-03                                                                                                                             | <b>Project/Grant Period:</b> 06/01/2014 - 05/31/2019                                                                                                                                                                                  |
| <b>Reporting Period:</b> 06/01/2015 - 05/31/2016                                                                                                                 | <b>Requested Budget Period:</b> 06/01/2016 - 05/31/2017                                                                                                                                                                               |
| <b>Report Term Frequency:</b> Annual                                                                                                                             | <b>Date Submitted:</b> 05/13/2016                                                                                                                                                                                                     |
| <b>Program Director/Principal Investigator Information:</b><br>PETER DASZAK , BS PHD<br><b>Phone number:</b> (b)(6)<br><b>Email:</b> (b)(6)                      | <b>Recipient Organization:</b><br>ECOHEALTH ALLIANCE, INC.<br>ECOHEALTH ALLIANCE, INC.<br>460 W 34TH ST<br>17TH FLOOR<br>NEW YORK, NY 100012320<br><br><b>DUNS:</b> 077090066<br><b>EIN:</b> 1311726494A1<br><br><b>RECIPIENT ID:</b> |
| <b>Change of Contact PD/PI:</b> N/A                                                                                                                              |                                                                                                                                                                                                                                       |
| <b>Administrative Official:</b><br>ALEKSEI CHMURA<br>460 W 34th St., 17th Floor<br>New York, NY 10001<br><br><b>Phone number:</b> (b)(6)<br><b>Email:</b> (b)(6) | <b>Signing Official:</b><br>ALEKSEI CHMURA<br>460 W 34th St., 17th Floor<br>New York, NY 10001<br><br><b>Phone number:</b> (b)(6)<br><b>Email:</b> (b)(6)                                                                             |
| <b>Human Subjects:</b> Yes<br>HS Exempt: No<br>Exemption Number:<br>Phase III Clinical Trial:                                                                    | <b>Vertebrate Animals:</b> Yes                                                                                                                                                                                                        |
| <b>hESC:</b> No                                                                                                                                                  | <b>Inventions/Patents:</b> No                                                                                                                                                                                                         |

## 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). 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.

#### B.1.a Have the major goals changed since the initial competing award or previous report?

No

### B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Year 2 NIAID CoV Report Final.pdf

### B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

### B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Year 2 NIAID CoV Report Professional Development.pdf

**B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?**

1) Conference and University lectures: PI Daszak, and Co-investigators Shi, Epstein, Olival, Ge, and Zhang gave >100 invited University and Conference lectures including Forum on Microbial Threats (National Academies of Science), Symposium at École du Val-de-Grâce in Paris, Leadership Roundtable at Concordia University Montreal, 1st annual Global Pandemic Policy Summit at Texas A&M Univ., Intl. Conf. of the Wildlife Disease Association in Australia, Intl. Conf. of Conservation Biol in Montpellier France, Michigan State University, Duke University, WDA, ISID conference, Zoological Society of London Symposium, Future Earth meeting, North American Bat Research Symposium, and others that included specific discussion of the current project and results.

2) Agency and other briefings: PI Daszak and Research Technician Dr. Guangjian Zhu introduced this project to potential collaborators within the following agencies: Forestry Dept of Peoples' Republic of China, FAO, TNC, TRAFFIC, China CDC, and TA Foundation in Beijing China in meetings (2015) and also at presentations at the first Wildlife and Public Health Workshop in China (2016) co-hosted by EcoHealth Alliance, the State Forestry Administration of China, and China CDC.

3) Public outreach: PI Daszak presented this work to members of the NIH, NSF, DoD, IUCN, EPA, and the general public, at an EcoHealth Alliance meeting hosted by the Cosmos Club, Washington D.C. (2015); PI Daszak and Co-investigator Zhu reported on this project at a Wildlife Trade and Public Health Seminar, Beijing (2016); PI Daszak introduced this project in a lecture on Pandemics at a New York Academy of Science Panel (2016); Co-PI Y-Z Zhang presented project and results-to-date to department heads and senior researchers at Infectious Disease Departments of four Yunnan Hospitals (2015)

**B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?**

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

- Given the reduced amount of wildlife in the local markets within Southern China, and the continued expansion of the Chinese wildlife trade within SE Asia, 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). EcoHealth Alliance has other activities in these countries which would provide leverage to reduce costs of fieldwork, and samples would be tested in Wuhan, China.

- Following the successful collection of ethnographic interviews and focus groups in Year 2, we will be analyzing the qualitative data collection from Years 1 and 2.

- Finalize and conduct survey collection tool for a network study of wildlife farmers using a questionnaire to characterize and map the wildlife value chain.

- After the success of our pilot studies in Year 2, we will continue targeted (at individuals with high risk of exposure to bats), integrated behavioral and biological survey work in Yunnan and expand to Guangxi and Guangdong provinces.

- We will commence our anonymized, surveillance data collection from acutely ill hospital in-patients who satisfy syndromic eligibility criteria; have complete medical records; non-normative laboratory confirmed diagnostic results; and suspected acute viral infection. Eligibility criteria are: (a) suspected acute viral infection; (b) fever > 38°C, and (c) presenting symptoms of at least one of the following:

- Encephalitis of unknown origin
- Hemorrhagic fever of unknown origin
- Respiratory disease
- oInfluenza-like illness (ILI)
- oSevere Acute Respiratory like Illness (SARI)
- Rash
- Diarrhea

Some patients with particular infections such as with HIV, HCV, and HBV, may be excluded from the study on that basis. Hospital surveillance has the advantage of monitoring an acutely ill population. Anonymized, passive hospital surveillance allows for data collection and viral testing from all eligible hospital patients thereby limiting population sample bias and increasing the likelihood of identifying positive cases. The strengths of this approach are enormous: an unbiased patient population; prospectively collected, anonymized patient data; a low resource effort with a high efficiency design; and impactful research potential for both case series and case control studies. We have already secured approval from the Institutional Review Boards of the Wuhan School of Public Health and Hummingbird IRB.

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:

- Test and implement our respondent-driven survey to collect specific data on the diversity, abundance, and turnover of species along the wildlife trade network in south China.

- Model viral mixing across the full range parameters found along the wildlife trade network to identify the trade nodes with highest mixing potential. This will include a network analysis of market facility/site connectivity including wild harvest sites, wildlife farming operations, transit holding facilities, and small and large wildlife markets.

- Phylogeographic study of bat-CoV to better understand the geographic distribution and evolution of bat-CoV genetic diversity in south

China.

- Phylogeographic study of bat host (*Rhinolophus*) species to assess the connectivity of bat populations and infer their historical movements and demographic history to improve our understanding of CoV transmission among bat populations in southern China. Preliminary sequences data has been generated and will be completed and analyzed.
- Cophylogenetic analyses of bat host and CoV phylogenies to assess frequency of cross-species transmission. Comparison of Alpha- and Beta-CoV cophylogenetic patterns building on Year 2 analyses using published sequences and also including Spike gene and additional sequences obtained in Year 2.
- Test and implement our respondent-driven survey to assess diversity, abundance, and turnover of species along the wildlife trade network.
- Examine co-evolutionary congruence of bat-CoVs and their hosts using both functional (receptor) and neutral genes;
- Parameterize mathematical models that predict CoV evolutionary and transmission dynamics
- Continued surveillances of SARS-like CoVs and lineage C betacoronaviruses (MERS-related CoVs) in Southern China;
- Full-length genome sequencing and evolution analysis of SARS-like coronaviruses identified from different bat species and different geographical locations across China;
- Full-length genome sequencing and evolution analysis of Lineage C betacoronaviruses identified from different bat species and different geographical locations across China;
- Full-length genome sequencing and evolution analysis of HKU9-related and HKU10-related bat coronaviruses in China;

Specific Aim 3: Testing predictions of CoV inter-species transmission. The following experiments will be undertaken in Year 2:

- Humanized mice with human ACE2 receptors will be infected with WIV1 and the two rescued chimeric SARS-like coronaviruses to determine the tissue tropism and pathogenicity of bat SL-CoV
- Isolation of novel bat coronaviruses. Live virus or pseudovirus will be used to infect cells of different origin or expressing different receptor molecules. Spillover potential for each isolated virus will be assessed.
- An infectious clone of full-length MERS-CoV will be constructed using reverse genetic method. Using the S sequence of different MERS-related viruses identified from Chinese bats, the chimeric viruses with S gene of bat MERS-related coronaviruses and backbone of the infectious clone of MERS-CoV will be constructed to study the receptor usage and infectivity of bat MERS-related coronavirus.
- Surveillance of infection in human populations by SARS-like CoVs. This work will be performed at locations in Yunnan, Guangxi, and Guangdong provinces, in previously identified areas with human populations of high risk of exposure to bats. PCR and ELISA will be used, respectively, for detection of viral replicase gene and antibodies against the viral nucleocapsid protein.

