From:	Hauguel, Teresa (NIH/NIAID) [E]
To:	Glowinski, Irene (NIH/NIAID) [E]; Dixon, Dennis M. (NIH/NIAID) [E]; Lambert, Linda (NIH/NIAID) [E]; Spiro, David (NIH/NIAID) [E]; Hauguel, Teresa (NIH/NIAID) [E]; Post, Diane (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Dugan, Vivien (NIH/NIAID) [E]; Mulach, Barbara (NIH/NIAID) [E]; Ford, Andrew (NIH/NIAID) [E]; Strickler-Dinglasan, Patricia (NIH/NIAID) [E]; Hanson, Christopher (NIH/NIAID) [E]; Delarosa, Patricia (NIH/NIAID) [E]; Santora, Kenneth (NIH/NIAID) [E]
Cc:	Beanan, Maureen (NIH/NIAID) [E]
Subject:	4/15 DURC/GoF Meeting Agenda
Date:	Wednesday, April 13, 2016 9:35:16 AM
Attachments:	image001.png Gain-of-Function Pause Review - Ralph Baric CETR Project.msg FW Request to Test Polymerase Mutants.msg FW Response from St. Judes to our request for clarification on their submission.msg MATERIALS NSABB WG teleconference.msg NIAID GoF Pause Implementation Guidelines 5-13-15.docx

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Hello Everyone,

Below is the agenda for tomorrow's DURC/GoF meeting.

Attached are documents for agenda items 1-4.

Weekly DURC/GoF Meeting Agenda

Friday, April 15, 2016
3:00-4:30pm
5601/7G31
Call in number: (b)(6)
Passcode: (b)(6)

1. Projects for GoF Review
    a. Baric (CETR) – MERS/SARS viruses – Maureen B.
    b. Kawaoka (CEIRS) – influenza polymerase mutants – Diane
2. Non-USG Funded Project for DURC Review
    a. St. Jude (PI: Russell) – All
3. NSABB WG Updates – Dennis/Diane/Teresa
    a. Revised GOFROC attributes (attachment 1 from NSABB WG email)
4. GOFROC Strawman – Teresa
    a. Experiments that should/should not be covered
    b. Experiments that should be excepted
5. Other Updates
    a. Erasmus RMP – Ken/Tricia
6. Round Robin/Other Items

Teresa M. Hauguel, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS

5601 Fishers Lane, Room 8E19
Bethesda, MD 20892
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.

Please consider the environment before printing this e-mail 

From:	Strickler-Dinglasan, Patricia (NIH/NIAID) [E]
To:	Hauguel, Teresa (NIH/NIAID) [E]; Post, Diane (NIH/NIAID) [E]; Spiro, David (NIH/NIAID) [E]; Lambert, Linda (NIH/NIAID) [E]
Cc:	Ford, Andrew (NIH/NIAID) [E]; Mulach, Barbara (NIH/NIAID) [E]
Subject:	FW: Response from St. Jude's to our request for clarification on their submission
Date:	Friday, April 8, 2016 12:26:32 PM
Attachments:	FW Dr. Charles Russell - Non-USG-Funded 30-Day Reporting of Research That Meets the Scope of the Policy for Institutional DURC Oversight.msg
Importance:	High

Hi again Flu Group,

Please see below for St. Jude's response to our request for clarification regarding the documentation provided for Dr. Russell's non-Federally funded research project involving HPAI. The original email has been attached for easy retrieval.

Maybe we can add this to the agenda for next Friday's meeting?

Thanks,
Trish

From: Viggiani, Christopher (NIH/OD) [E]
Sent: Friday, April 08, 2016 11:35 AM
To: NIAID BUGS <BUGS@niaid.nih.gov>
Cc: Harris, Kathryn (NIH/OD) [C] (b)(6) Ramkissoon, Kevin (NIH/OD) [C] (b)(6)
Subject: FW: Response from St. Jude's to our request for clarification on their submission
Importance: High

Hi BUGS,

We received a response from St. Judes.

cv

Christopher Viggiani, Ph.D.
National Institutes of Health
Office: (b)(6) || Mobile: (b)(6) (b)(6)

From: Harris, Kathryn (NIH/OD) [C]
Sent: Friday, April 08, 2016 7:52 AM
To: Viggiani, Christopher (NIH/OD) [E] (b)(6) Ramkissoon, Kevin (NIH/OD) [C] (b)(6)
Subject: Response from St. Jude's to our request for clarification on their submission
Importance: High

See below:

Thanks!

K

From: Henry, James (b)(6)
Sent: Thursday, April 07, 2016 4:40 PM
To: Harris, Kathryn (NIH/OD) [C] (b)(6)
Subject: RE: Dr. Charles Russell - Non-USG-Funded 30-Day Reporting of Research That Meets the Scope of the Policy for Institutional DURC Oversight
Importance: High

Dear Kathryn:

Please find listed below responses to your comments.

1) Can you clarify when the PI's initial assessment was conducted? The document providing the PI's initial assessment is not dated, and it is unclear as to whether this is an assessment of the current 3 year renewal (as discussed by the IBC on 2/11/2016) or whether it was for the prior project period.

The initial assessment was conducted 8/31/2012 and the most recent review of the project was 2/11/2016. The document provided was from the initial assessment in 2012.

2) On the PI's initial assessment document, 3 of the 7 categories of experiments listed in the DURC policy were identified (1.5, 4.0, and 5.0), but the 30 day reporting document identifies only 2 of the 7. Can you clarify?

The PI evaluation was based on his initial belief rather than actually data. However, after data was generated and compared to the parental wild-type virus it was determined that it did not enhance harmful consequences of the agent. This information could only be determined after experiments were performed, which predates the GOF hold. Therefore, 1.5 was eliminated from the most recent assessment resulting in only two out of seven being identified in the report.

If you require additional information please do not hesitate to contact me.

Best regards,
James

From:	Strickler-Dinglasan, Patricia (NIH/NIAID) [E]
To:	Hauguel, Teresa (NIH/NIAID) [E]; Post, Diane (NIH/NIAID) [E]; Spiro, David (NIH/NIAID) [E]; Lambert, Linda (NIH/NIAID) [E]
Cc:	Ford, Andrew (NIH/NIAID) [E]
Subject:	FW: Dr. Charles Russell - Non-USG-Funded 30-Day Reporting of Research That Meets the Scope of the Policy for Institutional DURC Oversight
Date:	Wednesday, March 16, 2016 2:03:24 PM
Attachments:	2009JVreedH5N1HApHofActivation.pdf 2010JVreedH5N1pHofActivationInDucks.pdf 2013JVzaraketH5N1CH58HAactivationpHPathogenesisMice.pdf 2013JVzaraketH5N1VN1203inMiceandFerretsNoTransmission.pdf Evaluation of Dr. Charles Russell"s instutional Biosafety Committee Project #O3A-372 (SA00000239) for Dual Use Research of Concern (DURC) - Highly Pathogenic Avian Influenza (HPAI) Viruses.pdf IBC Approval.pdf PI"s Iitial Evaluation.pdf Instutioanl Biosafety Committee Minutes February 11, 2016pdf.pdf Evaluation of Dr Charlie Russell"s Institutional Biosafety Committee Project #O3A-372 (SA00000239) for Dual Use Research of Concern (DURC) - Highly (rev) .pdf Non-USG-Funded 30 Day Report for Dr Charles Russell (rev).pdf
Importance:	High

Hi Flu Group,

Per Chris V's email below, St. Jude's sent to NIH OSP their IRE's assessment of a non-Federally funded research project that involves HPAI and is reported to involve 2 of the 7 categories, but ultimately NOT DURC. Chris V. thinks that the institution has been fairly thorough.

Please take a look at the attached material and, assuming you agree that this falls with NIAID's purview, let me know when you would be ready to discuss at our WG meeting. I know this Friday might be too short a turn-around, but feel free to add to the agenda if you would like to discuss then.

Thank you,
Trish

From: Viggiani, Christopher (NIH/OD) [E]
Sent: Wednesday, March 16, 2016 10:25 AM
To: NIAID BUGS <BUGS@niaid.nih.gov>
Cc: Harris, Kathryn (NIH/OD) [C] (b)(6) Ramkissoon, Kevin (NIH/OD) [C] (b)(6)
Subject: FW: Dr. Charles Russell - Non-USG-Funded 30-Day Reporting of Research That Meets the Scope of the Policy for Institutional DURC Oversight
Importance: High

Dear BUGS,

We have received a report from St. Judes of non-Federally funded research subject to the Institutional DURC policy. The project involves HPAI and is reported to involve 2 of the 7 categories but not constitute DURC. The institution has included a fairly thorough and well-referenced description of their projects and process.

Please let us know if this is a project that would fall within NIAID's preview and if so, if you concur with the institution's assessment.

Happy to discuss, thanks.

Chris

Christopher Viggiani, Ph.D.
National Institutes of Health
Office: (b)(6) || Mobile (b)(6)
(b)(6)

From: Harris, Kathryn (NIH/OD) [C]
Sent: Tuesday, March 15, 2016 11:31 AM
To: Viggiani, Christopher (NIH/OD) [E] (b)(6)
Subject: FW: Dr. Charles Russell - Non-USG-Funded 30-Day Reporting of Research That Meets the Scope of the Policy for Institutional DURC Oversight
Importance: High

St Jude DURC report for NIAID input

From: Henry, James (b)(6)
Sent: Monday, March 14, 2016 10:53 AM
To: DURC <DURC@od.nih.gov>
Cc: Russell, Charles (b)(6) Potter, Phil (b)(6) Webby, Richard (b)(6) Diaz, Robyn (b)(6) Marsh, McGehee (b)(6)
Subject: FW: Dr. Charles Russell - Non-USG-Funded 30-Day Reporting of Research That Meets the Scope of the Policy for Institutional DURC Oversight
Importance: High

To Whom It May Concern:

Please accept our apologies. It was noted that the contact information concerning the IRE and dates were omitted from the previous report submitted on 3/11/2016. Therefore, please accept this resubmission with corrections. Again, if you have any questions please do not hesitate to contact Dr. Philip Potter or me.

Best regards,
James

James Henry, MBA, BSO
Biological Safety Officer / ARO
Environmental Health and Safety
St. Jude Children's Research Hospital
262 Danny Thomas Place, Mail Stop 730

Memphis, TN 38105
T (b)(6)
F (901) 595-3055
(b)(6)

From: Henry, James
Sent: Friday, March 11, 2016 5:04 PM
To: 'DURC@od.nih.gov' <DURC@od.nih.gov>
Cc: Russell, Charles (b)(6) Potter, Phil (b)(6) Webby, Richard (b)(6) Diaz, Robyn (b)(6) Marsh, McGehee (b)(6)
Subject: Dr. Charles Russell - Non-USG-Funded 30-Day Reporting of Research That Meets the Scope of the Policy for Institutional DURC Oversight

To Whom It May Concern:

In accordance with the Policy for Institutional Dual Use Research of Concern (DURC) Oversight, please find attached a 30-day report from Dr. Charles Russell for project **#O3A-372** entitled **Highly Pathogenic and Other BSL-3+ Influenza Viruses**. If you have any questions please do not hesitate to contact Dr. Philip Potter or me.

Best regards,
James Henry

James Henry, MBA, BSO
Biological Safety Officer / ARO
Environmental Health and Safety
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JOURNAL OF VIROLOGY, Apr. 2009, p. 3568–3580 Vol. 83, No. 8
0022-538X/09/$08.00+0 doi:10.1128/JVI.02238-08

Amino Acid Residues in the Fusion Peptide Pocket Regulate the pH of Activation of the H5N1 Influenza Virus Hemagglutinin Protein▽

Mark L. Reed,[1] Hui-Ling Yen,[1]† Rebecca M. DuBois,[1] Olga A. Bridges,[1] Rachelle Salomon,[1]‡ Robert G. Webster,[1] and Charles J. Russell[1,2]*

Division of Virology, Department of Infectious Diseases, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, Tennessee 38105-3678,[1] and Department of Molecular Sciences, University of Tennessee, Memphis, Tennessee 38163[2]

Received 23 October 2008/Accepted 19 January 2009

The receptor specificity and cleavability of the hemagglutinin (HA) protein have been shown to regulate influenza A virus transmissibility and pathogenicity, but little is known about how its pH of activation contributes to these important biological properties. To identify amino acid residues that regulate the acid stability of the HA protein of H5N1 influenza viruses, we performed a mutational analysis of the HA protein of the moderately pathogenic A/chicken/Vietnam/C58/04 (H5N1) virus. Nineteen HA proteins containing point mutations in the HA2 coiled-coil domain or in an HA1 histidine or basic patch were generated. Wild-type and mutant HA plasmids were transiently transfected in cell culture and analyzed for total protein expression, surface expression, cleavage efficiency, pH of fusion, and pH of conformational change. Four mutations to residues in the fusion peptide pocket, Y23H and H24Q in the HA1 subunit and E105K and N114K in the HA2 subunit, and a K58I mutation in the HA2 coiled-coil domain significantly altered the pH of activation of the H5 HA protein. In some cases, the magnitude and direction of changes of individual mutations in the H5 HA protein differed considerably from similar mutations in other influenza A virus HA subtypes. Introduction of Y23H, H24Q, K58I, and N114K mutations into recombinant viruses resulted in virus-expressed HA proteins with similar shifts in the pH of fusion. Overall, the data show that residues comprising the fusion peptide pocket are important in triggering pH-dependent activation of the H5 HA protein.

Highly pathogenic influenza H5N1 viruses, first observed in humans in Hong Kong in 1997 and 1998 (8), have since been reported in repeated outbreaks in Asia, Africa, and Europe, resulting in the culling of millions of infected poultry and an estimated worldwide economic cost of more than $10 billion (reviewed in references 23 and 34). As of December 2008, the transmission of H5N1 influenza viruses from birds to humans has resulted in 246 fatalities from 389 reported cases (www.who.int/csr/disease/avian_influenza/en/). An understanding of the molecular properties of emerging H5N1 influenza viruses may assist in future surveillance and containment strategies.

The influenza A virus hemagglutinin (HA) protein helps determine transmissibility and pathogenicity by its receptor binding and membrane fusion functions during viral entry. The HA protein is synthesized as uncleaved HA0 monomers, with trimerization and correct folding of the protein necessary for its trafficking to the cell surface (14). Cleavage of the HA0 precursor into subunits HA1 and HA2 is a prerequisite for activation of the HA protein to cause membrane fusion (24, 26). In highly pathogenic H5 and H7 subtypes of influenza A viruses, the presence of a polybasic cleavage site allows ubiquitous enzymes in the trans-Golgi network to cleave the HA protein, thereby facilitating systemic infection and causing greater pathogenicity (12, 32, 47). During entry of the influenza virus, the HA protein binds to sialic acid-containing receptors on the surface of the host cell (48). Receptor specificity contributes to virus host range: avian influenza viruses typically bind with a higher affinity to α(2,3) sialosides, whereas human influenza viruses preferentially bind to the α(2,6) sialic acid form (5, 31). After binding to the receptor on the target cell membrane, the virion is internalized by endocytosis (48). Within the endosomal compartment, the virion is exposed to increasingly low pH. At a threshold pH, which varies among strains and is typically between 5 and 6 (7), the HA protein undergoes an irreversible conformation change from its metastable prefusion conformation to a low-pH hairpin structure, promoting fusion of the virion and endosomal membranes (3). A change in the pH of fusion can help influenza viruses adapt to different host species and cell lines (15, 27), as well as facilitate resistance to antiviral agents that raise endosomal pH at high concentrations (7, 9, 39–41). However, a very high pH of fusion may facilitate environmental inactivation of the virus (1), and a very low one may cause lysosome-mediated degradation of the virus (53).

Previous studies using amantadine selection and laboratory adaptation to different host cells identified mutations in the HA proteins of H3 and H7 influenza subtypes that alter the pH at which fusion is triggered (6, 7, 9, 13, 21, 27, 39–41, 46). Residues that regulate the acid stability of H3 and H7 HA proteins are located in four broad regions: (i) the fusion peptide comprising the first 25 N-terminal residues of the HA2

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† Present address: Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, Pokfulam Road, Hong Kong.

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▽ Published ahead of print on 4 February 2009.

subunit (6, 7, 21, 40); (ii) the fusion peptide pocket, comprising residues residing within both HA subunits that surround the fusion peptide within the neutral pH metastable conformation (7, 39, 46); (iii) the coiled-coil regions of the HA2 subunit (7, 39); and (iv) the interface between the HA1 and HA2 subunits (7). These residues may also contribute to the acid stability of the HA protein by changing local interactions in structural regions important in regulating intermediate steps in the fusion conformational change (49).

High-resolution structures have been determined for multiple HA subtypes in recent years (11, 16, 35, 42, 43, 51, 52). Alignments of structures of different subtypes have revealed marked structural differences, such as rotation of the HA1 subunit around the central axis of the HA2 subunit and the shape and orientation of smaller structural elements such as the HA2 subunit membrane distal loop (16). HA proteins from all 16 known subtypes can be classified into five structural clades on the basis of these structural differences and signature sequences (35). In this structural phylogeny, the H5 HA protein lies distinct from the H3 and H7 subtypes (42, 52), raising the possibility that the pH-dependent activation of the H5N1 HA protein may be regulated differently than the H3 and H7 HA proteins.

To investigate the role of individual amino acids and potential mechanisms that modulate the acid stability of the H5 HA protein, 19 point mutations were made within the H5 HA protein of A/chicken/Vietnam/C58/04 (C58). The effects of the mutations on HA protein expression, cleavage, and the pH of membrane fusion and HA protein conformational changes are consistent with amino acid residues in the fusion peptide pocket playing a major role in regulating the pH of activation of the H5N1 HA protein.

MATERIALS AND METHODS

Plasmids. Point mutations were introduced into plasmid pSH054-A/chicken/Vietnam/C58/04 HA (36) by using a QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions. Residues are identified by H5 numbering throughout the study. Wild-type and mutant HA genes were subcloned into a pCAGGS expression plasmid (45) using XhoI and ClaI restriction enzyme sites. Nucleotide sequences of all plasmids were verified by DNA sequencing at the Hartwell Center for Bioinformatics and Biotechnology, St. Jude Children's Research Hospital.

Cell culture. Monolayer cultures of Vero cells (ATCC CCL-81), BHK-21 cells (ATCC CCL-10), and BSR-T7/5 cells (2) were grown in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, 1% glutamine, 1% penicillin, and 1% streptomycin. BSR-T7/5 cells were also grown in the presence of G418 (final concentration, 1 mg/ml), which was added to the DMEM at every other passage. BHK-21 cells were also supplemented with 10% tryptose phosphate broth.

Viruses. Recombinant viruses containing mutations Y23H, H24Q, K58I, and N114K were generated as described previously (19, 36). Briefly, eight dual promoter pHW2000 plasmids containing each of the influenza A virus gene segments were used to transfect MDCK/293T cocultured cells. Virus stock was prepared by inoculation of 10-day-old embryonated chicken eggs. Viral RNA was isolated directly from allantoic fluid of inoculated eggs by using an RNA extraction kit (RNeasy; Qiagen). Reverse transcription-PCR of viral RNA used a universal primer set for influenza A virus (20), and subsequent sequencing was completed by the Hartwell Center for Bioinformatics and Biotechnology at St. Jude Children's Research Hospital. All experiments using reverse genetics viruses were undertaken in a U.S. Department of Agriculture-approved biosafety level 3+ containment facility. All assays utilizing recombinant viruses were undertaken in Vero cells infected at a multiplicity of infection of 3. Peak titers of reverse genetics viruses were determined by single-step growth analysis in MDCK cells. Cells were infected for 1 h and then washed with phosphate-buffered saline (PBS; pH 2.2) to remove free infectious virus particles. Cells were incubated at 37°C in minimal essential medium (containing 10% fetal bovine serum and 1% glutamine). Supernatants were collected 2, 4, 6, 8, and 10 h postinfection and stored at −70°C for titration.

Transient expression of HA proteins. Monolayers of Vero cells in six-well dishes (85 to 95% confluence) were transiently transfected with 1 μg of pCAGGS HA protein DNA by using a Lipofectamine Plus expression system (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Transfected Vero cells were incubated for 4 h at 37°C. DMEM (containing 10% fetal bovine serum and 1% glutamine) was then added to cells, and cells were incubated for 16 h at 37°C. Cells were then treated as indicated for each experiment.

Adjustment of pH in vitro. The pH of phosphate-buffered saline with magnesium and calcium (PBS+) was adjusted by 0.1 M citric acid. Cell monolayers were exposed for 5 min at 37°C for syncytium formation and luciferase reporter gene assays and for 15 min at 37°C prior to processing for conformational flow cytometry.

Measurement of HA protein expression and cleavage by Western blotting, radioimmunoprecipitation, biotinylation, and flow cytometry. Sixteen hours after transfection, cell monolayers were washed twice with PBS+ solution. The samples were lysed with 0.5 ml of ice-cold radioimmunoprecipitation assay buffer containing Complete protease inhibitor cocktail (Roche, Indianapolis, IN). The lysate was spun at 67,000 × *g* in an Optima TLX ultracentrifuge (Beckman Coulter, Fullerton, CA). Cleared lysate was mixed with sample dye buffer containing 200 mM Tris, 8% sodium dodecyl sulfate (SDS), 0.2% bromophenol blue, 40% glycerol, and 12% β-mercaptoethanol. Samples were boiled for 5 min before being separated on 4–12% NuPAGE Bis-Tris polyacrylamide-SDS gels (Invitrogen). Proteins were transferred onto a polyvinylidene difluoride membrane, blocked with 5% fat-free milk, and probed by using rabbit polyclonal antisera against the peptide sequence (AADKESTQKAIDGVTNKVNSIIDK) in the HA2 subunit (Harlan Bioproducts for Science, Indianapolis, IN). Alexa Fluor 488-goat anti-rabbit conjugate secondary antibody was used to visualize bands with a Typhoon 9200 imager (GE Healthcare, Waukesha, WI). The band intensity was measured by using ImageQuant TL software (Molecular Dynamics, Sunnyvale, CA). Equal loading of wells was confirmed by Western blotting with a rabbit polyclonal primary antibody against the cellular protein GAPDH (glyceraldehyde-3-phosphate dehydrogenase; Abcam, Cambridge, MA). Radioimmunoprecipitation and biotinylation experiments were performed as described previously (28), using 25 μl of rabbit anti-HA2 peptide polyclonal primary antibody (1:200 dilution). Flow cytometry was performed as described previously (28), using the primary monoclonal antibody VN04-2 (1:500 dilution) (22), which reacts equally to both neutral- and low-pH conformations of the H5N1 HA protein (unpublished observation). Mean fluorescence intensity (MFI) values were normalized to those of the A/chicken/Vietnam/C58/04 wild-type HA protein. Monoclonal antibodies VN04-9 and VN04-16 (22) were used to measure the pH dependence of conformational changes of the HA protein at a 0.1 pH unit resolution. The pH of conformational changes was determined as the point at which 50% change in signal occurred between baseline and maximum.

Luciferase reporter gene assay for cell-cell membrane fusion. To quantify membrane fusion, we performed a luciferase reporter gene assay as described previously (33). Briefly, six-well dishes containing Vero cells (70 to 80% confluence) were transfected with 1.0 μg of luciferase control DNA (Promega, Madison, WI) and 1.0 μg of pCAGGS HA DNA. At 16 h posttransfection, BSR-T7/5 target cells (expressing T7 RNA polymerase) were overlaid onto the Vero cells expressing the HA protein (2). After a 1-h incubation at 37°C, the monolayers were washed and pH treated at a 0.2 pH unit resolution. Cells were neutralized by using DMEM (containing 10% fetal bovine serum and 1% glutamine) and left at 37°C for 6 h (21). Samples were lysed in reporter lysis buffer (Promega) and clarified by centrifugation at 15,000 × *g* in a tabletop centrifuge (5417C; Eppendorf, Germany) at room temperature. From each clarified lysate, a 150-μl sample was transferred to a 96-well plate (Lumitrac 200; Promega). The luciferase activity resulting from fusion of the two cell populations was quantified with a Veritas luminometer (Promega), using 50 μl of luciferase assay substrate (Promega) injected into each sample.

Syncytium assay for cell-cell fusion by HA mutants. Monolayers of BHK-21 cells grown in six-well plates were transfected with 1 μg of pCAGGS HA as described above. Monolayers of Vero cells grown in six-well plates were infected with recombinant virus at a multiplicity of infection of 3. At 16 h posttransfection or 6 h postinfection, cell monolayers were pH treated as described above at a 0.1 pH unit resolution. Cells were neutralized by using DMEM (containing 10% fetal bovine serum and 1% glutamine) and incubated at 37°C for 2 h. Samples were fixed and stained with Hema-3 Stat Pack staining kit (Fisher) according to the manufacturer's instructions. Representative fields were captured with a Nikon D70 digital camera attached to a Nikon Eclipse TS100 inverted microscope.

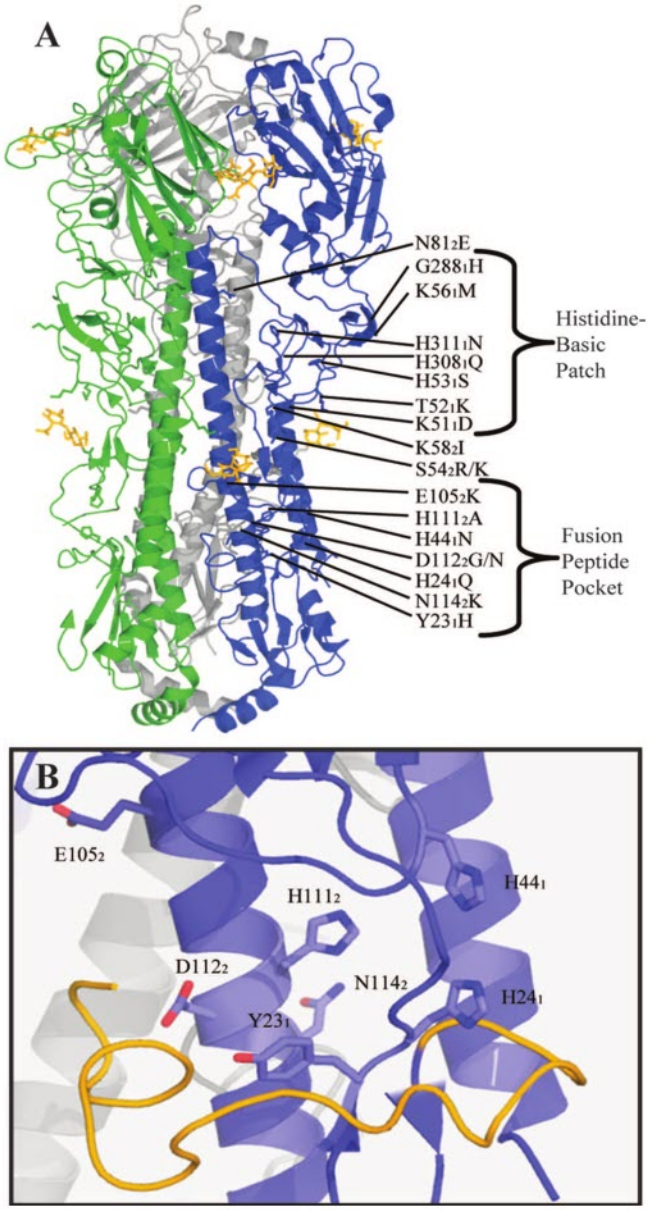

FIG. 1. (A) Locations of the 19 point mutations introduced into the HA protein of A/chicken/Vietnam/C58/04 (H5N1) influenza virus. Mutations are mapped to the available trimeric structure of the closely related A/Vietnam/1203/04 HA protein (42) (PDB:2fk0). (B) Fusion peptide (yellow) and fusion peptide pocket (blue). Residues mutated in the present study are shown with amino acid side chains. Subscript numbers denote the HA subunit in each mutation. H5 numbering is used throughout.

RESULTS

Selection of HA protein mutations. To identify amino acid residues that regulate the pH of activation of the HA protein of an H5N1 influenza virus, two sets of mutations were generated in the background of the HA protein of A/chicken/Vietnam/C58/2004 (H5N1) (Fig. 1). Eight mutations to HA2 coiled-coil residues that alter the pH of activation of H3 and H7 HA proteins were selected for the present study on the H5N1 HA protein (7, 39, 41, 49). Eleven mutations to introduce or remove basic residues were selected by sequence alignment of HA proteins from different subtypes. Y23$_1$H, K51$_1$D, G288$_1$H, and H308$_1$Q were selected based upon prevalence in the H1 HA protein; H44$_1$N, H53$_1$S, and H311$_1$N were selected based upon prevalence in the H3 HA protein; H111$_2$A was selected based upon prevalence in the H7 HA protein, and H24$_1$Q and K56$_1$M were selected based upon prevalence in the H9 HA protein. T52$_1$K was selected because it is one of the residues that differs between the A/chicken/Vietnam/C58/04 HA protein being studied, and the HA protein of A/Vietnam/1203/04 H5N1 virus being used as a structural model for comparison. This mutation is the only one that has been observed in circulating H5N1 influenza virus isolates.

Only the H111$_2$A mutation causes substantial changes in protein expression and cleavage. The effects of mutations on total HA protein expression were analyzed by radioimmunoprecipitation (15-min pulse and a 0-min chase) and Western blotting (Fig. 2). Densitometric analysis of HA0 band intensity showed that the initial expression of all mutant HA proteins was 64 to 125% that of the wild type (Fig. 2A and B and Table 1), showing that the mutations did not abrogate initial expression of the HA0 precursor protein.

In Western blot experiments, whole-cell lysates were collected 16 h posttransfection to determine the steady-state levels of expression and cleavage of the HA protein. SDS-polyacrylamide gel electrophoresis (PAGE) analysis showed that the HA protein containing an H111$_2$A mutation accumulated only as an HA0 precursor protein at a level 26% that of wild type; it was not detectable in the stable, cleaved form (Fig. 2C and Table 1). For the remaining 18 HA proteins containing mutations, the levels of cleavage were similar to that of the wild type, and the levels of expression were 42 to 186% that of the wild type (Fig. 2C and D and Table 1). A lower initial expression of the HA protein mutants G288$_1$H, H311$_1$N, and D112$_2$G, as measured by radioimmunoprecipitation, resulted in lower steady-state expression levels, as measured by Western blotting.

The effects of the mutations on the cell surface expression of HA proteins were studied by biotinylation and flow cytometry experiments. Intact cells were biotinylated 16 h posttransfection and analyzed by Western blotting. The levels of cleavage of all biotinylated HA proteins containing mutations were comparable to that of the wild type, except for the HA protein containing an H111$_2$A mutation (Fig. 3A and B and Table 1). Of the mutations in the histidine or basic patch, the H24$_1$Q, T52$_1$K, H53$_1$S, and H308$_1$Q mutations caused significant increases, and the G288$_1$H, H311$_1$N, and H111$_2$A mutations significant decreases in cell surface expression compared to the wild type. Of the HA2 coiled-coil mutations, the S54$_2$R, K58$_2$I, and D112$_2$N mutations resulted in significant increases, and the E105$_2$K and N114$_2$K mutations significant decreases in cell surface expression compared to the wild type.

In flow cytometry experiments, intact cells were labeled 16 h posttransfection with the anti-H5 HA protein monoclonal antibody VN04-2 (22), which binds with equal affinity to both neutral- and low-pH forms of the A/chicken/Vietnam/C58/2004 (H5N1) HA protein (data not shown). The surface expression levels of most HA proteins containing histidine or basic patch mutations were similar to those of the wild type, although the H111$_2$A mutation reduced the cell surface ex-

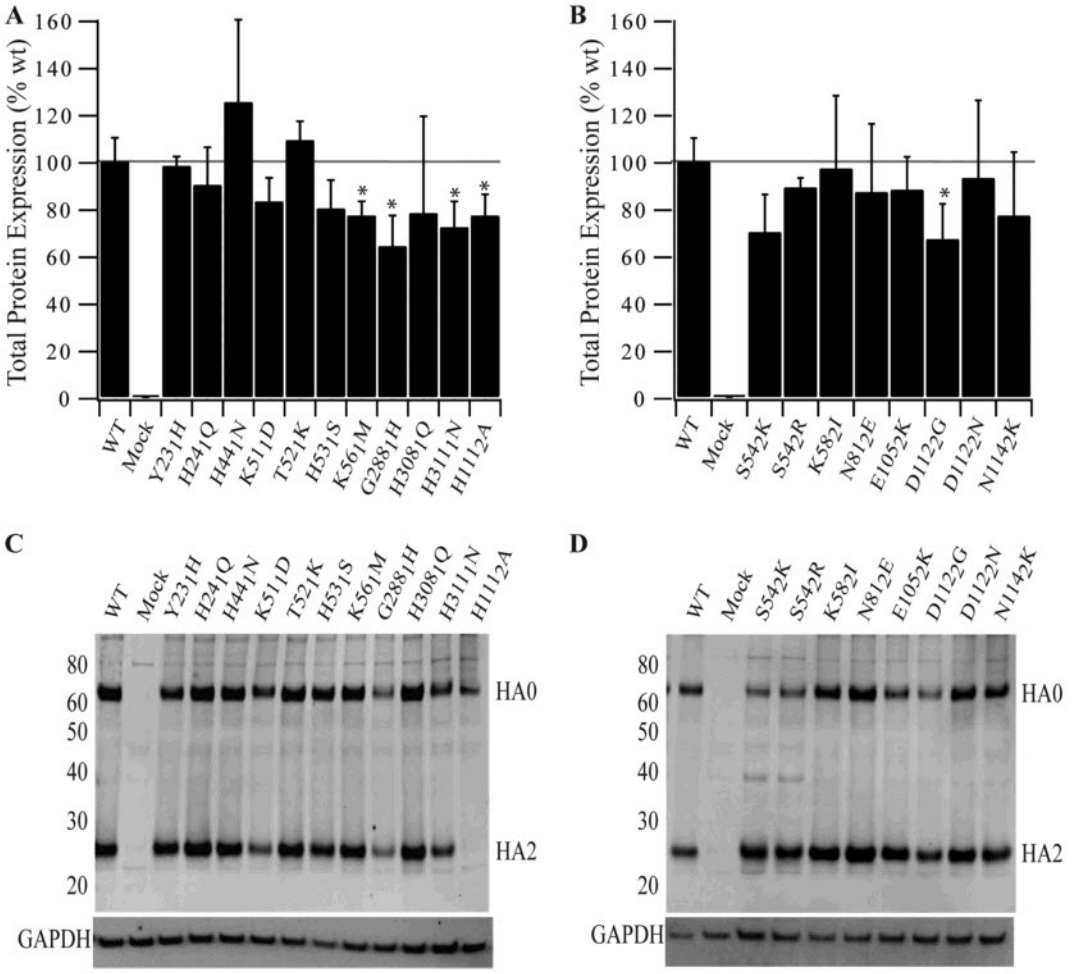

FIG. 2. Total expression of wild-type and mutant HA proteins in Vero cells. (A) Histidine or basic patch mutants; (B) HA2 coiled-coil mutants. Total initial expression was determined by immunoprecipitation analysis. At 16 h posttransfection, Vero cells expressing wild-type and mutant HA protein were serum starved for 30 min and subsequently labeled with [^{35}S]Promix for 15 min. HA0 expressed during the labeling pulse was immunoprecipitated by using the A0110 polyclonal antibody and analyzed by SDS-PAGE under reducing conditions. Initial expression was normalized to the wild-type HA protein. The horizontal line represents 100% expression. Error bars represent the standard deviation from triplicate experiments. Asterisks indicate a significant difference ($P < 0.05$) as determined by an independent group t test. (C and D) Total expression and cleavage of histidine or basic patch mutants (C) and HA2 coiled-coil mutants (D). Vero cells expressing wild-type and mutant HA proteins were processed 16 h posttransfection and analyzed by Western blotting with polyclonal antibody A0110 raised against a peptide motif in the HA2 subunit of H5 HA protein. Uncleaved HA0 precursor and the HA2 cleavage product are indicated. GAPDH loading controls are shown. WT, wild type.

pression of the HA protein to 31% that of the wild type (Fig. 3C and D and Table 1). Mutations in the HA2 coiled-coil region caused a more polarized effect. Both mutations at residue S54 resulted in increased cell surface expression. For the remaining HA2 coiled-coil mutations, cell surface expression levels were 62 to 92% compared to that of the wild type.

In general, cell surface expression levels from flow cytometry experiments were lower than those obtained by Western blot analyses of the biotinylated surface protein. Binding of the VN04-2 monoclonal antibody suggests that the HA proteins are correctly folded. However, we have yet to determine whether the VN04-2 antibody binds preferentially to either the cleaved or the uncleaved form of the HA protein or binds with equal affinity to both. The detection of some $H111_2A$ protein at the cell surface suggests that this antibody can recognize the uncleaved form of the HA protein. In contrast, the biotinylation analysis is expected to detect equally both uncleaved and cleaved forms of the HA protein at the cell surface but may not accurately measure the amount of functional protein at the cell surface (i.e., the protein competent for mediating membrane fusion). The collective results from both assays show that most mutant HA proteins were expressed at the cell surface of transfected cells at levels comparable to that of wild-type C58 HA protein. Moreover, except for $H111_2A$, the levels of cleavage of all other mutant HA proteins were similar to that of the wild type (Fig. 4 and Table 1). Levels of cleavage of surface expressed protein could be increased by ca. 11% with the presence of 5 μg of exogenous trypsin/ml for 30 min at 37°C (data not shown), suggesting that a population of HA protein reaches the cell surface uncleaved.

