



Dear Drs. Greer and Stemmy,

June 8, 2016

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

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

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

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

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

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

**Local conservation.
Global health.**

EcoHealth Alliance
460 West 34th Street, 17th Floor
New York, NY 10001-2320
212.380.4460

EcoHealthAlliance.org

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

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

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

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

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

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

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

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

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

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

Yours sincerely,

A handwritten signature in blue ink, appearing to read 'P. Daszak', with a horizontal line underneath.

Dr. Peter Daszak

PI
President and Chief Scientist
EcoHealth Alliance

Tel: +1-212-4473

e-mail: daszak@ecohealthalliance.org