1R01AI110964 Year 2 Report

PI: Daszak, Peter

**Year 1 Report:** Understanding the Risk of Bat Coronavirus Emergence**Award Number:** 1R01AI110964-02

\*\*\*\*\*

**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). 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.

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

**B.2 What was accomplished under these goals?**

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

In year 2, we continued and expanded the qualitative research begun at the end of Year 1. In addition, a community based integrated biological behavioral surveillance system was developed and pilot tested to identify specific animal exposure risk factors associated with biological evidence of exposure to SARS-like CoV (i.e., seropositive status).

**QUALITATIVE RESEARCH**

Targeted, in-depth ethnographic interviews were conducted with 47 individuals (18 women; 29 men) in rural Southern China where wildlife trade routes have been documented. Yunnan, Guangxi and Guangdong 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 Wuhan School of Public Health and Hummingbird IRB.

Recruitment sites in each province included forested areas or preserves, wildlife farms, hunting areas, wildlife restaurants, live animal markets, caves where people dwell or collect guano and residential areas/farms near known bat caves or roosts. Participants were recruited primarily through local contacts developed as part of wildlife conservation and health research conducted by team members over 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).

The five core themes that guided the in-depth discussions are: 1) human-animal contact, 2) unusual illness experience and response, 3) socioeconomics and daily living, 4) biosafety and 5) human environments and movement/travel. An ethnographic interview guide was developed with examples of questions that could be asked for each theme. In addition, field based participant-observation was ongoing throughout the study and involved observing and talking informally with people in their own natural setting. Field notes were maintained of these ongoing observations and discussions.

Table 1: Species Observed in Wetmarkets in Guangdong Province from 2015 - 2016

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

Interviews were conducted between March and June 2105 by 10 trained interviewers, none of whom had social science training. Interviewers conducted between one and 22 interviews; three interviewers conducted two thirds of all interviews. Interviews lasted between 20 and 60 minutes, and were tape-recorded and transcribed verbatim before they were translated into English. All participants received cooking oil valued at US\$10 in appreciation of their time.

The data are currently being coded and an analytic database is being constructed. Initial insights include observations by a number of participants, especially those who are older, that there has been a decrease in wildlife in the surrounding environment. This decrease is attributed to many factors including infrastructure development. The government has invested resources to build new roads and renovate local infrastructure with the intention of increasing tourism. This has reduced forested area.

Observations by research staff in live animal markets in Guangzhou found wildlife to be plentiful (see Table 1), although no bats were seen for sale during the observation period.

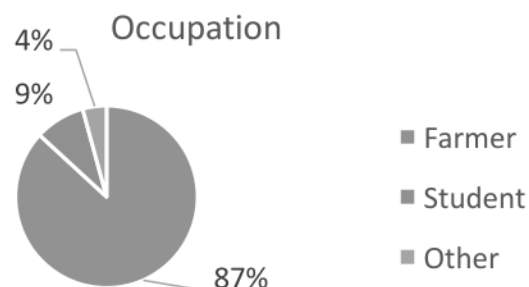
In contrast, wildlife was not found in live animal markets at the sites we visited in either Yunnan or Guangxi. This is a change from previous research visits to the same or similar communities, when bats, rodents and wild boar could be found. Locals in Yunnan and Guangxi attribute the change to conservation 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.

Preliminary analyses are underway. Three specific studies in support of Specific Aim 1 are being developed: the changing wildlife trade in Southern China, the economics of wildlife farming, and zoonotic disease risks resulting from a rapidly changing wildlife trade.

### INTEGRATED BIOLOGICAL BEHAVIORAL SURVEILLANCE PILOT STUDY

Currently, mechanisms of zoonotic viral spillover are unknown. In order to evaluate potential risk factors, it is necessary to measure both exposure and outcome data. Therefore, a behavioral risk survey was developed that assessed both animal exposure and experiences of unusual illness both during 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 SARS-like-CoV has been previously detected in the bat population in Jinning County, Yunnan. Please view the full survey here:

<https://www.dropbox.com/s/sv62neywuvl027r/Questionnaire%20Complete.docx?dl=0>



Of 218 participants, 139 (64%) were women and 79 (36%) were men, with a mean age of 48 (range: 12-80). Most reported being farmers (87%, and see chart to left); a majority were long term residents (97%). Animal exposures in the past year were extensive, including general (e.g., buying live animals at markets [61%]) and intimate (e.g., being scratched or bitten [9%], slaughter

[38%]). In fact, two-thirds of participants reported handling recently killed animal parts and 2 out of 5 reported slaughtering animals. Only 20 (9%) participants reported known exposure to bats.

Standardized syndromic case definitions informed questions concerning unusual illness experience (e.g. severe acute respiratory infections [SARI], influenza-like illness [ILI]). Lifetime, 12 month and unusual illness experience in family for the past 12 months were assessed for all participants. In the past year, SARI was reported by 4 (2%) respondents and for 4 additional family members. Table 2 provides data for all unusual illness experience assessed. None of the participants were found to be seropositive for SARS-like CoV.

Table 2. Unusual Illness Experience

| Symptoms                                   | Ever       | Past 12 months | Family (12m) |
|--------------------------------------------|------------|----------------|--------------|
| Severe Acute Respiratory Infections (SARI) | 15 (6.9%)  | 4 (1.8%)       | 4 (1.8%)     |
| Influenza Like Illness (ILI)               | 54 (24.8%) | 16 (7.3%)      | 26 (11.9%)   |
| Encephalitis                               | 19 (8.7%)  | 4 (1.8%)       | 3 (1.4%)     |
| Hemorrhagic Fever                          | 0 (0.0%)   | 0 (0.0%)       | 0 (0.0%)     |
| Fever with Diarrhea /Vomiting              | 12 (5.5%)  | 2 (0.9%)       | 3 (1.4%)     |
| Fever with Rash                            | 2 (0.9%)   | 2 (0.9%)       | 3 (1.4%)     |



Although the sample size was small, animal exposures among those who reported unusual illness experiences in the past 12 months were evaluated. Of the four respondents who reported SARI symptoms, 75% 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. Finally, 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 wet market.

Respondents were asked about the source of their unusual illnesses. None reported any kind of animal exposure as a potential source of infection and most stated they had no idea how they had become infected. However, when asked about potential behavior changes made at live animal markets in the last 12 months, participants reported a great deal of change. In particular, respondents reported buying live animals less often (38%), only buying farmed wildlife (54%) or buying meat at the supermarket (23%). (See Table 3).

**Table 3: Behavior Change at Wet Market in the last 12 months**

| Behavior                               | N  | (%)    |
|----------------------------------------|----|--------|
| Wear a mask                            | 4  | (3.0)  |
| Wear gloves                            | 5  | (3.8)  |
| Wash hands                             | 80 | (60.6) |
| Sometimes shop for meat at supermarket | 30 | (22.7) |
| Buy live animals less often            | 50 | (37.9) |
| Buy only farmed wildlife               | 71 | (53.8) |
| No longer buy wildlife at wet market   | 39 | (29.5) |

The results of this pilot study conducted with a largely female farmer population found high levels of unusual illness, as well as high levels of exposure to animals. There was a notable lack of knowledge of animals' ability to transmit infection. Despite this lack of knowledge, there may be a sense of unease about animal exposures, given the fairly dramatic behavior changes reported at live animal markets. The finding of a reduction in wildlife purchase may be due to sensitivity to the legality of wildlife trade, biasing respondents towards not admitting purchasing wildlife. Although, there were no participants seropositive for SARS-like CoV, serological data may add support to the findings from self-reported syndromic surveillance, once serological assays are optimized.

In preparation for full implementation of the integrated biological behavioral surveillance, the survey has been programmed as an application for use on either a mobile device or computer. Electronic data collection will facilitate survey implementation in the field and quality control of the data being collected. Four field team leads were trained on behavioral survey data collection, data collection technologies (the tablet application) and analysis.

Nucleic acid test results of human biological samples

*Testing High-Risk Human Populations for Coronavirus Infection*

Surveillance of CoV infections in human populations by SARS-like CoVs was significantly expanded in Year 2, including both custom-built ELISA serology (an assay developed by the Wuhan Institute of Virology to test antibodies against the N protein of SL-CoV) and PCR detection of viral RNA.

*Serological test for SL-CoV antibodies in human samples from Jinning, Yunnan Province*

In order to assess past exposure to bat CoVs, 223 human sera samples were collected in villages in proximity to the bat habitat from which two SL-CoVs with potential for interspecies infection, WIV1 and WIV16, were discovered in our previous research. An ELISA developed by the Wuhan Institute of Virology was used to test antibodies against the N protein of SL-CoV. A number of human specimens generated high OD values and neutralization test to WIV1 and WIV16 was then performed. These findings are encouraging; however, no neutralization antibodies were detected. In Year 3, we will continue to validate and optimize these ELISA assays and other serological tests to obtain data on past CoV exposure.