Five individual mutations to the HA protein change the pH of membrane fusion. The extents to which the wild-type and mutant HA proteins promote cell-to-cell membrane fusion were measured as a function of pH by syncytium and luciferase

TABLE 1. Phenotypes of wild-type and mutant influenza A HA proteins[a]

H5 numbering	H3 numbering	Total protein expression		Surface expression		Cleavage		pH of fusion		pH of conformation change	
		IP[b] (mean ± SD)	Western blot	MFI[c] (mean ± SD)	Biotinylation[d] (mean ± SE)	Total[e]	Surface[f] (mean ± SE)	Syncytia[g]	Luciferase[h]	VN04-9[i]	VN04-16[j]
Wild type		100 ± 10	100	100 ± 13	100 ± 23	0.45	0.55 ± 0.04	5.5	5.5	5.5	5.4
$Y23_1H$	$Y17_1H$	98 ± 4	72	95 ± 11	128 ± 16	0.49	0.60 ± 0.00	+0.4	+0.4	+0.3	+0.4
$H24_1Q$	$H18_1Q$	90 ± 16	96	91 ± 18	188 ± 41	0.48	0.49 ± 0.01	–0.3	–0.3	–0.4	–0.4
$H44_1N$	$H38_1N$	125 ± 35	91	88 ± 9	113 ± 21	0.49	0.60 ± 0.01	–0.1	0.0		
$K51_1D$	$K45_1D$	83 ± 10	55	95 ± 5	85 ± 14	0.41	0.69 ± 0.03	0.0	–0.1		–0.1
$T52_1K$	$T46_1K$	109 ± 8	104	98 ± 10	165 ± 19	0.45	0.56 ± 0.02	0.0	0.0		
$H53_1S$	$H47_1S$	80 ± 12	83	88 ± 5	146 ± 28	0.45	0.60 ± 0.02	0.0	0.0		0.0
$K56_1M$	$K50_1M$	77 ± 6	124	94 ± 4	134 ± 28	0.48	0.55 ± 0.05	0.0	–0.1		
$G288_1H$	$G275_1H$	64 ± 13	42	98 ± 6	52 ± 16	0.44	0.72 ± 0.02	0.0	–0.1		
$H308_1Q$	$H295_1Q$	78 ± 41	121	107 ± 8	166 ± 14	0.44	0.47 ± 0.07	0.0	–0.2		
$H311_1N$	$H298_1N$	72 ± 11	59	81 ± 4	32 ± 13	0.43	0.69 ± 0.02	0.0	0.0		
$H111_2A$	$H111_2A$	77 ± 9	26	31 ± 1	16 ± 4	0.07	0.10 ± 0.02	NA	NA		
$S54_2K$	$S54_2K$	70 ± 16	78	151 ± 20	113 ± 40	0.64	0.60 ± 0.07	0.0	0.0		
$S54_2R$	$S54_2R$	89 ± 4	91	138 ± 10	159 ± 52	0.61	0.54 ± 0.04	0.0	0.0		
$K58_2I$	$K58_2I$	97 ± 31	167	86 ± 10	136 ± 5	0.57	0.61 ± 0.02	–0.4	–0.4	–0.4	–0.4
$N81_2E$	$N81_2E$	87 ± 29	186	92 ± 7	103 ± 7	0.56	0.52 ± 0.04	0.0	–0.1		
$E105_2K$	$E105_2K$	88 ± 14	117	72 ± 10	70 ± 11	0.61	0.65 ± 0.04	–0.3	–0.2	–0.1	–0.2
$D112_2G$	$D112_2G$	67 ± 15	74	62 ± 8	96 ± 13	0.61	0.53 ± 0.05	0.0	0.0	+0.3	+0.3
$D112_2N$	$D112_2N$	93 ± 33	135	84 ± 10	150 ± 32	0.54	0.43 ± 0.06	0.0	+0.1	+0.2	+0.3
$N114_2K$	$N114_2K$	77 ± 27	108	78 ± 6	43 ± 11	0.49	0.43 ± 0.11	+0.2	+0.3	+0.5	+0.5

[a] Influenza A virus HA proteins expressed from pCAGGS DNA in Vero cells.
[b] Total HA0 expression after 15 min of [^{35}S]methionine pulse-labeling. Data are normalized to wild-type HA protein. IP, immunoprecipitation.
[c] Cell surface expression (expressed as the MFI) was determined by flow cytometry using monoclonal antibody VN04-2. Data are normalized to wild-type C58 HA protein. The reported error indicates the standard deviation from triplicate experiments.
[d] Cell surface expression determined by biotinylation. Data are normalized to wild-type C58 HA protein. The reported error indicates standard error from triplicate experiments.
[e] That is, the cleavage ratio of total cell lysates determined using the formula HA2/(HA0 + HA2).
[f] That is, the cleavage ratio determined by biotinylation. Data are normalized to the wild-type C58 HA protein. The reported error indicates standard error from triplicate experiments.
[g] A syncytium formation assay for the pH of membrane fusion was determined as the last pH point at which syncytium formation was within a representative field of view. NA, absence of syncytium formation.
[h] pH of membrane fusion derived from the luciferase reporter gene assay was determined as the point at which 50% of maximum increase in signal was achieved. NA, absence of fusion as determined by this assay.
[i] Monoclonal antibody VN04-9 favors the metastable conformation of the H5 HA protein. The pH of conformational change was determined as the pH at which a 50% decrease in signal was observed between baseline and maximum.
[j] Monoclonal antibody VN04-16 favors the low-pH conformation of the H5 HA protein. The pH of conformational change was determined as the pH at which a 50% increase in signal was observed between baseline and maximum.

reporter gene assays. Syncytium formation between BHK-21 cells was initiated 16 h posttransfection, and the pH of fusion was defined as the highest pH at which syncytium formation was observed (Fig. 5 and Table 1). The wild-type C58 HA protein promoted syncytium formation at pH 5.5. Eight of the eleven mutations in the histidine or basic patch caused a pH change of 0.1 U or less. The $Y23_1H$ mutation increased the pH of syncytium formation by 0.4 pH units, whereas the $H24_1Q$ mutation decreased it by 0.3 pH units. The $H111_2A$ mutant showed no syncytium formation at any pH over the range measured (pH 5.0 to 6.0), a finding consistent with this mutant not being present on the cell surface of transfected cells in a cleaved form. Five of the eight mutations in the HA2 coiled-coil group caused no change in the pH of syncytium formation. The $K58_2I$ and $E105_2K$ mutations decreased the pH of fusion by 0.4 and 0.2 pH units, respectively, whereas the $N114_2K$ mutation caused a pH increase of 0.2 pH units.

A luciferase reporter gene assay was also used to measure the effects of the mutations on the pH of HA-mediated membrane fusion (Table 1 and Fig. 6A and B). Similar to results from the syncytium assays, in the luciferase assay the $Y23_1H$ mutation increased the pH of fusion by 0.4 U, the $H24_1Q$ mutation decreased the pH by 0.3 U, the $H111_2A$ mutation eliminated membrane fusion, and the remaining eight mutations in the histidine or basic group had little effect on the pH of luciferase activity (Table 1). The $K58_2I$, $E105_2K$, and $N114_2K$ mutations in the HA2 coiled-coil group also caused similar shifts in the pH of membrane fusion in both the syncytium and the luciferase assays, and the remaining five HA2 mutations had little or no effect on the pH of acid stability of the C58 H5 HA protein. Unexpectedly, the mutations $D112_2G$ and $D112_2N$ did not increase the pH of membrane fusion of the H5 HA protein in either assay, despite substantially altering the pH of fusion in H3 and H7 subtypes (7, 39, 49). This assay was also used to measure the fusogenic efficiency of the mutants compared to the wild type. It showed that the majority of mutants had no significant effect on the proportion of cells fused under conditions of low pH (Fig. 6C and D). Mutants $K51_1D$ and $H53_1S$ had caused a significant change in fusogenic efficiency, similar to what has previously been hypothesized for basic residues in this region for H1 and H5 subtypes (42, 43). $E105_2K$ decreased the fusogenic potential of the HA protein, while $H111_2A$ demonstrated a very low level of fusion consistent with observations made in the syncytium formation assay. The very low signal demonstrated for the $H111_2A$

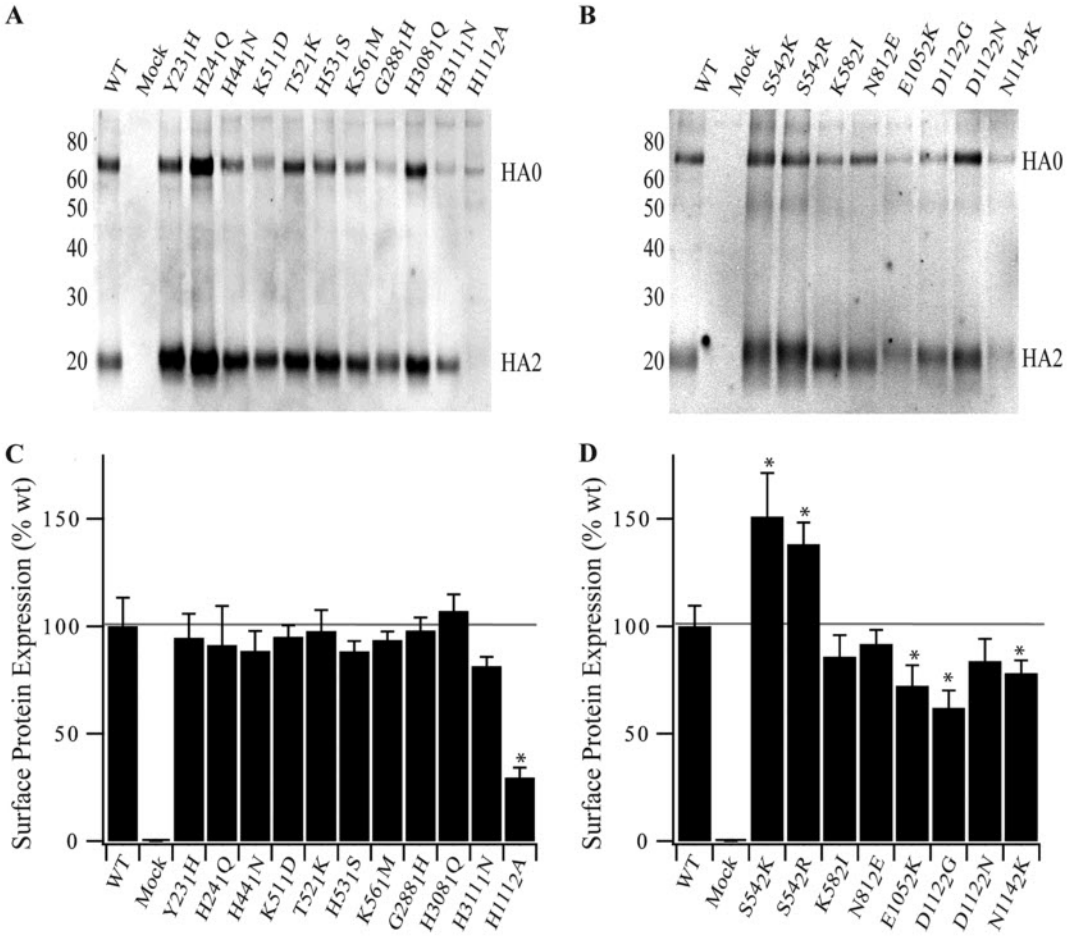

FIG. 3. Cell surface expression of wild-type and mutant HA proteins as determined by surface protein biotinylation assay. (A) Histidine or basic patch mutants; (B) HA2 coiled-coil mutants. Vero cells expressing wild-type or mutant HA protein were biotinylated for 30 min. The biotinylated protein was isolated by using streptavidin-conjugated Sepharose beads, analyzed by SDS-PAGE under reducing conditions, and subsequently analyzed by Western blotting. Western blotting with polyclonal antisera A0110 against a peptide sequence in HA2 shows the uncleaved HA0 precursor and the HA2 subunit cleavage product (indicated). (C and D) Surface expression of H5 HA protein using monoclonal antibody VN04-2 for histidine or basic patch mutants (C) and HA2 coiled-coil mutants (D) (22). MFI values were normalized to 100% surface expression for wild-type C58 HA protein. The horizontal line represents 100% expression. Error bars represent the standard deviation from triplicate experiments. Asterisks indicate a significant difference ($P < 0.05$), as determined by an independent group t test. WT, wild type.

mutant is consistent with the syncytium formation assay showing no syncytium formation for this mutant at any pH.

Mutations that alter the pH of membrane fusion also alter the pH of HA protein conformational changes. The influenza A virus HA protein is expressed on the surfaces of infected cells and virions in a metastable, spring-loaded conformation and undergoes a dramatic, pH-dependent molecular rearrangement that promotes membrane fusion (10, 37). To determine whether the histidine or basic patch mutations $Y23_1H$, $H24_1Q$, $K51_1D$, and $H53_1S$ and the HA2 coiled-coil mutations $K58_2I$, $E105_2K$, $D112_2G$, $D112_2N$, and $N114_2K$ change the pH of refolding of the HA protein, flow cytometry experiments were performed with conformation-based monoclonal antibodies (Fig. 7 and Table 1). The monoclonal antibodies VN04-9 and VN04-16 (22) bound preferentially to the native and low-pH conformations, respectively, of the wild-type HA protein of A/chicken/Vietnam/C58/04 (H5N1) (Fig. 7A and B). On the basis of these differences in binding preference, the pH dependence of conformational changes in the HA protein was determined by flow cytometry. Unexpectedly, the pH of conformational changes for $D112_2G$ and $D112_2N$ were 0.3 and 0.25 pH units higher, respectively, than the pH of the membrane fusion. Similarly, the $N114_2K$ mutation induced conformational changes at a pH ~0.3 U higher than its pH of membrane fusion. It is possible that the HA proteins containing $D112_2G$, $D112_2N$, or $N114_2K$ mutations undergo somewhat localized changes in conformation detected by the antibodies at a pH higher than that required to trigger the complete HA protein refolding necessary to promote membrane fusion. In contrast, the HA proteins containing mutations $Y23_1H$, $H24_1Q$, $K58_2I$, and $E105_2K$ showed shifts in the pH of conformational changes similar to those observed in the syncytia and luciferase assays (Fig. 7C and Table 1). Analysis of $K51_1D$ and $H53_1S$ with VN04-16 also confirmed that the pH of conformational change matched the findings of the pH of membrane fusion assays (Table 1).

Mutations that alter the pH of fusion in transfected cells have a conserved effect in reverse genetics virus. In order to

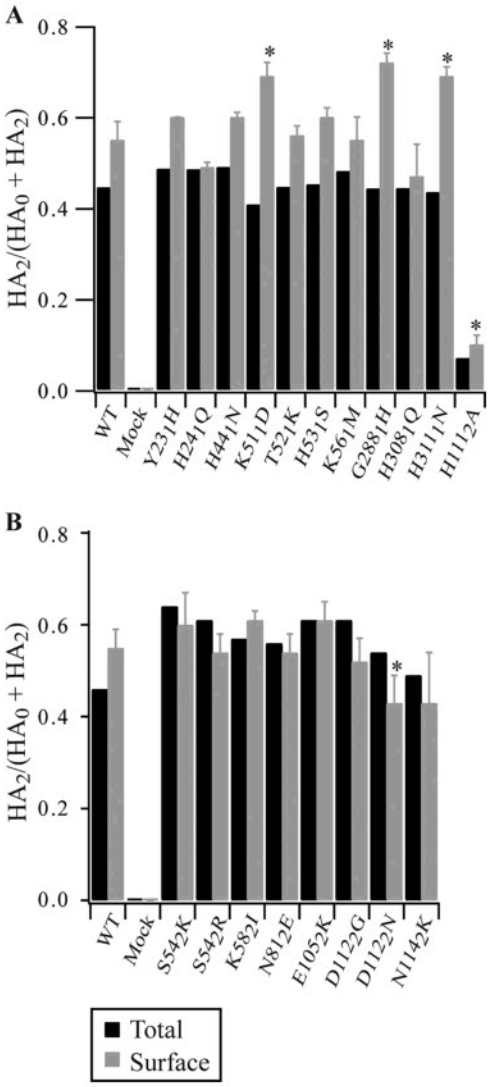

FIG. 4. Cleavage of wild-type and mutant H5 HA proteins. Intensities of the HA0 and HA2 bands from Fig. 2C and D were quantified, and the fraction of cleaved HA2 was calculated by dividing HA2 by total HA (represented by HA0 + HA2, since the HA1 protein is not observed in the assay). Error bars represent standard errors from three separate experiments. Asterisks indicate a significant ($P < 0.05$) difference as determined by an independent group t test. WT, wild type.

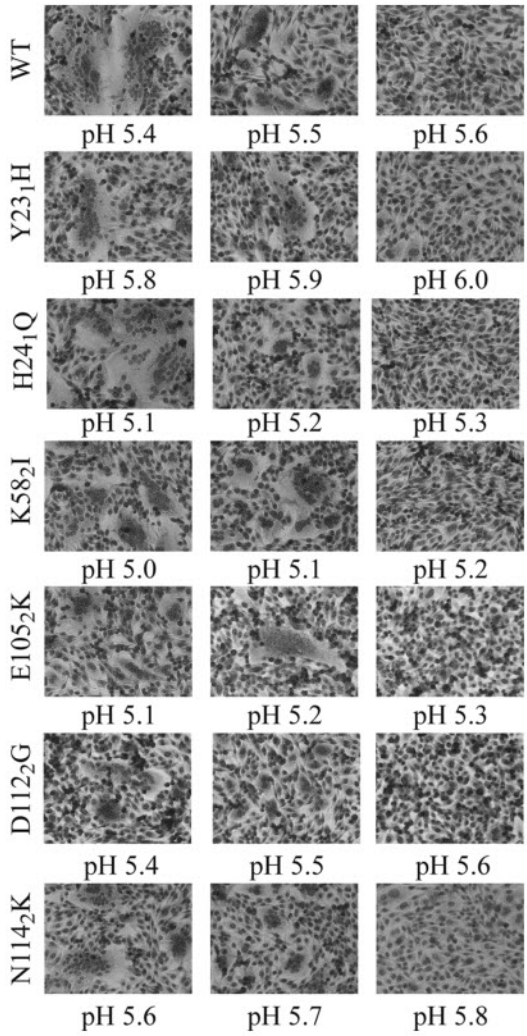

FIG. 5. Representative micrographs of the syncytium formation assay to observe the pH of membrane fusion for wild-type (WT) and mutant C58 HA proteins. The micrographs show the presence or absence of syncytium formation when BHK-21 cells expressing HA protein were incubated at the indicated pH. The pH of fusion was measured as the highest pH value at which syncytium formation was observed.

determine whether changes in the pH of fusion observed in the transient-transfection system were applicable to H5N1 recombinant virus, wild-type A/chicken/Vietnam/C58/04 virus, and viruses containing the mutations $Y23_1H$, $H24_1Q$, $K58_2I$, and $N114_2K$ in the HA gene were generated by using the eight-plasmid system (19). These four mutations were selected because they gave a range of changes in the pH of fusion encompassing a 0.8 pH unit range around the value observed for wild-type HA protein. All viruses were successfully rescued, and sequencing determined that only the mutation of interest was present in each case. Single-step growth analysis using the viruses showed that the peak titers obtained were analogous for all viruses tested. Furthermore, the expression and cleavage of virus-derived HA protein was confirmed by Western blotting of virus-infected Vero cells. The pH of fusion for each virus was determined by using a syncytium formation assay in Vero cells. Table 2 shows that mutant and wild-type HA proteins were successfully expressed and cleaved in virus-infected cells. Furthermore, the shift in the pH of fusion is similar to that observed in transfected cells. The pH of fusion for $N114_2K$ was closer to the value of pH of conformational change observed in transfected cells.

DISCUSSION

We have investigated how the pH of activation of the H5N1 HA protein is regulated by introducing 19 individual amino acid mutations into the HA protein of A/chicken/Vietnam/C58/04 (H5N1). We then characterized the mutational effects on expression, cleavage, conformational changes, and membrane fusion of the HA protein. Seven of the mutations were

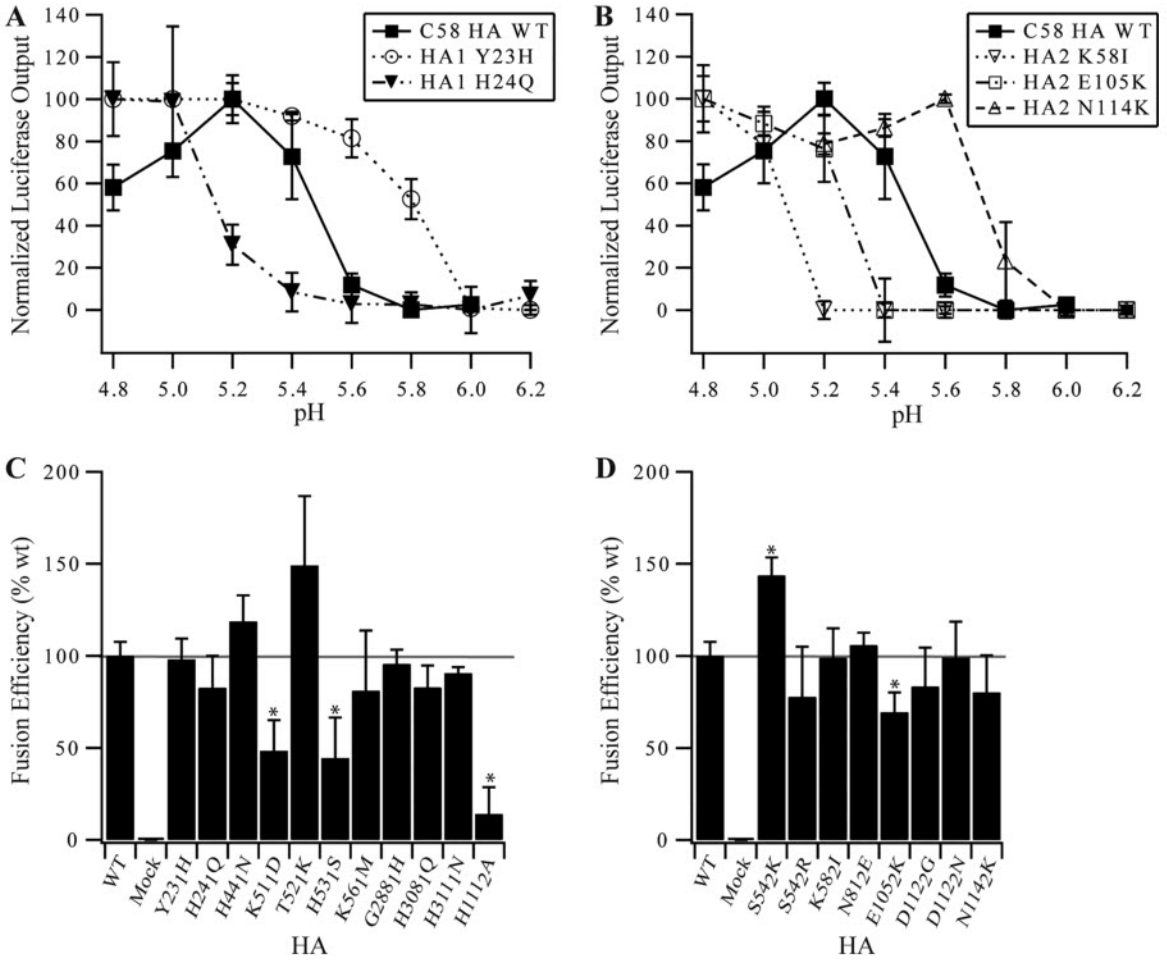

FIG. 6. Luciferase membrane fusion assay to measure the pH of fusion of H5 HA proteins. Vero cells were cotransfected with pCAGGS HA plasmids and T7 control DNA. At 16 h posttransfection, Vero effector cells were overlaid with BSR-T7/5 cells that express T7 polymerase (2). The two cell populations were then exposed to low-pH buffer conditions and coincubated for 6 h to allow cell-to-cell fusion to occur. The extent of membrane fusion was measured as the difference in signal between mock-transfected cells (T7 control DNA only) and the maximum signal acquired for the wild-type HA protein. The pH of fusion was determined as the point at which a 50% change in signal occurred. Error bars represent the standard deviation from triplicate experiments. Examples are shown for histidine or basic patch mutants (A) and HA2 coiled-coil mutants (B). (C and D) Comparative efficiency of fusion between mutant and wild-type HA proteins. Asterisks indicate a significant difference ($P < 0.05$) as determined by an independent group t test.

found to alter the pH of membrane fusion and/or protein refolding by ~0.2 pH units or more. Six of the mutations are located in the fusion peptide pocket, and the $K58_2I$ mutation is located in the "A" α-helix that buttresses the central HA2 coiled coil in the metastable structure (Fig. 1). An $H111_2A$ mutation in the fusion peptide pocket significantly decreased expression of the HA protein and completely eliminated cleavage and membrane fusion. Eleven other mutations had little effect on the pH of fusion of the H5N1 HA protein, including all of the mutations to residues in a membrane-distal histidine or basic patch at the interface of the HA1 and HA2 domains (Fig. 1). Four mutants capable of altering the pH of fusion in transfected cells were introduced into reverse genetics virus. All viruses were successfully rescued and demonstrated a shift in pH of fusion similar to that observed in transfected cells expressing mutant HA proteins.

Residues in the fusion peptide pocket may universally regulate HA acid stability across all HA subtypes. Consistent with our findings for the H5 HA protein, previous studies have shown that residues in the fusion peptide pocket regulate the acid stabilities of H3 and H7 HA proteins (7, 16, 35, 39–41, 46). For example, the $Y23_1H$ mutation in the H5 HA protein in our study increased the pH of activation by 0.4 pH units. The reverse mutation in the H3 HA protein $H17_1Y$ (H3 numbering) has the opposite effect of decreasing the pH of activation by 0.3 pH units (46).

High-resolution structures have been determined for HA proteins of A/Vietnam/1194/04 (H5N1) and A/Vietnam/1203/04 (H5N1) viruses, which differ from the HA protein of A/chicken/Vietnam/C58/04 (H5N1) by only four and five amino acids, respectively (42, 52). In the high-resolution structures of the H5 HA protein, the $Y23_1$ residue is nearly completely buried by the fusion peptide after cleavage, and the hydroxyl group of the $Y23_1$ side chain forms a hydrogen bond with the backbone amine of fusion peptide residue $G13_2$ (Fig. 8A). An $Y23_1H$ mutation may destabilize the H5 HA protein

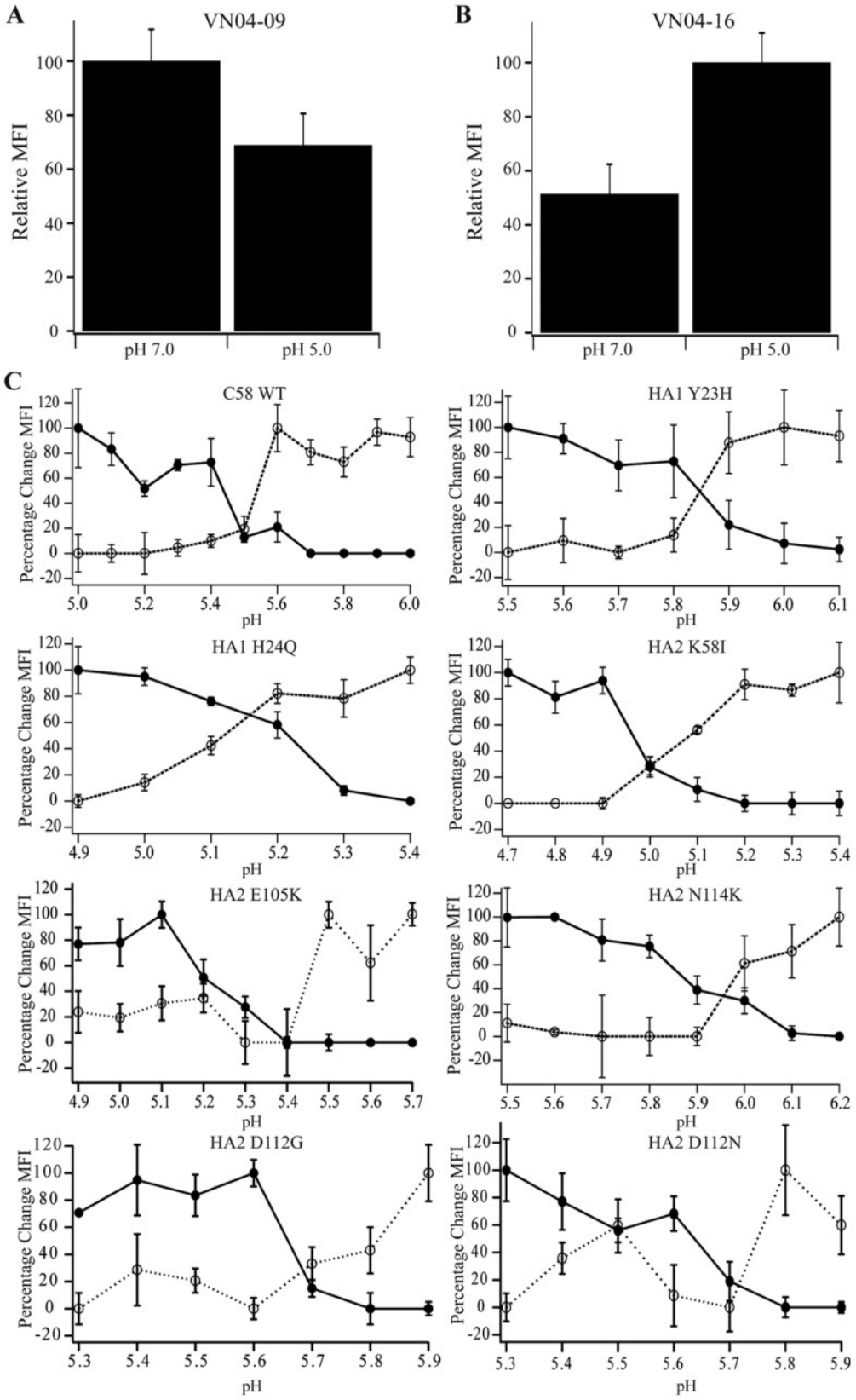

FIG. 7. Analysis of the pH of conformational changes by flow cytometry using monoclonal antibodies that bind preferentially to either the neutral or low-pH forms of the H5N1 HA protein. Vero cells were transfected with pCAGGS HA plasmids. At 16 h posttransfection, cells were stained and analyzed by flow cytometry. (A) Monoclonal antibody VN04-9 binding profile at neutral (7.0) and low (5.0) pH. (B) Monoclonal antibody VN04-16 binding profile at neutral (7.0) and low (5.0) pH. (C) Conformational change of wild-type and mutant HA proteins as characterized by the change in monoclonal antibody binding. The pH of the conformational change was determined as the point at which a 50% change in signal between maximum and baseline was observed. Dotted traces with open circles denote binding of VN04-9, and solid traces with filled circles show VN04-16 monoclonal antibody binding. The MFI was measured by flow cytometry.

TABLE 2. Initial characterization of mutant and wild-type rescued viruses

Virus	Mean ± SD			ΔpH[d]
	Peak titer[a]	Expression[b]	Cleavage[c]	
Wild type	6.03 ± 0.66	100 ± 1	0.66 ± 0.05	
$Y23_1H$	5.83 ± 0.25	103 ± 6	0.57 ± 0.04	0.4
$H24_1Q$	5.77 ± 0.47	98 ± 4	0.61 ± 0.09	−0.3
$K58_2I$	5.86 ± 0.28	98 ± 20	0.64 ± 0.09	−0.6
$N114_2K$	5.59 ± 0.24	99 ± 3	0.48 ± 0.06	0.5

[a] The peak titer determined by a single-step growth curve 12 h postinfection at multiplicity of infection of 3. Titers expressed as $\log_{10}$ PFU/ml.

[b] Expression was determined by Western blotting of whole-cell lysates of infected cells. The results represent the total number of HA0+HA2 bands as detected by A0110 polyclonal antibody. Expression data were normalized to the value for wild-type C58 HA protein.

[c] The cleavage ratio of total cell lysates was determined by using the formula HA2/(HA0 + HA2).

[d] The change in pH of fusion (ΔpH) was determined by syncytium formation assay.

by a loss of this important hydrogen bonding interaction between the fusion peptide and its pocket. A comparison of H5 and H3 HA structures shows that the fusion peptide and fusion peptide pocket residues adopt similar conformations in both subtypes, thus allowing for similar interactions in both subtypes (Fig. 8). Therefore, the $H17_1Y$ mutation may stabilize the H3 HA protein by introducing energetically favorable hydrogen bonding between residue 17 in the fusion peptide pocket and the fusion peptide. In fact, a molecular model of tyrosine at position 17 in the H3 HA protein and a comparison with H1 HA protein structures containing native tyrosine residues at position 17 are consistent with the hydroxyl group on the tyrosine side chain interacting with residues 10 and 12 of the fusion peptide in the context of the H3 HA protein (46). In the H3 HA structure, the native histidine at HA1 position 17 forms hydrogen bonds with the fusion peptide via water molecules. Although the analogous $Y23_1H$ residue in the H5 HA protein may also form similar hydrogen bonds with the fusion peptide via water molecules, the observation that this mutation makes the HA protein less acid stable is consistent with such potential interactions being weaker than direct interactions between the native tyrosine residue at position 23 in H5 HA1 subunit. Further evidence that H3 HA residue 17 (H5 HA residue 23) plays a critical role in activating the HA protein for membrane fusion is supported by the observations that mutation of H3 HA protein residue $H17_1$ to alanine, arginine, or glutamine increases the pH of membrane fusion by 0.4, 0.7, and 0.9 pH units, respectively (7, 39, 46, 50). On the basis of similarities between the H3 and H5 HA proteins in this region, $Y23_1A$, $Y23_1R$, and $Y23_1Q$ mutations in H5 are expected to destabilize the HA protein to a greater extent than the $Y23_1H$ mutation characterized here; however, such mutational analyses have not yet been performed.

HA2 residue 111 is also located in the fusion peptide pocket and is conserved along structural group-specific lineages (16, 35). For the H5 HA protein, an $H111_2A$ mutation decreased its expression, eliminated HA0 precursor cleavage, and blocked membrane fusion. Similar to the H5 HA protein, the H2 HA protein is also in the H1 structural clade and contains a histidine at HA2 residue 111. For the H2 HA protein, an $H111_2A$ mutation also eliminates its cell surface expression (46). The H3 HA protein belongs to a distinct structural lineage (H3 lineage) and contains a threonine residue at HA2 residue 111. For the H3 HA protein, a $T111_2A$ mutation has little effect on acid stability and fusogenicity, and $T111_2H$ and $T111_2V$ mutations increase the pH of activation and membrane fusion by 0.6 and 0.3 pH units, respectively (46).

The H3 and H7 structural clades contain a glutamate residue at HA2 residue 114 in the fusion peptide pocket, whereas the H1 structural clade (that includes the H5 HA protein) and the H9 structural clade contain an asparagine at HA2 residue 114. Despite clade-specific differences in wild-type residues at this position, mutation of HA2 residue 114 to a lysine residue in the H3, H7, and H5 HA proteins appears to cause in increase in the pH of conformational changes for all proteins by ~0.5 pH units (7). Although the overall effects of this mutation on acid stability are similar across the HA structural clades, the localized interactions that contribute to acid stability of the HA protein may differ for H5. Although the $N114_2$ residue of the H5 HA protein interacts with the hydroxyl group of tyrosine 22 of the fusion peptide, the $E114_2$ side chain of the H3 HA protein is twisted nearly 180° to interact with glutamine 47 of the HA2 subunit. The similar destabilization of H3, H5, and H7 HA proteins by a lysine mutation at HA2 residue 114 may

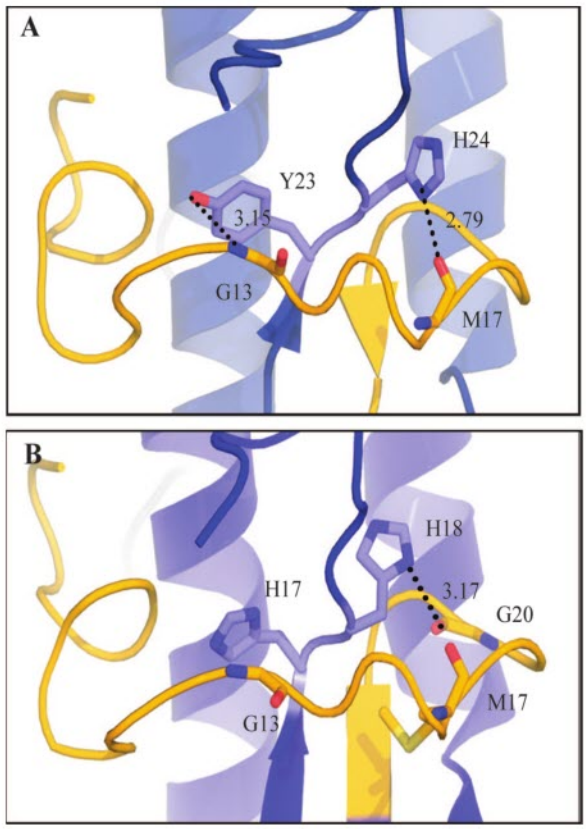

FIG. 8. Structural comparison of $Y23_1/H24_1$ residues in the H5 HA protein and the equivalent residues $H17_1/H18_1$ in the H3 HA protein. The HA1 subunit backbone is blue, and HA2 subunit fusion peptide backbone is yellow. Predicted bonding interactions are denoted by dashed lines with predicted bond lengths (in angstroms) given. Numbering in each diagram reflects that of the specific subtype in each case. (A) H5 HA protein using PDB:2fk0 (42); (B) H3 HA protein using PDB:1mql (17).

be due to steric hindrance or electrostatic disruptions, or both, of similar energetic magnitude in different local structural contexts.

