Dear Dr. Whitley, Dr. Baric, and Dr. Denison:

Thank you for your email of March 29, 2016, describing your proposed plans to generate novel viruses that may be considered as a gain of function experiments as outlined in the October 17, 2014 White House announcement of a U.S. Government-wide pause on certain gain-of-function experiments (http://www.whitehouse.gov/blog/2014/10/17/doing-diligence-assess-risks-and-benefits-life-sciences-gain-function-research). The generation and use of the proposed viruses was not included in the original award. The pause pertains to gain-of-function research projects that may be reasonably anticipated to confer attributes to influenza, SARS, or MERS viruses such that the resulting virus has enhanced pathogenicity and/or transmissibility in mammals via the respiratory route.

NIAID reviewed the information provided in your email as it relates to the U19AI109680 award, PI: Richard Whitley, Antiviral Drug Discovery and Development Center, and has made the following assessments regarding the proposed addition of new research components to Project 2 - Inhibitors of Coronavirus Fidelity and Cap Methylation as Broadly Applicable Therapeutics (PIs:Dr. Mark Denison and Dr. Ralph Baric).

A. Two proposed Recombinant Strains: MERS-like CoV HKU5 Mav-nsp-12 and MERS-CoV MA-S (MERS-15S)

The U19AI109680 cooperative agreement supports the Antiviral Drug Discovery and Development Center. Project 2 is focused on the identification and development of inhibitors of coronavirus high fidelity replication. A candidate therapeutic has been identified – GS-5734 - which has been shown to have activity against SARS-CoV and MERS-CoV *in vitro* and was identified as a good candidate for *in vivo* testing. Initial experiments revealed that the GS-5734 compound (and related nucleoside analogs) are very sensitive to esterase 1 activity and require testing in esterase 1 -/- deficient mice.

GS-5734 has been shown to inhibit the SARS-CoV nsp12 gene product, RNA dependent RNA polymerase (RdRp), and protect mice, when using a mouse-adapted SARS-CoV virus (SARS-CoV MA15) to infect esterase 1 -/- mice. The investigators would like to assess *in vivo* activity of GS-5734 against the MERS-CoV nsp-12 gene RdRp, however the esterase 1 -/- mouse strain does not express a permissive dipeptidyl peptidase receptor for MERS-CoV docking entry, thus they are not able to infect esterase 1 -/- mice with the existing mouse adapted MERS-CoV strain. The investigators have the option to generate a genetically appropriate mouse strain by backcrossing the esterase 1 -/- genotype into the existing 288/330 DPP4 mouse strain, which is susceptible to the existing mouse adapted MERS-CoV virus. The investigators note that this approach is estimated to take one year to obtain sufficient mice for testing. To address the possible lengthy delay, the investigators are proposing to generate two possible recombinant strains that could allow them to test the *in vivo* activity of GS-5734 against the MERS-CoV virus in a mouse model within a month or so if these experiments are not subject to the pause on gain-of-function research.

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1. New recombinant MERS-like CoV HKU5 Mav-nsp-12 strain

The investigators are proposing to include new research to modify the existing recombinant MERS-like KHU5-S MAv strain, which is a mouse-adapted MERS-like virus that has the capability to infect mouse cells. They are proposing to replace the nsp12 RdRp gene in MERS-like KHU5-S MAv strain with the MERS-CoV nsp 12 RdRp gene. A challenge to this approach is the prospect of non-viable viruses for two reasons:

1) Substitution of nsp12 involves swapping of a ribosomal frameshift RNA pseudoknot structure in the 5'end of the region encoding nsp12in increases the likelihood of generating detrimental or lethal alterations in RNA structure and an effect of the translation of ORF 1a/b
2) Other studies with coronavirus replicase proteins have demonstrated that substitution of outside a closely related genogroup has not been possible.

NIAID assessment: Given that other studies have demonstrated that this genetic modification has failed to generate viable viruses, this research is not reasonably anticipated to confer attributes to the SARS-CoV virus such that the virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route. Therefore, the experiments are not subject to the pause on gain-of-function research

Commented [BM1]: Not sure about this

However, given the potential major scientific weaknesses and likelihood of failure, NIAID does not support the performance of this proposed new research under the cooperative agreement research program.

Commented [BM2]: Just my thoughts at this point. I will have to get my section's input on this

2. New recombinant MERS-CoV MA-S (MERS-15S) strain

The investigators are proposing to include new research to modify the existing mouse-adapted strain of MERS-CoV (MERS-15). They are proposing to replace the MERS-CoV S (spike) gene with the SARS-CoV S glycoprotein to generate a new MERS-15S strain that will be capable of infecting esterase 1 -/- mice.

NIAID assessment: This research is reasonably anticipated to confer attributes to the MERS-15 virus such that the resulting virus has enhanced pathogenicity and/or transmissibility in mammals via the respiratory route. At a minimum, it will enable the MERS-15 virus to infect a new genetic background in mice and therefore, the experiments are subject to the pause on gain-of-function research.

Commented [BM([3]: Need to get Erik's thoughts

While it will involve a longer period of time, it is fortunate that an alternative approach is available to enable the investigators to test the *in vivo* activity of GS-5734 against the MERS-CoV virus in a mouse model via generation of a 288/330 DPP4 esterase 1 -/- mouse strain.

B. Proposed Development of two Mouse Adapted SARS-like Prepandemic Viruses

The U19AI109680 cooperative agreement supports the Antiviral Drug Discovery and Development Center. Project 2 is focused on the identification and development of inhibitors of coronavirus high fidelity replication. The investigators are focusing on two SARS-like strains which circulate in bats (WIV1 and SHC014) and are capable of efficient replication in human airway epithelial cells. Because these strains replicate poorly in mice, the investigators are proposing to include new research to generate mouse-adapted WIV1 and SHC014 stains that are more pathogenic. While these specific strains are not included in the U.S. government-wide pause on gain-of-function research projects that may be reasonably anticipated to confer attributes to influenza, SARS, or MERS viruses, the resulting SARS-like viruses will have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route.

NIAID assessment: WIV1 and SHC014 are not currently human pathogens. Project 2 is focused on the on the identification and development of inhibitors of coronavirus high fidelity replication to be used as treatments for human diseases. While it may have implications for future outbreaks, the proposed new research does not sufficiently align with the translational goals of therapeutics development for the Antiviral Drug Discovery and Development Center and NIAID does not support the performance of this proposed new research under the cooperative agreement award.

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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 pause or you observe enhanced pathogenicity and/or transmissibility of SARS-CoV virus in mammals via the respiratory route at any time during the course of conducting these experiments, you are encouraged to voluntarily pause these research activities and provide the NIAID Program Officer and Grants Management Specialist with the relevant data and information related to this change in anticipated outcomes.

Please let us know if you have any questions, or if you require any additional information.

Sincerely yours,

Maureen Beanan, NIAID Program Officer

Jorge Machuca, NIAID Grants Management Specialist