*PCR test for CoV Nucleic Acid in human samples from several Provinces*

We tested 405 individual human samples for CoV RNA to identify evidence of active infection in human populations and to obtain sequence data on strain variation. Individual samples (4 each) were pooled prior to nucleic acid extraction then tested using PCR. When a group tested positive, we then conducted the confirmation test in the individual samples. One single sample (14XN611) from someone who had identified as having had a fever and suffered both a cough and headache in the past 7-days was then identified to be positive for HCoV-HKU1. The low number of PCR detections in human specimens is not unexpected, and will be improved in Year 3-5 by better targeting syndromic individuals for specimen collection and continuing to optimize PCR assays. Refined serological assays (above) will provide sufficient data to assess past exposure to specific CoV lineages, and optimizing of PCR detections will allow for more CoV positive human sequences moving forward.

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

*Bat CoV PCR detection and sequencing from live-sampled bat populations*

We collected 1,714 anal swab samples, 677 fecal samples, 53 blood samples, and 38 serum samples from 15 bat genera in Guangdong, Yunnan, Sichuan, Hubei, Hunan, Guizhou, Guangxi provinces (Table 4).

**Table 4 Bat Samples collected for CoV surveillance in 2015**

| Sample date | Sample location    | Anal | Fecal | Blood | Serum |
|-------------|--------------------|------|-------|-------|-------|
| Mar. 2015   | Huidong, Guangdong | 69   | --    | --    | --    |
| Jun. 2015   | Guangdong          | 495  | --    | 12    | --    |
| Apr. 2015   | Menglun, Yunnan    | 51   | --    | --    | --    |
| May 2015    | Jinning, Yunnan    | --   | 193   | --    | --    |
| May. 2015   | Mojiang, Yunnan    | 93   | --    | --    | --    |
| Oct. 2015   | Jinning, Yunnan    | 30   | --    | --    | --    |

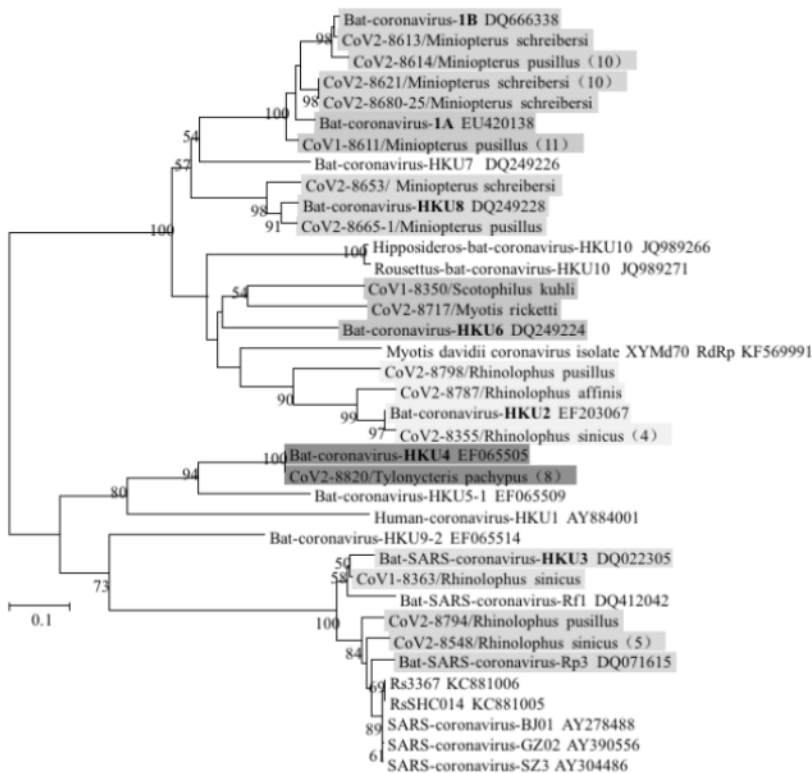
|               |                   |      |     |    |    |
|---------------|-------------------|------|-----|----|----|
| Dec, 2015     | Jingna, Yunnan    | 15   | 15  | 13 | 13 |
|               | Miaoxin, Yunnan   |      | 42  | 28 | 25 |
| Jul, 2015     | Zigong, Sichuan   | 128  | --  | -- | -- |
| Aug, 2015     | Hubei             |      | 332 |    |    |
| Sep, 2015     | Xianning, Hubei   |      | 95  |    |    |
| Aug, 2015     | Jishou, Hunnan    | 204  |     |    |    |
| Aug-Sep, 2015 | Tongren, Guizhou  | 438  |     |    |    |
| Dec, 2015     | Longzhou, Guangxi | 191  |     |    |    |
|               | Total             | 1714 | 677 | 53 | 38 |

We tested 2,256 samples for CoV RNA and 280 tested positive. The total positive rate is 12.4% (Table 5). Diverse alphacoronaviruses related to Bat CoV 1A, 1B, HKU2, HKU6, HKU7, HKU8 and HKU10 were identified; SARS-like coronaviruses were detected in *Rhinolophus* bats in both Yunnan and Guangdong (Fig 1). Novel lineage B betacoronaviruses more distantly related to SARS-CoV than other SL-CoVs were detected in *Vespertilio superans* in Sichuan. HKU4-related coronaviruses were found in *Tynolycteris pachypus* in Guangdong and Guangxi while HKU5-related coronaviruses were found to be highly prevalent in *Vespertilio superans* in Zigong, Sichuan (41 bats out of 128 tested positive).

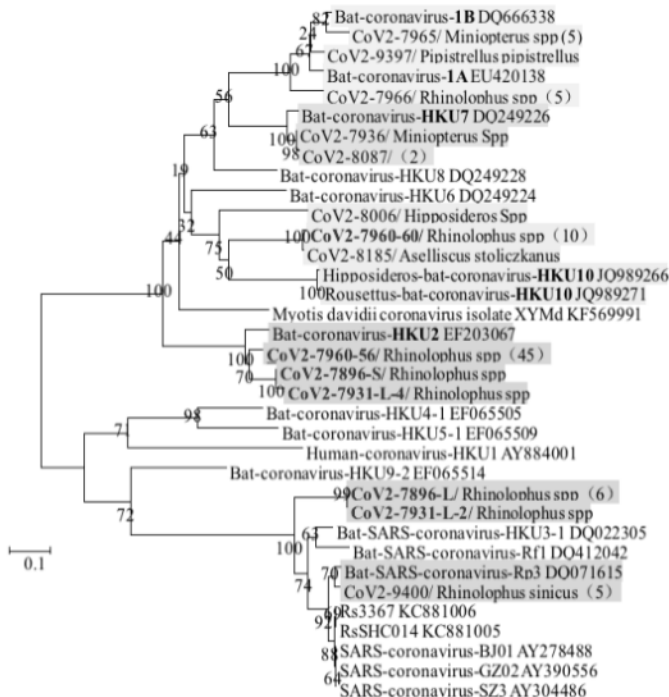
**Table 5 Test result of bat CoV surveillance in 2015 – 12% positive (280/2,256)**

|                              | Yunnan                       | Guangdong | Hubei  | Sichuan | Guangxi | Guizhou | Hunan | Total           |
|------------------------------|------------------------------|-----------|--------|---------|---------|---------|-------|-----------------|
| <b>Bat species</b>           | <b>No.positive/No.tested</b> |           |        |         |         |         |       |                 |
| <i>Rhinolophus spp.</i>      | 47/98                        | 12/103    |        |         |         | 16/225  | 8/63  | 83/489          |
| <i>Hipposideros spp.</i>     | 0/35                         | 0/51      | 26/152 |         |         | 0/131   | 0/91  | 26/460          |
| <i>Ia io</i>                 |                              |           |        |         |         | 0/3     |       | 0/3             |
| <i>Pipistrellus spp.</i>     | 1/1                          | 0/19      |        |         |         | 0/2     | 0/4   | 1/26            |
| <i>Miniopterus spp.</i>      | 6/7                          | 34/83     |        |         |         | 2/6     |       | 42/96           |
| <i>Eonycteris spp.</i>       | 0/3                          |           |        |         |         |         |       | 0/3             |
| <i>Vespertilio superans</i>  |                              |           |        | 41/128  |         |         |       | 41/128          |
| <i>Myotis spp.</i>           |                              | 1/38      |        |         |         | 0/70    | 0/35  | 1/143           |
| <i>Taphozous spp.</i>        | 0/25                         |           |        |         |         | 0/1     |       | 0/26            |
| <i>Tynolcyteris pachypus</i> |                              | 8/25      |        |         | 27/191  |         |       | 35/216          |
| <i>Scotophilus kuhlii</i>    |                              | 1/1       |        |         |         |         |       | 1/1             |
| <i>Eptesicus fuscus</i>      |                              | 0/1       |        |         |         |         |       | 0/1             |
| <i>Tadrida spp.</i>          |                              | 0/5       |        |         |         |         |       | 0/5             |
| <i>Barbastella</i>           |                              |           |        |         |         |         | 0/1   | 0/1             |
| <i>Nyctalus velutiaus</i>    |                              |           |        |         |         |         | 0/10  | 0/10            |
| Fecal samples                | 28/468                       |           | 22/180 |         |         |         |       | 50/648          |
| <b>Sub-total</b>             | 82/637                       | 56/326    | 48/332 | 41/128  | 27/191  | 18/438  | 8/204 | <b>280/2256</b> |