In the H3 and H7 HA proteins, $D112_2G$ or $D112_2N$ mutations increase the pH of fusion by 0.4 and 0.35 pH units, respectively (7, 49). Mutation of $D112_2$ in the present study to either a glycine or asparagine residue increased the pH of conformational changes by ~0.3 pH units. However, both mutations caused membrane fusion at a pH similar to that for the wild-type C58 HA protein. An important role for HA2 residue D112 in regulating HA protein activation is consistent with its universal conservation across all known HA subtypes and selection on multiple occasions for H3 and H7 influenza viruses in the presence of drugs that elevate endosomal pH (7, 39, 46). A high-resolution structure of the H3 HA protein containing a $D112_2G$ mutation shows that a water molecule partially replaces the aspartate side chain, the mutation does not cause changes in the surrounding structure, and the mutation results in the loss of four intrachain hydrogen bonds with the fusion peptide (49). The data in our study suggest that the putative loss of similar intrachain hydrogen bonds in the H5 HA protein is not sufficient to destabilize the metastable conformation and trigger full membrane fusion. Previous studies of acid inactivation of H1, H2, and H3 HA proteins have provided evidence for conformational intermediates of the HA protein that undergo changes in tertiary structure but remain capable of fusion at low pH (25). This might be possible if the conformational intermediate involves limited movement of the HA1 subunit, which in turn facilitates release of the fusion peptide from its buried location (30). All monoclonal antibodies used for conformational flow cytometry had epitopes within the HA1 subunit (22). Therefore, discrepancies between the pH of conformational change and the pH of membrane fusion may reflect a transition between two structurally distinct metastable forms of the protein. Equally, the pH pulse used for the conformational flow cytometry was extended from 5 to 15 min. It has previously been shown that certain transitions of the HA protein conformational change are pH reversible (4) and that mutations in the HA can affect fusion kinetics (29). Therefore, the differences observed between the pH of conformational change and the pH of fusion assays may represent a change in fusion kinetics as a result of changes in the rate of transition between conformational intermediates of fusion, all of which are required for full fusion to occur. Further work would have to be undertaken to resolve the mechanisms involved in this process.

Not all mutations had similar effects on the acid stability of the HA protein for the H5, H3, and H7 subtypes. In fact, the mutation of residue 105 in HA2 to lysine had opposite effects on the acid stabilities of H5 and H3 HA proteins, most likely due to subtype-specific differences in sequence and interactions at this position. H3, H7, and H9 structural clades have a glutamine residue at position 105 of HA2, whereas H1 and H5 structural clades have a glutamate at this position. Moreover, a $Q105_2K$ mutation increases the pH of activation of the H3 HA protein by 0.3 pH units (7), whereas our study shows that an $E105_2K$ mutation decreases the pH of activation of the H5 HA protein by ~0.2 pH units. In the H3 HA protein structure (17), the glutamine side chain forms hydrogen bonds with the backbone amide of HA1 residue 29 and with the aspartate side chain of HA2 residue 109 by a water molecule and has van der Waals contact with the histidine side chain of HA2 residue 106 from an adjacent monomer. The loss of such energetically favorable interactions because of a mutation to a lysine residue may explain why this mutation increases the pH of activation of the H3 HA protein. A $Q105_2R$ mutation also increases the pH of activation of the H3 HA protein by 0.3 pH units, whereas $Q105_2A$ and $Q105_2E$ mutations have less pronounced effects (46). In the H5N1 HA protein structures (42, 52), the glutamate side chain at position 105 in HA2 does not have stabilizing interactions that are as extensive but may have electrostatic repulsion between residues $E105_2$ and $D109_2$. The reversal of side chain charge due to an $E105_2K$ mutation may increase the acid stability of the H5 HA protein by introducing electrostatic attraction between residue 105 and 109 in HA2.

The introduction of $Y23_1H$, $H24_1Q$, $K58_2I$, and $N114_2K$ mutations into the HA proteins of reverse genetics virus was tolerated. Examination of the pH of fusion for the mutant and wild-type viruses confirmed that the magnitude and direction of changes in the pH of fusion observed in cells expressing HA expressed as a result of transient transfection was similar to that observed in cells that had been infected with virus. Expression and cleavage of the mutant HA proteins was similar to that observed in the wild-type virus, suggesting that these shifts in pH of fusion were not a result of changes in either of these traits. Furthermore, the mutant viruses were able to replicate over a single cycle to titers analogous to wild-type virus. These observations underscore that the mutant viruses were capable of virus entry, membrane fusion, protein expression, cleavage, assembly, and budding over a single cycle of replication, despite demonstrating an altered pH of fusion. Although the mutations used in the present study represent changes between subtype, and not those present in isolated H5N1 influenza viruses, further characterization of these viruses will provide a model for the contribution of the pH of fusion to the phenotype of the virus with respect to transmissibility, host adaptation, and pathogenesis.

In summary, we have found that residues in the fusion peptide pocket play an important role in regulating the pH of activation of the HA protein from an H5N1 influenza A virus. Residues in other regions of the H5 HA molecule, such as K58 in HA2, can also regulate its acid stability. A comparison of our data on the H5 HA protein to previously reported findings on the H3 and H7 HA proteins shows that residues regulating acid stability are subtype specific and depend on local structure and interactions that occur between noncovalent bonding partners within this local structure. In cases where the orientation of local structural elements is similar between subtypes or strains, some mutations, such as at position 17 of HA1 (H3 numbering) and position 58 of HA2 (46), have similar effects on acid stability of the HA protein. However, in cases where the local structure may differ between strains or subtypes, the relative contribution of similar mutations to HA protein acid stability may differ, as seen for residue 105 in the HA2 subunit. When four residues capable of changing the pH of fusion were introduced into reverse genetics virus, they had a conserved effect on the pH of fusion with respect to the virus, while not having any substantial effects on initial virus growth or protein expression.

A potential role for changes in the acid stability of the HA

protein in the adaptation of influenza A viruses to different species has already been established for H3N2 and H7N3 viruses (15, 27). The importance of the HA protein in virus pathogenesis and host range has also been established. The presence of a polybasic cleavage site within H5 and H7 HA proteins results in intracellular cleavage by ubiquitous proteases and leads to systemic infection and greater pathogenicity in vivo (12, 32, 47). Changes in the receptor binding specificity of the HA1 subunit also alter the host range and cell tropism of influenza A viruses (18, 38). In our study, two mutations ($Y23_1H$ and $N114_2K$) increased the pH of activation of individually expressed H5 HA proteins in vitro and three mutations ($H24_1Q$, $K58_2I$, and $E105_2K$) decreased the pH of activation. The contribution of the pH of fusion to influenza virus phenotype has yet to be fully determined. However, the stability of the HA protein may contribute to the longevity of the virus in the environment (1) and therefore the ease with which the virus is transmitted. Equally, changes in the acid stability of the virus may determine the organs in which the virus can readily replicate. For example, a virus's ability to spread into the low-pH environment of the digestive tract of the host may contribute to the lethality of the virus due to an increase in the number of organs where the virus can productively replicate. Equally, this may also determine the route of shedding of the virus, since this is a trait that has been shown to differ between H5N1 viruses (44). These are some of the factors that will be investigated in more detail in future work.

ACKNOWLEDGMENTS

We thank Elena Govorkova for generously providing the monoclonal antibodies. We thank Robert Lamb for the pCAGGS influenza virus Udorn HA plasmid and Karl-Klaus Conzelmann for the BSR-T7/5 cells. We thank Elena Govorkova for helpful discussions and Vani Shanker for editing the manuscript. We also thank Scott Krauss, Patrick Seiler and David Carey for technical support in the biosafety level 3 facility and the Hartwell Center shared resource at St. Jude Children's Research Hospital for sequencing data.

This project has been funded with federal funds from the National Institute Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract HHSN266200700005C and was supported by the American Lebanese Syrian Associated Charities.

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JOURNAL OF VIROLOGY, Feb. 2010, p. 1527–1535 Vol. 84, No. 3
0022-538X/10/$12.00 doi:10.1128/JVI.02069-09

The pH of Activation of the Hemagglutinin Protein Regulates H5N1 Influenza Virus Pathogenicity and Transmissibility in Ducks▽

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Received 30 September 2009/Accepted 7 November 2009

While the molecular mechanism of membrane fusion by the influenza virus hemagglutinin (HA) protein has been studied extensively *in vitro*, the role of acid-dependent HA protein activation in virus replication, pathogenesis, and transmission *in vivo* has not been characterized. To investigate the biological significance of the pH of activation of the HA protein, we compared the properties of four recombinant viruses with altered HA protein acid stability to those of wild-type influenza virus A/chicken/Vietnam/C58/04 (H5N1) *in vitro* and in mallards. Membrane fusion by wild-type virus was activated at pH 5.9. Wild-type virus had a calculated environmental persistence of 62 days and caused extensive morbidity, mortality, shedding, and transmission in mallards. An N114K mutation that increased the pH of HA activation by 0.5 unit resulted in decreased replication, genetic stability, and environmental stability. Changes of +0.4 and −0.5 unit in the pH of activation by Y23H and K58I mutations, respectively, reduced weight loss, mortality, shedding, and transmission in mallards. An H24Q mutation that decreased the pH of activation by 0.3 unit resulted in weight loss, mortality, clinical symptoms, and shedding similar to those of the wild type. However, the HA-H24$_1$Q virus was shed more extensively into drinking water and persisted longer in the environment. The pH of activation of the H5 HA protein plays a key role in the propagation of H5N1 influenza viruses in ducks and may be a novel molecular factor in the ecology of influenza viruses. The data also demonstrate that H5N1 neuraminidase activity increases the pH of activation of the HA protein *in vitro*.

Highly pathogenic H5N1 influenza viruses were transmitted to humans in 1997 in Southeast Asia (7) and have subsequently spread across Asia, Europe, and Africa (53). Millions of poultry have been culled to control outbreaks (19), and more than 250 human lives have been lost (http://www.who.int/csr/disease/avian_influenza/en/). These viruses appear currently to lack the molecular properties required for sustained transmission among humans. There is an urgent need to understand the molecular properties that contribute to the transmission and host range of these viruses for their effective surveillance and containment.

The transmissibility and pathogenicity of influenza A viruses, including the H5N1 subtype, in avian and mammalian species are determined by both viral and host factors (8, 39). One key factor is the multifunctional hemagglutinin (HA) protein. During viral entry, the HA protein binds to sialic acid-containing receptors on host cells; the virus then undergoes endocytosis, and its HA protein is activated at a low pH to cause the fusion of the viral and endosomal membranes (11, 41). The host range of influenza A viruses depends in large part on the receptor specificity of the HA protein. Avian influenza viruses generally bind to α(2,3) sialosides with greater affinity, while human influenza viruses usually bind to α(2,6) sialosides with greater affinity (4, 37). The receptor binding affinities and specificities of HA proteins also depend on internal linkages and modifications of inner oligosaccharides, and glycan microarray profiling has revealed differences in receptor binding between seasonal human influenza viruses and H5N1 viruses (23, 45, 46). Thus, the natural distribution of various sialosides in different tissues of different species helps to determine both tissue tropism and species specificity (31, 40, 50, 58). The posttranslational cleavability of the HA0 precursor protein into the fusion-capable HA1–HA2 complex is a critical determinant of the virulence of influenza viruses (16, 22, 55). The presence of a polybasic cleavage site in H5 and H7 influenza viruses allows HA protein cleavage in the *trans*-Golgi network by ubiquitous furin-like enzymes and is a marker of high pathogenicity (12, 38, 55).

During entry into host cells, influenza viruses are exposed to increasingly lower pHs until a threshold is reached at which HA protein trimers undergo irreversible conformational changes that promote membrane fusion (11, 41). Threshold pH values differ among influenza viruses, and a change in the pH of fusion of the HA protein can help influenza viruses to adapt to

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▽ Published ahead of print on 18 November 2009.

different cell lines (5, 25) and host species (13) or to the higher endosomal pH induced by high concentrations of the antiviral drug amantadine (6, 9, 42–44). In general, a high pH of HA protein activation could result in influenza virus inactivation in the environment or during transport to the cell surface for intracellularly cleaved HA proteins (2, 44). On the other hand, a low pH of HA protein activation could result in degradation in the lysosome as the pH of the endocytic pathway decreases from early endosomes to late endosomes to lysosomes (61). Therefore, for efficient propagation within a biological host and ecological niche, an influenza virus may have an optimal range of pHs of activation for the HA protein. Moreover, the optimal activation pH may change upon introduction of an influenza virus into a new host species or environment.

Aquatic birds are a natural reservoir of influenza viruses, but surprisingly little is known about the molecular basis of influenza virus propagation in these species. To test the hypothesis that the pH of activation of the HA protein contributes to the pathogenicity and transmissibility of H5N1 influenza viruses in the mallard, a prototypic aquatic bird, we previously generated four recombinant H5N1 viruses containing mutations that altered the acid stability of the HA protein without changing its level of expression, cleavage, receptor binding, or membrane fusion efficiency (36). Two of the mutations increased the pH of membrane fusion of the H5N1 HA protein ($Y23_1H$ and $N114_2K$), and the other two mutations reduced the pH of fusion ($H24_1Q$ and $K58_2I$). HA1 mutations $Y23_1H$ and $H24_1Q$ (H5 numbering with subscripts denoting HA1 and HA2 subunits) are located in the fusion peptide pocket and were originally chosen because of their presence in H1 and H9 subtypes, respectively. The $K58_2I$ mutation in the A-helix of HA2 was chosen because it decreases the pH of membrane fusion of the H3 HA protein by 0.7 unit (44). The $N114_2K$ mutation in the fusion peptide pocket was chosen because it increases the pH of membrane fusion by approximately 0.5 unit in H3 and H7 subtypes (6). Here we measured the effects of the H5 HA protein mutations on virus replication *in vitro*, on genetic stability after repeated passage in eggs, and on environmental stability. Mallards were inoculated with the recombinant viruses and were housed with contact ducks in order to determine the effects of the mutations on virus shedding, pathogenesis, and transmissibility. An $H24_1Q$ mutation in the HA protein was found to decrease the pH of activation by 0.3 pH unit, to increase the titers of infectious virus recovered from ducks' water dishes, and to prolong the persistence of infectious virus in the environment. In general, changes in the acid stability of the HA protein were found to alter H5N1 influenza virus replication, pathogenicity, and transmissibility.

MATERIALS AND METHODS

Viruses, plasmids, and cell culture. Recombinant viruses and plasmids containing HA protein mutations $Y23_1H$, $H24_1Q$, $K58_2I$, and $N114_2K$ were generated previously (36). All experiments using H5N1 influenza viruses were performed in a USDA-approved biosafety level 3+ containment facility. Monolayer cultures of Vero cells (ATCC CCL-81) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% glutamine, 1% penicillin, and 1% streptomycin. Monolayers of Madin-Darby canine kidney (MDCK) cells (ATCC CCL-34) were grown in minimum essential medium (MEM) supplemented with 10% fetal bovine serum, 1% glutamine, 1% penicillin, and 1% streptomycin.

Virus growth kinetics. Single-step growth curves in MDCK cells were determined for each of the recombinant viruses. Confluent monolayers were infected at a multiplicity of infection (MOI) of ~3 PFU per cell. After 1 h of incubation, cells were first washed with a 0.9% aqueous NaCl solution (pH 2.2) to remove any free infectious virus particles and were then washed twice with phosphate-buffered saline (PBS) to adjust the pH. Cells were incubated at 37°C in MEM (containing 4% bovine serum albumin and 1% glutamine). Supernatants were collected 2, 4, 6, 8, and 10 h postinfection and were stored at −70°C. To determine multiple-step growth kinetics, MDCK cells were infected at an MOI of ~0.01 PFU/cell. After 1 h of incubation, cells were washed twice with PBS and were incubated at 37°C in MEM (containing 4% bovine serum albumin and 1% glutamine). Supernatants were collected 12, 24, 36, 48, 60, and 72 h postinfection and were stored at −70°C. The virus was titrated as described previously (60). Briefly, confluent MDCK cells were incubated for 1 h at 37°C with 10-fold serial dilutions of virus in 1 ml infection medium. The cells were then washed and overlaid with freshly prepared MEM containing 0.3% bovine serum albumin and 0.9% Bacto agar. After incubation at 37°C for 3 days, plaques were visualized by using a 0.1% crystal violet solution containing 10% formaldehyde.

Genetic stability. The H5N1 influenza viruses were serially passaged in 10-day-old embryonated chicken eggs to assess the genetic stability of the introduced mutations. Eggs were infected with 1 HA unit of sequence-confirmed virus. Allantoic fluid was collected, and the HA titer was measured to determine the dilution for subsequent passage of the virus. RNA was extracted and sequenced as described above.

Environmental stability. Stocks of recombinant viruses were diluted 1:50 in distilled water (pH 7.4) containing 2 mM HEPES buffer. Aliquots were incubated at 28°C (the approximate environmental temperature in Louisiana during the summer, allowing comparison with data from similar studies) (2). Aliquots were removed daily for 8 days, and their titers measured by plaque assay were compared to the initial virus titer. The sequential data were $\log_{10}$ transformed and analyzed by linear regression using GraphPad Prism software (GraphPad Software, La Jolla, CA). The gradient from this model was then used to calculate the estimated persistence of 1×10^6 PFU/ml of recombinant virus and the time required to reduce the infectivity of the initial inoculum by 90% (1 $\log_{10}$). Differences in the linear regression models were measured by using GraphPad Prism software.

Inoculation and transmission studies of mallards. Groups of three 4-week-old mallards (*Anas platyrhynchos*) were inoculated via intranasal, intraocular, and intratracheal instillation of $\sim 10^6$ 50% egg infective doses (EID_{50}) of virus in a 1-ml volume, as described previously (21). Two uninoculated contact ducks were placed in the cage with the inoculated ducks 24 h postinoculation (p.i.), and shared a common food and water source. Birds were weighed and observed daily for signs of morbidity or mortality over a period of 14 days. Birds that did not eat or drink on their own due to severe disease signs were euthanized, and their deaths were recorded on the following day of observation. Tracheal and cloacal swabs were collected from all ducks on days 3, 5, 7, and 10 p.i., and 0.5 ml of drinking water was sampled on days 1, 3, 5, 7, and 10 p.i. Influenza virus was detected by virus isolation in 10-day-old embryonated chicken eggs as previously described (14, 47). The virus was titrated in positive samples by calculating the EID_{50}, using the method of Reed and Muench (35); the lower limit of quantification was 0.75 $\log_{10}$ EID_{50}/ml. Swab samples with detectable influenza virus but titers below the limit of quantification were reported as having a titer of $<10^1$ EID_{50}/ml. All data shown were derived from two separate experiments. All animal experiments were approved by the Animal Care and Use Committee of St. Jude Children's Research Hospital (Memphis, TN) and were performed in compliance with relevant institutional policies, the Association for the Accreditation of Laboratory Animal Care guidelines, the National Institutes of Health regulations, and local, state, and federal laws.

Transient expression of HA and NA proteins. Monolayers of Vero cells in 6-well dishes (85 to 95% confluence) were transiently transfected with 1 μg of pCAGGS A/chicken/Vietnam/C58/04 HA DNA by using the Lipofectamine Plus expression system (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Transfected Vero cells were incubated for 4 h at 37°C. DMEM (containing 10% fetal bovine serum and 1% glutamine) was then added to cells, and cells were incubated for 16 h at 37°C. Cells were then treated as indicated for each experiment. Neuraminidase (NA) protein was expressed by using 0.1 to 1.0 μg of the pCAGGS A/chicken/Vietnam/C58/04 NA plasmid.

Syncytium assay. Monolayers of Vero cells grown in 6-well plates were transfected with 1.0 μg pCAGGS HA as described above or were infected with recombinant virus at an MOI of ~3 PFU per cell. At 16 h posttransfection or 6 h postinfection, cell monolayers were overlaid for 5 min with phosphate-buffered saline with magnesium and calcium (PBS+) that was adjusted to the reported pH with a 0.1 pH unit resolution using 0.1 M citric acid. Cells were neutralized by using DMEM (containing 10% fetal bovine serum and 1% glutamine) and were incubated at 37°C for 2 h. Samples were fixed and stained with a Hema 3 stat pack staining kit (Fisher) according to the manufacturer's instructions. Repre-

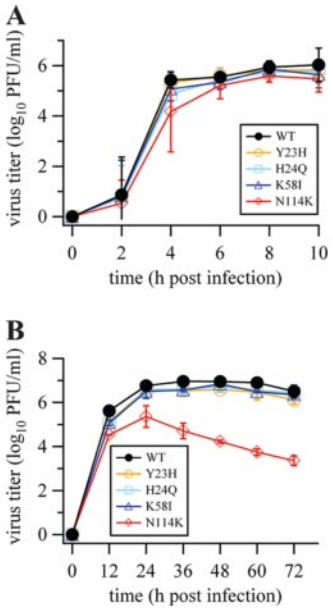

FIG. 1. Replication kinetics of recombinant A/chicken/Vietnam/C58/04 (H5N1) influenza viruses in MDCK cells. (A) For single-step growth curves, cells were infected at an MOI of 3 PFU/cell with wild-type virus or viruses containing HA protein mutation $Y23_1H$, $H24_1Q$, $K58_2I$, or $N114_2K$. (B) For multiple-step growth curves, cells were infected with the recombinant viruses at an MOI of 0.01 PFU/cell. The supernatant was collected at the indicated times, and the virus was quantified by a plaque assay. Each point represents the mean ± standard deviation from three experiments.

sentative microscopic fields were captured with a Nikon D70 digital camera attached to a Nikon Eclipse TS100 inverted microscope (26).

NA activity assay and NA inhibition. A modified fluorometric assay was used to determine the enzymatic activity of the NA protein present in transfected cell lysates with the fluorogenic substrate 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MUNANA; Sigma, St. Louis, MO) (15, 34, 59). The fluorescence of the released 4-methylumbelliferone was measured in a Fluoroskan II spectrophotometer (Labsystems, Helsinki, Finland) using excitation and emission wavelengths of 355 and 460 nm, respectively. The enzymatic activity of NA protein was standardized to 0.1 mg total protein by using a bicinchoninic acid assay (Sigma, St. Louis, MO) and was expressed as the quantity of substrate (in picomoles) converted during a 30-min incubation at 37°C. The NA inhibitor oseltamivir carboxylate ([3*R*,4*R*,5*S*]-4-acetamido-5-amino-3-[1-ethylpropoxy]-1-cyclohexene-1-carboxylic acid) was provided by Hoffmann-La Roche, Ltd. (Basel, Switzerland). The compound was dissolved in distilled water, and aliquots were stored at −20°C until use. NA activity was inhibited by using a 4 μM concentration of oseltamivir carboxylate added immediately posttransfection or 1 h before the assay. The effect of an NA protein inhibitor on the pH of fusion was determined by performing a syncytium formation assay in parallel.

RESULTS

The HA protein mutations have little effect on the *in vitro* replication kinetics of recombinant H5N1 influenza viruses. In a previous study, we identified four mutant H5 HA proteins whose pH values of membrane fusion differed from that of wild-type HA protein when expressed from transiently transfected plasmid DNA (36). Here we determined whether the HA protein mutations affected the *in vitro* replication kinetics of recombinant H5N1 influenza viruses by generating single-step and multiple-step growth curves. Single-step growth curves showed that mutant and wild-type viruses grew at similar rates over the 10-h time course (Fig. 1A). In multiple-step growth curves, viruses containing the HA protein mutations $Y23_1H$, $H24_1Q$, and $K58_2I$ had replication rates similar to that of wild-type virus (Fig. 1B). Titers of the virus containing an $N114_2K$ mutation in the HA protein were similar to those of wild-type virus at the 12-h time point but were later reduced by 1 to 3 $\log_{10}$ units.

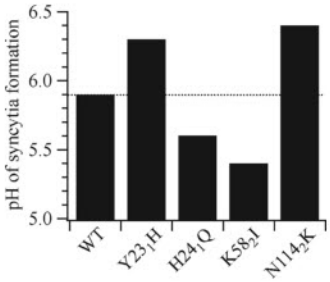

FIG. 2. The pH of HA-mediated membrane fusion by wild-type and mutant H5N1 influenza viruses in Vero cells was measured at 0.1 pH increments and is expressed as the highest pH value at which syncytium formation was observed.

The HA protein mutations alter the pH of membrane fusion *in vitro*. The cell surface expression, cleavage, receptor binding affinities, and membrane fusion efficiencies of the mutant HA proteins in Vero cells were previously found to be similar to those of wild-type virus (36). Moreover, infection of DF-1 primary chicken embryonic fibroblasts with the viruses resulted in HA protein properties similar to those found when Vero cells were infected with the viruses. To determine the effects of the mutations on the pH of membrane fusion, monolayers of MDCK cells were first infected with recombinant H5N1 influenza viruses at an MOI of ~3 PFU/cell and then exposed to PBS solutions of varying pHs, at a resolution of 0.1 unit. The highest pH at which cell-cell membrane fusion was induced in cells infected with wild-type virus was 5.9 (Fig. 2). The $H24_1Q$ and $K58_2I$ mutations reduced the pH of membrane fusion to 5.6 and 5.4, respectively, while the $Y23_1H$ and $N114_2K$ mutations increased the pH of membrane fusion to 6.3 and 6.4, respectively. The $N114_2K$ mutation, which increased the pH of HA activation by 0.5 pH unit, resulted in decreased virus fitness over several cycles of replication *in vitro*, while the other mutations did not alter *in vitro* replication kinetics.

The $N114_2K$ mutation is genetically unstable over multiple passages in eggs. To test the genetic stability of the HA protein mutations, wild-type and mutant viruses were passaged 10 times in 10-day-old embryonated chicken eggs. The sequence identity of each of the passage 1 (P1) recombinant viruses had been confirmed previously (36). Purified viral RNA sampled from allantoic fluid at P5 and P10 was sequenced. In parallel, syncytium formation assays were performed using Vero cells infected with P10 viruses to determine whether repeated passage in eggs resulted in any mutations that might alter the acid stability of the viral HA proteins (Table 1). Wild-type virus and viruses containing the HA protein mutation $H24_1Q$ or $K58_2I$ showed no additional mutations over the course of 10 passages and no change in the pH of membrane fusion. The virus containing the $Y23_1H$ mutation maintained the mutation for at least 5 passages in eggs and acquired an additional HA protein mutation, $R228_1I$, between P5 and P10. Residue R228 (H5 numbering) is located in the receptor-binding pocket of the HA1 subunit with its side chain facing away from the pocket

TABLE 1. Genetic stability of recombinant H5N1 influenza viruses containing HA protein mutations after serial passages in embryonated chicken eggs

P1 virus	ΔpH[a] at P1	Mutation[b] at:		ΔpH at P10
		P5	P10	
Wild type		—	—	
$Y23_1H$	+0.4	—	$R228_1I$	+0.4
$H24_1Q$	−0.3	—	—	−0.3
$K58_2I$	−0.5	—	—	−0.5
$N114_2K$	+0.5	$K114_2N$	$K114_2N$	+0.2

[a] ΔpH, change in the pH of membrane fusion from that of the wild-type virus as measured by a syncytium formation assay.
[b] —, no change in the HA protein sequence from that of the P1 virus. All of the mutations reported are in the HA gene (H5 numbering), and there were no amino acid sequence changes in the other genes.

(46, 57) such that the $R228_1I$ mutation may enhance receptor binding in eggs (56). Despite the extra $R228_1I$ mutation, the P10 virus caused membrane fusion at a pH of 6.3, as did P1 $Y23_1H$ virus without the additional $R228_1I$ mutation. The virus containing the $N114_2K$ mutation in the HA protein was the only recombinant virus whose pH of membrane fusion was altered at P10 from that for the P1 stock virus, a decrease from pH 6.4 to 6.1 (Table 1). Both P5 and P10 $K114_2N$ viruses showed reversion mutations, demonstrating that the $N114_2K$ mutation was not genetically stable and was selected against within 5 passages.

Changes in the pH of activation of the HA protein can alter the environmental stability of H5N1 influenza viruses. The environmental stability of highly pathogenic H5N1 isolates has been found to be lower than that of lower-pathogenicity viruses (1, 2). To determine whether changes in the pH of fusion of the HA protein alter the environmental stability of H5N1 viruses, we incubated the viruses in the present study at 28°C for 8 days and measured the virus titer as a function of time by a plaque assay. Data from each series were plotted, and the gradient of virus degradation was calculated by linear regression analysis (Table 2). The wild-type virus and the virus containing a $Y23_1H$ mutation in the HA protein showed similar rates of titer reduction (1 $\log_{10}$ unit every 10 days), a rate of degradation that matches those of other highly pathogenic H5N1 isolates (2). This result suggests that changes in the pH of fusion as great as +0.4 pH unit can be tolerated without a loss in environmental stability. Viruses containing the $H24_1Q$ or $K58_2I$ mutation, both of which promoted membrane fusion at lower pH values than wild-type virus,

TABLE 2. Environmental stability of H5N1 influenza viruses in water at 28°C

Virus	LRM[a]	R^2	Estimated persistence (days)[b]
Wild type	$6.8742 - 0.0974x$	0.7625	62 (10)
$Y23_1H$	$6.1717 - 0.0991x$	0.7599	61 (10)
$H24_1Q$	$6.9496 - 0.0775x$	0.8364	77 (13)
$K58_2I$	$6.6408 - 0.0761x$	0.7151	79 (13)
$N114_2K$	$5.6297 - 0.4234x$	0.9680	14 (2)

[a] LRM, linear regression model, where y is the virus titer ($\log_{10}$ PFU/ml) and x is persistence (in days).
[b] With a starting virus titer of 1×10^6 PFU/ml. Numbers in parentheses are days required to reduce the initial virus titer by 1 $\log_{10}$ unit.

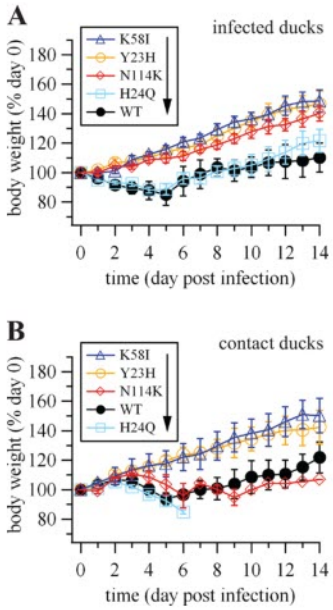

FIG. 3. Weight change in mallards infected with mutant and wild-type H5N1 influenza viruses. (A) Groups of ducks were inoculated with 10^6 EID_{50} of recombinant virus. (B) Contact ducks were introduced into each group's cage 1 day p.i. Ducks were weighed daily for 14 days. Data points (and error bars) represent the mean (± standard deviation) weight changes. Viruses listed in the figure keys are ordered by increasing weight loss.

were calculated to lose 1 $\log_{10}$ unit in their titers every 13 days. Thus, the two mutant viruses with lower pH values of activation retained infectivity longer than the wild-type virus. The virus containing an $N114_2K$ mutation rapidly lost infectivity in the environmental stability experiment, losing 1 $\log_{10}$ unit in its titer approximately every 2 days (Table 2). Therefore, an increase in the pH of HA activation to 6.4 due to the $N114_2K$ mutation resulted in greatly reduced environmental stability, and a decrease in the pH of activation of the HA protein to 5.6 or 5.4 due to the $H24_1Q$ or $K58_2I$ mutation, respectively, moderately increased environmental stability.

The pH of activation of the HA protein contributes to the pathogenicity and transmissibility of H5N1 viruses in mallards. To measure the biological properties of the mutant viruses in mallards, we inoculated duplicate groups of three animals and introduced two contact animals into the cage of each group after 1 day. The wild-type and $H24_1Q$ viruses induced considerable weight loss in both inoculated and contact animals (Fig. 3) and caused death in 60% and 70% of animals, respectively (Table 3). In contrast, the $Y23_1H$ and $K58_2I$ viruses did not induce weight loss or death in either inoculated or contact animals. Moreover, the $Y23_1H$ virus caused only cloudy eyes for 50% of the inoculated ducks, while the $K58_2I$ virus caused cloudy eyes only for one contact duck. While the virus containing an $N114_2K$ mutation in the HA protein did not induce weight loss or death in inoculated ducks, contact animals in this group unexpectedly showed weight loss after 4 days, and three of the four contact animals died. Neurological signs were observed in these contact animals, whereas none were observed in the inoculated group. Because of these unexpected findings, we sequenced viral RNA isolated from pos-

TABLE 3. Morbidity and mortality caused by the recombinant H5N1 influenza viruses in mallards[a]

Virus and infection route	No./total:		
	Dead	With cloudy eyes	With neurological signs[b]
Wild type			
Inoculation	4/6	5/6	3/6
Contact	2/4	4/4	1/4
$Y23_1H$			
Inoculation	0/6	3/6	0/6
Contact	0/4	0/4	0/4
$H24_1Q$			
Inoculation	3/6	4/6	3/6
Contact	4/4	2/4	2/4
$K58_2I$			
Inoculation	0/6	0/6	0/6
Contact	0/4	1/4	0/4
$N114_2K$			
Inoculation	0/6	2/6	0/6
Contact	3/4	2/4	2/4

[a] Data are from two separate experiments. In each, 3 ducks were inoculated with 10^6 EID_{50} of virus and 2 naïve contact birds were introduced into the cage 24 h p.i. Birds were observed daily.
[b] Twitching head, ataxia, violent tremors, severe torticollis, and/or loss of balance.

itive swabs from surviving inoculated and contact birds on days 7 and 10. In all cases, the $N114_2K$ mutation had reverted to the wild-type $N114_2$, as it had after serial passage in eggs (Table 1). This reversion offers the most plausible explanation for the increased transmissibility and pathogenicity in contact birds in the $N114_2K$ group. The reversion mutation also explains the greater weight loss, morbidity, and mortality in the contact birds than in the infected birds in this group. No other reversion mutations were sequenced from swabs of contact ducks infected with the other viruses, including the $H24_1Q$ virus.

To assess replication and transmission potential, titers of virus shed from the trachea and cloaca were measured (Table 4). Inoculated birds in all groups shed virus on day 3; therefore, it is clear that all of the viruses were capable of initial infection and replication. However, even at this early time point, the viruses containing HA protein mutation $Y23_1H$ or $K58_2I$ productively infected fewer ducks than wild-type and $H24_1Q$ viruses. Wild-type and $H24_1Q$ viruses were shed at similar levels on days 3 and 5. On day 7, wild-type virus was not shed, whereas the $H24_1Q$ virus continued to be shed. All contact birds in the wild-type and $HA24_1Q$ groups were shedding virus by day 3 p.i. (100% transmission). All contact birds in the $H24_1Q$ group succumbed to infection, whereas only half of the contact birds in the wild-type group died. The $Y23_1H$ virus was not detected in any contact birds throughout the experiment, showing that the mutation results in attenuated transmission compared to that of the wild-type virus. Five days p.i., the $K58_2I$ virus was detected in one inoculated bird and one contact bird, showing that its fitness and transmissibility were lower than that of the wild-type virus. The $N114_2K$ virus showed inconsistent shedding in inoculated birds. Inoculated

TABLE 4. Tracheal and cloacal shedding of H5N1 influenza viruses by inoculated and contact ducks

Virus	No. of inoculated or contact ducks shedding virus/total no. of ducks (mean titer of shed virus in positive swabs [$\log_{10}$ EID_{50}/ml][a])															
	Day 3 p.i.				Day 5 p.i.				Day 7 p.i.				Day 10 p.i.			
	Inoculated		Contact		Inoculated		Contact		Inoculated		Contact		Inoculated		Contact	
	Trachea	Cloaca	Trachea	Cloaca	Trachea	Cloaca	Trachea	Cloaca	Trachea	Cloaca	Trachea	Cloaca	Trachea	Cloaca	Trachea	Cloaca
Wild type	6/6 (2.5)	4/6 (1.4)	4/4 (3.1)	4/4 (1.6)	1/4 (<1)	2/4 (2.4)	3/3 (2.2)	2/3 (2.6)	0/2	0/2	1/2 (2.0)	0/2	0/2	0/2	0/2	0/2
$Y23_1H$	4/6 (1.6)	1/6 (1.3)	0/4	0/4	0/6	0/6	0/4	0/4	0/6	0/6	0/4	0/4	0/6	0/6	0/4	0/4
$H24_1Q$	6/6 (3.1)	5/6 (1.9)	4/4 (2.4)	4/4 (2.4)	3/5 (<1)	2/5 (1.6)	4/4 (2.3)	2/4 (1.4)	1/4 (1.5)	2/4 (2.0)	—[b]	—	0/2	0/2	—	—
$K58_2I$	4/6 (2.4)	3/6 (1.4)	1/4 (1.3)	0/4	0/6	1/6 (<1)	1/4 (1.3)	0/4	0/6	0/6	0/4	0/4	0/6	0/6	0/4	0/4
$N114_2K$	5/6 (3.2)	2/6 (3.1)	1/4 (1.8)	1/4 (1.5)	0/6	0/6	3/4 (1.3)	2/4 (2.0)	1/6 (2.5)	3/6 (1.1)	2/2 (2.1)	1/2 (1.8)	0/6	1/6 (1.8)	0/1	0/1

[a] Where swabs were positive but below the threshold of accurate measurement, a value of <1 $\log_{10}$ EID_{50}/ml was recorded.
[b] —, no animals survived.