A



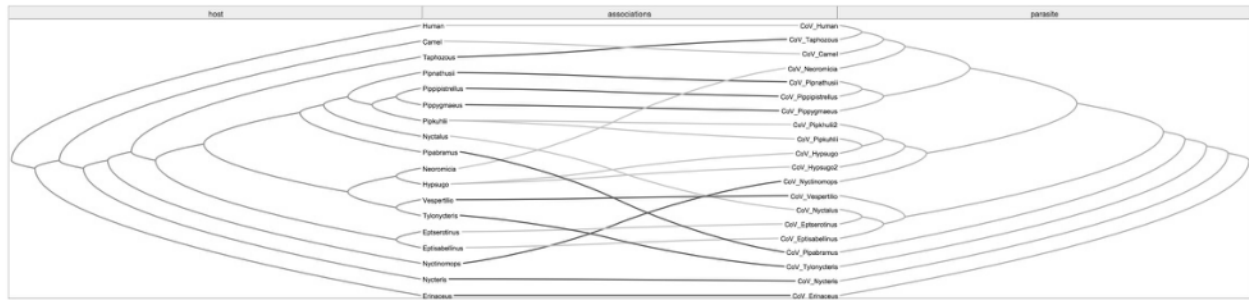
B



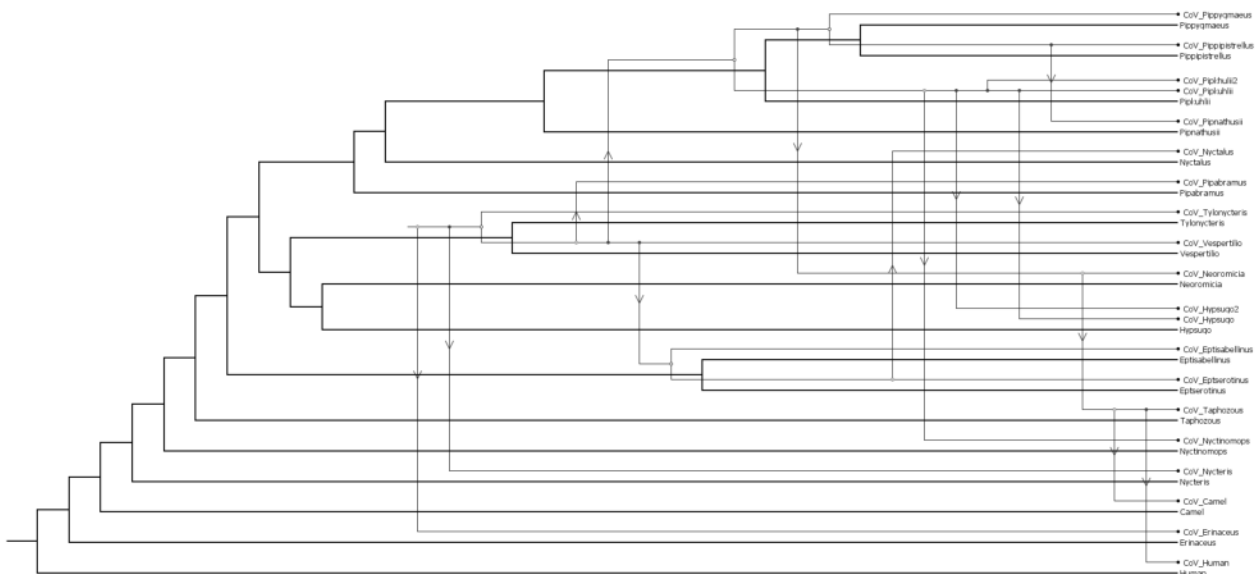
**Fig 1** Phylogenetic analysis of partial RdRp gene of CoV (440-nt partial sequence). CoVs identified in 2015 are named by the sample numbers. Sequence amplified from samples co-infected with two CoV strains are indicated in red. (A) CoVs detected in Guangdong. (B) CoVs detected in Yunnan.

Cophylogenetic analysis of CoV host switching

We completed preliminary cophylogenetic analysis of bat host – CoV sequences using data published in the literature and available on Genbank. Two figures from these analyses are highlighted below (Figs 2 and 3) and these methods are currently being extended using partial RdRp CoV and bat mitochondrial DNA sequences from a large number of bat specimens found CoV positive in Year 2 (Table 5, above).



**Figure 2:** Tanglegram depicting the pattern of infection of bats (and outlier mammalian hosts) by CoVs. The CoV tree was reconstructed from DNA sequences available in GenBank (partial RdRp gene) using Bayesian inference (MrBayes). The topology of host tree was reconstructed using the mammal and bat phylogenies available in Asher & Helgen (2010) and Agnarsson et al. (2011), using methods our group has previously applied to bat parasite cophylogenetic analyses (Lei and Olival 2014). Both ParaFit (ParaFitGlobal = 64957.61, p-value = 0.001) and PACo (m2 = 366.44, p-value = 0.013) provided evidence for significant global congruence between the two topologies, and evidence for coevolution. Lines connecting taxa indicate host-CoV associations. Red lines indicate significant host-CoV associations as indicated by ParaFit ( $p \leq 0.05$ , 999 permutations).



**Figure 3:** Reconstruction of one of 3 potentially optimal solutions of reconciled host-CoV trees recovered from a Jane analysis. Black and blue lines represent the host and CoV trees, respectively. For each solution, the number of co-speciation events inferred by Jane was always significantly greater than expected by chance. Jane inferred 4 co-speciation events (hollow colored circles), 1 duplication (solid

colored circle), 14 host switches (solid colored circle with arrow), 0 loss and 0 failure to diverge.

Our findings demonstrate co-speciation alone is not sufficient to explain the observed co-phylogenetic pattern and several host switches can be specifically identified. This is the case even if a significant global signal of co-speciation has been detected. This work highlights, the need for these types of detailed cophylogenetic analyses to best explain the evolutionary history and host-switching of bat-CoVs.

*References cited for the above analysis:* Agnarsson, I., Zambrana-Torrel, C.M., Flores-Saldana, N.P. & May-Collado, L.J. (2011) A time-calibrated species-level phylogeny of bats (Chiroptera, Mammalia). *PLOS Currents*, 3:RRN1212. Asher, R.J. & Helgen, K.M. (2010) Nomenclature and placental mammal phylogeny. *BMC Evolutionary Biology*, 10, 1-9. Lei BR, Olival KJ (2014) Contrasting Patterns in Mammal–Bacteria Coevolution: *Bartonella* and *Leptospira* in Bats and Rodents. *PLoS Negl Trop Dis* 8(3): e2738.

#### Market Characterization Model Parameterization

Our ongoing observational research and mapping of farms and markets suggests that rapid changes in the market and regulatory environment are changing the nature and location of the wildlife market trade. The nexus of the wildlife trade and the potential hotspots of interspecies viral mixing is now in many cases in animal storage facilities and transport between high-volume customers. To define realistic parameters for intermixing wildlife species in areas of high potential mixing, we have developed a preliminary survey and sampling protocol to assess these values as animals move along the value chain – through these storage facilities - using respondent-driven questionnaires to follow and sample along the wildlife trade network and reveal hidden nodes and sites of intermixing of species.

We have expanded our intermixing modeling framework to incorporate the variations along this value chain, where the diversity, abundance, residence time, and contact rates between species change as animals move through the trade network.

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

In Year 2, we continued surveillance for novel SARS-like CoVs from bats in Yunnan and Guangdong provinces and obtained full genome sequence for 11 CoV isolates. Full genome analysis of these CoV isolates was completed, including phylogenetic and recombination analyses. Importantly, recombination analysis of the full-length SL-CoV genome sequences from a single bat population revealed that frequent recombination events among different SL-CoV strains occur. Several SL-CoVs that are more genetically similar to SARS-CoV (2003) than any previously discovered were also identified from bat populations in Yunnan province. Full genome analysis suggests that an epicenter of SL-CoV occurs in rhinolophid bats and provides more insight into the evolutionary origin of SARS-CoV.

#### Full-length genome sequencing of SL-CoVs identified from a single bat colony

To date, including preliminary data submitted for this R01 that we are now analyzing under the current funding, we have conducted 5-years of surveillance of SL-CoV in a single bat colony in Yunnan Province (from 2011 to 2015), leading to the discovery of diverse novel SL-CoVs. Based on genotyping of these SL-CoVs by the region corresponding to the receptor-binding domain (RBD) of SARS-CoVs, 11 isolates were selected and full-length genome sequencing was performed in Year 2.

These SL-CoVs, including four others isolated previously from this colony, Rs3367, RsSHC014, WIV1 and WIV16, are highly diversified in the S gene, but share similar sequence identity to SARS-CoV in ORF1ab (Fig 4). Genomic phylogenetic analysis showed that the SL-CoVs detected in this colony are more closely

related to SARS-CoVs from other geographic regions, especially three isolates, WIV16, Rs4874 and Rs4231 (Fig 5). Notably, among the 15 SL-CoVs, two isolates, Rs4084 from *Rhinolophus sinicus* and Rf4092 from *Rhinolophus ferrumequinum*, are highly similar to SARS-CoV in the ORF8 region (Fig 5). Rf4092 possessed a single ORF8 of the same length (369bp) as that in civet SARS-CoV SZ3, and the sequence showed only 10 nucleotide substitution (Fig 6). The ORF8 sequence of Rs4084 is highly similar to that of Rf4092, however in the region corresponding to the 29-bp deletion acquired in human SARS CoVs (e.g Tor2), a shorter deletion of only 5-bp is present, resulting in two overlapping ORF8s, ORF8a and ORF8b. The position of start codon and stop codon of the two ORFs were consistent with those in human strains (Fig 6).

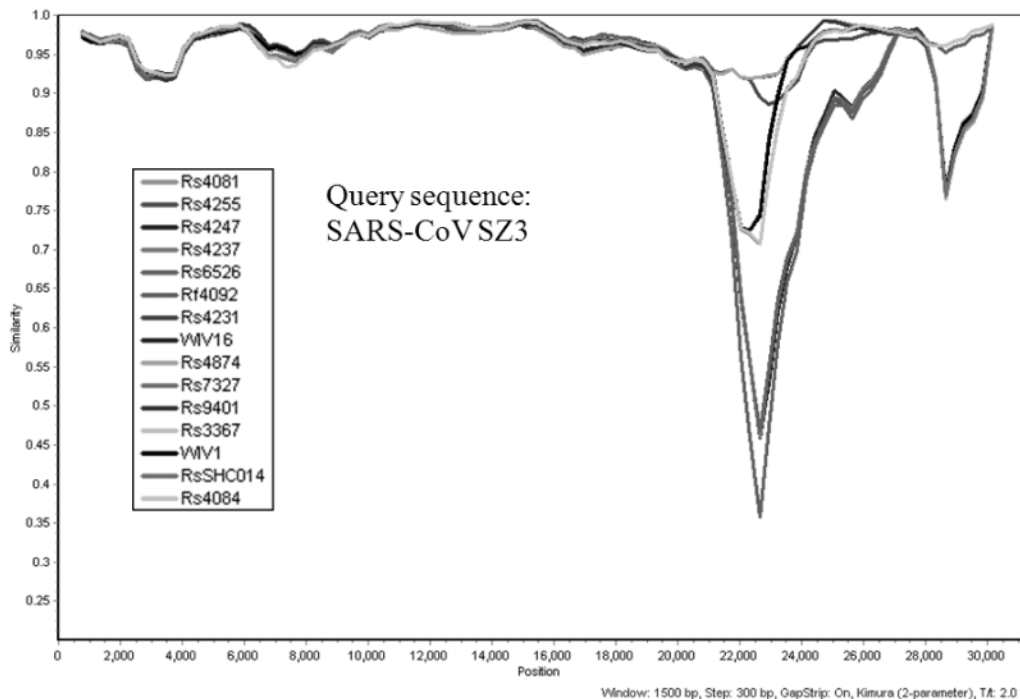


Fig 4. Simplot analysis of the 15 SL-CoVs identified from a single bat colony in Yunnan. SARS-CoV SZ3 is used as query sequence.





Fig 5. Phylogenetic analysis of full-length genome sequences of SL-CoVs and SARS-CoVs. Isolates identified in the single investigated bat colony in Yunnan in bold.

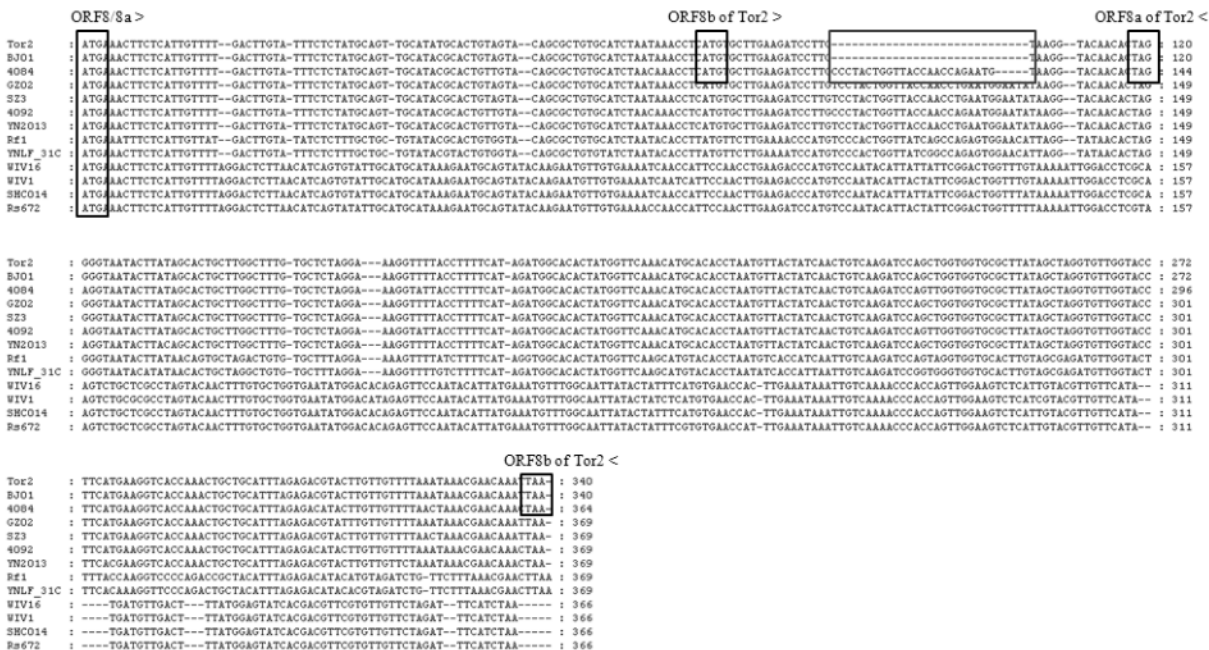
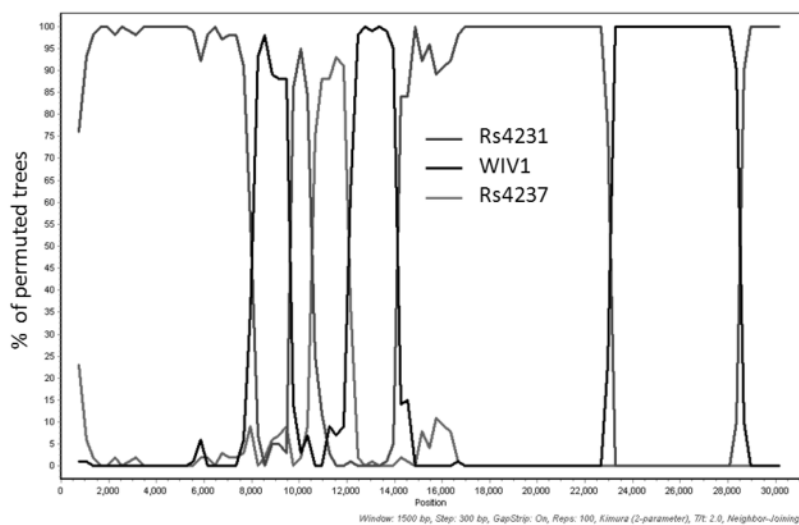


Fig 6. Alignment of ORF8 nucleotide sequences of SARS-CoV and bat SL-CoVs. The red box indicates the 29-nt deletion present in SARS-CoV of middle and late phase.

Recombination analysis of the full-length genome sequences reveals frequent recombination events among different SL-CoV strains circulating in this bat population. For example, WIV16 appears to be a recombination product of WIV1 and Rs4231. An important breakpoint is identified between the N-terminal domain (NTD) and RBD region in the S gene (Fig 7A). Consequently, WIV16 is identical to Rs4231 and WIV1 in NTD and RBD of the spike protein, respectively, and is highly homologous to SARS-CoV in both NTD and RBD. This makes it the SL-CoV most closely related to the direct progenitor of SARS-CoV discovered to date. Moreover, evidence is found to support the hypothesis that the direct progenitor of SARS-CoV was generated from recombination of WIV16 with Rf4092 at the site near ORF8. This work, which identifies diverse SL-CoVs highly homologous to SARS-CoV in different regions of the genome, suggests that rhinolophid bats are an evolutionary epicenter of SL-CoV and offers more insights into the evolutionary origin of SARS-CoV.