TABLE 5. Titers of H5N1 influenza viruses in the water dishes of mallards[a]

Virus	Titer ($\log_{10}$ EID_{50}) on:				
	Day 1	Day 3	Day 5	Day 7	Day 10
Wild type	0.63	3.25	1.50	0	0
$Y23_1H$	0	0	0	0	0
$H24_1Q$	1.89	3.38	4.25	4.13	0
$K58_2I$	1.38	2.29	0	0	0
$N114_2K$	0	1	0.75	0	0

[a] Data are means from two separate experiments in which 3 ducks per group were inoculated with 10^6 EID_{50} of virus and 2 naïve contact birds were introduced into the cage 24 h p.i. Samples of drinking water (0.5 ml) were collected on days 1, 3, 5, 7, and 10 p.i. for virus titration in eggs.

birds shed virus on day 3 but not on day 5, yet some birds again shed virus on days 7 and 10. This result suggests that transmission to contact birds was mediated by the reverted $K114_2N$ virus, which was then transmitted back to inoculated ducks before being detected on days 7 and 10. On day 3 p.i., tracheal shedding was generally observed more often and at higher titers than cloacal shedding, consistent with previous work (48). The $H24_1Q$ and $K58_2I$ mutations did not appear to increase cloacal virus shedding; thus, small decreases in the pH of activation (and inactivation) of the HA protein may be insufficient to enhance virus replication in the low-pH environment of the duck digestive tract (49).

We also investigated shedding of the recombinant viruses by titrating virus in the ducks' water dishes. Wild-type virus was detected on days 1, 3, and 5 p.i. and had a peak titer of 3.25 $\log_{10}$ EID_{50} on day 3 (Table 5). No $Y23_1H$ virus was detected on any day, consistent with low shedding of this virus on day 3 and none on days 5, 7, and 10 p.i. The $K58_2I$ virus titer in the water dishes was comparable to that of the wild-type virus on days 1 and 3 but was subsequently undetectable, consistent with the pattern of virus shedding from the tracheae and cloacae of ducks (Table 4). The presence of the $N114_2K$ virus in water dishes on days 3 and 5 but not on day 1 is consistent with low-level shedding until after reversion. Higher titers of the $H24_1Q$ virus than of wild-type virus were detected in the water dishes on days 1 through 7, consistent with this mutant's greater environmental stability and lethality in contact ducks. Overall, our results show that the reduction of the pH of membrane fusion for the virus containing an $H24_1Q$ HA mutation enhances two properties that could promote H5N1 virus transmission in aquatic birds: shedding of virus into water and persistence of virus infectivity in water.

The properties of H5N1 influenza viruses reported in Fig. 1 to 3 and Tables 1 to 5 are summarized in Table 6.

NA activity promotes pH-mediated membrane fusion induced by the HA protein. The highest pH at which wild-type virus caused membrane fusion was 5.9 (Fig. 2); however, we previously found that wild-type HA protein expressed from transiently transfected plasmid DNA caused membrane fusion only when the pH was decreased to 5.5 (36). The occurrence of this change in the context of virus infection (with expression of all viral proteins) in the present study suggested that one or more of the other viral proteins promote acid-induced activation of the H5N1 HA protein. Previous studies have shown that the NA protein facilitates the entry of H3N2 influenza viruses (28, 32). To determine whether NA protein expression increases the pH of membrane fusion by the HA protein, we transfected Vero cells with the pCAGGS HA wild-type plasmid in the presence and absence of cotransfection with the pCAGGS NA wild-type plasmid. Titration showed that 0.1 μg of plasmid DNA produced neuraminidase activity similar to that in 10 μl of allantoic fluid containing virus (data not shown); therefore, a 1:0.1-μg ratio of HA to NA was used in all follow-up experiments. Transfected cells expressing the wild-

TABLE 6. Summary of properties of H5N1 influenza viruses[a] *in vitro* and in ducks

Mutation by H5 numbering[b]	Mutation by H3 numbering[c]	pH of membrane fusion[d]	*In vitro* growth rate[e]	Genetic stability[f]	Estimated environmental persistence[g]	Weight loss in directly infected ducks[h]	Mortality (%)[i]	Rank order of virus shedding[j]	Days on which virus was detected in ducks' water dishes[k]
$N114_2K$	$N114_2K$	6.4	+	No	14	−			
$Y23_1H$	$Y17_1H$	6.3	+++	Yes	61	−	0	4	None
Wild type		5.9	+++	Yes	62	+	60	2	1, 3, 5
$H24_1Q$	$H18_1Q$	5.6	+++	Yes	77	+	70	1	1, 3, 5, 7
$K58_2I$	$K58_2I$	5.4	+++	Yes	79	−	0	3	1, 3

[a] Recombinant influenza viruses in the background of A/chicken/Vietnam/C58/04 (H5N1).
[b] According to the number in the amino acid sequence of the H5 HA protein. The subscript "1" refers to numbering in HA1, and the subscript "2" refers to numbering in HA2, after cleavage.
[c] The number of the mutation in H5 has been converted to the conventional H3 numbering scheme.
[d] The highest pH at which syncytium formation was observed in Vero cells *in vitro*.
[e] Multiple-step growth rate in MDCK calls after infection with an MOI of 0.01 PFU/cell. Symbols represent a peak titer of ~5 $\log_{10}$ PFU/ml (+) or ~7 $\log_{10}$ PFU/ml (+++). Detailed data are reported in Fig. 1B.
[f] "No" means that the sequence reverted within 5 serial passages in the allantoic cavities of embryonated chicken eggs. "Yes" means that there were no mutations after 5 passages in eggs and no changes in the pH of membrane fusion after 10 passages in eggs.
[g] Expressed as the calculated number of days of virus persistence at 28°C (starting virus titer, 1×10^6 PFU/ml).
[h] −, continuous weight gain during the 14-day experiment; +, loss of 15 to 20% of the starting weight over the course of the first 5 days of infection.
[i] Calculated for a total of 6 directly infected ducks and 4 contact ducks. Data for the $N114_2K$ virus are excluded because this virus reverted to the wild-type sequence during the experiment.
[j] From tracheal and cloacal swabs taken from both directly infected and contact ducks. Data for the $N114_2K$ virus are excluded because this virus reverted to the wild-type sequence during the experiment. Detailed data are given in Table 4. Numbers are in descending rank order; i.e., 1 represents the highest level of shedding, and 4 represents the lowest.
[k] None, no detectable virus on days 1, 3, 5, 7, and 10. Data for the $N114_2K$ virus are excluded because this virus reverted to the wild-type sequence during the experiment. Detailed data are given in Table 5.

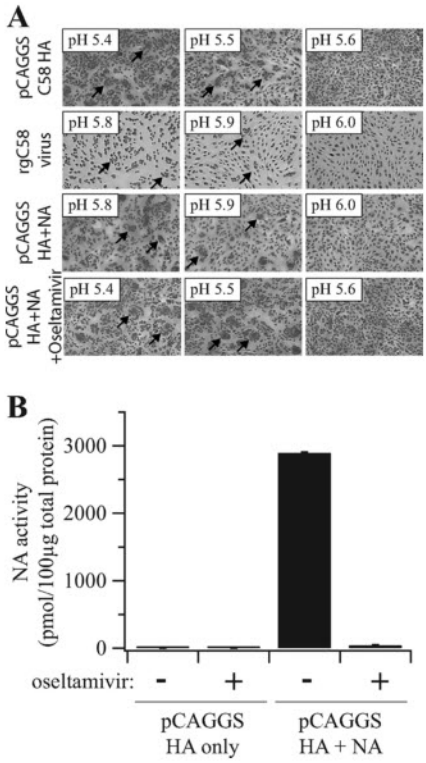

FIG. 4. Contribution of NA enzymatic activity to the pH of membrane fusion mediated by the HA protein. (A) Representative photomicrographs of syncytia showing the contribution of the NA protein to HA-mediated membrane fusion. The pH values are given in the top left corner of each micrograph. The arrows point to examples of syncytia. rgC58, reverse-genetics wild-type C58 strain of H5N1 influenza virus. (B) Mean neuraminidase activity as measured by a fluorescence-based assay using MUNANA as the substrate. Error bars represent the standard deviations from three independent determinations. Oseltamivir carboxylate (4 μM) was used to inhibit the enzymatic activity of the NA protein.

type H5N1 HA and NA surface proteins showed syncytium formation at pH 5.9, the same pH as that for wild-type virus (Fig. 4A). Having established that expression of the NA protein accounted for the observed increase in the pH of HA-mediated membrane fusion, we next examined whether NA enzymatic activity was responsible for the increase. NA enzymatic activity was eliminated in Vero cells cotransfected with pCAGGS HA and NA plasmids by treatment with oseltamivir carboxylate (3, 51) (Fig. 4B). The syncytium formation assay was repeated using cells coexpressing the HA and NA proteins in the presence of oseltamivir carboxylate. When NA enzymatic activity was inhibited by the drug, the pH of HA-mediated membrane fusion decreased to pH 5.5, the same value observed in cells expressing HA protein alone (Fig. 4A). These results are consistent with the promotion of H5 HA-mediated membrane fusion by N1 neuraminidase activity.

DISCUSSION

To investigate how the pH of activation of the HA protein influences the *in vitro* and *in vivo* properties of influenza viruses, we compared four recombinant viruses with altered pH-dependent HA protein stability to wild-type A/chicken/Vietnam/C58/04 (H5N1) virus. An $N114_2K$ mutation in the HA2 fusion peptide pocket region increased the activation pH of the HA protein from 5.9 to 6.4, allowing activation under mildly acidic conditions. This mutation dramatically reduced the fitness of the virus in three ways: (i) multiple-step replication *in vitro* was reduced by a factor greater than 10; (ii) infectivity in the environment decreased four times as rapidly as that of wild-type virus; and (iii) the virus reverted to the wild-type sequence within 5 passages in chicken eggs and after inoculation in mallards. The $N114_2K$ mutation may increase the pH of activation of the HA protein above the threshold pH at which a significant portion of intracellularly cleaved HA trimers become prematurely triggered, and inactivated, during transport to the cell surface (44). The HA protein mutations $Y23_1H$ and $K58_2I$ changed the activation pH of the HA protein to 6.3 and 5.4, respectively. While these two mutations had opposite effects on the activation pH, the recombinant viruses bearing the mutations had similar phenotypes. Despite *in vitro* replication rates similar to that of wild-type virus, the viruses bearing a $Y23_1H$ or $K58_2I$ mutation did not induce weight loss, neurological signs, or mortality in mallards, were not efficiently transmitted, and were shed significantly less. Overall, the data show that efficient and sustainable infection of mallards by H5N1 influenza virus is not supported by HA protein activation pH values less than 5.5 or greater than 6.2.

The results of experiments with the virus bearing an HA-$H24_1Q$ mutation suggest that robust infection in mallards is supported by activation pH values between 5.6 and 5.9. The data also raise the possibility that natural mutations that slightly reduce the pH of activation of the HA protein could increase the transmission of H5N1 influenza viruses among mallards. The wild-type virus and the HA-$H24_1Q$ virus had similar replication kinetics *in vitro* and induced similar weight loss, mortality, clinical signs, and shedding in mallards, but higher titers of the $H24_1Q$ virus were found in the ducks' water dishes, and the $H24_1Q$ virus retained infectivity ~20% longer than wild-type virus in an environmental stability experiment. The fact that all of the contact ducks succumbed to infection with transmitted $H24_1Q$ virus while only half died from transmitted wild-type virus also suggests that contact ducks were exposed to a larger inoculum of the $H24_1Q$ virus.

Our results demonstrate that the pH of activation of the HA protein plays a key role in the pathogenicity and transmissibility of H5N1 influenza viruses in mallards. Natural H5N1 virus isolates are highly pathogenic in many, but not all, duck species (21, 47, 48), and their transmission among wild ducks and from wild ducks to domestic poultry and mammals, including humans, has been a key element in their natural ecology (10, 33, 54). Moreover, wild ducks are thought to be a main reservoir of low-pathogenicity avian influenza viruses (33). The intraspecies and interspecies transmission of influenza viruses depends on at least four factors: (i) the amount of virus shed by the donor, (ii) the stability of the virus in the environment over time, (iii) the time between donor shedding and acceptor exposure, and (iv) the infectivity of the virus in the acceptor animal. Since the pH of activation of the HA protein was found here to determine both the amount of shedding from ducks and the stability of virus in the environment, this molecular property may have an essential role in the propagation of

H5N1 viruses in aquatic birds. Furthermore, HA mutations that maximize virus shedding and environmental stability via altered HA acid stability may be expected to promote both intraspecies and interspecies transmission. A broad survey of the environmental stability of 12 low-pathogenicity avian influenza viruses of various subtypes revealed that they were generally most stable at a slightly basic pH (7.4 to 8.2), a low temperature, and fresh to brackish salinity (1). The viruses lost infectivity much more rapidly after incubation under acidic conditions (pH <6.6), warmer temperatures, and higher salinity. Among the HA mutations characterized in the present study, the $N114_2K$ mutation increased the pH of activation to 6.4 while significantly reducing environmental stability, and the $H24_1Q$ and $K58_2I$ mutations reduced the pH of activation to 5.6 and 5.4, respectively, and moderately increased environmental stability. Thus, the pH of activation of the HA protein contributes to the duration of H5N1 influenza virus infectivity in the environment.

In the present study, an optimal range in the pH of activation of the HA protein supported the propagation of H5N1 influenza viruses in ducks. The adaptation of other subtypes of influenza viruses to different host tissues and species has been found to involve the selection of viruses with altered pH values for membrane fusion. A few passages of egg-grown recombinant X-31 influenza virus (H3N2 with the internal genes of A/PR/8/34 [H1N1]) in mammalian MDCK and Madin-Darby bovine kidney (MDBK) cells consistently resulted in HA protein mutations that increased the pH of HA-mediated membrane fusion from 5.2 to 5.6 to 5.8, and similar results were found after the passage of egg-grown A/Japan/305/57 (H2N2) virus in MDCK cells (25). The natural adaptation of H7N3 influenza viruses from wild ducks to turkeys coincided with two amino acid mutations in and near the HA2 stalk and a decrease in the pH of activation of the HA protein without a change in receptor binding (13). However, it is not known whether these mutations exclusively caused the reduction in the pH of membrane fusion, because the adaptation also resulted in a 23-amino-acid deletion in the NA stalk that reduced neuraminidase activity. We showed here that the absence of neuraminidase activity results in a lower pH of membrane fusion by the HA protein. Moreover, decreased neuraminidase activity in H3N2 influenza viruses has been shown to reduce virus entry (28, 32). In general, there may be a cooperative interaction between the neuraminidase activity of the NA protein and the fusogenicity of the HA protein. A functional balance between neuraminidase activity and the receptor binding activity of the HA protein is well known in many influenza virus subtypes (17, 20, 29, 30, 52).

In influenza viruses of the H3N2, H7N1, and H7N7 subtypes, an increase in the pH of activation of the HA protein results in resistance to high concentrations of amantadine (>0.1 mM), which raise the endosomal pH (6, 9, 18, 44). In a recombinant virus bearing the envelope glycoproteins of A/Netherlands/219/03 (H7N7), an $HA\text{-}G23_2C$ mutation in the fusion peptide that reduced the pH of membrane fusion from 5.4 to 4.4 reduced *in vitro* replication by more than 2 $\log_{10}$ units and increased the 50% mouse lethal dose by more than 3 $\log_{10}$ units (18). Thus, in mammalian species there may also be an optimal range of HA protein activation pHs that supports efficient virus replication, infection, and pathogenicity. Since high- and low-pathogenicity influenza viruses differ in their tissue tropism in avian and mammalian species (24, 27), the optimum pH values at which their HA proteins are activated to support successful infection and transmission may differ according to the influenza virus and the host species. Future investigation of the biological properties of the recombinant viruses from the present study with mouse and ferret models may reveal whether changes in the pH of activation of the HA protein support the adaptation and transmission of H5N1 influenza viruses in mammalian species, which are significant factors in the pandemic potential of these viruses.

ACKNOWLEDGMENTS

We thank Scott Krauss, Heather Forrest, David Carey, and Sharon Lokey for technical support in the BSL3+ facility. We thank Robert Lamb for the pCAGGS plasmid. We thank Rebecca DuBois and Stephen White for helpful discussions on protein structure and Sharon Naron for editing the manuscript. We thank the Hartwell Center for Bioinformatics & Biotechnology at St. Jude Children's Research Hospital for DNA sequencing.

This project was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract HHSN266200700005C, and by the American Lebanese Syrian Associated Charities (ALSAC).

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Journal of Virology

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J. Virol. **2013, 87(9):4826. DOI: 10.1128/JVI.03110-12.**
Published Ahead of Print 28 February 2013.

JVI
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The pH of Activation of the Hemagglutinin Protein Regulates H5N1 Influenza Virus Replication and Pathogenesis in Mice

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After receptor binding and internalization during influenza virus entry, the hemagglutinin (HA) protein is triggered by low pH to undergo irreversible conformational changes that mediate membrane fusion. To investigate how mutations that alter the activation pH of the HA protein influence the fitness of an avian H5N1 influenza virus in a mammalian model, we infected C57BL/6J or DBA/2J mice and compared the replication and virulence of recombinant A/chicken/Vietnam/C58/04 (H5N1) HA-Y23$_1$H mutant, wild-type, and HA-H24$_1$Q and HA-K58$_2$I mutant viruses that have HA activation pH values of 6.3, 5.9, 5.6, and 5.4, respectively. The HA-Y23$_1$H mutant virus was highly susceptible to acid inactivation *in vitro* and was attenuated for growth and virulence in mice, suggesting that an H5N1 HA protein triggered at pH 6.3 is too unstable for the virus to remain fit. Wild-type and HA-H24$_1$Q viruses were similar in pathogenicity and grew to similar levels in mice, ducks, and cell cultures derived from both avian and mammalian tissues, suggesting that H5N1 HA proteins triggered at pH values in the range of 5.9 to 5.6 broadly support replication. The HA-K58$_2$I mutant virus had greater growth and virulence in DBA/2J mice than the wild type did, although the mutant virus was highly attenuated in ducks. The data suggest that adaptation of avian H5N1 influenza virus for infection in mammals is supported by a decrease in the HA activation pH to 5.4. Identification of the HA activation pH as a host-specific infectivity factor is expected to aid in the surveillance and risk assessment of currently circulating H5N1 influenza viruses.

Highly pathogenic avian influenza (HPAI) H5N1 viruses were first detected in geese in 1996 in Guangdong Province, China. In 1997, Hong Kong reported the first human outbreak of H5N1 influenza, which caused six deaths (1). Since 2003, H5N1 influenza viruses have spread across Asia and into Europe and Africa (2), causing 360 deaths in 610 reported human cases as of 17 December 2012 (http://www.who.int/influenza/human_animal_interface/en/). H5N1 has become endemic in domestic poultry in Indonesia and Egypt, causing large economic losses (3, 4). Surveillance studies suggest that currently circulating H5N1 viruses may lack the ability to be transmitted efficiently between humans (4, 5). Nevertheless, H5N1 remains a pandemic threat, as H5N1 viruses continue to circulate in domestic poultry, frequently infecting humans. Recently, H5 influenza viruses have been shown to be capable of acquiring airborne transmissibility in ferrets (6–8), highlighting the potential threat of circulating H5 viruses. For surveillance, risk assessment, and preventive control measures directed toward HPAI viruses of the H5N1 subtype, there is an urgent need to understand the molecular properties required for replication and pathogenesis in mammalian hosts.

The replication efficiency, pathogenicity, and transmissibility of influenza viruses depend on multiple viral genetic and host factors (9). The present study focused on the hemagglutinin (HA) protein, which binds receptors and mediates viral-cellular membrane fusion during viral entry and is the major antigenic target during infection (10, 11). The HA protein is a trimeric class I membrane fusion protein (11, 12) that contains in its ectodomain a membrane-proximal, metastable stalk domain capped by a membrane-distal receptor-binding domain (RBD) (13, 14). The HA protein is primed for membrane fusion activity, and consequently infectivity, by posttranslational cleavage of the HA0 precursor into the fusion-capable HA1-HA2 complex (11). Intracellular furin-like proteases can cleave the polybasic cleavage sites of some H5 and H7 HA proteins, enabling systemic virus spread and enhancing the virulence of these highly pathogenic avian influenza (HPAI) viruses (15–17). Infection by influenza viruses is initiated when the HA surface glycoprotein binds sialic acid-containing receptors on the surface of the host cell. The receptor-binding specificity of the HA protein has been shown to be a major determinant of the host range, tissue tropism, pathogenicity, and transmissibility of influenza viruses (18). Currently circulating H5N1 influenza viruses have HA proteins that tend to bind preferentially to α(2,3)-linked sialosides and thus are poorly adapted for growth in the upper respiratory tracts of humans (19). Alternatively, human-adapted influenza viruses tend to bind preferentially to α(2,6)-linked sialosides that are predominant in the human upper respiratory tract (16, 20). A switch from α(2,3) receptor binding specificity to α(2,6) receptor binding specificity is generally thought to be a necessary, but not necessarily sufficient, step in the adaptation of avian influenza viruses for efficient growth in the upper respiratory tracts of mammals and airborne transmissibility (6, 7, 21).

After binding to cellular receptors, influenza viruses are internalized by endocytosis. As the pH is progressively decreased, a threshold is eventually reached at which the metastable HA surface protein is triggered to undergo irreversible structural changes that facilitate fusion of the viral envelope with the endosomal membrane (22, 23). The HA proteins from different strains and subtypes can vary in their activation pH values, which range from approximately 4.6 to 6.0 (24). The HA proteins from HPAI viruses

Received 5 November 2012 **Accepted** 1 February 2013
Published ahead of print 28 February 2013
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doi:10.1128/JVI.03110-12

tend to have activation pH values near the higher end of the range, toward 6.0, whereas those from human seasonal viruses tend to have lower pH values, nearer to 5.0. For a limited sampling of H5N1 influenza virus isolates, the HA activation pH has been measured to range from 5.3 to 5.9 (7, 25–27). For H1, H3, and H7 influenza viruses, mutations that alter the HA activation pH have been associated with changes in virulence in mice (28–31). For experimental infection of H5N1 influenza viruses in ducks and chickens, the highest levels of replication and pathogenesis appear to correlate with HA activation pH values that range between 5.6 and 6.0, while HA activation pH values lower than 5.6 have been found to attenuate replication and pathogenesis (25, 26, 32). In contrast, the replication of attenuated or reassorted H5 viruses in the upper respiratory tracts of mice and ferrets was enhanced by mutations that lowered the activation pH of the H5 HA protein to 5.6 or lower (7, 34).

Knowing the factors and molecular signatures that govern the efficient growth of a virus in one host species, tissue, or cell culture versus another is of fundamental importance in viral infectious disease. Such an understanding is an essential requirement to effectively conduct surveillance, perform risk assessments of viruses, make decisions to cull animals or quarantine humans, develop therapeutics that alleviate pathogenesis, identify and validate suitable drug targets, decide which virus seed stocks to prepare, efficiently and rapidly produce vaccines, and even decide which avenues of research are worthy of pursuit. The rationale for this and related studies is to understand how one such fundamental molecular property, the HA activation pH, governs the growth of H5N1 influenza virus in various species and cell types so as to benefit public health and agriculture in the aforementioned ways.

Here we investigated how the pH of activation of the HA protein regulates the replication and virulence of H5N1 influenza virus in mice. The wild-type (WT) virus selected for the present study was A/chicken/Vietnam/C58/04 (H5N1), a clade 1 influenza virus that has avian-virus-like α(2,3) receptor binding specificity and a polymerase poorly suited for replication in mammals (33) but was not engineered to be attenuated, reassorted, or mammal adapted, as had been done in previous studies (6, 7, 34). As a result of these molecular properties, the WT C58 H5N1 influenza virus does not cause weight loss, death, transmission (either contact or airborne), systemic spread, or robust nasal shedding in ferrets (33), further mitigating the risks involved in the use of the moderately pathogenic C58 strain for H5N1 research.

In the present study, mice were infected either with the WT C58 virus (HA activation pH of 5.9) or with a C58 virus containing a single point mutation in the HA1 subunit, HA-$Y23_1$H (HA activation pH of 6.3) or HA-$H24_1$Q (HA activation pH of 5.6), or in the HA2 subunit, HA-$K58_2$I (HA activation pH of 5.4) (26). These mutations in the HA stalk domain have been previously shown to alter the pH of activation of the C58 HA protein without altering HA protein expression, cleavage, or receptor-binding affinity (27), and viruses containing these mutations have replication rates in MDCK cells similar to that of the WT C58 virus (26). Another advantage of using the C58 viruses to study avian H5N1 infection in mice is that these same viruses were previously used to investigate replication, pathogenesis, and transmission in mallards (26). Therefore, the present results for infection in a mammalian model can be compared to those obtained for infection in an avian model. The results from the present study show that the C58 HA-$Y23_1$H mutant virus, which has an HA activation pH higher than that of the WT, is attenuated for replication and pathogenesis in mice, just as it was in ducks. In contrast, the C58 HA-$K58_2$I mutant virus, which has a decreased HA activation pH, promoted high levels of replication in the lungs and pathogenesis in mice despite being severely attenuated in ducks. The C58 HA-$K58_2$I mutant virus also replicated better in the murine nasal cavity than did the C58 WT virus, albeit to maximal levels that were relatively low, most likely because of an avian-like polymerase complex. Overall, the data from both the present study and a previous study (26) on the C58 viruses support the notion that a decrease in the activation pH of the HA protein that is detrimental to H5N1 replication in avian species may be necessary, but not sufficient, for adaptation to a mammalian host. Thus, this work provides evidence that the HA activation pH is an important molecular factor involved in the interspecies adaptation of highly pathogenic H5N1 influenza virus.

MATERIALS AND METHODS

Viruses. Recombinant influenza viruses of the A/chicken/Vietnam/C58/04 (H5N1) strain (33) were generated by reverse genetics and characterized previously (26, 27). These viruses were C58 WT, C58 HA-$Y23_1$H (Y23H mutation in the HA1 subunit, H5 numbering), C58 HA-$H24_1$Q (H24Q mutation in the HA1 subunit, H5 numbering), and C58 HA-$K58_2$I (K58I mutation in the HA2 subunit, H3 and H5 numbering). All viruses were grown in eggs and plaque titrated in eggs and MDCK cells. All experiments with HPAI H5N1 viruses were conducted before the moratorium on avian influenza virus transmission research (35).

Biosafety and biosecurity. All work with highly pathogenic H5N1 influenza virus was performed in an enhanced animal biosafety level 3 (ABSL-3+) laboratory that is select agent approved and routinely inspected by both institutional biosafety and USDA officials. The ABSL-3+ facility has entry and exit access control with both a card scanner and a biometric fingerprint reader. Personnel enter through a shower area and then take off all items and wear a scrub suit, a Tyvek suit, a disposable outer gown, gloves, and powered air-purifying respirators that HEPA filter the breathing air. All rooms are under negative air pressure, and there is a double-door autoclave and a double-HEPA-filtered air exhaust, and security cameras are placed throughout the laboratory. All *in vitro* work is performed in class II biosafety cabinets, and animal work is performed in negatively pressurized flexible-film isolators. All personnel are required to shower upon exit and comply with a quarantine policy to prevent outside contact with birds or immunocompromised hosts. Only personnel who receive training with H5N1 HPAI virus and who receive select agent security clearance can access the facility. ABSL-3+ personnel also receive annual refresher training to ensure adherence to regulations. Emergency plans are in place, and annual drills are performed to minimize biological risks and ensure personnel safety. The virus inventory is secured in locked freezers and is under constant security monitoring. The lab manager controls access to the virus inventory, and a logbook and database of all inventory are kept up to date. The ABSL-3+ laboratory is inspected biannually by the USDA, is in compliance with all USDA regulations, and meets or exceeds all standards outlined in *Biosafety in Microbiological and Biomedical Laboratories, 5th edition* (http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf).

Virus growth kinetics. Multiple-step growth kinetics of the WT and mutant viruses were determined in the following cell lines: MDCK (Madin-Darby canine kidney), A549 (CCL-185, human lung carcinoma), NHBE (normal human bronchial epithelium), DF1 (CRL-12203, chicken embryo fibroblast), and CCL-141 (duck embryo fibroblast). Confluent monolayers of cells were infected with a multiplicity of infection (MOI) of approximately 0.01 PFU/cell (the PFU titer was determined in MDCK cells). After 1 h of incubation at 37°C, cells were washed twice with phosphate-buffered saline (PBS) plus calcium and magnesium (PBS+) to remove nonbound virus particles and reincubated at 37°C. Culture super-

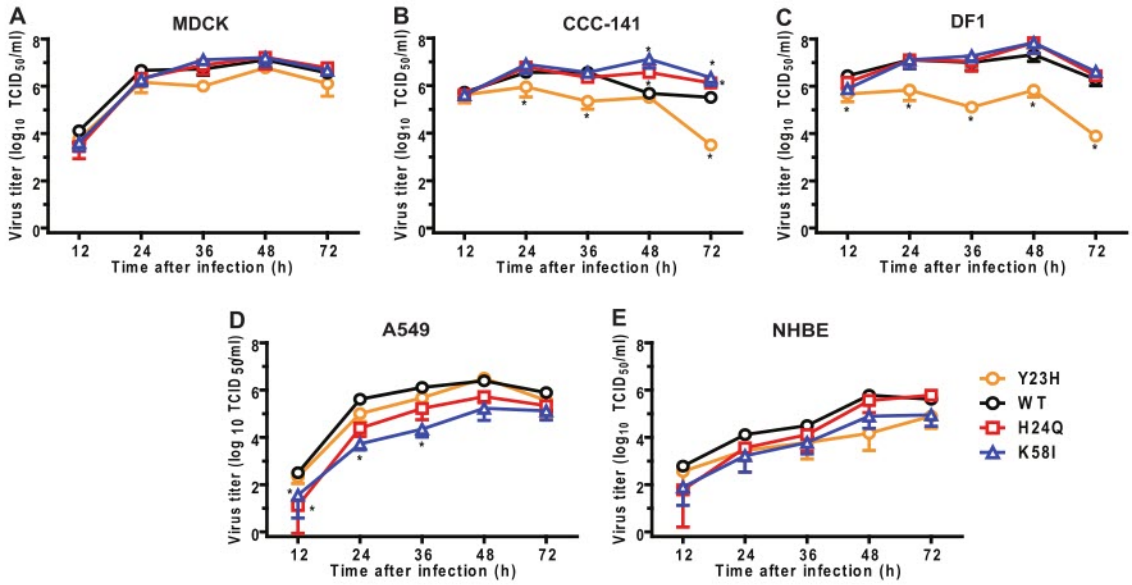

FIG 1 *In vitro* replication kinetics of reverse genetic C58 WT and mutant viruses. MDCK (A), CCL-141 (B), DF-1 (C), A549 (D), or NHBE (E) cells were infected with reverse genetic C58 WT or mutant virus at an MOI of 0.01 PFU/cell. Supernatants were collected at the indicated time points, and virus infectious titers in MDCK cells were quantified by performing $TCID_{50}$ assays. Error bars represent the standard deviations of triplicate samples. Graphs are representative of two independent experiments. Statistical analysis was performed by two-way ANOVA. Asterisks indicate *P* values of <0.05.

natants were collected at indicated time points and stored at −80°C. Samples were titrated in MDCK cells by using a 50% tissue culture infective dose ($TCID_{50}$) assay, and virus titers were calculated by using the Reed and Muench method (36).

Animal experiments. Seven-week-old female DBA/2J or C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were inoculated intranasally under isoflurane anesthesia. DBA/2J mice were inoculated with 2.8×10^4 50% egg infective doses (EID_{50}; equivalent to ~1 50% minimum lethal dose [MLD_{50}] determined by a pilot experiment) contained in 50 μl of PBS. In the case of C57BL/6J mice, we used 1.6×10^6 EID_{50} in 50 μl, which is the highest concentration we could attain with our virus stock. Mice were then observed daily for survival and weight loss for 17 days. Animals having signs of severe illness (e.g., paralysis) or more than 25% weight loss were euthanized for humane reasons. Virus titers in tissues were determined only for DBA/2J mice. For determination of tissue titers, groups of mice were euthanized at 2, 4, and 7 days postinfection. Tissues were collected and homogenized in PBS, and aliquots were stored at −80°C until further use. Samples were titrated in 10-day-old embryonated chicken eggs, and titers were expressed as EID_{50}/ml calculated by the Reed and Muench method (36).

Acid stability. To measure the effect of acid exposure on the retention of infectivity *in vitro*, virus stocks were diluted in PBS+, adjusted to the desired pH by using 0.1 M citric acid, and incubated at 37°C for 1 h. The infectivities of these viruses were then determined by measuring $TCID_{50}$s.

Statistical analysis. All statistical analyses were performed with GraphPad Prism5 software. One-way analysis of variance (ANOVA), two-way ANOVA, or a log-rank chi-square test was used to test differences between different groups. *P* values of less than 0.05 were considered statistically significant.

RESULTS

***In vitro* replication kinetics of H5N1 influenza viruses containing HA mutations.** In a previous study, we found that a C58 virus containing activation pH-altering mutation HA-$Y23_1$H (activation pH, 6.3) or HA-$K58_2$I (activation pH, 5.4) had single- and multistep replication kinetics in MDCK cells similar to those of the WT C58 virus (activation pH, 5.9), despite the two mutant viruses having reduced growth and earlier clearance in the trachea and cloaca of mallards (26). To examine how the pH of activation of the HA protein contributes to the growth kinetics of the C58 viruses in cultured cells derived from various species, we determined multistep growth curves in (i) mammalian MDCK and A549 cell monolayers, (ii) avian DF1 and CCL-141 monolayers, and (iii) differentiated NHBE cells (Fig. 1).

In MDCK cells, a prototypic mammal-derived cell type for *in vitro* assays of influenza virus growth kinetics, the HA-$Y23_1$H, HA-$H24_1$Q, and HA-$K58_2$I mutant C58 strain viruses generally had replication rates similar to that of the WT virus (Fig. 1A). In CCL-141 duck and DF1 chicken embryo fibroblast cells (Fig. 1B and C), the mutant viruses containing the activation pH-lowering mutations HA-$H24_1$Q and HA-$K58_2$I had replication rates similar to that of the WT virus. The mutant virus containing the activation pH-increasing mutation HA-$Y23_1$H had significantly reduced replication rates in the two avian-derived cell lines (*P* values of <0.01 for both cell lines, two-way ANOVA), consistent with the previously reported attenuation of this virus in mallards (26). In A549 human lung carcinoma cells (Fig. 1D), the WT and HA-$Y23_1$H and HA-$H24_1$Q mutant viruses had replication kinetics that were not statistically significantly different (*P* values of >0.05, two-way ANOVA), except for the HA-$H24_1$Q mutant at the 12-h time point. In contrast, the virus with the HA-$K58_2$I mutation and the lowest activation pH had significantly lower titers between 12 and 36 h after infection (*P* values of <0.01, two-way ANOVA) before reaching a maximum after 72 h of infection that was less than 1 $\log_{10}$ lower than that of the WT virus. In NHBE cells (Fig. 1E), all four viruses grew at relatively similar rates.