A.



B.

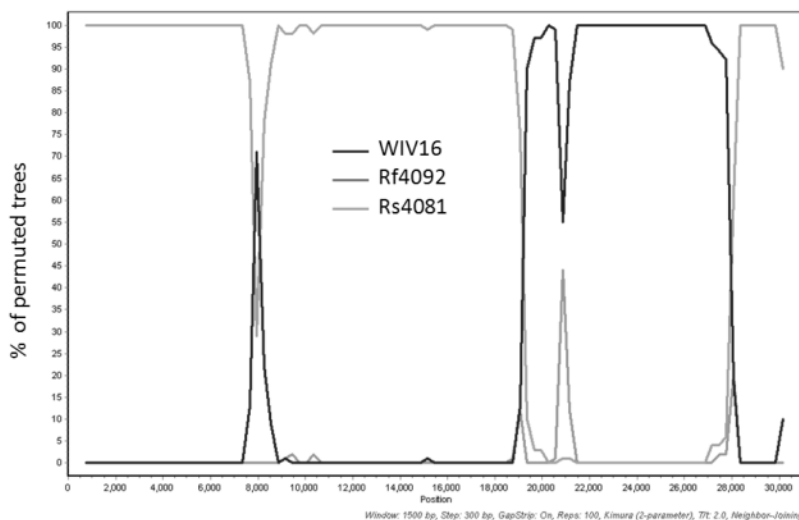


Fig 7 Bootscan analysis of full-length genome sequences of SL-CoVs. (A) WIV16 is used as query sequence. (B) SARS-CoV SZ3 is used as the query sequence. (Kimura model, window size, 1500bp, step size, 300bp)

Additional Year 2 items for Specific Aim 3:

- The infectious clone of WIV1 was successfully constructed using reverse genetic methods;
- Two chimeric bat SARS-like coronavirus strains were constructed by replacing the S gene in the backbone of WIV1;
- Permission to import mice with human ACE2 to China was obtained, so as to conduct the experimental infections proposed in our R01 specific aims.

Specific Goals Not Met.

- 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 (This will be conducted in year 3);
- Animal infection experiments of SARS-like coronaviruses were not done, because of the unavailability of mice with human ACE2 in Year 2. We now have secured these mice and will begin this work in year 3.
- Sampling of bat and other mammalian species in markets to screen for CoVs. We will begin this work in year 3.

**Section C: Accomplishments: Publications**PUBLISHED

Xing-Yi Ge, Ning Wang, Wei Zhang, Ben Hu, Bei Li, Yun-Zhi Zhang, Ji-Hua Zhou, Chu-Ming Luo, Xing-Lou Yang, Li-Jun Wu, Bo Wang, Yun Zhang, Zong-Xiao Li, and Zheng-Li Shi. Coexistence of multiple coronaviruses in several bat colonies in an abandoned mineshaft. *Virologica Sinica* 31, 31–40 (2016).

Mei-Niang Wang, Wei Zhang, Yu-Tao Gao, Ben Hu, Xing-Yi Ge, Xing-Lou Yang, Yun-Zhi Zhang, Zheng-Li Shi. Longitudinal surveillance of SARS-like coronaviruses in bats by quantitative real-time PCR, *Virologica Sinica* 31(1): 78-80 (2016).

Cristin C. W. Young and Kevin J. Olival. Optimizing Viral Discovery in Bats. *PLoS ONE* 11(2) (2016).

Kevin J. Olival. To Cull, or Not To Cull, Bat is the Question. *Ecohealth* 13, 6–8 (2015).

Xing-Lou Yang, Ben Hu, Bo Wang, Mei-Niang Wang, Qian Zhang, Wei Zhang, Li-Jun Wu, Xing-Yi Ge, Yun-Zhi Zhang, Peter Daszak, Lin-Fa Wang, Zheng-Li Shi. Isolation and characterization of a novel bat coronavirus closely related to the direct progenitor of Severe Acute Respiratory Syndrome Coronavirus, *Journal of Virology* 90(6): 3253-6 (2015).

Ben Hu, Xingyi Ge, Lin-Fa Wang, Zhengli Shi. Bat origin of human coronaviruses. *Virology Journal* 12 (1): 221 (2015)

ACCEPTED, IN PRESS

Lei-Ping Zeng, Yu-Tao Gao, Xing-Yi Ge, Qian Zhang, Cheng Peng, Xinglou Yang, Bin Tan, Jing Chen, Aleksei Chmura, Peter Daszak, and Zheng-Li Shi. Bat SARS-like coronavirus WIV1 encodes an extra accessory protein ORFX involving in modulation of host immune response. *Journal of Virology* (in press, 2016)

**B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?**

1R01AI110964 Year 2 Report

PI: Daszak, Peter

**B.4 What opportunities for training and professional development has the project provided?**

We presented our project to graduate students, laboratory personnel, directors, and doctors from three Hospitals in Yunnan Province: Yunnan Provincial Institute of Endemic Diseases Control & Prevention (YNCDC); Dali Provincial Hospital; and The Third People's Hospital of Kunming. Select doctors at YNCDC (1) and Dali Provincial Hospital (3) were trained in the passive Hospital surveillance project protocols.

We trained graduate students from Dali School of Public Health (1) and the Wuhan University School of Public Health (3) in qualitative behavioral risk data collection methodologies and data collection technologies, survey data collection and analysis. These were also enrolled in and passed the Human Subjects Research Course provided by the Collaborative Institutional Training Initiative (CITI Program) at the University of Miami (<http://citiprogram.org>). The CITI Program is a leading provider of research education content with web based training materials serving millions of learners at academic institutions, government agencies, and commercial organizations in the U.S. and around the world.

## C. PRODUCTS

## C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

## Publications Reported for this Reporting Period

| Public Access Compliance | Citation                                                                                                                                                                                                                                                                                                                                              |
|--------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Complete                 | Yang XL, Hu B, Wang B, Wang MN, Zhang Q, Zhang W, Wu LJ, Ge XY, Zhang YZ, Daszak P, Wang LF, Shi ZL. Isolation and Characterization of a Novel Bat Coronavirus Closely Related to the Direct Progenitor of Severe Acute Respiratory Syndrome Coronavirus. J Virol. 2015 Dec 30;90(6):3253-6. PubMed PMID: 26719272; PubMed Central PMCID: PMC4810638. |
| Complete                 | Olival KJ. To Cull, or Not To Cull, Bat is the Question. Ecohealth. 2016 Mar;13(1):6-8. PubMed PMID: 26631385; PubMed Central PMCID: PMC4833651.                                                                                                                                                                                                      |

## Non-compliant Publications Previously Reported for this Project

| Public Access Compliance | Citation |
|--------------------------|----------|
| Non-Compliant            | (b)(4)   |

## C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

## C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

## C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

## C.5 OTHER PRODUCTS AND RESOURCE SHARING

## C.5.a Other products

NOTHING TO REPORT

## C.5.b Resource sharing

NOTHING TO REPORT

## D. PARTICIPANTS

## D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

| Commons ID | S/K  | Name                   | SSN    | DOB               | Degree(s)                      | Role                                                                 | Cal    | Aca   | Sum | Foreign Org | Country | SS |
|------------|------|------------------------|--------|-------------------|--------------------------------|----------------------------------------------------------------------|--------|-------|-----|-------------|---------|----|
| (b)(6)     | Y    | DASZAK, PETER          | (b)(6) |                   | BS,PHD                         | PD/PI                                                                | (b)(6) |       |     |             |         | NA |
|            | N    | HOSSEINI, PARVIEZ RANA |        | BS,PHD            | Co-Investigator                |                                                                      |        |       | NA  |             |         |    |
|            | Y    | Ross, Noam Martin      |        | PhD               | Co-Investigator                |                                                                      |        |       | NA  |             |         |    |
|            | N    | OLIVAL, KEVIN J        |        | PHD               | Co-Investigator                |                                                                      |        |       | NA  |             |         |    |
|            | N    | KE, CHANGWEN           |        | PHD               | Co-Investigator                | Center for Disease Control and Prevention of Guangdong Province      |        | CHINA | NA  |             |         |    |
|            | N    | ZHANG, SHUYI           |        | PHD               | Co-Investigator                | East China Normal University                                         |        | CHINA | NA  |             |         |    |
|            | N    | ZHANG, YUNZHI          |        | PHD               | Co-Investigator                | Yunnan Provincial Institute of Endemic Diseases Control & Prevention |        | CHINA | NA  |             |         |    |
|            | N    | ZHU, GUANGJIAN         |        | PHD               | Co-Investigator                | East China Normal University                                         |        | CHINA | NA  |             |         |    |
|            | N    | GE, XINGYI             |        | PHD               | Co-Investigator                | Wuhan Institute of Virology                                          |        | CHINA | NA  |             |         |    |
|            | N    | EPSTEIN, JONATHAN H    |        | MPH, DVM, BA, PHD | Co-Investigator                |                                                                      |        |       | NA  |             |         |    |
|            | N    | CHMURA, ALEKSEI A      |        | BS                | Non-Student Research Assistant |                                                                      |        |       | NA  |             |         |    |
| N          | SHI, | PhD                    | Co-    | Wuhan             | CHINA                          | NA                                                                   |        |       |     |             |         |    |

|        |         |        |              |        |                       |  |  |
|--------|---------|--------|--------------|--------|-----------------------|--|--|
| (b)(6) | ZHENGLI | (b)(6) | Investigator | (b)(6) | Institute of Virology |  |  |
|--------|---------|--------|--------------|--------|-----------------------|--|--|

|                                                                                                                                                                             |                                                                                                                                                                        |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Glossary of acronyms:</b><br>S/K - Senior/Key<br>DOB - Date of Birth<br>Cal - Person Months (Calendar)<br>Aca - Person Months (Academic)<br>Sum - Person Months (Summer) | Foreign Org - Foreign Organization Affiliation<br>SS - Supplement Support<br>RE - Reentry Supplement<br>DI - Diversity Supplement<br>OT - Other<br>NA - Not Applicable |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**D.2 PERSONNEL UPDATES**

**D.2.a Level of Effort**

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

**D.2.b New Senior/Key Personnel**

Are there, or will there be, new senior/key personnel?

Yes

File uploaded: Noam Ross CV 2016.pdf

**D.2.c Changes in Other Support**

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

**D.2.d New Other Significant Contributors**

Are there, or will there be, new other significant contributors?

No

**D.2.e Multi-PI (MPI) Leadership Plan**

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

Page 245 of 260

Withheld pursuant to exemption

(b)(6)

of the Freedom of Information and Privacy Act



Page 246 of 260

Withheld pursuant to exemption

(b)(6)

of the Freedom of Information and Privacy Act

Page 247 of 260

Withheld pursuant to exemption

(b)(6)

of the Freedom of Information and Privacy Act

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

| Dollar Amount | Country |
|---------------|---------|
| 211699        | CHINA   |

## F. CHANGES

|                                                                                                                                                    |
|----------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE</b><br>Not Applicable                                                                            |
| <b>F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM</b><br>NOTHING TO REPORT                                    |
| <b>F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS</b><br><b>F.3.a Human Subjects</b><br>No Change |
| <b>F.3.b Vertebrate Animals</b><br>No Change                                                                                                       |
| <b>F.3.c Biohazards</b><br>No Change                                                                                                               |
| <b>F.3.d Select Agents</b><br>No Change                                                                                                            |

## G. SPECIAL REPORTING REQUIREMENTS

## G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

## G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

## G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

## G.4 HUMAN SUBJECTS

## G.4.a Does the project involve human subjects?

Yes

## Is the research exempt from Federal regulations?

No

## Does this project involve a clinical trial?

No

## G.4.b Inclusion Enrollment Data

Report Attached: Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

## G.4.c ClinicalTrials.gov

## Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

No

## G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

## Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

No

## G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

## Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

## G.7 VERTEBRATE ANIMALS

## Does this project involve vertebrate animals?

Yes

## G.8 PROJECT/PERFORMANCE SITES

| Organization Name: | DUNS | Congressional | Address |
|--------------------|------|---------------|---------|
|--------------------|------|---------------|---------|

|                                   |           | District |                                                                    |
|-----------------------------------|-----------|----------|--------------------------------------------------------------------|
| Primary: EcoHealth Alliance, Inc. | 077090066 | NY-010   | 460 West 34th Street<br>17th Floor<br>New York NY 100012317        |
| Wuhan Institute of Virology       | 529027474 |          | Xiao Hong Shan, No. 44<br>Wuchang District<br>Wuhan                |
| East China Normal University      | 420945495 |          | 3663 Zhongshan Beilu<br>Shanghai                                   |
| ECOHEALTH ALLIANCE                | 077090066 |          | ECOHEALTH ALLIANCE, INC.<br>460 W 34TH ST<br>NEW YORK NY 100012320 |
| EcoHealth Alliance, Inc.          | 077090066 | NY-010   | 460 West 34th Street<br>17th Floor<br>New York NY 100012317        |
| Wuhan Institute of Virology       | 529027474 |          | Xiao Hong Shan, No. 44<br>Wuchang District<br>Wuhan                |
| East China Normal University      | 420945495 |          | 3663 Zhongshan Beilu<br>Shanghai                                   |

**G.9 FOREIGN COMPONENT**

**Organization Name:** Wuhan Institute of Virology

**Country:** CHINA

**Description of Foreign Component:**

Principal Laboratory for all Research in China as per section G8 (above) and detailed in our Specific Aims

**Organization Name:** East China Normal University

**Country:** CHINA

**Description of Foreign Component:**

Principal Coordinating Team for all project field work as per section G8 (above) and detailed in our Specific Aims

**G.10 ESTIMATED UNOBLIGATED BALANCE**

**G.10.a** Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

**G.11 PROGRAM INCOME**

Is program income anticipated during the next budget period?

No

**G.12 F&A COSTS**

Is there a change in performance sites that will affect F&A costs?

No

### Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 166195

Using an Existing Dataset or Resource: No

Delayed Onset Study?: No

Clinical Trial: No

Enrollment Location: Foreign

NIH Defined Phase III Clinical Trial: No

Study Title: Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

### Planned Enrollment

**Planned Enrollment Total:** 2,460

**NOTE:** Planned enrollment data exists in the previous format; the PD/PI did not enter the planned enrollment information in the modified format and was not required to do so. Only the total can be provided.

### Cumulative Enrollment

| Racial Categories                         | Ethnic Categories      |            |                      |                    |          |                      |                                |          |                      | Total      |
|-------------------------------------------|------------------------|------------|----------------------|--------------------|----------|----------------------|--------------------------------|----------|----------------------|------------|
|                                           | Not Hispanic or Latino |            |                      | Hispanic or Latino |          |                      | Unknown/Not Reported Ethnicity |          |                      |            |
|                                           | Female                 | Male       | Unknown/Not Reported | Female             | Male     | Unknown/Not Reported | Female                         | Male     | Unknown/Not Reported |            |
| American Indian/Alaska Native             | 0                      | 0          | 0                    | 0                  | 0        | 0                    | 0                              | 0        | 0                    | 0          |
| Asian                                     | 157                    | 108        | 0                    | 0                  | 0        | 0                    | 0                              | 0        | 0                    | 265        |
| Native Hawaiian or Other Pacific Islander | 0                      | 0          | 0                    | 0                  | 0        | 0                    | 0                              | 0        | 0                    | 0          |
| Black or African American                 | 0                      | 0          | 0                    | 0                  | 0        | 0                    | 0                              | 0        | 0                    | 0          |
| White                                     | 0                      | 0          | 0                    | 0                  | 0        | 0                    | 0                              | 0        | 0                    | 0          |
| More than One Race                        | 0                      | 0          | 0                    | 0                  | 0        | 0                    | 0                              | 0        | 0                    | 0          |
| Unknown or Not Reported                   | 0                      | 0          | 0                    | 0                  | 0        | 0                    | 0                              | 0        | 0                    | 0          |
| <b>Total</b>                              | <b>157</b>             | <b>108</b> | <b>0</b>             | <b>0</b>           | <b>0</b> | <b>0</b>             | <b>0</b>                       | <b>0</b> | <b>0</b>             | <b>265</b> |

**From:** Aleksei MacDurian  
**Sent:** Fri, 13 May 2016 21:57:36 +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,

Dr. Daszak submitted his report yesterday.

We received a warning that one of the publications (Brierley et al.) listed from the past year is non-compliant. We have been in touch with NCBI about removing the non-compliant reference as we are not able to remove it via Dr. Daszak's account. As of this week, Dr. Daszak's My NCBI bibliography is correct, but it appears that the eRA Commons form has not yet populated or updated?

Please let me know any time (b)(6) if there are any questions or additional details necessary.

Many thanks!

**Aleksei Chmura**  
*Senior Coordinator of Operations*  
*Authorized Organizational Representative*

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

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

[www.ecohealthalliance.org](http://www.ecohealthalliance.org)

Visit our blog: [www.ecohealthalliance.org/blog](http://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.*

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>

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 original 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. National Institute of Allergy and Infectious Diseases shall not accept liability for any statements made that are sender's own and not expressly made on behalf of the NIAID by one of its representatives.

**From:** Umerah, Nina  
**Sent:** Wed, 20 Apr 2016 17:28:45 +0000  
**To:** Cockrell, Adam; Stemmy, Erik (NIH/NIAID) [E]  
**Cc:** Baric, Ralph; Leyva-Grado, Victor; Sims, Amy C  
**Subject:** RE: AMC call for Monday 04-25-16

Hi Adam,

That is the correct information for the conference call.