In summary, the HA-$Y23_1$H mutation, which raised the activation pH from 5.9 to 6.3, contributed to an H5N1 virus with reduced replication in avian-derived cells (Fig. 1B and C) and in mallards (26). The HA-$H24_1$Q mutation, which lowered the activation pH to 5.6, did not produce attenuation in any of the cell

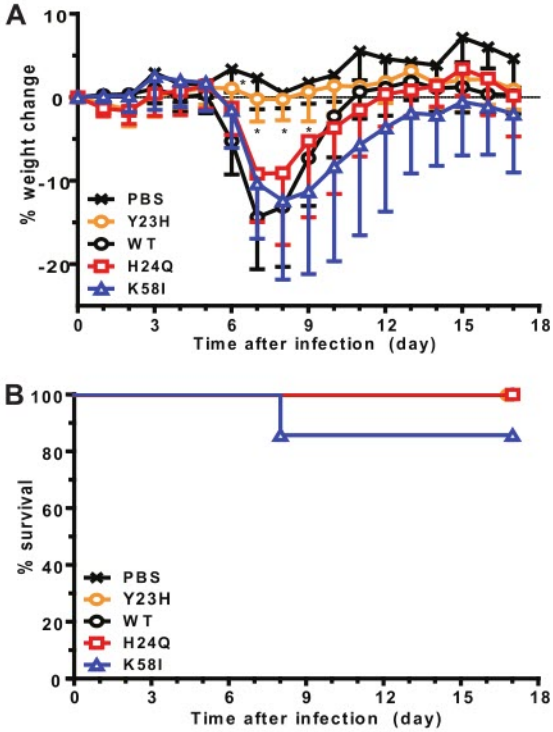

FIG 2 Virulence of reverse genetic C58 WT and mutant viruses in C57BL/6J mice. Shown are the mean percent weight change (A) and survival (B) of C57BL/6J mice ($n = 7$) after intranasal inoculation with 1.6×10^6 EID_{50} of reverse genetic C58 WT or mutant virus. Error bars represent the standard deviations. Statistical analysis was performed by two-way ANOVA for weight loss and log-rank chi-square test for survival curves. Asterisks indicate P values of <0.05.

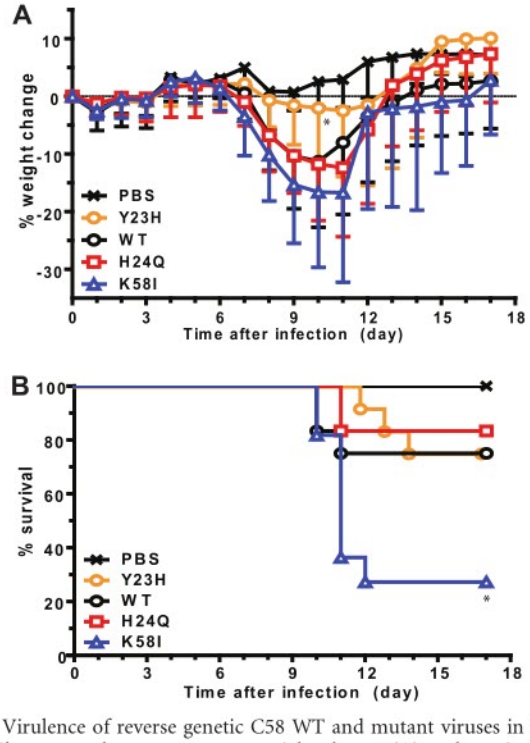

FIG 3 Virulence of reverse genetic C58 WT and mutant viruses in DBA/2J mice. Shown are the mean percent weight change (A) and survival (B) of DBA/2J mice ($n = 12$) after intranasal inoculation with 28,000 EID_{50} of reverse genetic C58 WT and mutant viruses. Error bars represent the standard deviations. Statistical analysis was performed by two-way ANOVA for weight loss and log-rank chi-square test for survival curves. Asterisks indicate P values of <0.05.

lines tested (Fig. 1), just as it did not produce attenuation in mallards (26). Finally, the HA-$K58_2I$ mutation, which lowered the activation pH to 5.4, contributed to attenuation in A549 cells (Fig. 1D) and in mallards (26) but, unexpectedly, not in avian CCL-141 or DF1 cells (Fig. 1B and C).

HA-$Y23_1H$, with an increased activation pH, reduces the virulence of the C58 H5N1 strain in C57BL/6J mice. We have previously discovered that efficient replication, virulence, and transmission of C58 strain H5N1 viruses in mallards are promoted by HA proteins that have activation pH values of 5.9 (WT) and 5.6 (HA-$H24_1Q$) but not by an HA protein that has an activation pH value of 6.3 (HA-$Y23_1H$) or 5.4 (HA-$K58_2I$) (26). In the present study, we investigated how the pH-altering mutations might alter the virulence of C58 viruses in mice, first by using the C57BL/6J strain, which is more resistant to H5N1 influenza viruses than the DBA/2J strain is (37). We inoculated groups of C57BL/6J mice intranasally with 50 μl of PBS containing 1.6×10^6 EID_{50} of WT or mutant C58 virus and then monitored the mice for weight loss and survival for 17 days (Fig. 2). All (100%) of the C57BL/6J mice inoculated with this relatively high dose of WT C58 virus survived the infection and had an average maximum weight loss of less than 15% of their starting weight 7 days after inoculation (Fig. 2A). The low virulence of the WT virus in C57BL/6J mice is consistent with the C58 virus being highly attenuated in mammalian species because of its avian-like polymerase complex (33). All of the mice infected with the HA-$H24_1Q$ mutant virus also survived and suffered an average maximum weight loss of ~10% 7 days after inoculation. The virulence of the HA-$K58_2I$ mutant virus in C57BL/6J mice was largely similar to that of the WT, as the mutant virus caused a slightly lesser extent (~12%) and delay (by ~1 day) of weight loss and recovery yet, on the other hand, increased the mortality rate by 15%, a difference not significant by log rank chi-square test. All of the C57BL/6J mice inoculated with the HA-$Y23_1H$ mutant survived, and the animals did not suffer substantial weight loss compared to a group mock inoculated with PBS. Thus, the C58 virus containing an HA-$Y23_1H$ mutation that raises the activation pH from 5.9 to 6.3 was avirulent in C57BL/6J mice (Fig. 2A), just as it was in mallards (26). In contrast, the HA-$K58_2I$ mutation, which lowered the activation pH to 5.4 and eliminated virulence in mallards, was not found here to have attenuated virulence in C57BL/6J mice compared to that of the WT C58 virus.

HA-$K58_2I$, with a decreased activation pH, contributes to the increased virulence of the C58 H5N1 strain in DBA/2J mice. As infection of the relatively resistant C57BL/6J strain of mice with the C58 strain viruses resulted in relatively low levels of weight loss even at a high dose, we next compared the viruses for pathogenicity in the relatively susceptible DBA/2J strain of mice (37). We inoculated groups of DBA/2J mice intranasally with 50 μl of PBS containing 2.8×10^4 EID_{50} of WT or mutant C58 virus and then monitored the mice for weight loss and survival for 17 days (Fig. 3). DBA/2J mice inoculated with either WT or HA-$H24_1Q$ virus had similar average maximum weight losses of ~12% of their starting weight and had mortality rates of 25% and 17%, respectively. Just as in the resistant C57BL/6 mice (Fig. 2A), in the susceptible DBA/2J mice, the HA-$Y23_1H$ mutant virus was atten-

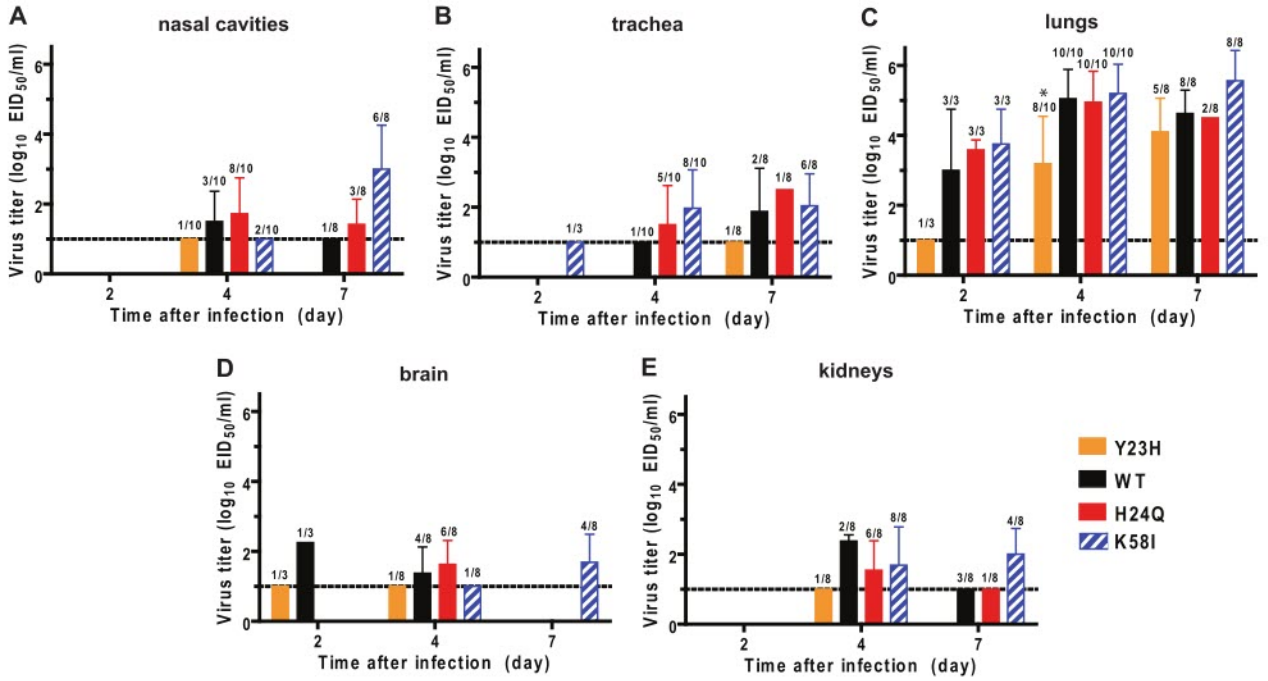

FIG 4 Viral titers of reverse genetic C58 WT and mutant viruses in tissues of infected DBA/2J mice. Shown are the titers of reverse genetic C58 WT and mutant viruses in the nasal cavities (A), tracheas (B), lungs (C), brains (D), and kidneys (E) of infected DBA/2J mice at days 2, 4, and 7 postinfection. The ratio above each bar represents the number of mice with detectable virus titers divided by the total number of infected mice. The dotted horizontal line indicates the assay detection limit ($TCID_{50}$/ml = 1 $\log_{10}$). Data from two independent experiments were combined, and the error bars represent the standard deviations. Statistical analysis was performed by one-way ANOVA. Asterisks indicate *P* values of <0.05.

uated compared to the WT virus, contributing to a maximum weight loss of ~5% compared to PBS-inoculated mice (Fig. 3A). DBA/2J mice inoculated with the HA-$K58_2$I mutant virus had an average maximum weight loss of ~17% (~5% greater weight loss than the WT group) and a mortality rate of 73% (~50% higher mortality rate than the WT group, *P* value of <0.05 by log-rank chi-square test). In summary, the rank order of pathogenicity of the C58 viruses in DBA/2J mice was HA-$Y23_1$H (activation pH, 6.3) $\ll$ WT (activation pH, 5.9) $\approx$ HA-$H24_1$Q (activation pH, 5.6) $<$ HA-$K58_2$I (activation pH, 5.4).

The pH of HA activation influences the growth of the C58 H5N1 strain in the murine respiratory tract. To investigate how the activation pH of the HA protein may influence tissue-specific replication of the avian C58 H5N1 influenza viruses, we intranasally inoculated groups of DBA/2J mice with 50 μl of PBS containing 2.8×10^4 EID_{50} (~1 MLD_{50}) of virus and collected tissues 2, 4, and 7 days later so that tissue virus titers could be measured in the nasal cavities, tracheas, lungs, brains, and kidneys (Fig. 4). While all four viruses disseminated to the brain and kidneys, none of the viruses grew to high levels ($>10^3$ EID_{50}/ml), perhaps because of their inefficient polymerase complex activity in mice (33). Just as the WT and HA-$H24_1$Q mutant viruses induced similar weight losses and death rates in DBA/2J mice (Fig. 3), these two viruses also grew to similar levels in the lungs, with an average peak of ~10^5 EID_{50}/ml 4 days after inoculation (Fig. 4C). The WT and HA-$H24_1$Q mutant viruses grew to similarly low levels ($<10^2$ EID_{50}/ml) in the trachea and nasal cavities (Fig. 4A and B), consistent with these viruses having avian-like polymerase activity (26, 27, 33).

In contrast to the HA-$H24_1$Q mutant, notable differences in virus growth in respiratory tract tissues were observed between the WT virus and the HA-$Y23_1$H and HA-$K58_2$I mutants. Just as the HA-$Y23_1$H mutant virus induced less weight loss in DBA/2J mice than did the WT virus (Fig. 3A), the HA-$Y23_1$H mutant virus also grew to significantly lower levels in the lungs 4 days after inoculation (nearly 2 $\log_{10}$ titer reduction; *P* value of <0.01; one-way ANOVA) (Fig. 4C). The average virus titer and percentage of virus positivity after 2 and 7 days of infection were also substantially lower in mice inoculated with the HA-$Y23_1$H mutant than in mice inoculated with the WT. The opposite trend was observed for the HA-$K58_2$I mutant virus. In the lungs, the HA-$K58_2$I mutant continued to grow to a titer approximately 10-fold higher than that of the WT 7 days after inoculation (Fig. 4C), consistent with the HA-$K58_2$I mutant virus inducing greater weight loss and death in DBA/2J mice (Fig. 3). Moreover, the average virus titers and percentages of virus positivity after 7 days of infection in the trachea and nasal cavities were also substantially greater in mice inoculated with the HA-$K58_2$I mutant than in those inoculated with the WT. Notably, the HA-$K58_2$I mutant virus grew in the nasal cavities to an average peak titer of 10^3 EID_{50}/ml, 100-fold higher than the peak titer of the WT virus in the nasal cavities. In summary, the rank order of C58 virus growth in the respiratory tracts of DBA/2J mice was HA-$Y23_1$H (activation pH, 6.3) $\ll$ WT (activation pH, 5.9) $\approx$ HA-$H24_1$Q (activation pH, 5.6) $<$ HA-$K58_2$I (activation pH, 5.4).

An increase in the pH of HA activation coincides with increased sensitivity to acid inactivation. Murine nasal epithelium is surrounded with glands similar to those surrounding human nasal epithelium, and both are slightly acidic (38). Acid secretions in the respiratory tract increase upon irritation or infection with

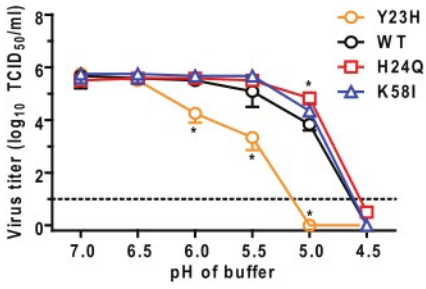

FIG 5 Acid stability of H5N1 influenza virus. Prestandardized virus stock was diluted in PBS buffer adjusted to the indicated pH and incubated for 1 h at 37°C. The remaining infectious virus titer was quantified by performing $TCID_{50}$ assays with MDCK cells. Statistical analysis was performed by two-way ANOVA. Asterisks indicate *P* values of <0.05.

influenza virus (39). Therefore, we hypothesized that a greater sensitivity to acid inactivation may contribute to the attenuation of the HA-$Y23_1H$ mutant virus and that a greater resistance to acid inactivation may contribute to the enhanced fitness of the HA-$K58_2I$ mutant virus in the murine respiratory tract. To test this, we incubated aliquots of each virus in pH-adjusted buffers ranging from pH 7.0 to pH 4.5 for 1 h and then after neutralization measured the titers of the viruses (Fig. 5). As might have been expected from its relatively high activation pH of 6.3, the HA-$Y23_1H$ mutant virus's infectivity was reduced by >10-fold after exposure to pH 6.0 buffer, reduced by >100-fold after exposure to pH 5.5 buffer, and completely eliminated by pH 5.0 buffer. The WT virus was more resistant to acid inactivation than the HA-$Y23_1H$ mutant was, as the WT did not lose infectivity after exposure to pH 6.0 buffer, lost <1 log_{10} infectivity due to pH 5.5 buffer, and lost ~2 log_{10} infectivity due to pH 5.0 buffer. Similar to the WT virus, the HA-$H24_1Q$ and HA-$K58_2I$ mutant viruses did not lose infectivity after exposure to pH 6.0 and were completely inactivated after exposure to pH 4.5. After incubation at pHs 5.5 and 5.0, the HA-$H24_1Q$ and HA-$K58_2I$ mutant viruses retained slightly more infectivity (<1 log_{10}) than the WT virus, consistent with these two mutant viruses having a lower pH of HA activation. In summary, the rank order of C58 virus resistance to acid inactivation was HA-$Y23_1H$ (activation pH, 6.3) ≪ WT (activation pH, 5.9) < HA-$H24_1Q$ (activation pH, 5.6) ≈ HA-$K58_2I$ (activation pH, 5.4).

DISCUSSION

The goal of this study was to investigate how mutations that alter the pH of activation of the HA protein influence avian H5N1 influenza viral infection in a mouse model. We infected groups of C57BL/6J and DBA/2J mice with either WT A/chicken/Vietnam/C58/04 (H5N1) virus or a recombinant virus containing a $Y23_1H$, $H24_1Q$, or $K58_2I$ mutation in the HA protein. Compared to the WT C58 HA protein, which is activated to undergo irreversible conformational changes and cause membrane fusion at pH 5.9, the $Y23_1H$, $H24_1Q$, and $K58_2I$ mutant HA proteins have been previously shown to be activated at pHs 6.3, 5.6, and 5.4, respectively (26), yet have expression, receptor binding, and cleavage phenotypes similar to those of the WT (27). Infection of mallards with these four C58 strain viruses has been investigated previously (26), thereby allowing one to compare and contrast the roles of HA activation pH in the fitness of an avian H5N1 influenza virus in avian and mammalian models. Overall, the data support the notion that a decrease in the pH of activation of the HA protein supports the adaptation of an avian H5N1 influenza virus to a mammalian host. However, the data also suggest that a mammalian-preferred HA activation pH is insufficient for robust growth in the mammalian upper respiratory tract in the absence of mammalian-adapted polymerase activity and α(2,6) receptor binding specificity, two well-established characteristics of mammalian-adapted influenza viruses.

Our present study and a previous (26) study have shown that the destabilizing HA-$Y23_1H$ mutation, which increases the HA activation pH from 5.9 to 6.3, attenuates virus replication in avian-derived cell lines, the trachea and cloaca of ducks, and the respiratory tracts of mice. The overall lack of fitness of the C58 HA-$Y23_1H$ mutant virus is most likely due to the HA protein of this virus being rather susceptible to inactivation, as the destabilizing mutation was also shown to increase susceptibility to acid inactivation in the present study. In general, an HA protein with an activation pH of 6.3 may be too unstable to support efficient replication *in vivo*, consistent with the activation pH values of HA proteins from diverse influenza virus subtypes ranging from 4.8 to 6.0 (24).

The C58 WT and HA-$H24_1Q$ mutant viruses have HA activation pH values of 5.9 and 5.6, respectively, and these two viruses grow to similar levels in mice, ducks, and cell cultures derived from both avian and mammalian hosts. Both the WT and HA-$H24_1Q$ mutant viruses were found to be highly pathogenic and transmissible in ducks (26) yet only moderately pathogenic in mice. Previous studies have shown that robust replication and high pathogenicity of H5N1 influenza viruses in chickens are supported by HA activation pH values of 5.7 and 6.0, while HA activation pH values lower than 5.5 are associated with decreased virulence (25, 26). Overall, these studies suggest that the preferred HA activation pH range for H5N1 influenza virus infection in avian species may be approximately 5.6 to 6.0, although additional studies with a broader array of viruses and avian hosts are needed to test this notion comprehensively.

The pH of activation of the H5N1 HA protein appears to be a host-specific replication and pathogenicity factor. While an HA activation pH of less than 5.5 substantially attenuates H5N1 influenza virus replication and virulence in avian species (25, 26), the HA-$K58_2I$ mutant virus, with an HA activation pH of 5.4, was shown here to have enhanced replication and virulence in mice compared to those of the WT C58 virus, whose HA is activated at pH 5.9. The attenuated replication, pathogenicity, and transmission of the HA-$K58_2I$ mutant virus in ducks (26) are not consistent with the mutant virus being shown here to replicate with WT-like efficiency in duck and chicken embryo fibroblasts, although it is possible that pH gradients resident in the endocytic pathways of respiratory and enteric tissues of mallards differ from those of duck-derived cultured cells. On the other hand, the increased fitness of the HA-$K58_2I$ mutant virus in mice may be due in part to a small, but perhaps biologically important, increase in its resistance to inactivation by exposure to mildly acidic environments in the respiratory tract. Airway epithelium is a primary line of innate defense against inhaled pathogens (40), and mice have a cellular and glandular composition similar to that of humans (41). Normal human airway epithelial tissue, especially in the nasal cavity, is acidic (pH 5.5 to 6.9) because of secretions by submucosal glands (38, 42). Moreover, acid secretions into the airway are increased upon irritation, inflammation, or infection with influenza viruses, decreasing the pH in nasal passages to 5.2 (39, 43). Thus,

better growth of the HA-K58$_2$I mutant virus (activation pH, 5.4) than the WT (activation pH, 5.9) in the nasal cavity and lungs 7 days after inoculation may be due to increased resistance to extracellular acid inactivation. On the other hand, the attenuated growth of the HA-Y23$_1$H mutant virus (activation pH, 6.3) is most likely due to its greatly enhanced susceptibility to extracellular acid inactivation.

As the optimal HA activation pH for influenza virus growth differs in various hosts and tissues, it may be possible to optimize live attenuated influenza virus vaccines by introducing mutations that yield a suitable HA activation pH value for vaccine virus growth both in eggs or Vero cells and in the respiratory tract. Several recent reports are consistent with this notion. Introduction of the previously described HA-K58$_2$I mutation (26, 27) into a live attenuated (with NS1 deleted) H5N1 vaccine candidate was shown to lower the HA activation pH to 5.3, lower the 50% mouse infective dose by 25-fold, and induce greater systemic and mucosal antibody responses in mice (34). In another recent study (44), an HA-N117$_2$D stalk mutation in PR8 virus was found to increase the HA activation pH from 5.2 to 5.4 and, consequently, increase virus growth in Vero cells 10,000-fold, most likely because Vero cells have a relatively high endosomal pH (45). Introduction of the HA-N117$_2$D mutation into various 2009 pandemic H1N1, H3N2, and seasonal H1N1 viruses was also shown to increase virus growth 100- to 1,000-fold in Vero cells (44), suggesting that the production of live attenuated vaccine viruses in Vero cells with human-adapted influenza viruses may, in general, be enhanced via mutations that increase the HA activation pH. Of course, care should be taken not to increase the activation pH of a live attenuated vaccine too much, otherwise the infectivity, growth, and immunogenicity of the vaccine may be reduced. For example, the introduction of an HA-G75$_2$R mutation into an A/Vienna/28/06 (H3N2) virus with NS1 deleted raised the HA activation pH from 5.4 to 5.8 and simultaneously impaired the immunogenicity of this vaccine candidate in ferrets (46).

The activation pH of the HA protein may regulate the replication and virulence of a wide variety of high- and low-pathogenicity influenza viruses in mice. A G23$_2$C mutation in the fusion peptide of the HA protein from HPAI A/Netherlands/219/03 (H7N7) has been shown to decrease the HA activation pH from 5.4 to 4.4 and simultaneously reduce virulence and virus growth in mice (29). The adaptation of low-pathogenicity avian influenza virus A/Hong Kong/1/68 (H3N2) to the lungs of mice led to the discovery of several HA mutations that increase the pH of hemolysis (a surrogate for membrane fusion) from 5.2 to 5.6 while simultaneously increasing HK68 virulence and growth in the lungs (30). Similarly, the serial passage of A/PR/8/34 (H1N1) in Mx1-positive mice (47) led to the discovery that a combination of HA mutations P78$_1$L and H354$_1$Q increases the pH of hemolysis from 5.3 to 5.8 while simultaneously increasing PR8 virulence in mice (48).

The adaptation of H5 influenza viruses to support airborne transmission in ferrets has recently been associated with a decrease in the HA activation pH, along with changes in receptor-binding specificity and glycosylation (7). Three sequential mutations were required before airborne transmissibility was acquired by a reassortant influenza virus that contains seven genes from a 2009 H1N1 pandemic virus and the H5 HA-encoding gene from A/Vietnam/1203/04: (i) N224$_1$K/Q226$_1$L in the receptor-binding pocket to switch from α(2,3)- to α(2,6)-linked receptor binding specificity, (ii) N158$_1$D to remove a glycosylation site from the RBD head, and (iii) T318$_1$I in the stalk domain to decrease the HA activation pH from 5.8 to 5.6 (7). Three functionally similar mutations were also sequentially introduced into A/Indonesia/5/2005 (H5N1) before this virus acquired airborne transmissibility in ferrets: (i) Q222$_1$L/G224$_1$S in the receptor-binding pocket to switch from α(2,3)- to α(2,6)-linked receptor binding specificity, (ii) N182$_1$K to remove a glycosylation site from the RBD head, and (iii) H103$_1$Y at the interface of the HA1 RBD and the HA2 coiled-coil stalk adjacent to a residue shown to regulate the acid stability of the H5N1 HA protein (6, 25). Thus, in both cases, after receptor-binding specificity was switched from α(2,3) to α(2,6) and a glycosylation site was deleted, a final mutation required for airborne transmission in ferrets was one that has been directly shown to decrease the HA activation pH or was one that likely decreases the HA activation pH.

In the present study, the K58$_2$I stalk mutation that decreased the HA activation pH from 5.9 to 5.4 was associated with an increase in C58 H5N1 virus growth and virulence in DBA/2J mice. Describing increased virulence and stability of highly pathogenic H5N1 influenza virus qualifies as dual-use research (DUR), but we do not consider knowledge of this work or the C58 HA-K58$_2$I mutant virus itself to constitute DUR of concern (DURC). The C58 HA-K58$_2$I mutant virus is not a threat to agriculture because the mutant virus is attenuated and loses transmissibility in avian species compared to that of the WT C58 virus (26). We believe that the C58 HA-K58$_2$I mutant virus does not increase the risk of H5N1 influenza virus to human health for several reasons. First, it lacks the ability to bind to human α(2,6) receptors and has an avian-like polymerase deficient for growth in mammalian hosts; therefore, the virus does not have the capacity to be transmitted in humans. Second, the virulence of the C58 HA-K58$_2$I mutant virus in mice is orders of magnitude weaker than naturally occurring H5N1 viruses and is on a par with the virulence of currently circulating human H1N1 viruses that are considered to be clinically mild (33, 49). Third, the C58 HA-K58$_2$I mutant virus is susceptible to oseltamivir and is antigenically matched to an A/Vietnam/1203/04 (H5N1) experimental vaccine. Fourth, the mutant viruses were not actively adapted during animal experiments and tissues were destroyed after titers were measured.

We also do not believe that a knowledge of the biological importance of the HA activation pH could be directly misapplied to pose a significant threat to public health and is therefore not DURC. Some might reasonably question whether the introduction of an HA-K58$_2$I mutation into a ferret-transmissible virus (7) would be expected to yield a human-transmissible H5N1 virus with enhanced pathogenicity. The final mutation required for the acquisition of airborne transmissibility in ferrets in a study by Imai et al. (7) was an HA-T318$_1$I mutation that lowered the HA activation pH from 5.8 to 5.6. In the context of the C58 strain, we find that the HA-K58$_2$I and HA-H24$_2$Q mutations decrease the HA activation pH by 0.5 and 0.3 unit, respectively (26, 27), and in combination, the effect is additive, with the two mutations decreasing the HA activation pH of C58 WT by 0.8 unit, from 5.9 to 5.1 (unpublished data). The additive nature of activation pH-altering mutations in other strains of H5N1 influenza viruses has also been described previously (25). Therefore, one would expect the introduction of an HA-K58$_2$I mutation into the ferret-transmissible virus in the study by Imai et al. (7) to reduce the HA activation pH to ~5.1, which is most likely too low for efficient

HA activation during entry. H3N2 and H7N7 viruses with HA activation pH values of less than 5.3 have been shown to have lower replication and virulence in mice than related viruses that have HA activation pH values ranging from 5.4 to 5.6 (29, 30).

As H5N1 influenza viruses are currently endemic in Egypt and Indonesia, continuing to spread among domestic poultry and often infecting humans (3, 4), H5N1 constitutes an ever-present threat to both agriculture and human health. The key finding in this paper is that a decrease in the HA activation pH (from 5.9 to 5.4) supports H5 influenza virus growth in a mammalian model while it has a deleterious effect on H5 growth in avian species (25, 26). Thus, the data show that the HA activation pH is a novel interspecies adaptation marker, helping us understand the properties necessary for influenza viruses to cross the species barrier.

This work may benefit public health in several ways. First, this work assists surveillance by identifying individual mutations and specific HA activation pH values that promote adaptation to mammals. Second, risk assessment will be enhanced through the realization that avian H5N1 influenza viruses with low pathogenicity in avian species because of a relatively low HA activation pH (such as the C58 HA-K58$_2$I mutant) may constitute a greater risk to mammals. Third, the knowledge of molecular markers for increased adaptation to mammals should assist scientists and public health authorities in making decisions to cull animals, quarantine humans, select prepandemic vaccine seed stocks, rapidly produce immunogenic vaccines, and identify viable drug targets such as the HA stalk (a region of the protein known to regulate its HA activation pH and a target of experimental small-molecule drugs and universal antiviral antibodies). Finally, this work also has implications for viral infectious diseases in general. Many enveloped viruses invade cells after their fusion glycoprotein is triggered by a low pH, including hepatitis C virus, Epstein-Barr virus, vesicular stomatitis virus, avian leukemia virus, human rhinovirus, dengue virus, and severe acute respiratory syndrome coronavirus (50). Thus, the tropism and host range of other important human and agricultural pathogens may also be influenced by the pH of activation of their fusion protein.

ACKNOWLEDGMENTS

We thank Elena Govorkova and Tatiana Baranovich for providing the NHBE cells, Cherise Guess for editing the manuscript, and Robert Webster for generously providing plasmids and viruses. We thank the Animal Resource Center (ARC) at St. Jude Children's Research Hospital for help with animal experiments.

This work was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, under contract HHSN266200700005C of the Centers of Excellence for Influenza Research and Surveillance (CEIRS) and by the American Lebanese Syrian Associated Charities (ALSAC).

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Journal of Virology

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J. Virol. **2013, 87(17):9911. DOI: 10.1128/JVI.01175-13.**
Published Ahead of Print 3 July 2013.

JVI
Journals.ASM.org

Increased Acid Stability of the Hemagglutinin Protein Enhances H5N1 Influenza Virus Growth in the Upper Respiratory Tract but Is Insufficient for Transmission in Ferrets

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Influenza virus entry is mediated by the acidic-pH-induced activation of hemagglutinin (HA) protein. Here, we investigated how a decrease in the HA activation pH (an increase in acid stability) influences the properties of highly pathogenic H5N1 influenza virus in mammalian hosts. We generated isogenic A/Vietnam/1203/2004 (H5N1) (VN1203) viruses containing either wild-type HA protein (activation pH 6.0) or an HA2-K58I point mutation (K to I at position 58) (activation pH 5.5). The VN1203-HA2-K58I virus had replication kinetics similar to those of wild-type VN1203 in MDCK and normal human bronchial epithelial cells and yet had reduced growth in human alveolar A549 cells, which were found to have a higher endosomal pH than MDCK cells. Wild-type and HA2-K58I viruses promoted similar levels of morbidity and mortality in C57BL/6J mice and ferrets, and neither virus transmitted efficiently to naive contact cage-mate ferrets. The acid-stabilizing HA2-K58I mutation, which diminishes H5N1 replication and transmission in ducks, increased the virus load in the ferret nasal cavity early during infection while simultaneously reducing the virus load in the lungs. Overall, a single, acid-stabilizing mutation was found to enhance the growth of an H5N1 influenza virus in the mammalian upper respiratory tract, and yet it was insufficient to enable contact transmission in ferrets in the absence of additional mutations that confer α(2,6) receptor binding specificity and remove a critical N-linked glycosylation site. The information provided here on the contribution of HA acid stability to H5N1 influenza virus fitness and transmissibility in mammals in the background of a non-laboratory-adapted virus provides essential information for the surveillance and assessment of the pandemic potential of currently circulating H5N1 viruses.

Influenza A virus is a negative-sense, single-stranded RNA virus of the family *Orthomyxoviridae*. Its genome consists of eight segments encoding at least 16 proteins (1–6). Being an RNA virus with a segmented genome, influenza virus is characterized by a high mutation rate and the ability to reassort its genome segments with other viruses (3, 7). These two properties allow the virus to constantly evolve and sustain in its original host and to adapt to new hosts (5, 8). These two properties also contribute to the large diversity among influenza A viruses and their propensity to infect a broad range of hosts, including members of avian (e.g., shorebirds, ducks, chickens, etc.) and mammalian (e.g., horses, pigs, dolphins, seals, humans, etc.) species (5).

Annual outbreaks in humans are currently caused by influenza A viruses of the H1N1 and H3N2 subtypes. Periodically, a new influenza virus subtype crosses the species barrier and causes a pandemic; the 2009 H1N1 pandemic is the most recent example (8, 9). Viruses that occasionally cross the host barrier and cause infections in humans are of concern due to their potential to adapt to humans and become pandemic. H5, H7, and H9 influenza viruses have recently been reported in humans (10–14). Of great concern is avian H5N1 influenza virus.

Since 2003, H5N1 influenza viruses have been actively evolving and diversifying, causing outbreaks in wild and domestic birds in Asia, Africa, and Europe and becoming endemic in poultry in Egypt and Indonesia (15–17). As of 26 April 2013, 628 human infections of H5N1 have been documented, of which approximately 60% have resulted in death (http://www.who.int/influenza/human_animal_interface/). While most of the human cases were due to contact with poultry or consumption of undercooked poultry meat or blood (18), a few cases of human-to-human transmission have been reported (18–20). Despite a limited number of cases of human-to-human transmission, H5N1 influenza virus remains a pandemic threat, and determining the molecular properties that contribute to its ability to adapt to humans is critical for monitoring the virus and implementing effective control measures (e.g., culling birds and selecting vaccine seed stocks). Mutations influencing the interspecies adaptation of H5N1 influenza viruses have been identified in the HA (hemagglutinin), NA (neuraminidase), and PB2 (basic polymerase) proteins (8, 21, 22). Of these, the HA protein is reportedly the most important determinant of host adaptation and transmission of H5N1 influenza virus (21, 23–25) and is an essential component in the emergence of pandemic influenza.

The HA protein is a metastable class I membrane fusion protein. It is posttranslationally cleaved into two subunits: the HA1 subunit, which harbors the receptor binding globular head domain, and the HA2 subunit, which comprises the majority of the fusogenic stalk domain (26–28). During viral entry, the HA pro-

Received 30 April 2013 **Accepted** 27 June 2013
Published ahead of print 3 July 2013
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doi:10.1128/JVI.01175-13

tein binds to sialic acid-containing cell-surface receptors, and the virus is internalized into the endosomes (26, 29). Low pH in the endosomes triggers irreversible conformational changes in the HA protein that mediate fusion of the viral and endosomal membranes, enabling delivery of viral RNA into the host cell (26).

For various influenza virus subtypes, mutations altering the pH at which the HA protein is activated for membrane fusion have been associated with altered virulence in mice (30–35) and interspecies adaptation (35–40). Our previous work has shown that efficient growth and transmission of H5N1 influenza viruses in avian species is associated with a relatively high HA activation pH, ranging from pH 5.6 to 6.0 (39, 41, 42). Moreover, we found that an HA2-K58I mutation (K to I at position 58) that decreases the HA activation pH of A/chicken/Vietnam/C58/2004 (H5N1) from 5.9 to 5.4 supports enhanced and prolonged replication in the murine upper respiratory tract (URT) while simultaneously reducing replication and eliminating transmission in mallards (35, 43). Similarly, a decrease in the HA activation pH also coincided with enhanced replication of NS1 deletion mutant H5 and H3 influenza viruses in the URT of mice (44, 45).

Avian influenza viruses such as H5N1 tend to bind preferentially to α(2,3)-linked sialic acid receptors, while human viruses favor sialic acid receptors in an α(2,6) orientation (46–49). Nonetheless, some recent H5N1 viruses have acquired the ability to bind α(2,6)-linked sialic acid receptors while still maintaining high binding affinity to the α(2,3) form (48). A gain of α(2,6) receptor binding specificity alone has not been sufficient to promote H5N1 transmission in ferrets, the standard model for human transmission of influenza viruses (21, 50, 51). A few studies investigated whether a complete switch to α(2,6) binding is sufficient to support efficient airborne transmission of H5N1 influenza virus. Chen et al. showed that, with the proper HA-NA balance, a mutated H5 virus with α(2,6) receptor binding specificity supported only partial transmission via respiratory droplets (21).

Two recent studies showed that efficient airborne transmission of H5-containing influenza viruses occurred following a series of mutations that first switched receptor binding specificity to α(2,6), then deleted a glycosylation site, and last decreased the pH of activation of the HA protein (determined either by direct measurement or by structural considerations) (24, 25). While a decrease in the activation pH of the HA protein was found to be necessary for airborne transmissibility of H5 viruses in ferrets, it is unknown whether this adaptation alone is sufficient to increase the transmissibility of circulating H5N1 influenza viruses in mammals. The goal of the current study was to determine how a single acid-stabilizing HA protein mutation influences *in vivo* infection in mice and ferrets when introduced into the background of an unmodified H5N1 influenza virus that has not been laboratory adapted, reassorted, or mutated to have α(2,6) receptor binding specificity.