Nina

**Nina Umerah**

(b)(6)

---

**From:** Cockrell, Adam [mailto:(b)(6)]  
**Sent:** Tuesday, April 19, 2016 3:35 PM  
**To:** Stemmy, Erik (NIH/NIAID) [E]  
**Cc:** Baric, Ralph S; Umerah, Nina; Leyva-Grado, Victor; Sims, Amy C  
**Subject:** RE: AMC call for Monday 04-25-16

Hi Erik,

Will do. Is this the correct call in number/passcode?

(b)(6) The participant passcode is (b)(6)

Thanks,  
Adam

---

**From:** Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]  
**Sent:** Tuesday, April 19, 2016 3:25 PM  
**To:** Cockrell, Adam (b)(6)  
**Cc:** Baric, Ralph S (b)(6); Umerah, Nina (b)(6); Leyva-Grado, Victor (b)(6)  
**Subject:** RE: AMC call for Monday 04-25-16

Hi Adam,

Yes, We've still got the call scheduled for Monday. It would be great if you could provide the update.

Thanks!  
Erik

Erik J. Stemmy, Ph.D.  
Program Officer  
Respiratory Diseases Branch

Division of Microbiology and Infectious Diseases NIAID/NIH/HHS

5601 Fishers Lane, (b)(6)

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.

\*\*\*\*\*

NOTE: This material is intended for the individual or entity to which it is addressed. It may contain privileged, confidential information that is protected from disclosure under applicable laws. If you are not the addressee, or a person authorized to deliver the document to the addressee, please note that you are strictly prohibited from reviewing, copying, disclosing, disseminating or distributing this material 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:** Cockrell, Adam [mailto:(b)(6)]  
**Sent:** Tuesday, April 19, 2016 2:54 PM  
**To:** Stemmy, Erik (NIH/NIAID) [E] (b)(6)  
**Cc:** Baric, Ralph (b)(6)  
**Subject:** AMC call for Monday 04-25-16

Hi Erik,

Hope all is well.

Checking to see if the AMC meeting is still planned for Monday 04-25-16 at 11am?

Ralph is out of the country for the AMC call on Monday, and asked if I could provide the update for the call.

Thanks,

Adam Cockrell  
Post-Doctoral Fellow  
Department of Epidemiology  
University of North Carolina at Chapel Hill  
Chapel Hill, NC, 27599  
Phone: (b)(6)

**From:** Umerah, Nina  
**Sent:** Tue, 5 Apr 2016 17:43:26 +0000  
**To:** Stemmy, Erik (NIH/NIAID) [E]; Leyva-Grado, Victor  
**Cc:** 'Amy Sims'  
**Subject:** RE: NCE for A57?

Hi Erik,

We are requesting an extension through May 31<sup>st</sup>, 2016. I'm going to call my grants office now and find out what the delay is.

Thanks for following up,  
Nina

**Nina Umerah**

(b)(6)

---

**From:** Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]  
**Sent:** Tuesday, April 05, 2016 1:40 PM  
**To:** Umerah, Nina; Leyva-Grado, Victor  
**Cc:** 'Amy Sims'  
**Subject:** RE: NCE for A57?

Hi Nina,

I just wanted to check in on the NCE. Doesn't look like OA has received it yet. Can you let me know what the status is? We are getting close to the end of the performance period, and I need to process the Modification. If you let me know the new end date you're requesting I can get the paperwork underway while we wait to receive the NCE request officially.

Thanks!  
Erik

---

**From:** Umerah, Nina [mailto:(b)(6)]  
**Sent:** Tuesday, March 29, 2016 10:36 AM  
**To:** Stemmy, Erik (NIH/NIAID) [E] (b)(6) Leyva-Grado, Victor (b)(6)  
(b)(6)  
**Cc:** 'Amy Sims' (b)(6)  
**Subject:** RE: NCE for A57?

Hi Erik,

For some reason the request was sent to our finance office. It was forwarded it to me last week so I will push my grants office to send it to you ASAP.

Thanks,  
Nina

**Nina Umerah**

Manager, Grants and Contracts  
Department of Microbiology  
Icahn School of Medicine at Mount Sinai  
One Gustave L. Levy Place, Box 1124  
New York, NY 10029  
Tel.: (b)(6)  
Fax: 212-534-1684  
Email: (b)(6)

---

**From:** Stemmy, Erik (NIH/NIAID) [E] [mailto:(b)(6)]  
**Sent:** Tuesday, March 29, 2016 9:26 AM  
**To:** Leyva-Grado, Victor; Umerah, Nina  
**Subject:** NCE for A57?

Hi Victor and Nina,  
Just checking in on the NCE. Have you had a chance to submit it? Ralph mentioned they submitted it to you guys about two weeks ago.

Erik

Erik J. Stemmy, Ph.D.  
Program Officer  
Respiratory Diseases Branch  
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS  
5601 Fishers Lane, (b)(6)  
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.

\*\*\*\*\*

NOTE: This material is intended for the individual or entity to which it is addressed. It may contain privileged, confidential information that is protected from disclosure under applicable laws. If you are not the addressee, or a person authorized to deliver the document to the addressee, please note that you are strictly prohibited from reviewing, copying, disclosing, disseminating or distributing this material 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:** Yao, Alison (NIH/NIAID) [E]  
**Sent:** Tue, 5 Apr 2016 13:25:12 -0400  
**To:** Baric, Toni C; Baric, Ralph; Beisel, Christopher (NIH/NIAID) [E]; Damania, Blossom A; Spiro, David (NIH/FIC) [E]; Stemmy, Erik (NIH/NIAID) [E]; Graham, Rachel; Mathur, Punam (NIH/NIAID) [E]; Sims, Amy C; Dugan, Vivien (NIH/NIAID) [E]  
**Cc:** Hoffmann, Megan (NIH/NIAID) [C]  
**Subject:** RE: Cancel UNC call today

Thank you. - Alison

---

**From:** Baric, Toni C [mailto:(b)(6)]  
**Sent:** Tuesday, April 05, 2016 12:29 PM  
**To:** Yao, Alison (NIH/NIAID) [E] (b)(6) Baric, Ralph (b)(6) Beisel, Christopher (NIH/NIAID) [E] (b)(6) Damania, Blossom A (b)(6) Spiro, David (NIH/NIAID) [E] (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) Graham, Rachel (b)(6) Mathur, Punam (NIH/NIAID) [E] (b)(6) Sims, Amy C (b)(6) Dugan, Vivien (NIH/NIAID) [E] (b)(6)  
**Cc:** Hoffmann, Megan (NIH/NIAID) [C] (b)(6)  
**Subject:** RE: Cancel UNC call today

Hi Alison,  
Let's reschedule for 2-3pm on Thursday 4/7. The calling numbers are:  
Phone: (b)(6)  
Passcode: (b)(6)

Thank you so much for your flexibility with rescheduling.  
Best regards,  
Toni

---

**From:** Yao, Alison (NIH/NIAID) [E] [mailto:(b)(6)]  
**Sent:** Tuesday, April 05, 2016 12:21 PM  
**To:** Baric, Toni C; Baric, Ralph S; Beisel, Christopher (NIH/NIAID) [E]; Damania, Blossom A; Spiro, David (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Graham, Rachel; Mathur, Punam (NIH/NIAID) [E]; Sims, Amy C; Dugan, Vivien (NIH/NIAID) [E]  
**Cc:** Hoffmann, Megan (NIH/NIAID) [C]  
**Subject:** RE: Cancel UNC call today

Hi Toni,

Vivien and I are available:  
4/7 1-3 (preferred)  
4/6 before noon

For your information, Punam is currently out of the office due to a family emergency. Megan Hoffmann, who is a program analyst in our office and copied here, will help out in Punam's absence.

Thank you,  
Alison

---

**From:** Baric, Toni C [mailto:(b)(6)]  
**Sent:** Tuesday, April 05, 2016 9:04 AM  
**To:** Baric, Ralph (b)(6); Beisel, Christopher (NIH/NIAID) [E] (b)(6)  
Damania, Blossom A (b)(6); Spiro, David (NIH/NIAID) [E]  
(b)(6); Stemmy, Erik (NIH/NIAID) [E] (b)(6); Graham, Rachel  
(b)(6); Mathur, Punam (NIH/NIAID) [E] (b)(6); Sims, Amy C  
(b)(6); Dugan, Vivien (NIH/NIAID) [E] (b)(6); Yao, Alison  
(NIH/NIAID) [E] (b)(6)  
**Subject:** Cancel UNC call today

Hi All,  
Ralph is out of town today and we will need to reschedule our monthly call. Alternative times this week are:  
4/6 before noon  
4/7 12-3  
4/8 after 11 am.

Do any of these times fit into your schedule?

Sorry for the late notice.  
Best regards,

## Toni Baric

Department of Microbiology and Immunology  
9025 Burnett Womack  
CB# 7292  
Chapel Hill, NC 27599-7292  
Office: (b)(6)  
(b)(6)