The rationale for this work was to enhance our ability to assess the likelihood that currently circulating avianlike H5N1 influenza viruses may acquire the ability to jump species to humans and potentially cause a pandemic. This is important in conducting surveillance, performing risk assessment of currently circulating viruses, making decisions to quarantine humans, and selecting prepandemic vaccine seed stocks. Furthermore, knowledge of the molecular properties that govern the efficient growth of H5N1 influenza virus in one cell type, tissue, or host species versus another is expected to help optimize vaccine yield and efficacy and may suggest novel ways to treat infection.

MATERIALS AND METHODS

Ethics statement. All animal studies were approved by the Animal Care and Use Committee of St. Jude Children's Research Hospital (protocol 464) and were performed in compliance with relevant institutional policies, Association for the Accreditation of Laboratory Animal Care guidelines, National Institutes of Health regulations, and local, state, and federal laws.

Plasmids. pHW2000 plasmids containing individual genome segments of the wild-type (WT) A/Vietnam/1203/04 (H5N1) (VN1203) influenza virus have been described previously (52). The HA2-K58I point mutation was introduced using a QuikChange site-directed mutagenesis kit (Stratagene) according to the manufacturer's instructions. To perform *in vitro* expression of the surface glycoproteins, the HA and NA genes were subcloned into the pCAGGS expression vector using gene-specific primers, with ClaI and XhoI site overhangs at the 5′ and 3′ end, respectively (43).

Viruses. rg-VN1203-HA-WT and rg-VN1203-HA2-K58I viruses were generated by using reverse genetics (rg) as previously described (53). Briefly, eight pHW2000 plasmids, each containing an individual gene of the eight influenza A virus genes, were transfected into cocultured MDCK (Madin-Darby canine kidney) and 293T cells. Virus was then harvested, plaque purified on MDCK cells, and propagated in 10-day-old embryonated chicken eggs. Virus identity was confirmed by performing full-genome sequencing at the Hartwell Center for Bio-informatics and Biotechnology at St. Jude Children's Research Hospital.

Transient expression of HA and NA proteins. Vero cells at 70 to 80% confluence were transfected with pCAGGS HA (1.0 μg) and pCAGGS NA (0.1 μg) plasmids by using a Lipofectamine plus expression system (Invitrogen) (54). The cells were then incubated at 37°C for 4 h before the transfection medium was replaced with Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum. The cells were incubated for 16 h at 37°C to allow the expression of the HA and NA proteins.

HA protein activation pH. Monolayers of Vero cells expressing the HA protein on their surface were washed with phosphate-buffered saline plus calcium and magnesium (PBS+) and treated for 5 min with 500 μl PBS+ that was adjusted to the desired pH using 0.1 M citric acid. The cells were then neutralized with DMEM and incubated for another 2 h at 37°C. The cells were then fixed and stained by using a Hema 3 stat pack staining kit (Fisher) according to the manufacturer's instructions. The pH of conformational change was determined by using Vn04-16 conformation-specific monoclonal antibody as previously described (41, 43).

Total and surface expression. HA-expressing Vero cells were lysed using radioimmunoprecipitation (RIPA) buffer containing 10 mg/ml iodoacetamide and protease inhibitors (43). Clarified lysates were resolved on 4 to 12% NuPAGE Bis-Tris polyacrylamide-SDS gels (Invitrogen) and transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were blotted using polyclonal HA antibody A0110 (43), and bands were visualized using horseradish peroxidase (HRP)-conjugated anti-rabbit antibody on X-ray films. Flow cytometric analysis of the surface expression of the HA protein was performed by using VN04-02 monoclonal antibody as described previously (43).

Hemadsorption. Vero cells expressing the HA protein on their surface were washed two times with PBS+, overlaid with 1% chicken or turkey erythrocytes, and incubated at 37°C for 30 min. Monolayers were washed 3 times with DMEM (phenol red free) to remove unbound red blood cells (RBS) and lysed with RBC lysis buffer. The amount of bound erythrocytes was determined by measuring the absorbance of clarified lysate at 415 nm by using a Synergy-2 multimode microplate reader (BioTek).

Receptor binding specificity assay. We used a solid-phase binding assay to measure the receptor binding specificity of the HA protein for α(2,3)- or α(2,6)-linked sialic acid. Briefly, plates were coated with 10 mg/ml fetuin (Sigma) overnight at 4°C and then washed with PBS (without calcium and magnesium) and blocked with 0.1 ml of PBS containing 2% bovine serum albumin (Sigma) at room temperature for 1 h. Plates were washed 3 times with PBS and incubated with 100 μl virus (64 HA

units) overnight at 4°C to allow binding of the virus to the plates. Unbound virus was aspirated, and the plates were washed three times with PBS before the addition of a biotinylated sialylglycopolymer containing either an α(2,3) (Neu5Acα2,3Galβ1,4GlcNAcβ1-pAP) or an α(2,6) (Neu5Acα2,6Galβ1,4GlcNAcβ1-pAP) sialic acid for 3 h at 4°C. After washing three times with PBS, plates were incubated with horseradish peroxidase (HRP)-conjugated streptavidin (diluted 1:1,000; Invitrogen) for 1 h at room temperature. After washing with PBS 5 times, the plates were incubated with 50 μl of tetramethylbenzidine (TMB) substrate for 10 min at room temperature. The reaction was stopped with 50 μl of 50 mM HCl, and the optical density was measured at 450 nm.

Resistance to acid inactivation. The acid stabilities of the viruses were measured by determining the susceptibility of each virus to acid inactivation. Virus was diluted in PBS+ adjusted to the desired pH (4.5 to 6.0 pH) by using 0.1 M citric acid. Then, samples were incubated at 37°C for 1 h. The titer of remaining infectious virus was determined in MDCK cells by using the 50% tissue culture infectious dose ($TCID_{50}$) assay. The method of Reed and Muench was used to estimate the $TCID_{50}$/ml of virus (55).

***In vitro* virus growth kinetics.** MDCK (ATCC CRL-2936), A549 (human lung carcinoma; ATCC CCL-185), and NHBE (normal human bronchial epithelium; Lonza CC-2540) cells were infected with WT and HA2-K58I viruses at a multiplicity of infection (MOI) of 0.01 PFU/cell. One hour postincubation, cells were washed twice with PBS+ to remove nonbound virus particles and incubated at 37°C. To compare the growth kinetics of the two viruses, 100-μl samples of culture supernatant were collected at 10, 24, 48, and 72 h postinfection and titrated in MDCK cells by using the $TCID_{50}$ assay.

Endosomal pH. The endosomal pHs of MDCK and A549 cell lines were compared by using a pH-sensitive endosomal dye, pHrodo red dextran (Invitrogen), according to the manufacturer's recommendations. Briefly, MDCK or A549 cells were washed and suspended in warm PBS+ buffer. The cells (10^6) were then incubated at 37°C in the presence of 40 μg/ml of the dye for 15 min. The cells were washed and suspended in warm PBS+ buffer, and the intensity of fluorescence was measured by using flow cytometry. Quantitative measurement of the endosomal pH was performed as previously described (56), with some modifications. Briefly, cells were washed twice with PBS and incubated with 5 mg/ml fluorescein-TMR (tetramethylrhodamine)-tagged dextran (Invitrogen) for 20 min at 37°C, and then cells were washed 5 times with PBS and imaged in phenol red-free DMEM medium. For endosomal pH calibration, after the dye uptake, the cells were incubated in freshly prepared calibration buffer (120 mM KCl, 20 mM NaCl, 1 mM $CaCl_2$, 1 mM Mg_2Cl, and 10 mM HEPES for pH values 6.5 to 7.0 or 10 mM MES [morpholineethanesulfonic acid] for pH values 4.0 to 6.0) containing 10 μM nigericin and 10 μM monensin ionophores for 20 min. The cells were imaged on a Nikon TE2000 E2 microscope equipped with a Nikon C2 confocal scan head. Excitation was with 488-nm and 561-nm diode-pumped solid-state (DPSS) lasers, and emission was collected through 515/30 and 605/75 band pass filters. Images were acquired with a Nikon 40× 1.3 numerical aperture Plan Fluor objective and using Nikon NIS Elements software. Cells were maintained at 37°C, 5% CO_2 during imaging. The pH was estimated as the intensity ratio of TMR (red) and fluorescein (green) fluorescence. Curve fitting was performed as previously described (56).

Animal experiments. To determine the 50% mouse lethal dose (MLD_{50}), groups of 7-week-old female C57BL/6J mice (Jackson Laboratory) were intranasally inoculated with 50 μl of 10-fold serial dilutions of WT VN1203 virus. To compare the virulence and replication of the WT and HA2-K58I viruses, mice were infected with 50 μl or 5 μl of PBS+ containing ~1 MLD_{50} (~150 50% egg infectious doses [EID_{50}/ml]). The mice were then weighed and observed daily. Mice showing severe weight loss (>25%) or illness (e.g., hind limb paralysis) were euthanized for humane reasons. To determine virus dissemination, mice were euthanized and tissues, including nasal cavities, trachea, lungs, brain, and kidneys, were collected. Tissues were homogenized in PBS and titrated in 10-day-old embryonated chicken eggs. Viral loads were expressed as EID_{50}/ml, calculated by the method of Reed and Muench (55). For contact transmission experiments, 3-month-old male ferrets (Triple F farms) that were seronegative to influenza A viruses circulating in humans were used. The ferrets were anesthetized with isoflurane and inoculated intranasally with 1,000 $TCID_{50}$ in 0.5 ml PBS. Twenty-four hours postinfection, two naive ferrets were introduced into a cage containing one inoculated donor ferret. Ketamine was used to induce sneezing in the ferrets to collect nasal washes. The ferrets were observed daily. Ferrets that lost >25% of their starting weight and/or were severely ill (e.g., paralysis) were humanely euthanized. On day 5 postinfection, three directly inoculated ferrets from each virus group were sacrificed and tissues were collected to determine viral loads. All animal experiments were carried out under applicable laws and guidelines and were approved by St. Jude Children's Research Hospital Animal Care and Use Committee.

Serologic testing. To test for potential seroconversion, serum was collected from contact ferrets on day 20 postcontact. Serum samples were treated with receptor-destroying enzyme (Seiken) overnight at 37°C to destroy nonspecific receptors, heat inactivated at 56°C for 30 min, and tested using the hemagglutination inhibition (HI) assay as described in the WHO animal influenza training manual (57). The HI assay was performed using VN1203 virus and 0.5% chicken red blood cells.

Biosafety and biosecurity. All work with highly pathogenic H5N1 influenza was performed in an enhanced animal biosafety level 3 (ABSL3+) laboratory that is select agent approved and routinely inspected by both institutional biosafety and USDA officials. The ABSL3+ facility has entry and exit access control with both a card scanner and biometric fingerprint reader. Personnel enter through a shower area and then take off all items and wear a scrub suit, Tyvek suit, disposable outer gown, gloves, and powered air-purifying respirators with HEPA filters for the breathing air. All rooms are under negative air pressure, and there is a double-door autoclave, double-HEPA-filtered air exhaust, and security cameras placed throughout the laboratory. All *in vitro* work is performed in class II biosafety cabinets, and animal work is performed in negative-pressurized flexible-film isolators. All personnel are required to shower upon exit and comply with a quarantine policy to prevent outside contact with birds or immunocompromised hosts. Only personnel who receive training with highly pathogenic avian H5N1 influenza virus and who receive select agent security clearance can access the facility. ABSL3+ personnel also receive annual refresher training to ensure adherence to regulations. Emergency plans are in place, and annual drills are performed to minimize biological risks and ensure personnel safety. The virus inventory is secured in locked freezers and is under constant security monitoring. The laboratory manager controls access to the virus inventory, and a logbook and database of the inventory are kept up-to-date. The ABSL3+ laboratory is inspected biannually by the USDA, is in compliance with all USDA regulations, and meets or exceeds all standards outlined in *Biosafety in Microbiological and Biomedical Laboratories* (58).

DUR. All experiments with ferrets were conducted in 2011 before the moratorium on H5N1 influenza virus research. Before initiation, the Institutional Biosafety Committee of St. Jude Children's Research Hospital reviewed and approved the experiments and recommended mitigation strategies, which were subsequently implemented by the investigators. Upon completion of the studies and also after preparation of the manuscript, an internal Dual-Use Research of Concern (DURC) Committee at St. Jude Children's Research Hospital reviewed this work and concluded that the agents and results described herein are DUR but not DURC. The manuscript was also reviewed by the NIH/NIAID, the funding agency, which likewise judged this work to be DUR but not DURC.

Statistical analysis. All statistical analyses were performed by using GraphPad Prism5 software. The *t* test, one-way analysis of variance (ANOVA), two-way ANOVA, or log-rank chi-square test was used to test differences between different groups. *P* values of <0.05 were considered statistically significant.

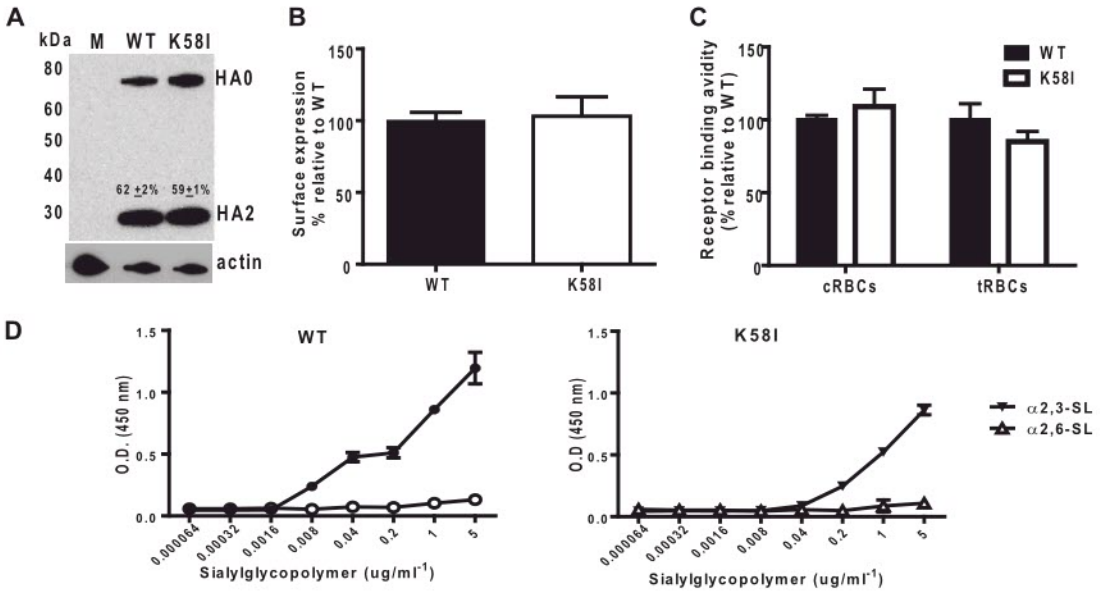

FIG 1 Characterization of the WT and HA2-K58I HA proteins. (A) Western blot of HA expression. Values above the HA2 band express the mean percentage of HA cleavage ± standard deviation, estimated by dividing the HA2 band intensity by that of the total HA (i.e., HA0 + HA2). (B) HA surface expression as measured by flow cytometry. (C) Binding avidities of cell surface-expressed HA protein to chicken (cRBCs) or turkey (tRBCs) red blood cells, measured as the amount of hemoglobin released following lysis of bound RBCs. (D) Binding of WT or K58I virus to either an α(2,3) or α(2,6) sialylglycopolymer. O.D. (450 nm), optical density at 450 nm. Error bars show standard deviations.

RESULTS

An HA2-K58I mutation decreases the HA activation pH without altering other properties. For these studies, we selected the prototypic influenza virus A/Vietnam/1203/2004 (H5N1). VN1203 has avianlike α(2,3) receptor binding specificity and a human-like PB2 K627 polymorphism (52). In ferrets, these two properties of VN1203 contribute to robust virus growth in the lungs and high virulence but no contact or airborne transmission (51, 52). We introduced into the VN1203 HA protein an HA2-K58I mutation, which we previously found to decrease by 0.5 pH units the HA activation pH of the related isolate A/chicken/Vietnam/C58/2004 (H5N1) (39, 43). Wild-type and K58I HA proteins, transiently coexpressed in Vero cells along with the NA protein, had similar levels of total and cell surface HA protein expression as determined by Western blotting and flow cytometry, respectively (Fig. 1A and B). Western blot analysis also showed no significant difference in HA protein cleavage (Fig. 1A). Both HA proteins had similar binding avidities to chicken and turkey red blood cells (Fig. 1C), suggesting similar receptor binding affinities. We compared the receptor binding specificities of the wild-type and K58I HA proteins using a solid-phase binding assay and found that both HA proteins bound exclusively to α(2,3)-linked sialic acid receptors and not to α(2,6)-linked sialic acid (Fig. 1D). Overall, the K58I mutation did not result in substantial differences in HA protein expression, cleavage, or receptor binding.

We next compared the activation pH values of the wild-type and K58I HA proteins. Vero cells expressing either the wild-type or K58I HA protein, along with the VN1203 NA protein, were pulsed with pH-adjusted buffers and either allowed to fuse for microscopic examination of syncytia (cell-to-cell fusion) or detached from tissue culture plates for flow cytometric analyses using the HA protein conformation-specific monoclonal antibody Vn04-16 (43, 59). The syncytium assay showed that wild-type HA protein was activated for membrane fusion after buffer pulses of pH 6.0 or lower, while the K58I mutant was not triggered for membrane fusion unless the pH pulse was reduced to pH 5.5 or lower (Fig. 2A). Flow cytometry showed that the midpoint of acid-induced conformational changes for wild-type HA protein was pH 5.9, while the midpoint for the K58I HA protein was reduced to pH 5.4 (Fig. 2B and C). Therefore, the average HA activation pH values for wild-type and K58I HA proteins were 5.95 and 5.45, respectively (Fig. 2D). To probe whether differences in the activation pH values of the HA proteins affected their susceptibilities to acid inactivation, prestandardized reverse genetic-engineered viruses rg-VN1203-WT and rg-VN1203-HA2-K58I were exposed to buffers ranging in pH from 4.5 to 7.0 for 1 h at 37°C. The titers of the remaining infectious virus that survived the pH treatment were quantified using $TCID_{50}$ (Fig. 2E). The HA2-K58I virus retained its infectivity upon exposure to buffers with pH values as low as 5.5, while the WT virus lost ~90% (~1 $\log_{10}$) of its infectious titer at pH 5.5. The infectious titers of both WT and HA2-K58I viruses declined nearly 100-fold after exposure to pH 5.0 buffer and were completely lost after exposure to pH 4.5 buffer. Overall, the results show that the K58I mutation decreases the HA activation pH of the VN1203 HA protein by 0.5 units without altering other biochemical properties, just as was observed when K58I was introduced into the background of the C58 virus isolate (39, 43).

***In vitro* growth of HA2-K58I virus is reduced in A549 cells due to a relatively high endosomal pH.** In the background of the C58 strain, we have previously shown that the HA2-K58I mutation attenuates the growth of H5N1 virus in A549 cells (human lung carcinoma cells) but does not alter the virus replication kinetics in MDCK (canine kidney epithelial cell line), NHBE (normal human bronchial epithelial), DF1 (chicken embryo fibroblast), and CCL-141 (duck embryo fibroblast) cells (35, 39).

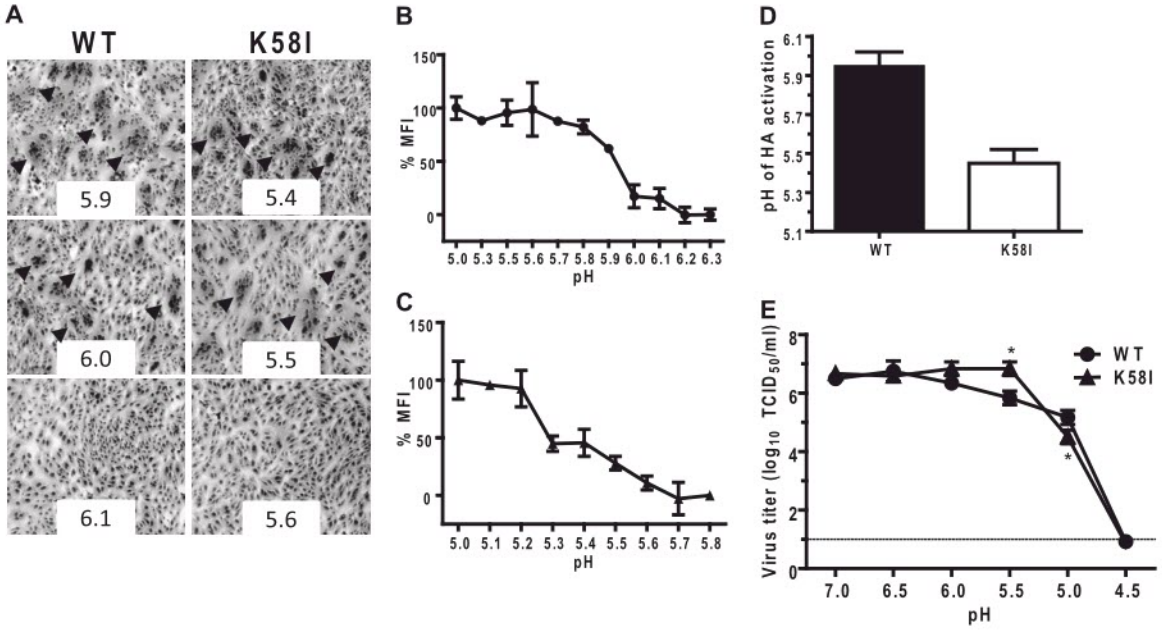

FIG 2 Acid stabilities of the HA proteins. (A) Micrographs of syncytium formation due to HA protein activation at various pH points in Vero cells. The arrowheads indicate syncytia. (B and C) Quantification of the HA protein conformational change at indicated pH points by using conformation-specific monoclonal antibody (VN04-16) and flow cytometry. MFI, mean fluorescence intensity. (D) Activation pHs of the HA proteins expressed as the average of the pH of syncytium formation and the pH at which 50% conformational change occurs. (E) Residual titers upon treatment of WT or HA2-K58I virus with the indicated pH buffers. Asterisks indicate P values of <0.05 using two-way ANOVA. Error bars show standard deviations.

Similarly, we found here that the rg-VN1203-HA2-K58I virus had wild-type-like growth kinetics in MDCK (Fig. 3A) and NHBE (Fig. 3C) cells and yet grew to significantly lower virus titers (P values of <0.01, two-way ANOVA) than the wild-type after 24 h of infection in A549 cells (Fig. 3B). The decreased growth of rg-VN1203-HA2-K58I virus in A549 cells but not in MDCK cells suggested that the two cell lines may differ in their endosomal pHs. To test this hypothesis, we probed MDCK and A549 cell lines using pHrodo red, a pH-sensitive fluorescent dextran conjugate. Upon uptake into the endosomes, the fluorescence intensity of pHrodo increases as endosomal pH decreases. The mean fluorescence intensity of the pHrodo probe was 2-fold higher ($P < 0.05$, t test) in MDCK cells than in A549 cells (Fig. 4A), suggesting that A549 cells have a higher endosomal pH than MDCK cells. We then determined the endosomal pH quantitatively by using fluorescein-TMR double-conjugated dextran. The intensity of the fluorescein is quenched in a predictable manner under acidic conditions, while that of the TMR remains stable, thus allowing one to track endosomes and measure their pH values. We incubated MDCK and A549 cells with the double-conjugated dextran to allow uptake of the dye and then calibrated the *in situ* fluorescein emissions as a function of clamped cytoplasmic pH. A plot of the red/green ratio on the calibration curve revealed that MDCK cells had an endosomal pH of 5.4 and A549 cells had an endosomal pH of 5.9 (Fig. 4B). These results are consistent with the HA2-K58I virus, which has a lower HA activation pH than wild-type virus, being attenuated in A549 but not MDCK cells, while the wild-type virus is not attenuated in A549 cells.

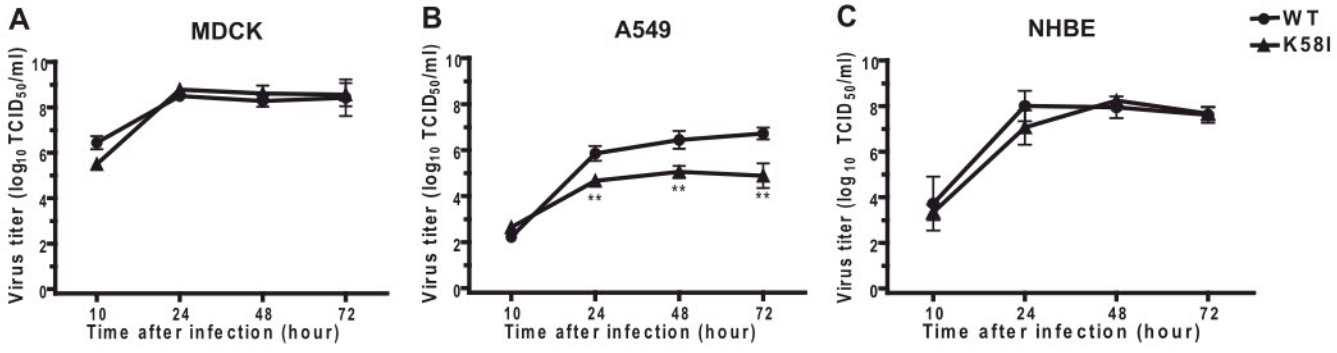

FIG 3 *In vitro* replication kinetics of rg-VN1203 wild-type (WT) and mutant viruses. MDCK (A), A549 (B), or NHBE (C) cells were infected with rg-VN1203 WT or HA2-K58I virus at an MOI of 0.01 PFU/cell. Virus titers were determined at the indicated time points in MDCK cells by using $TCID_{50}$ assays. The detection limit was 1 $\log_{10}$ $TCID_{50}$/ml. Graphs are representative of two independent experiments. Statistical analysis was performed by using two-way ANOVA. Asterisks indicate P values of <0.01. Error bars show standard deviations.

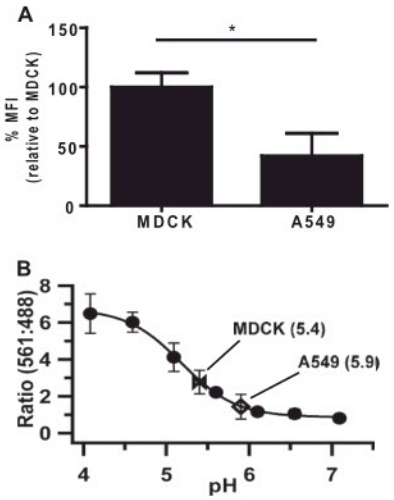

FIG 4 Endosomal acidification of MDCK and A549 cells. (A) pH-sensitive pHrodo dextran was used to compare the endosomal acidity of MDCK and A549 cells. Following uptake of the dye, cells were analyzed using flow cytometry to measure the intensity of pHrodo fluorescence. Data are expressed as the percentage of mean fluorescence intensity (MFI) relative to that of the MDCK cells. A higher fluorescence intensity of pHrodo correlates with a lower pH value. (B) Determination of the endosomal pH of MDCK (bowtie) and A549 (open diamond) cells. Cells were incubated with fluorescein-TMR double-conjugated dextran to allow uptake of the dye into the endosomes and then were washed and imaged by using confocal microscopy. Measurements of pH were done by using *in situ* calibration of fluorescein emission (closed circles) as a function of clamped endosomal pH. Clamping of endosomal pH was attained by using potassium ionophores as previously described (56). The average red (TMR, 561 nm)/green (fluorescein, 488 nm) intensities were obtained from six fields per cell line. The endosomal pH values of MDCK and A549 cells indicated on the curve were obtained by interpolation. Error bars represent standard deviations.

The HA2-K58I mutation does not alter the virulence of VN1203 in C57BL/6J mice. To investigate the effect of the HA2-K58I mutation on VN1203 virulence in mice, we inoculated C57BL/6J mice with 50 μl of PBS containing 150 EID_{50} of either rg-VN1203-WT or rg-VN1203-HA2-K58I and then either monitored the animals for weight loss and mortality (Fig. 5A and B) or euthanized the mice after 4 days of infection to measure tissue titers (Fig. 5C). Infection with HA2-K58I virus resulted in an average maximum weight loss of 8% after 11 days of infection, compared to an average maximum weight loss of 15% after 10 days of infection with WT virus; however, this difference was not statistically significant (Fig. 5A). Only 13% of mice infected with HA2-K58I virus survived infection, compared to 20% surviving infection with WT virus, a difference that was not statistically significant (Fig. 5B). Four days after the 50-μl inoculation in C57BL/6J mice, the wild-type and HA2-K58I viral loads were similar, and not robust, in the nasal cavity, trachea, and brain (Fig. 5C). Only wild-type virus was detected in the kidneys, although at low levels. In the lungs, significantly higher viral loads were detected in HA2-K58I virus-infected mice than in those infected with wild-type virus ($P < 0.01$, two-way ANOVA).

Low viral loads of rg-VN1203-HA2-K58I in the murine nasal cavity were unexpected, as we had previously found that the HA2-K58I mutation increased the viral load of the C58 isolate in the URT of mice (35). However, the C58 isolate is highly attenuated in mammals compared to the infectivity of VN1203 (52), so our previous studies on rg-C58-HA2-K58I required the inoculation of a more-susceptible strain of mice (DBA/2J) with 50 μl of 2.8×10^4 EID_{50} of virus to observe >50% mortality (35). We considered one possible explanation for the low virus load of rg-VN1203-HA2-K58I in the nasal cavity in the present study to be the combination of a relatively low virus inoculum (150 EID_{50}) in a rather high volume (50 μl), which would result in the retention of only a small number of infectious particles in the nasal cavity during the initiation of infection.

To investigate further the effect of the HA2-K58I mutation on VN1203 virus growth in the URT of mice, we inoculated C57BL/6J mice with 5 μl PBS containing 150 EID_{50} of either the WT or HA2-K58I virus. The small volume allowed maximum delivery of the inoculum to the nasal cavity at the expense of delivery to the lungs (60). A 5-μl inoculation with either the WT or HA2-K58I virus resulted in little (<10%) to no weight loss (Fig. 6A). Only one mouse in the HA2-K58I virus-infected group succumbed to infection, due to hind limb paralysis, compared to none in the WT group (Fig. 6B). After the 5 μl-inoculation, systemic spread was only observed for the HA2-K58I virus, which also had significantly higher viral loads ($P < 0.05$, two-way ANOVA) in the nasal cavity and trachea than did wild-type virus (Fig. 6D). Therefore, when infectious virus was delivered predominantly to the nasal cavity in a 5-μl volume, the HA2-K58I virus was found to enhance VN1203 virus growth in the murine URT.

The HA2-K58I mutation enhances early growth of VN1203 in the ferret nasal cavity but does not promote productive contact transmission. We next investigated the effect of the HA2-K58I mutation on VN1203 virus growth, virulence, and contact transmission in ferrets. For these studies, we inoculated groups of 5 ferrets intranasally with 0.5 ml of PBS containing 1,000 $TCID_{50}$ of either rg-VN1203-WT or rg-VN1203-HA2-K58I. Nasal washes were performed on all 5 ferrets on days 1, 2, and 4 postinoculation. After 5 days of infection, three of the five directly inoculated ferrets were euthanized so that tissues could be recovered and viral loads could be measured. The other two inoculated ferrets were used as donor animals in contact transmission experiments in which one directly inoculated ferret was cohoused with two naive contact animals 1 day after inoculation. Ferrets directly inoculated with the HA2-K58I virus had slightly less weight loss, albeit not a significant difference, than those directly inoculated with WT virus (Fig. 7A). All ferrets directly inoculated with either virus succumbed to infection within 7 days due to severe weight loss or were euthanized because of hind limb paralysis (Fig. 7B).

The viral loads of the HA2-K58I virus in ferret nasal washes were 100- to 1,000-fold higher ($P < 0.01$) than those of the wild-type virus on days 1 and 2 postinfection (Fig. 7C). Thus, we observed a correlation between increased HA acid stability (or a lower pH of activation) and early H5N1 growth in the ferret URT. By day 4 postinfection, both wild-type and HA2-K58I virus-infected ferrets had similar viral loads in their nasal washes (Fig. 7C), and by day 5 postinoculation, both groups had similar viral loads in the nasal tissues collected from euthanized animals (Fig. 7D). Similarly, the brains and large intestines from the wild-type and HA2-K58I virus-infected ferrets had comparable viral loads on day 5 postinoculation. Relatively large amounts ($>10^4$ $TCID_{50}$/g of tissue) of wild-type VN1203 virus were detected in the lungs and the livers of inoculated ferrets; however, only low levels (<10 $TCID_{50}$/g of tissue on the average) of HA2-K58I virus were detected in the lungs and no HA2-K58I virus was detected in the

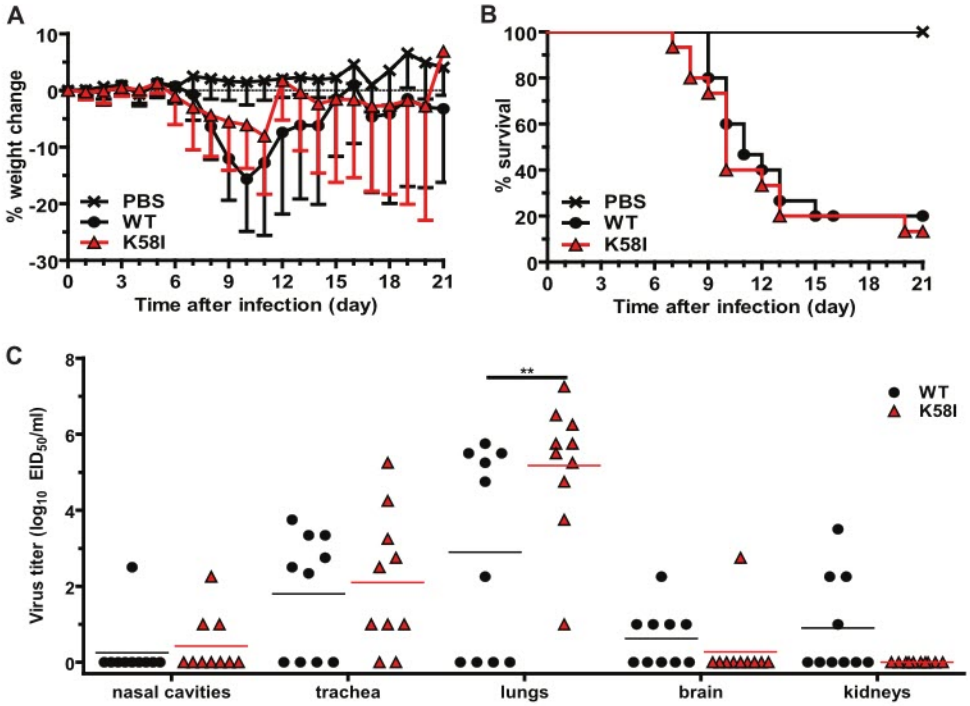

FIG 5 Effect of the K58I mutation on virulence and virus growth in C57BL/6J mice following inoculation with a large volume. (A and B) Mean percentages of weight change (A) and survival (B) of C57BL/6J mice ($n = 15$) following intranasal inoculation with 50 μl PBS containing 1 MLD_{50} of the WT or HA2-K58I viruses. The control (PBS) group was inoculated with 50 μl PBS only. Error bars show standard deviations. (C) Replication of the rg-VN1203 WT and HA2-K58I viruses in different mouse tissues. Tissues were harvested from mice ($n = 10$) on day 4 following infection with 1 MLD_{50}/50 μl, and the EID_{50}/ml titers were determined in 10-day-old embryonated chicken eggs. The detection limit was 1 $\log_{10}$ EID_{50}/ml. Horizontal lines within groups show mean values. Statistical analysis was performed by using two-way ANOVA for comparison of weight loss and virus titers and the log-rank chi-square test for survival curves. Asterisks indicate P values of <0.01.

livers of inoculated ferrets (Fig. 7D). The low viral loads of HA2-K58I virus in the lungs of ferrets were unexpected, as the mutant virus had greater viral loads in the lungs of C57BL/6J mice (Fig. 5C).

None of the four naive contact ferrets from either the wild-type or the HA2-K58I virus-infected group were observed to lose weight, die, have neurological symptoms, or shed virus (Fig. 7A to C). The absence of weight loss and detectable virus in the nasal washes of contact ferrets suggested that neither wild-type nor HA2-K58I virus was able to efficiently transmit between ferrets. To confirm that contact transmission did not occur even at minimal levels, serum was collected from contact ferrets 20 days after they had first been exposed to directly inoculated animals. For the wild-type group, none of the four contact ferrets seroconverted (limit of detection, anti-H5 titer of 10). In contrast, one of the four contact ferrets in the HA2-K58I group seroconverted, albeit to a low level (anti-H5 titer of 20) that is suggestive of nonproductive contact transmission. As productive transmission between co-housed ferrets was not observed, airborne transmission experiments between separated donor/recipient ferret pairs were not conducted.

DISCUSSION

In this study, we investigated the effect of a single mutation (i.e., HA2-K58I, which decreases the pH of activation of the HA protein) on the growth, virulence, and transmissibility of an H5N1 influenza virus in mice and ferrets. The virus chosen for this work was A/Vietnam/1203/2004 (H5N1). The VN1203 virus was chosen over the previously characterized in A/chicken/Vietnam/C58/2004 (35) due to its ability to replicate and cause disease in ferrets (52). This human isolate from clade 1 has an avian preferred α(2,3) receptor binding specificity but a mammalian-adapted polymerase (i.e., PB2-E627K) (52). As the VN1203 virus was not laboratory adapted or reassorted and contained no mutations other than HA2-K58I, the results described here bear directly on the potential of a single, acid-stabilizing mutation to enhance the capability of a circulating H5N1 virus to grow, disseminate, and transmit in mammalian hosts. Our biochemical analyses showed that the K58I mutation in the HA2 stalk decreases the pH of activation of the VN1203 HA protein from pH 6.0 to 5.5 without affecting HA protein expression, cleavage, or receptor binding. *In vitro*, the HA2-K58I mutation did not affect VN1203 replication kinetics in MDCK and NHBE cells but was attenuating in A549 cells, which were found to have a higher endosomal pH than MDCK cells. In mice and ferrets, the HA2-K58I mutation did not alter the virulence of VN1203. However, the acid-stabilizing mutation altered the tropism of the virus by promoting 100- to 1,000-fold greater growth in the ferret URT early in infection (days 1 to 2 after inoculation) while simultaneously reducing the viral load 1,000- to 100,000-fold in the lungs later in infection (day 5 after inoculation). Robust influenza virus growth in the URT is necessary for transmission between ferrets (61). Yet, the enhanced growth of VN1203 in the URT due to the acid-stabilizing HA2-K58I mutation was not sufficient to enable productive transmission between contact ferrets. We have found previously that an

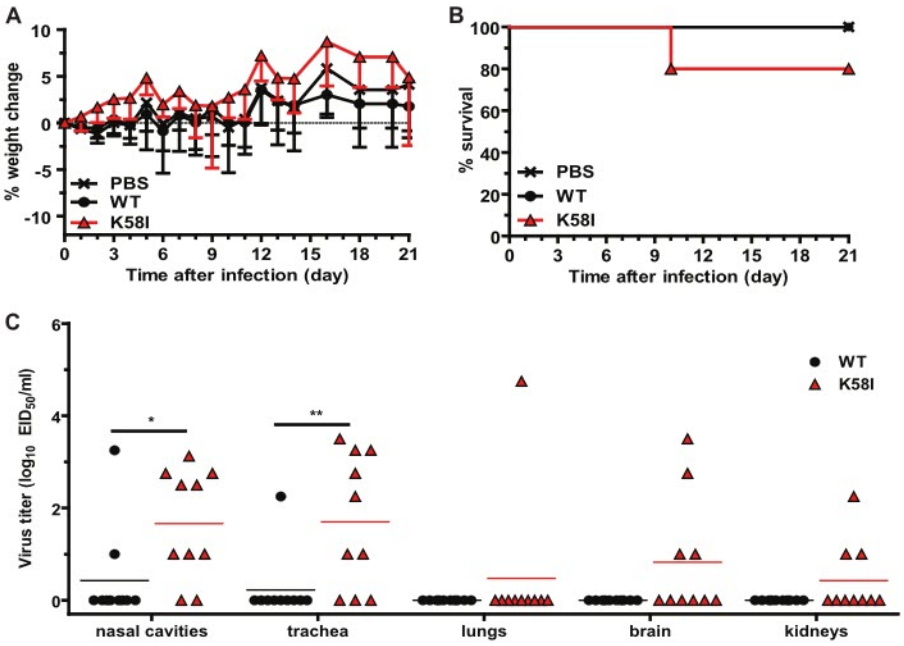

FIG 6 Effect of the HA2-K58I mutation on virulence and virus growth in C57BL/6J mice following inoculation with a small volume. (A and B) Mean percentages of weight change (A) and survival (B) of C57BL/6J mice ($n = 5$) following inoculation with a small volume (5 μl) of PBS containing 1 MLD_{50} of the rg-VN1203 WT or HA2-K58I virus. The control (PBS) group was inoculated with 5 μl PBS only. Error bars show standard deviations. (C) Replication of WT and HA2-K58I viruses in different mouse tissues. Tissues were harvested from mice ($n = 10$) on day 4 following infection with 1 MLD_{50}/5 μl, and the EID_{50}/ml titers were determined in 10-day-old embryonated chicken eggs. The detection limit was 1 $\log_{10}$ EID_{50}/ml. Horizontal lines within groups show mean values. Statistical analysis was performed by using two-way ANOVA for comparison of weight loss and virus titers and the log-rank chi-square test for survival curves. *, $P < 0.05$; **, $P < 0.01$.

HA2-K58I mutation in A/chicken/Vietnam/C58/2004 (H5N1) is attenuating in mallards (39) but enhancing in mice (35). Here we report that HA2-K58I enhances the early growth of the related VN1203 virus in the ferret URT. Overall, these studies reveal that a single acid-stabilizing mutation in the HA protein can switch the preference of a nonadapted/nonreassorted H5N1 virus (which has avian preferred receptor binding specificity) from an avian to a mammalian host; however, such an adaptation appears to be insufficient for transmissibility in mammals in the absence of other molecular factors.

For influenza virus to transmit, either by contact or by aerosol, it must efficiently infect, replicate in, and expel from the URT and be stable in the environment and/or respiratory droplets. An E627K mutation in the PB2 protein was found to support H5N1 influenza virus replication in the URT of mammalian species (62, 63). However, the presence of this mutation was not permissive for transmission of VN1203 H5N1 virus among ferrets (51). The HA protein receptor binding preference for α(2,6)-linked sialic acid receptors is considered a requirement for efficient influenza virus transmission between humans (64). A switch from α(2,6)- to α(2,3)-linked sialic acid receptor specificity abolishes the ability of the 1918 pandemic H1N1 influenza virus to transmit among ferrets (65). Despite some recent H5N1 influenza viruses acquiring the ability to bind α-(2,6) receptors (48), these viruses remain unable to transmit among ferrets and, presumably, humans (50, 51). This suggests that a decrease in α(2,3) receptor binding affinity in addition to increased α-2,6 receptor binding affinity may be required for efficient airborne transmission of H5N1 influenza viruses between ferrets or humans. Nonetheless, one study introduced mutations known to switch the receptor binding specificity of the H5 HA protein to α(2,6)-linked sialic acid, and yet the engineered H5N1 virus still failed to transmit, implying that other molecular changes are needed (50). Similarly, another study showed that an H5 virus with α(2,6) binding preference was able to partially transmit among ferrets via respiratory droplets only when it was coupled with a human N2 neuraminidase (21). Another study using an H5 reassortant virus showed that adding an acid-stabilizing HA mutation promoted contact transmission in ferrets (66), although the implications of the study for circulating H5N1 viruses are unclear, as the reassortant virus contained 6 PR8 internal genes, a 2009 H1N1 pandemic NA gene, and an H5N1 HA gene that had α(2,6) receptor specificity and lacked both a polybasic cleavage site and glycosylation on residue 158.

Recently, two laboratories were able to engineer and adapt H5 HA-containing influenza viruses that transmit between ferrets by the airborne route (24, 25). One study used A/Indonesia/5/2005 (H5N1) (24), and the other study used a reassortant virus containing the HA gene from A/Vietnam/1203/04 (H5N1) and the other seven genes from A/California/04/09 (H1N1) (25). In both cases, three sequential groups of mutations were needed before efficient airborne transmissibility was achieved, as follows: (i) two mutations in the HA receptor binding pocket that switched the receptor binding specificity from α(2,3) to α(2,6), (ii) a single mutation that removed a glycosylation site from the top of the HA receptor binding domain, and (iii) a single HA1 mutation in or adjacent to the metastable stalk domain that decreased the pH of activation of the HA protein (either measured directly [25] or as judged by structural considerations [24, 41]). Thus, a decrease in the pH of activation of the H5 HA protein to ~5.6 (25) was necessary for airborne transmissibility in ferrets. The data from the present

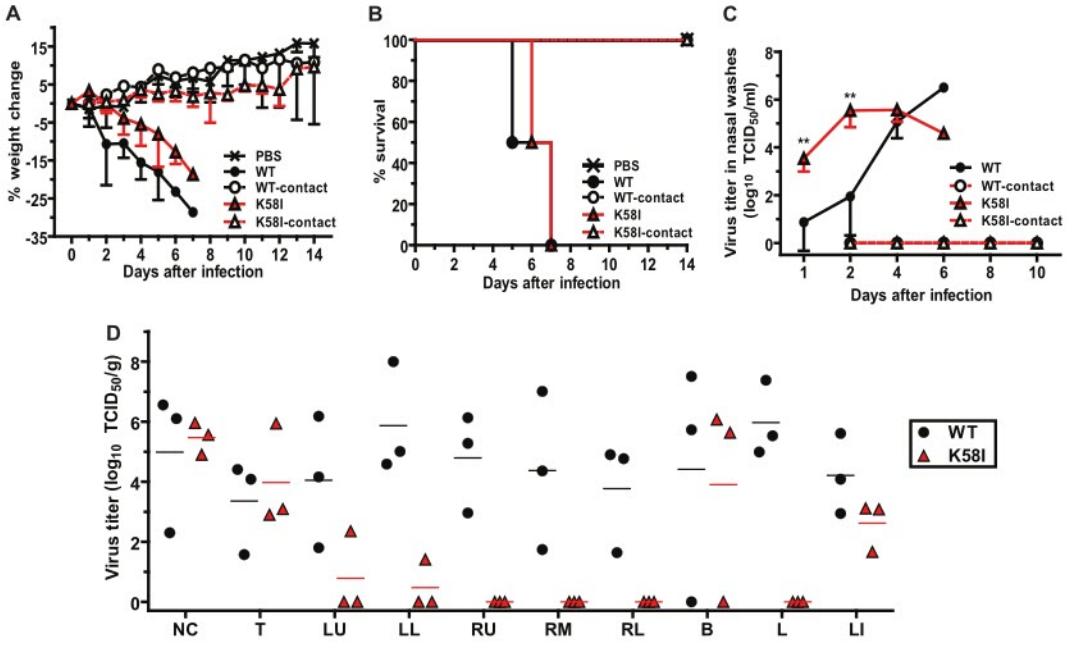

FIG 7 Effects of the K58I mutation on virulence, replication, and contact transmission of H5N1 influenza virus in ferrets. (A and B) Ferrets ($n = 5$) were inoculated with 0.5 ml PBS containing 10^3 $TCID_{50}$ of the WT or HA2-K58I viruses. Two of the 5 directly inoculated ferrets and all contact ferrets ($n = 4$) were observed for (A) weight loss and (B) survival. (C) Virus replication in the nasal cavities. Nasal washes were collected from all ferrets on the indicated days until death or termination of the experiment. On day 5, 3 of the 5 directly inoculated ferrets were euthanized to collect tissues, and on days 5 and 6, one ferret each from the WT and the HA2-K58I group, respectively, died from illness. (D) Replication of the WT and HA2-K58I viruses in different body tissues on day 5 after infection. The detection limit was 1 $\log_{10}$ $TCID_{50}$/ml. Closed symbols indicate directly inoculated ferrets. Open symbols indicate contact ferrets. NC, nasal cavities; T, trachea; LU, left lung's upper lobe; LL, left lung's lower lobe; RU, right lung's upper lobe; RM, right lung's middle lobe; RL, right lung's lower lobe; B, brain; L, liver; LI, large intestine. Horizontal lines within groups show mean values. Statistical analysis was performed by using two-way ANOVA for comparison of weight loss and virus titers and the log-rank chi-square test for survival curves. The differences in the titers of WT and HA2-K58I viruses in the left lower lung were statistically significant ($P < 0.01$). Statistical analyses could not be performed to compare WT and K58I groups in tissues where no K58I was detected, including the liver and right upper, middle, and lower lung.

study show that a single mutation in the HA protein that lowers the HA activation pH to 5.5 is not sufficient to promote contact transmission in ferrets in the background of a nonreassorted/non-laboratory-adapted H5N1 influenza virus that retains its avian-like α(2,3) receptor binding specificity and intact glycosylation sites.

On one hand, the results from the present study suggest a lower risk that circulating avian-like H5N1 influenza viruses will jump species to humans, as H5N1 viruses are now expected to require more than one acid-stabilizing HA mutation to acquire pandemic potential. Other required changes include the acquisition of α(2,6) receptor binding specificity and/or the deletion of a glycosylation site (24, 25). Furthermore, our previous studies show that circulating avian H5N1 influenza viruses tend to have HA activation pH values ranging from 5.7 to 6.0 (39, 41, 54) and are unlikely to acquire acid-stabilizing mutations that lower the HA activation pH below pH 5.6 in species such as chickens and ducks (39, 41, 42). On the other hand, during H5N1 infection in mammals, the likelihood of a virus acquiring an acid-stabilizing mutation may be relatively high. The present and previous studies (35, 44, 45, 66, 67) show that acid-stabilizing mutations promote H5N1 growth in the URT of mammals. We propose that numerous acid-stabilizing mutations are functionally equivalent, thereby increasing the likelihood of such a mutation being naturally selected. Eight mutations in H5 viruses have already been reported to decrease the HA activation pH: HA1-H18Q (35, 39, 43, 66), HA1-H103Y (24), HA1-D104N/I115T, HA1-E216K, and HA1-S221P (41), HA1-T318I (25), HA2-K58I (35, 39, 44), and HA2-E105K (43). These residues are located throughout the prefusion HA protein in four broad regions that undergo dramatic changes in secondary and tertiary structure after acid-induced, irreversible activation. The four regions include (i) the interface between the HA1 receptor binding domain trimer, (ii) the HA1-HA2 interface, (iii) the spring-loaded HA2 stalk, and (iv) the pocket surrounding the hydrophobic fusion peptide (Fig. 8). Dozens of other acid-stabilizing mutations to numerous H5 HA residues could potentially arise, just as a wide variety of activation pH-altering mutations in these four regions of the HA molecule have been selected for in H3N2 and H7N7 viruses (69–71). As mutations that alter the acid stability of the HA protein appear to be functionally equivalent, we propose that future surveillance efforts to identify H5N1 viruses with increased potential for transmissibility in mammals include functional assays for HA acid stability, in addition to using sequence data to identify known acid stabilizers, such as HA1-H103Y and HA1-T318I.

Even though the HA2-K58I mutation did not increase the virulence of highly pathogenic H5N1 influenza virus or permit contact transmission in ferrets, this mutation altered the tropism of the VN1203 virus in the ferret respiratory tract and increased the stability of the virus in the presence of acid at pH 5.5. Therefore, HA2-K58I is a gain-of-function mutation, and the results described here qualify as dual-use research (DUR). We do not be-

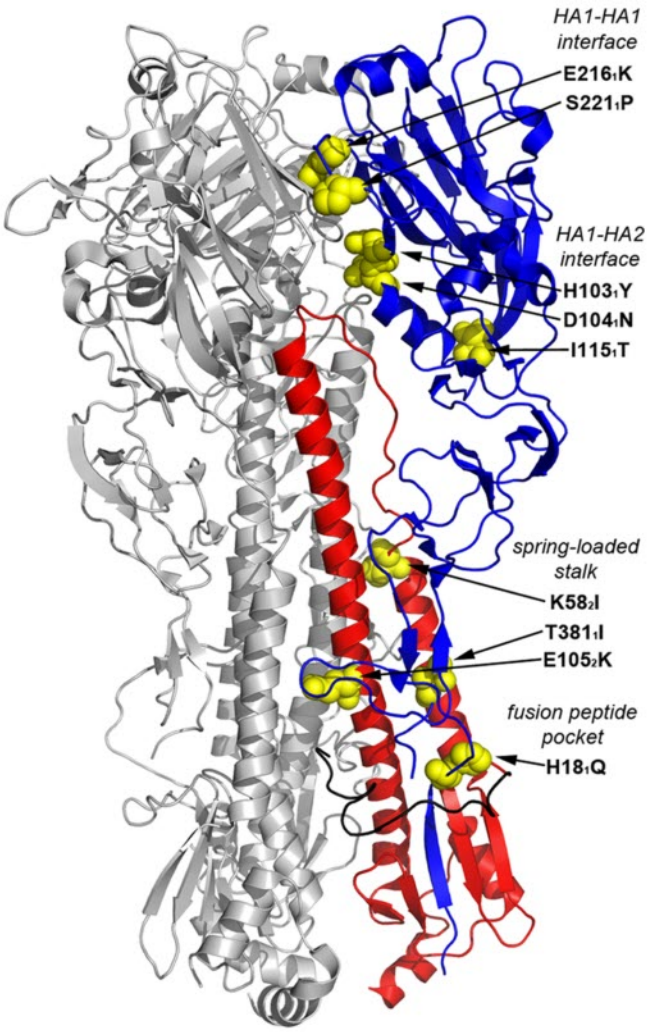

FIG 8 Structure of the H5N1 HA protein, identifying the locations of acid stabilizing mutations. In this crystal structure of the H5N1 HA protein (PDB 3S11), two protomers are colored gray. In the third protomer, residues in the HA1 subunit are colored blue, residues in HA2 are red, and the hydrophobic fusion peptide in HA2 is black. Amino acid residues for which acid stabilizing mutations have been discovered are presented as yellow spheres. The mutations are listed to the right of the molecule, along with the four structural regions that contain the mutations (denoted by italics). H3 numbering is used, and subscripts denote HA1 (1) and HA2 (2) subunits.

lieve that the VN1203-HA2-K58I virus itself or the results described here could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety or to agriculture. Therefore, we contend that this work is not dual-use research of concern (DURC) (72). The VN1203-HA2-K58I agent itself poses no enhanced risk to public health or agriculture compared to unmodified VN1203 or other circulating H5N1 viruses because (i) the HA2-K58I mutation does not increase virulence or transmissibility in ferrets, a model for infection in humans, and (ii) the HA2-K58I mutation has been shown previously to reduce H5N1 virus growth, virulence, and transmission in mallards, an avian model (39). Additionally, the VN1203-HA2-K58I virus is susceptible to oseltamivir, and a vaccine containing the parental VN1203 HA protein is available (44). The VN1203-HA2-K58I virus was not actively adapted during experiments in ferrets, which were conducted in 2011 before the moratorium on H5N1 research (73), and tissues were destroyed after titers were measured. Knowledge of the HA2-K58I mutation could not be directly misapplied to threaten public health by introducing HA2-K58I into the ferret airborne-transmissible viruses (24, 25), which already have functionally equivalent acid-stabilizing mutations and would most likely be attenuated by the addition of an HA2-K58I mutation (35). These studies have been reviewed and approved before, during, and after completion both internally by the Institutional Biosafety Committee and externally by the funding agency. All work was conducted in a secure biocontainment ABSL3+ facility (see Materials and Methods) by highly qualified and trained scientists who abide by a quarantine policy that prohibits contact with immunocompromised patients, zoos, pet shops, and avian species within 1 week of accessing the ABSL3+ facility.

Highly pathogenic H5N1 influenza viruses continue to circulate in domestic poultry and wild birds, and these viruses occasionally infect humans and other mammals (22). If H5N1 viruses adapt to mammals and acquire the ability for sustained human-to-human transmission, a pandemic will be both inevitable and devastating to public health and economic welfare. Therefore, there is an urgent need and responsibility to assess the risk of this threat and take precautionary steps to prevent, contain, or minimize it. This work describes a mutation and a molecular property that support the adaptation of H5N1 influenza viruses from avian to mammalian hosts and provides a better understanding of the requirements for H5N1 influenza viruses to cross the species barrier.

Knowledge of the results described here may benefit public health in several ways. First, this work provides a mammalian preferred HA activation pH (~5.5) for H5N1 and specific HA mutations that enhance its acid stability (Fig. 8). This information will enhance the effectiveness of influenza virus surveillance activities. Second, understanding interspecies adaptation will assist in risk assessment, prepandemic vaccine selection, and decisions to cull animals or quarantine humans. Third, the HA2-K58I mutation may be used to engineer vaccines for optimal growth in culture (egg or cell-based) and *in vivo*, thereby enhancing vaccine production and efficacy, as has already been demonstrated in two proof-of-concept studies (38). Fourth, this work bolsters novel approaches to develop anti-influenza virus inhibitors that prevent or induce HA conformational changes by binding to the stalk domain. Mutations conferring resistance to several experimental fusion inhibitors have been shown to increase the activation pH of the HA protein (74, 75), which the present study suggests may be counterproductive for efficient early growth in the mammalian URT. Finally, the results described here may provide new directions for research on other important human and animal pathogens, such as SARS coronavirus, dengue virus, hepatitis C virus, Epstein-Barr virus, human rhinovirus, vesicular stomatitis virus, and avian leukemia virus, which also contain envelope glycoproteins that are activated by low pH (76). The host range and tropism of these other viruses may also be influenced by mutations that alter the pH of activation of their fusion glycoproteins.

ACKNOWLEDGMENTS

We thank Elena Govorkova for providing the NHBE cells, Patrick Seiler for help with tissue collection, and Beth Little, David Carey, Scott Krauss, and Lisa Kercher for help with animal care and ABSL3+ training and procedures. We thank Deborah Nelson for advice on endosomal pH measurement and Jennifer Peters and Jama Temirov for help with confocal

imaging. We thank the Animal Resources Center and the Hartwell Center Macromolecular Synthesis and Sequencing facilities at St. Jude Children's Research Hospital for providing support.

This work was supported by the National Institutes of Health, National Institute of Allergy and Infectious Diseases, Centers of Excellence for Influenza Research and Surveillance (CEIRS), under contract no. HHSN266200700005C and by the Children's Infection Defense Center (CIDC) at St. Jude Children's Research Hospital and the American Lebanese Syrian Associated Charities (ALSAC).

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MEMORANDUM
Office of the Scientific Director

TO: Dr. Charlie Russell, Department of Infectious Diseases

FROM: Dr. James Downing, EVP & Scientific Director, Institutional Official
Dr. Philip Potter, Chair Dual Use Research of Concern (DURC) Subcommittee

DATE: November 16, 2012

SUBJECT: **Evaluation of Dr. Charlie Russell's Institutional Biosafety Committee Project #O3A-372 (SA00000239) for Dual Use Research of Concern (DURC) - Highly Pathogenic Avian Influenza (HPAI) Viruses**

On August 31, 2012, the IBC Dual Use Research of Concern (DURC) subcommittee met to review and discuss experiments that you are currently conducting, which involves highly pathogenic avian influenza (HPAI) viruses. During your presentation, you provided a thorough review of your current activities that involve life science research that has been identified as having the potential of providing knowledge, information, products, or technologies that could be directly misapplied, thus posing a significant threat with broad potential consequences to public health and safety, agricultural crops, and other plants, animals, the environment, material, or national security. However, after careful consideration it was determined by the Dual Use Research of Concern (DURC) subcommittee and subsequently concurred by the Institutional Biosafety Committee (IBC) that the current project does not present such risk and therefore does not meet the definition of DURC as specified in the policy.

Sincerely,

(b)(6)

Jan
EVP & Scientific Director, Institutional Official

11/26/12
Date

(b)(6)

Philip Potter, Ph.D.
Chair Dual Use Research of Concern (DURC) Subcommittee

11/30/12
Date

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MEMORANDUM
Department of Environmental Health and Safety

To: Dr. Charles Russell Department of Infectious Diseases
From: Dr. Phillip Potter, V. Chair, Institutional Biosafety Committee
Date: 2/11/2016
Subject: IBC Approval of Biological Project # SA00001553 - **Highly pathogenic and other BSL3+ influenza viruses (RE)**

The Institutional Biosafety Committee has reviewed and approved your Renewal Application on project **#O3A-372** entitled **Highly pathogenic and other BSL3+ influenza viruses (RE)**. In addition to precautions considered good laboratory practice, Biosafety Level 3 precautions should be taken. Please review Biosafety Level 3 requirements and the PI's responsibilities under the *NIH rDNA Guidelines*. This approval is specific for the following:

Objectives: The purpose of this project is to (a) understand mechanisms of replication, infectious disease, inter-species adaptation, and inter-host transmission of highly pathogenic influenza viruses; and (b) exploit an understanding of influenza virology to develop novel vaccines and therapeutics. The primary viral protein of interest in this project is the hemaglutinin (HA) surface glycoprotein--the primary antigenic determinant of influenza viruses, a central player in the emergence of pandemic influenza, and the viral protein responsible for invasion of influenza viruses into host cells. As influenza virus properties are determined by a constellation of viral and host factors, studies on the HA protein also involve characterization of the properties of the other viral genes.

Medical Surveillance Required: Annual seasonal influenza immunization

PPE Required: Refer to BMBL 5th edition

Other Special Requirements: After careful deliberation on the proposed work and taking into consideration the recommendations from the IBC DURC subcommittee the following special requirements must be observed. They are as follows:

1. While the current research would be classified as dual use (DUR), it is not DUR "of concern" (DURC).
2. If publication(s) go forward, we suggest including a statement in the Abstract and Methods sections conveying the dates that the experiments were conducted. It should be noted in any publications that these experiments were conducted during an 18-month period (January 2010--June 2011), which precedes any moratoriums that have since been established. Similar statements are typically included in clinical trial reports, and this would alleviate any concerns that such experiments are ongoing at SJ during the moratorium.
3. The PI must submit an annual report about the ongoing research to the IBC DURC subcommittee.
4. Unexpected findings that might result in enhancements considered harmful to public health, animals or agriculture must be immediately reported to the IBC DURC subcommittee via the IBC Chair, V. Chair or BSO.
5. Gain of function (GOF) experiments shall not be conducted at this time.
6. Both ABSL-3 practices and containment must be observed.

Personnel or Room Modifications: None

This approval covers the duration of the project within a 1-year period unless substantial changes occur in materials or methods, at which time, you must submit an amendment for Institutional Biosafety Committee review of the changes. If your project has not been completed within 1-year, it will require continuing review on or before 2/21/2017.

If you have questions or need further assistance to ensure the safe conduct of these experiments, please do not hesitate to contact the Biological Safety Officer or me.

Philip Potter, Ph. D., V. Chair
Institutional Biosafety Committee

CC: Environmental Health and Safety

National Security Advisory Board for Biosecurity(NSABB)

Certain research projects that do or do not involve Select Agents may be considered dual use research depending on the nature of the particular experiments and the potential for misuse of the results and/or technology. The National Security Advisory Board for Biosecurity(NSABB) provides advice regarding biosecurity oversight of dual use research. Therefore, identification of such technologies/applications at the local level is critical. For that reason and to prevent the use of technology that could be misapplied to threaten public health or national security, it is necessary that the following additional questions be addressed so that an appropriate determination can be achieved.

Dual Use Research Help: Help

1.0 * Does the proposed research have the highest potential for yielding knowledge, products, or technology that could be misapplied to threaten public health or national security?
(●) Yes ( ) No

1.5 * Will the research enhance the harmful consequences of a biological agent or toxin?
(●) Yes ( ) No

2.0 * Does the research have the potential of disrupting immunity or the effectiveness of an immunization without clinical and/or agricultural justification?
( ) Yes (●) No

3.0 * Can the proposed research confer to a biological agent or toxin, resistance to clinically, and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitate their ability to evade detection methodologies?
( ) Yes (●) No

4.0 * Will the proposed research increase the stability, transmissibility, or the ability to disseminate a biological agent or toxin?
(●) Yes ( ) No

5.0 * Will the research alter the host range or tropism of a biological agent or toxin?
(●) Yes ( ) No

6.0 * Will the research render the host populations to be more susceptible to the consequences of an agent or toxin?
( ) Yes (●) No

7.0 * Will the research generate a novel pathogenic agent or toxin or reconstitute an eradicated or extinct biological agent?
( ) Yes (●) No

8.0 If you answered yes to any of the above listed effects then please provide a detailed scientific explanation as to why you believe your conclusion to be true. Please be precise in your response.

We previously generated mutant H5N1 viruses for which "yes" has been noted above. These viruses have been reviewed by the IBC and the DURC subcommittee. We are not planning on generated new mutant viruses unless we first consult with the IBC and DURC subcommittee.

MINUTES
Institutional Biosafety Committee
St. Jude Children's Research Hospital

MEETING: 2016-02

DATE: **Thursday, February 11, 2016**

PRESENT: Richard Webby, Jason Rosch, Helen Morrow, Rob Throm, Scott Long, Peter Vogel, Elroy Fernandes, Mike Meagher, Amali Samarasinghe, Phil Potter, Mark Hatley, Jim Gaut, and James Henry

ABSENT: Lisa Kercher, Scott Shanker, Richard Rahija and McGehee Marsh

GUESTS: Charles Russell and Stacey-Schultz-Cherry

TOPIC	DISCUSSION/ACTION	FOLLOW-UP
Welcome	Dr. Webby presided over the meeting.	
Minutes of last meeting	A motion was made to approve the minutes from the January 14, 2016. The motion passed.	No follow up needed.
Regulatory reviews	Nothing to report.	No follow up needed.
Adverse events	Nothing to report.	No follow up needed.
Variance Report	Nothing to report.	No follow up needed.
Reportable events	An employee of the Department of Therapeutics Production & Quality sustained a splash to their brow and eyelid while disconnecting a line. The initial notification was submitted to the NIH-OBA on February 1, 2016 and a formal report will be submitted within the thirty day requirement.	Follow up will continue.
ICC report	Nothing to report.	No follow up needed.
GMP Report	Nothing to report.	
Select Agent Report	The amendment to remove a room from the registration is still pending. It	No follow up needed.

	is anticipated that the approval will be received shortly.	
New Business		
[redacted]	This is a new project to compare the event-free survival (EFS) in patients with newly diagnosed metastatic Ewing sarcoma treated with multiagent chemotherapy with and without the addition of ganitumab (AMG 479). After assessment of the biosafety risks, there were no biosafety concerns. This project is exempt from NIH Guidelines. A motion was made to approve this biosafety level 1 project. The motion passed.	Project status; project approved.
SA00001529 (O1A-660) g3bp1-gfp reporter [redacted] Principal Investigator: Dr. Joseph Taylor	This is a new project to create a reporter [redacted] to directly observe stress granule formation and dynamics within a mammalian, whole organism context. After assessment of the biosafety risks, there were no biosafety concerns. This project is subject to *NIH Guidelines Section III-E-3*. A motion was made to approve this biosafety level 1 project. The motion passed.	Project status; project approved.
SA00001539 (O2-662) Determining the function of MAGE proteins. Principal investigator: Dr. Patrick Potts	This is a new project to elucidate the biochemical, molecular, and cellular functions of the MAGE protein family. The committee advised Dr. Potts that HIV surveillance will be necessary for work involving lentivirus and Hepatitis B vaccination is also required for individuals working with human cells. It was recommended to change to a four plasmid system in the future to further decrease the potential for RCL formation and increase the biosafety profile of these vector preps. The committee also asked for more detail on the nature of the human cell lines to be used. The committee reviewed Dr. Potts responses prior to the meeting. After assessment of the biosafety risks, there were no biosafety concerns and the committee was satisfied with Dr. Potts response. This project is subject to *NIH Guidelines Section III-E-1*. A motion was made to approve this biosafety level 2 project. The motion passed.	Project status; project approved.
SA00001555 (O2A-284) Lentiviral Deletion of MCL-1 and Anti-Apoptotic BCL-2 Family Members. Principal Investigator: Dr. Joseph	This is an amendment to an approved project. The committee asked for the project description and generation of lentivirus sections be updated to reflect that the vector core no longer provides services as previously indicated in the application.	Project status; amendment approved.

Opferman	The committee reviewed Dr. Opferman's responses prior to the meeting. After assessment of the biosafety risks, there were no biosafety concerns and the committee was satisfied with the response. This project is subject to *NIH Guidelines Sections III-D-2, III-E-1 and III-D-3*. A motion was made to approve the amendment to this biosafety level 2 project. The motion passed.	
SA00001583 (O2-418) Production of Lentiviral gene therapy vectors. Principal Investigator: Dr. Michael Meagher	This is an amendment to an approved project to update safety precautions and risk analysis. Dr. Meagher explained to the committee the proposed changes. The committee asked for more information in the rDNA section and that questions 4 and 5 in the retrovirus section be answered. After assessment of the biosafety risks, there were no biosafety concerns. This project is subject to *NIH Guidelines Section III-D-2-a and III-E-1*. A motion was made to approve the amendment to this biosafety level 2 project pending FDA approval. The motion passed. Dr. Meagher was not present for discussion and voting. Dr. Throm also abstained from voting.	Project status; amendment approved pending FDA approval.
SA00001557 (O2A-227) Role of anti-apoptotic MCL-1 in hematopoiesis and Leukemia. Principal Investigator; Dr. Joseph Opferman	This is an amendment to an approved project. After assessment of the biosafety risks, the committee had no biosafety concerns. This project is subject to *NIH Guidelines Section III-D-2-a and III-E-1*. A motion was made to approve the amendment to this biosafety level 2 project. The motion passed.	Project status; amendment approved.
SA00001576 (O2A-586) ALK as a therapeutic target in rhabdomyosarcoma. Principal Investigator: Dr. Philip Potter	This is an amendment to an approved project to update genes and viral vectors in this project. After assessment of the biosafety risks, there were no biosafety concerns. This project is subject to *NIH Guidelines Section III-D-3-a*. A motion was made to approve the amendment to this project. The motion passed. Dr. Potter abstained from voting.	Project status; amendment approved.
SA00001541 (O2A-460) Transcriptional regulation of NK cell development.	This is an amendment to an approved project to transfer principal investigator responsibilities from Dr. Wing Leung to Dr. Lea Cunningham.	Project status; amendment approved pending response from Dr. Cunningham.

Principal Investigator: Dr. Lea Cunningham	The committee asked for retrovirus questions 4 and 5 be answered. After assessment of the biosafety risks, there were no biosafety concerns. This project is subject to *NIH Guidelines Sections III-E-1 and III-D-3*. A motion was made to approve the amendment to this biosafety level 2 project pending response from Dr. Cunningham. The motion passed.	
SA00001553 (O3A-372) Highly pathogenic and other BSL3+ influenza viruses. Principal Investigator: Dr. Charles Russell	This is a three year renewal of an approved project. Because this is a biosafety level 3 project, Dr. Russell was present to explain changes to the project. The committee asked that the DURC annual report be attached to the project and to confirm the studies proposed in the project will only use naturally occurring HPAI. There were also revisions needed in the personnel occupational health requirement section. Finally, a report will be submitted to the funding agency in accordance with the Policy for Institutional Oversight. After assessment of the biosafety risks, there were no biosafety concerns and the committee was satisfied with Dr. Russell's response. This project is subject to *NIH Guidelines Sections III-D-7, III-D-7-b, III-D-4, III-D-2, III-D-2-a and III-D-1-b*. A motion was made to approve the renewal of this biosafety level 3 project. The motion passed.	Project status: renewal approved. Report will be submitted in thirty days to funding agency in accordance with the Policy for Institutional Oversight.
[redacted]	This is a three year renewal of an approved project. The committee asked for Dr. Evans to clarify the use of human subjects in this project. After assessment of the biosafety risks, there were no biosafety concerns and the committee was satisfied with Dr. Evans' response. This project is exempt from NIH Guidelines. A motion was made to approve the renewal to this biosafety level 2 project. The motion passed.	Project status; renewal approved.
SA00001547 (O2A-245) Mechanisms of cell death and cell survival. Principal Investigator: Dr. Douglas	This is a three year renewal of an approved project. The committee informed Dr. Green that HIV surveillance is required for work involving lentivirus. Also, to clarify the production system that will be used and source of the cells that will be transduced.	Project status; renewal approved pending response to committee questions.

Green	After assessment of the biosafety risks, there were no biosafety concerns. This project is subject to *NIH Guidelines Sections III-E-1 and III-D-3*. A motion was made to approve the renewal to this biosafety level 3 project pending response to committee questions. The motion passed.	
SA00001561 (O3A-487) Influenza virus infection in obese [redacted]. Principal Investigator: Dr. Stacey Schultz-Cherry	This is a three year renewal of an approved project. Because this is a biosafety level 3 project, Dr. Schultz-Cherry was present to explain changes to the project. The committee asked Dr. Schultz-Cherry to redact a statement that was noted in the description section of the application. Also, it was noted that baseline serum collection will be required. After assessment of the biosafety risks, there were no biosafety concerns, and the committee was satisfied with Dr. Schultz-Cherry responses. This project is subject to *NIH Guidelines Sections III-D-7-b*. A motion was made to approve the renewal to this biosafety level 3 project. The motion passed.	Project status; renewal approved.
[redacted]	This is an annual review of an approved clinical project. After assessment of the biosafety risks, there were no biosafety concerns. This project is exempt from NIH Guidelines. A motion was made to approve the continuing review of this biosafety level 2 project. The motion passed.	Project status; continuing review approved.
[redacted]	This is an annual review of an approved clinical project. After assessment of the biosafety risks, there were no biosafety concerns. This project is exempt from NIH Guidelines. A motion was made to approve the continuing review of this biosafety level 1 project. The motion passed.	Project status; continuing review approved.

[redacted]		
[redacted]	This is an annual review of an approved clinical project. After assessment of the biosafety risks, there were biosafety concerns. This project is exempt from NIH Guidelines. A motion was made to approve the continuing review of this biosafety level 1 project. The motion passed.	Project status; continuing review approved.
SA00001527 (O3A-508) Host Responses to Highly Pathogenic Influenza Infection. Principal Investigator: Dr. Paul Thomas	This is an annual review of an approved biosafety 3 project. After assessment of the biosafety risks, there were no biosafety concerns. This project is subject to *NIH Guidelines Section III-D-7*. A motion was made to approve the continuing review of this biosafety level 3 project. The motion passed.	Project status; continuing review approved.
Other Business		
Declination for Hepatitis and Lentivirus Medical Surveillance	The committee discussed Hepatitis B and lentivirus medical surveillance requirements. More information will be requested from Human Resources and the Office of Legal Services as it relates to requirements and declination criteria.	Follow up will continue.
Biological Safety Manual	The final draft of the Biological Safety Manual has be sent to the committee for review. A motion was made to approve the document. The motion passed.	No follow up needed.
Children in the Workplace	The committee was informed of a policy that would be published in the Biological Safety Manual. In brief, the policy is as follows: **To protect children, no one under the age of 18 is permitted in laboratories or [redacted] facilities at St. Jude unless in a St. Jude-approved, formal learning activity and only after having completed EHS-reviewed safety measures specific for the hazardous work area to be entered.** This policy ensures children will not be exposed to biological, chemical, radiological or physical safety hazards present in these work areas. Hazardous work areas include any location identified by the responsible	No follow up needed.

	Principal Investigator, Director, Supervisor or Manager in a Work Hazard Assessment Tool (WHAT) or similar hazard assessment based on the Biosafety, General Safety and Radiation Safety Manuals and the Chemical Hygiene Plan. After an in-depth discussion the committee agreed with the policy and decision to include it in the Biological Safety Manual.	

The meeting was adjourned at 12:27.

Richard Webby, Ph.D., Chair
Institutional Biosafety Committee

St. Jude Children's Research Hospital
ALSAC · Danny Thomas, Founder
Finding cures. Saving children.

MEMORANDUM
Office of the Scientific Director

TO: Dr. Charlie Russell, Department of Infectious Diseases

FROM: Dr. James Downing, EVP & Scientific Director, Institutional Official
Dr. Philip Potter, Chair Dual Use Research of Concern (DURC) Subcommittee

DATE: November 16, 2012

SUBJECT: **Evaluation of Dr. Charlie Russell's Institutional Biosafety Committee Project #O3A-372 (SA00000239) for Dual Use Research of Concern (DURC) - Highly Pathogenic Avian Influenza (HPAI) Viruses**

On August 31, 2012, the IBC Dual Use Research of Concern (DURC) subcommittee met to review and discuss experiments that you are currently conducting, which involves highly pathogenic avian influenza (HPAI) viruses. During your presentation, you provided a thorough review of your current activities that involve life science research that has been identified as having the potential of providing knowledge, information, products, or technologies that could be directly misapplied, thus posing a significant threat with broad potential consequences to public health and safety, agricultural crops, and other plants, animals, the environment, material, or national security. However, after careful consideration it was determined by the Dual Use Research of Concern (DURC) subcommittee and subsequently concurred by the Institutional Biosafety Committee (IBC) that the current project does not present such risk and therefore does not meet the definition of DURC as specified in the policy.

Sincerely,

(b)(6)

Jame
EVP & Scientific Director, Institutional Official

11/26/12
Date

(b)(6)

Philip Potter, Ph.D.
Chair Dual Use Research of Concern (DURC) Subcommittee

11/30/12
Date

Evaluation of Dr. Charlie Russell's Institutional Biosafety Committee Project #O3A-372 (SA00000239) Dual Use Research of Concern (DURC) - Highly Pathogenic Avian Influenza (HPAI) Viruses

Research:
The goals of Dr. Russell's research are as follows:

1. Determine the contribution of the membrane fusion activity of the HA proteins of HPAI A viruses to adaptation acid stability of the HA protein in the interspecies adaptation of influenza viruses.

    - **Experiment 1:** Measure the pH dependence of membrane fusion of influenza viruses of different subtypes that were isolated from various host species.
    - **Experiment 2:** Identify the amino acid residues that regulate the membrane fusion activities of the HA proteins from avian influenza subtypes in a virus-free system (BL2).

2. Determine the contribution of membrane fusion activity to influenza virus replication and pathogenesis.
    - **Experiment 1**: Analyze the infectivity and replication efficiency of recombinant viruses containing HA mutations in the membrane fusion domain.
    - **Experiment 2**: Evaluate the virulence of these recombinant viruses in animal models.

Findings:
Based on the data and information shown, the DURC subcommittee concluded that Dr. Russell's experiments do not confer enhancements that could be construed as being immediately enabling if published. In addition, the information will enhance scientific knowledge in his field. The current practice and containment are adequate for the experiments that are being conducted. Finally, the current research does not meet the definition of DURC; namely, the nature of Dr. Russell's project does not meet all criteria to be classified as "of concern," as reflected in the policy.

Furthermore, we do not believe knowledge of the biological importance of the HA activation pH could be directly misapplied to pose a significant threat to public health for several reasons. They are as follows:

1. The scientific community is already fully aware that a decrease in the activation pH of the HA protein has been associated with adaptation of H5 influenza viruses to ferrets in the context of an H5 virus that already has mammalian-adapted polymerase and receptor binding specificity.

2. The C58 strain selected for the present study mitigates concerns about adapting H5N1 influenza viruses to mammals because the parental C58 virus has the following properties: (a) α(2,3) receptor-binding specificity; (b) a PB2-E627 residue that confers inefficient polymerase activity in mammals; (c) presence of glycosylation sites in the RBD head; (d) low growth and rapid clearance of the virus in the ferret nasal cavity; (e)

no contact or airborne transmission of the virus in ferrets; (f) the virus does not cause weight loss or mortality in ferrets; (f) the virus is susceptible to oseltamivir; (g) the virus is antigenically matched to an A/Vietnam/1203/04 experimental vaccine; and (h) the mutations investigated in this study were laboratory-engineered and have not been observed in H5 viruses in nature.

3. The mutant viruses were not actively adapted during animal experiments, and tissues were destroyed after titers were measured.

4. The present study establishes that a decrease in the HA activation pH is not sufficient to convert an avian H5N1 influenza virus into one that is highly pathogenic in mice or grows efficiently in the murine upper respiratory tract when the virus contains avian-preferred polymerase and receptor binding specificity. Nasal titers of the C58-HA-K58$_2$I virus were less than 10^4 EID_{50}/ml in DBA/2J mice, a relatively low level, and MLD_{50} values of the C58-HA-K58$_2$I virus were comparable those obtained previously for the prototypic 2009 pandemic virus A/California/04/09 (H1N1), reported to be 10^6 and 10^4 EID_{50} in C57BL/6J and DBA/2J mice, respectively. Thus, the present study establishes that the C58-HA-K58$_2$I virus is not highly pathogenic and does not grow to high titers in the upper respiratory tract of mice, and a previous study shows that the C58-HA-K58$_2$I virus is deficient for replication, virulence, and transmission in ducks. Based on current understanding, we believe that the insights of this study into the biological importance of the HA activation pH for an avian H5N1 influenza virus cannot be reasonably anticipated to be directly misapplied or accidently result in a significant threat to public health or agriculture.

Recommendations:
After careful deliberation, the DURC subcommittee recommends the following:

1. That the current research be classified as DUR and not DURC "of concern."

2. If publication(s) goes forward, we suggest including a statement in the Abstract and Methods sections conveying the dates that the experiments were conducted. For example, "These experiments were conducted during an __-month period (starting month, year – concluding month, year), which preceded the moratorium that has since been established. Similar statements are typically included in clinical trial reports, and this would alleviate any "raised eyebrows" that such experiments are ongoing at St. Jude during the moratorium.

3. The PI must submit an annual report about the ongoing research to the IBC DURC subcommittee.

4. Unexpected findings that might result in enhancements considered harmful to public health, animals, or agriculture must be immediately reported to the IBC DURC subcommittee via the IBC Chair, V. Chair, or BSO.

IBC Final Discussion and Approval:
During a convened meeting on September 13, 2012, the Institutional Biosafety Committee reviewed the findings provided by the DURC subcommittee. After deliberations, the IBC agreed with the proposed recommendations and moved to approve the project. The project was put to a motion, second, and passed by a majority.

Evaluated against the Seven Effects in the DURC Policy

1. **Enhances the harmful consequences of the agent or toxin?**
   After careful review of the proposed project and data it was determined that gain of function compared to the parental wild-type virus did not enhance harmful consequences of the agent. While the HA-K58I mutation was shown to increase the pathogenicity of the lower-pathogenic CH58-strain virus, this mutation actually caused less weight loss after infection in the background of the higher-pathogenic VN1203 strain in mice and in ferrets.

2. **Disrupts immunity or the effectiveness of an immunization against the agent or toxin without clinical and/or agricultural justification?**
   Based on the data reviewed the mutations are not located in the antigenic sites and therefore do not alter prefusion structure. In addition, the CH58 backbone is antigenically similar to VN1203 vaccine. Therefore, there is no indication that the proposed work will disrupt immunity or the effectiveness of immunization against the agent in use.

3. **Confers to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies?**
   It is believed that based on the proposed work that no expected changes in hemagglutinin (HA) antigenicity, oseltamivir susceptibility of CH58 or VN1203 NA protein, or amantadine resistance of CH58 or VN1203 M2 protein will confer resistance against currently known clinically or agriculturally useful prophylactic or therapeutic interventions.

4. **Increases the stability, transmissibility, or the ability to disseminate the agent or toxin?**
   The evidence shown to date has failed to demonstrate an increased rate of transmission (for HPAI, in ferrets or in avian species) than the parental wild-type virus.

5. **Alters the host range or tropism of the agent or toxin?**
   Compared to a parental wild-type lower-pathogenic CH58 virus, the HA-K58I virus caused an increase in virus growth in the nasal cavity but was unable to support high growth of the virus in the nasal cavity, most likely because of its avian-like polymerase and receptor-binding properties. Compared to the parental wild type higher-pathogenic VN1203 virus, the HA-K58I virus had earlier growth and earlier clearance in the nasal washings of ferrets and lost the ability to grow efficiently in the lungs. These minor alterations in the tropism of the parental viruses are not thought to constitute a threat to public health.

6. **Enhances the susceptibility of a host population to the agent or toxin?**
   Based on the data reviewed HA stalk mutations are not expected to enhance susceptibility.

7. **Generates or reconstitutes an eradicated or extinct agent or toxin listed in Section 3(1)?**
   After careful consideration of the proposed work it was determined that this question is not applicable.

Reports of federally funded research should be submitted directly to the relevant USG funding agency.

Reports of non-USG-funded research should be submitted to the National Institutes of Health via one of the following:

1. U.S. mail, courier service, or facsimile to:
   Attention: Institutional DURC Oversight Policy Reporting
   NIH Program on Biosecurity and Biosafety Policy
   6705 Rockledge Drive, Suite 750
   Bethesda, MD 20892-7985
   (For all non-USPS US Postal Service deliveries use Zip Code 20817)
   Telephone 301-496-9838
   Fax 301-496-9839

2. Email: DURC@od.nih.gov

Template for 30-Day Reporting of Research That Meets the Scope of the *Policy for Institutional DURC Oversight*

Date of Report: 3/11/2016

1. Contact Information

1.1 Institutional Contact for Dual Use Research (ICDUR)

Name: Philip Potter, Ph.D.	Phone number: (b)(6)
Email: (b)(6)	Fax: 901-595-4293

1.2 Person Completing This Form (If Different from ICDUR)

Name: Charles J. Russell, Ph.D.	Phone number: (b)(6)
Email: (b)(6)	Fax: 901-595-8559

2. Project Information

2.1 Principal Investigator (PI) or Other Scientist Responsible for This Research

Name (Last, First, MI): Russell, Charles, J.	
Mailing address: Charles J. Russell Associate Member St. Jude Children's Research Hospital 262 Danny Thomas Place Memphis, TN 38105-3678	Phone number: (b)(6) Fax: 901-595-8559 Email: (b)(6)
Department (if applicable): Department of Infectious Diseases, MS330	

2.2 Funding Source(s)

U.S. Government agency funding this research *(If more than one source, list all that apply. For non-USG-funded research, please provide the name of the funding entity and point of contact)*: none
Grant/contract number *(For non-USG-funded research, please provide a project identifier)*: none

2.3 Project Title(s)

Highly pathogenic and other BSL3+ influenza viruses

2.4 Project Description (Non-USG-Funded Research Only)

If the project is not supported with U.S. Government funds, please provide sufficient detail describing the nature of this research *(e.g., description of agent and how it is to be used, animal models, methods and procedures, biosafety and biosecurity measures)* that will allow for complete and accurate review by the designated USG funding agency. Alternatively, this information may be provided as supplemental material *(see Section 4)*.

Avian influenza A viruses pose a significant threat to global agriculture and human health. To determine the role of HA-mediated membrane fusion in influenza virus pathogenesis, the membrane fusion activities of highly pathogenic avian influenza viruses and other BSL3+ influenza viruses will be studied in tissue BSL3+ facility.

Virus isolates will be grown in embryonated chicken eggs and/or cell culture, and plaque assays in tissue culture cells will be performed to measure viral titers and plaque sizes. To

3. Institutional Review

3.1 Institutional Review Entity

Name of entity:	Date(s) of review:
St. Jude Children's Research Hospital	8/31/2012, 11/16/2012, 4/9/2013, 2/11/2016
Mailing address: 262 Danny Thomas Place Mail Stop 730 Memphis, TN 38105-3678	Phone number: (b)(6)
	Fax: 901-595-3055
	Email: (b)(6)

3.2 Agent or Toxin Involved in Project (Check All That Apply)

- ■ Avian influenza virus (highly pathogenic)
- ☐ *Bacillus anthracis*
- ☐ Botulinum neurotoxin (any quantity)
- ☐ *Burkholderia mallei*
- ☐ *Burkholderia pseudomallei*
- ☐ Ebola virus
- ☐ Foot-and-mouth disease virus
- ☐ *Francisella tularensis*
- ☐ Marburg virus
- ☐ Reconstructed 1918 influenza virus
- ☐ Rinderpest virus
- ☐ Toxin-producing strains of *Clostridium botulinum*
- ☐ Variola major virus
- ☐ Variola minor virus
- ☐ *Yersinia pestis*

3.3 Assessment by the IRE for Experimental Effects

Please indicate whether the research produces, aims to produce, or can be reasonably anticipated to produce any of the experimental effects listed below. Check all that apply.

☐ Enhances the harmful consequences of the agent or toxin.

If checked, please explain below:

☐ Disrupts immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification.

If checked, please explain below:

☐ Confers to the agent or toxin resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates its ability to evade detection methodologies.

If checked, please explain below:

■ Alters properties of the agent or toxin in a manner that would enhance its stability, transmissibility, or ability to be disseminated.

If checked, please explain below:

H5N1 with a K58I mutation, which lowers the HA activation pH by 0.5 pH units, increases environmental persistence. WT virus is persistent for 10 days at 28 degrees C, and the K58I mutant is persistent for 13 days. This is published in Reed et al. J. Virol. 84: 1527-1535, 2010. PMCID: PMC2812356

■ Alters the host range or tropism of the agent or toxin.

If checked, please explain below:

H5N1 with a K58I mutation, which lowers the HA activation pH by 0.5 pH units, increases early virus growth in the nasal cavities of mice and ferrets. This is published in two Zaraket et. al publications from 2013:
J. Virol 87: 4826-4834, 2013, and
J. Virol 87: 9911-9922, 2013.

☐ Enhances the susceptibility of a host population to the agent or toxin.

If checked, please explain below:

☐ Generates or reconstitutes an eradicated or extinct agent or toxin listed in Section 3.2 of this form.

If checked, please explain below:

3.4 Determination by the IRE of Whether the Research Meets the Definition of DURC

Please provide the IRE's rationale for why the research does or does not meet the definition of DURC. The *USG Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern* defines DURC as "life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security."

The K58I mutant virus does not increase pathogenicity or transmissibility in mammals. In fact, it decreases H5N1 growth in ferret lungs. It eliminates transmissibility and pathogenicity in ducks. The H5N1 virus it was introduced into is oseltamivir sensistive and is antigenically identical to a vaccine (VN1203). It's enhanced environmental stability was published and known before DURC policy (Reed et al. J. Virol. 84: 1527-1535, 2010.

2.4 Project Description (Non-USG-Funded Research Only)

If the project is not supported with U.S. Government funds, please provide sufficient detail describing the nature of this research (e.g., description of agent and how it is to be used, animal models, methods and procedures, biosafety and biosecurity measures) that will allow for complete and accurate review by the designated USG funding agency. Alternatively, this information may be provided as supplemental material (see Section 4).

Avian influenza A viruses pose a significant threat to global agriculture and human health. To determine the role of HA-mediated membrane fusion in influenza virus pathogenesis, the membrane fusion activities of highly pathogenic avian influenza viruses and other BSL3+ influenza viruses will be studied in tissue BSL3+ facility.

Virus isolates will be grown in embryonated chicken eggs and/or cell culture, and plaque assays in tissue culture cells will be performed to measure viral titers and plaque sizes. To measure membrane fusion, monolayers of tissue culture cells will be infected at equal multiplicities of infection, incubated with buffers ranging in pH from 4.0 to 6.0, then re-neutralized and later analyzed of syncytia (multi-nucleated cells). In parallel studies, equivalent amounts of virus will be incubated with erythrocytes at neutral pH, incubated in buffers ranging in pH from 4.0 to 6.0, and analyzed activity by spectrophotometry. These analyses will help to establish the role of the acid stability of the HA protein in the adaptation of avian influenza viruses. In experiments studying pathogenesis in the chicken model, standardized dilutions of viruses will be injected intravenously into chickens and the intravenous pathogenicity index (IVPI) will period for clinical signs of disease by standard methods. In experiments in ducks, viruses will be inoculated by the natural route (intraocular, intratracheal, and intranasal) and both tracheal be taken while monitoring weight changes and survival. Alternatively, ducks will be sacrificed at defined timepoints post infection and tissue will be harvested to measure virus growth. In experiments mouse model, serial dilutions of viruses will be inoculated intranasally into mice. Weight loss, clinical symptoms, intranasal titers and survival will be observed over a 21 day period. In other experiments, morbidity will be measured and viral titers in the lungs, brain, blood and spleen will be determined. In experiments using the ferret model, viruses will be inoculated intranasally and signs of infectious disease will be determined including viral titers after nasal sneezing, relative inactivity, weight loss, temperature, and survival. From harvested tissues, viral titers in the nose, olfactory bulb, spleen, and intestine will be determined by inoculation of embryonated chicken eggs. Histologic changes in these tissues will be examined.

In this protocol, we will use naturally occurring HPAI viruses and naturally occurring low pathogenic avian influenza viruses that require BSL3+ containment. We will also use H5N1 viruses containing HA protein mutations that alter the HA acid stability--these viruses were previously generated, characterized, and reviewed by the IBC and DURC sub-committee. They were judged to be DUR but not DURC. The viruses have previously been published in the following manuscripts:

Reed ML, Yen H-L, DuBois RM, Bridges OA, Salomon R, Webster RG, Russell CJ. Amino acid residues in the fusion peptide pocket regulate the pH of activation of the H5N1 influenza virus hemagglutinin (HA) protein. J. Virol. 83: 3568-3580, 2009. PMCID: PMC2663236.

Reed ML, Bridges OA, Seiler P, Kim J-K, Yen H-L, Salomon R, Govorkova EA, Webster RG, Russell CJ. The pH of activation of the hemagglutinin protein regulates H5N1 influenza virus pathogenicity and transmissibility in ducks. J. Virol. 84: 1527-1535, 2010. PMCID: PMC2812356

Zaraket H, Bridges OA, Russell CJ. The pH of activation of the hemagglutinin protein regulates H5N1 influenza virus replication and pathogenesis in mice. J. Virol. 87: 4826-4834, 2013. PMCID: PMC3624295.

Zaraket H, Bridges OA, Duan S, Baranovich T, Yoon S-W, Reed ML, Salomon R, Webby RJ, Webster RG, Russell CJ. Increased acid stability of the hemagglutinin protein enhances H5N1 influenza virus growth in the upper respiratory tract but is insufficient for transmission in ferrets. J. Virol. 87: 9911-9922, 2013. PMCID: PMC3754100.

These viruses do not enable H5N1 to become airborne transmissible in ferrets. They boost early virus growth in the upper respiratory tracts of mice and ferrets and they increase environmental stability. They do not increase pathogenicity in mammals. Highly pathogenic avian BL3+ agents will be handled appropriately according to the standards of the SJCRH "Guidelines for reassortment of influenza viruses" and the HHS "Biosafety in Microbiological Laboratories".

3.4 Determination by the IRE of Whether the Research Meets the Definition of DURC

Please provide the IRE's rationale for why the research does or does not meet the definition of DURC. The USG Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern defines DURC as "life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security."

The K58I mutant virus does not increase pathogenicity or transmissibility in mammals. In fact, it decreases H5N1 growth in ferret lungs. It eliminates transmissibility and pathogenicity in ducks. The H5N1 virus it was introduced into is oseltamivir sensistive and is antigenically identical to a vaccine (VN1203). It's enhanced environmental stability was published and known before DURC policy (Reed et al. J. Virol. 84: 1527-1535, 2010. PMCID: PMC2812356). It's effect on nasal growth in mammals was published in two 2013 Journal of Virology papers.

NIAID's Implementation Guidelines for Determining Whether Research is Subject to the GoF Funding Pause[1]

General Principles & Definitions

- As a general principle, studies on genes and proteins of unknown function, or for which there is not definitive existing data, should **not** fall under the gain-of-function (GoF) funding pause. However, in the event that the studies unexpectedly result in a virus with enhanced pathogenicity and/or transmissibility in mammals via the respiratory route, they should be immediately halted as the findings would now meet the GoF funding pause criteria.
- "Reasonably anticipated" will be defined as when the **majority of evidence** from previous studies supports that similar experiments/techniques resulted in enhanced pathogenicity and/or transmissibility in mammals via the respiratory route. To help make the determination of what is a "reasonably anticipated" outcome, NIAID will request that investigators provide a balanced summary of background data with references on previously conducted studies.
- Proposed experiments will be evaluated based on whether they would result in a GoF as compared to the **original wild-type virus isolates and/or currently circulating wild-type viruses which evolved from the original wild-type virus isolates**.[2] For those experiments in which multiple parental viruses could be chosen as the comparator strain, the most pathogenic and/or transmissible parental virus in the mammalian animal species under investigation will be selected as the comparator strain for assessing GoF outcomes.[3]

Research Areas

- **Altering genes of unknown function and assessing the resulting phenotype *in vitro* and *in vivo*, or performing mutagenesis or reassortment studies with unknown potential for GoF phenotypes**
    - Example: Investigators are mutating MERS viral nucleases such as nsp-14 which are involved in proofreading RNA replication. The goal of the work is to attenuate the virus; however mutations may either increase or decrease the fidelity of replication and may impact pathogenesis.
        - NIAID Recommendation: NIAID recommends that these studies should **not** fall under the scope of the GoF funding pause. This recommendation is because the intent of the study is a "loss-of-function" with the anticipation that the resulting viruses will be attenuated. NIAID recommends that as **a general principle**, studies on genes and proteins of unknown function and studies for which there is a lack of definitive existing data to reasonably predict whether GoF phenotypes will result, should **not** fall under the GoF funding pause. However, in the event that the studies unexpectedly result in a virus with enhanced pathogenicity and/or transmissibility in mammals via the respiratory route, they should be immediately halted as the findings would now meet the GoF funding pause criteria.
        - Related Projects:
            - 5R01AI108197-02; PI: Dennison (Vanderbilt)
            - 1R01AI110700-01A1; PI: Baric (UNC); Jan Council – score (b)(6)

[1] Approved by Dr. Hugh Auchincloss on November 10, 2014 and November 21, 2014

[2] Currently circulating wild-type viruses which evolved from the original wild-type virus isolates may be used as comparator strains when the original wild-type virus isolates are unavailable or when there is limited existing data on the original wild-type virus isolates.

[3] Clarification language approved by Dr. Hugh Auchincloss on May 13, 2015

- **Performing phenotypic characterization studies with existing laboratory-generated viruses (e.g., PR8 expressing HPAI genes, transmissible HPAI, MERS viruses with increased replicative abilities)**
    - Example 1: Investigators would like to assess the *in vivo* efficacy of influenza antivirals and vaccines against the mammalian respiratory droplet transmissible HPAI H5N1 influenza viruses previously generated by Dr. Kawaoka and Dr. Fouchier.
    - Example 2: In the course of MERS animal model development, investigators passaged wild type MERS-CoV *in vitro* to select for strains that bind more efficiently to the MERS-CoV receptor. These viruses are reasonably anticipated to replicate more efficiently and may or may not have increased pathogenicity. The investigators would like to characterize these novel strains *in vivo*.
        - NIAID Recommendation: NIAID recommends that these studies should **not** fall under the scope of the GoF funding pause because they are not reasonably anticipated to enhance the pathogenicity and/or transmissibility in mammals via the respiratory route of these previously generated viruses. However, in the event that the studies unexpectedly result in a virus with enhanced pathogenicity and/or transmissibility in mammals via the respiratory route, they should be immediately halted as the findings would now meet the GoF funding pause criteria.
        - Related Project: Task Order A57; PI: Palese (MSSM), Sub: Baric (UNC)

- **Placing human genes/mutations from influenza or MERS viruses back into naturally occurring animal precursor viruses to study the natural evolution process of such viruses**
    - Example: Investigators would like to: (1) place human MERS genes/mutations back into a bat coronavirus; (2) place human H3N2V influenza genes/mutations back into a swine H3N2 virus; and (3) place human HPAI H5N1 polymerase genes/mutations back into an avian HPAI H5N1.
        - NIAID Recommendation: The USG GoF funding pause is limited to influenza, MERS, and SARS viruses. Therefore, GoF studies on bat coronaviruses would **not** fall within the scope of the funding pause. NIAID recommends that these types of studies with influenza, MERS, or SARS viruses should **not** fall under the GoF funding pause because viruses with these attributes already exist in nature. However, in the event that the studies unexpectedly result in a virus with enhanced pathogenicity and/or transmissibility in mammals via the respiratory route, they should be immediately halted as the findings would now meet the GoF funding pause criteria.
        - Related Project: 1R01AI110700-01A1; PI: Baric (UNC); Jan Council – score (b)(6)

- **Generating viruses through mutagenesis or reassortment such that they gain one but not all of the properties previously identified to be important for mammalian respiratory droplet transmission of HPAI H5N1 viruses (i.e., human-type receptor binding, HA glycosylation providing enhanced binding to receptors, HA stability, enhanced replication)**
    - Example: Investigators may seek to generate a HPAI H5N1 virus containing only the mutations previously identified to confer human-type receptor binding or only the mutations previously identified to enhance HA stability, and then characterize the resulting viruses and phenotypes *in vivo*.
        - NIAID Recommendation: NIAID recommends that studies proposing to introduce only one of these properties into a virus that is not currently transmissible in mammals by respiratory droplets, and that are not reasonably anticipated to result in a virus with

enhanced pathogenicity, should **not** fall under the GoF funding pause because previous studies support that addition of one property alone is not sufficient to confer respiratory droplet transmission to non-transmissible influenza viruses. However, in the event that the studies unexpectedly result in a virus with enhanced pathogenicity and/or transmissibility in mammals via the respiratory route, they should be immediately halted as the findings would now meet the GoF funding pause criteria.

- **Use of comparator viruses in assessing gain-of-function**
  - Example 1: Investigators would like to introduce mutations into the NS1 gene of the mouse-adapted attenuated PR8 [A/PR8/34 (H1N1)] influenza virus to examine the innate immune response to influenza infection in the lung. The mutant PR8 viruses may be more pathogenic in mice compared to the mouse-adapted attenuated PR8 virus, but they are not reasonably anticipated to be as pathogenic as the original A/PR8/34 human H1N1 influenza virus isolate or currently circulating human seasonal H1N1 influenza strains.
  - Example 2: To generate samples for high-throughput OMICS studies focusing on the role of the immune-related viral genes during infection, investigators will introduce the pH1N1 influenza NS1 gene into an attenuated H5N1 and the resulting reassortant virus will be used to infect cells and mice. The attenuated H5N1/pH1N1 reassortant may be more pathogenic than the attenuated H5N1 virus but is not reasonably anticipated to be more pathogenic than the original HPAI H5N1 virus isolate or currently circulating wild-type H5N1 strains.
  - Example 3: Investigators commonly compare genetically-related strains of highly pathogenic avian influenza (HPAI) viruses that differ by only a few amino acids but nevertheless demonstrate differing pathogenic phenotypes. By placing the amino acids from more pathogenic viruses into less pathogenic viruses they can identify molecular markers of pathogenicity. The resulting mutant viruses may exhibit enhanced pathogenicity relative to the less pathogenic wild-type viruses but are not reasonably anticipated to be more pathogenic than currently circulating HPAI viruses.
  - Example 4: To determine the contribution of a host factor to influenza virus species tropism, investigators will generate and characterize a recombinant 6:2 influenza virus composed from influenza viruses that do and do not affect the host factor's activity. This 6:2 recombinant virus will contain the internal genes of a low pathogenic avian influenza virus (LPAI) and the HA and NA genes from the mouse-adapted attenuated PR8 influenza virus. Either parental virus could be considered an appropriate comparator strain, but the pathogenicity outcome evaluation would differ depending on which virus is chosen. The resulting recombinant virus may exhibit enhanced pathogenicity in mice compared to the LPAI influenza virus but is not reasonably anticipated to be more pathogenic in mice than the mouse-adapted PR8 virus
    - NIAID Recommendation: As indicated above, NIAID recommends that proposed experiments be evaluated based on whether they would result in a GoF as compared to the original wild-type virus isolates and/or currently circulating wild-type viruses which evolved from the original wild-type virus isolates.[2] For those experiments in which multiple parental viruses could be chosen as the comparator strain, the most pathogenic and/or transmissible parental virus in the mammalian animal species under investigation will be selected as the comparator strain for assessing GoF outcomes.[3] In line with this recommendation:
      - GoF studies using lab-adapted or attenuated strains of influenza, MERS, or SARS, that are reasonably anticipated to result in viruses with enhanced pathogenicity

and/or transmissibility in mammals via the respiratory route as compared to the original wild-type virus isolates and/or currently circulating wild-type strains which evolved from the original wild-type virus isolates, **should** fall under the GoF funding pause. If this outcome is not reasonably anticipated, the studies should **not** fall under the GoF funding pause. However, in the event that the studies unexpectedly result in a virus with enhanced pathogenicity and/or transmissibility in mammals via the respiratory route, they should be immediately halted as the findings would now meet the GoF funding pause criteria.

- For GoF studies using influenza, MERS, or SARS strains in which multiple parental viruses could be chosen as the comparator strain, those that are reasonably anticipated to result in viruses with enhanced pathogenicity and/or transmissibility in mammals via the respiratory route as compared to the most pathogenic and/or transmissible parental virus in the mammalian animal species under investigation, **should** fall under the GoF funding pause. If this outcome is not reasonably anticipated, the studies should **not** fall under the GoF funding pause. However, in the event that the studies unexpectedly result in a virus with enhanced pathogenicity and/or transmissibility in mammals via the respiratory route, they should be immediately halted as the findings would now meet the GoF funding pause criteria.
- Experiments using standard genetic manipulation to compare the virulence of genetically-related strains of influenza, MERS, or SARS viruses should **not** fall under the GoF funding pause because they are not reasonably anticipated to generate viruses with new or enhanced traits as compared to currently circulating wild-type viruses. In the event that the studies unexpectedly result in a virus with enhanced pathogenicity and/or transmissibility in mammals via the respiratory route, they should be immediately halted as the findings would now meet the GoF funding pause criteria. By analogy, this recommendation is consistent with NSABB's application of DURC policies.[4]
  - Related Projects:
    - HHSN272201400006C; CEIRS – St. Jude
    - HHSN272201400008C; CEIRS – MSSM
    - 5R00AI095320-03; PI: Balaji Manicassamy (Univ. Chicago)
    - 1R21AI115308-01A1; PI: Balaji Manicassamy (Univ. Chicago)

- **Performing *in vivo* characterization studies of genes or mutations shown to exhibit different phenotypes in different animal models**
  - Example: There is scientific evidence that the PB1-F2 influenza gene can contribute to replication, pathogenesis, and modulation of the immune response to influenza but the effects described are dependent on multiple variables including protein length, amino acid sequence, viral strain,

[4] http://osp.od.nih.gov/sites/default/files/resources/Framework%20for%20transmittal%20duplex%209-10-07.pdf "An example of information that would fall under this category, but is unlikely to be dual use of concern, includes routine techniques for restoring the virulence of viral stocks by back-passaging in animal hosts, identification of virulence factors through genome-wide screening or gene knockout techniques, and standard genetic manipulation to study the virulence of an organism."

infectious dose, and animal model. For example, full-length PB1-F2 has been shown to increase viral replication and spread in mice and ducks, but at low doses has been shown to decrease mortality in chickens. Additionally, the length of the protein can affect its function, as seen in swine where full-length PB1-F2 supports secondary bacterial infection but a truncated form prevents secondary bacterial infection. Given these variations, the PB1-F2 phenotype cannot always be predicted. Investigators would like to perform *in vivo* characterization studies of PB1-F2 variants.

- NIAID Recommendation: NIAID recommends that complex *in vivo* experiments in which the outcome is dependent on multiple scientific variables (see above) or for which there is contradictory published or preliminary data, be evaluated on a case-by-case basis. Investigators will be asked to provide a balanced summary of background data with references for NIAID's assessment and determination of the reasonably anticipated outcome of the proposed experiments.

- **Performing gain-of-function studies *in vitro* that may be reasonably anticipated to result in viruses that demonstrate enhanced pathogenesis and/or mammalian respiratory droplet transmission *in vivo*, without doing the follow-up *in vivo* studies to further characterize the viruses**
  - Example: Investigators would like to generate and characterize viruses with enhanced replication in mammalian cells without performing *in vivo* studies with the viruses. It is noted that if these generated viruses are not subsequently tested *in vivo*, the impact on pathogenesis and/or transmissibility in mammals via the respiratory route could not be definitively predicted because *in vitro* cell culture studies do not always predict *in vivo* phenotypes.
    - NIAID Recommendation: NIAID recommends that if it can be reasonably anticipated that *in vivo* studies using the *in vitro* generated viruses would enhance the pathogenicity and/or transmissibility in mammals via the respiratory route, the *in vitro* work **should** fall under the GoF funding pause. If this outcome is not reasonably anticipated, *in vitro* work should **not** fall under the GoF funding pause. However, in the event that the studies unexpectedly result in a virus with enhanced pathogenicity and/or transmissibility in mammals via the respiratory route, they should be immediately halted as the findings would now meet the GoF funding pause criteria.
    - Related Projects:
      - HHSN272201400006C; CEIRS – St. Jude
      - HHSN272201400008C; CEIRS – MSSM
      - 5U19AI106772-02; PI: Yoshihiro Kawaoka (UW-Madison)
      - 1F31AI115968-01; PI: Byrd-Leotis (Emory); Oct Council – score (b)(6)
      - 1R01AI110700-01A1; PI: Baric (UNC); Jan Council – score (b)(6)

From: Hauguel, Teresa (NIH/NIAID) [E]
To: Glowinski, Irene (NIH/NIAID) [E]; Dixon, Dennis M. (NIH/NIAID) [E]; Lambert, Linda (NIH/NIAID) [E]; Spiro, David (NIH/NIAID) [E]; Hauguel, Teresa (NIH/NIAID) [E]; Post, Diane (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Dugan, Vivien (NIH/NIAID) [E]; Mulach, Barbara (NIH/NIAID) [E]; Ford, Andrew (NIH/NIAID) [E]; Strickler-Dinglasan, Patricia (NIH/NIAID) [E]; Hanson, Christopher (NIH/NIAID) [E]; Delarosa, Patricia (NIH/NIAID) [E]; Santora, Kenneth (NIH/NIAID) [E]
Subject: 4/29 DURC/GoF Meeting Agenda
Date: Wednesday, April 27, 2016 4:05:23 PM
Attachments: image001.png
1a-Li 2R01AI 089728-06 GOF Response 2016-v3-1.pdf
1b-GoF Assessment - Fidelity Variants - 04-21-2016.docx
2-DURC Reach-Through Provision Email.pdf
2-Review of Non-Fed-funded-DURC-SOPs 41816.docx
2-CV Email.pdf
MATERIALS NSABB WG call .msg
3b(1)-DURC Implementation Metrics_institutional metrics.docx
3b(2)-iDURC_USG questions.docx

Hello Everyone,

Below is the agenda for Friday's DURC/GoF meeting.

Attached are documents for agenda items 1-3.

Weekly DURC/GoF Meeting Agenda

Friday, April 29, 2016
3:00-4:00pm
5601/7G31
Call in number: (b)(6)
Passcode: (b)(6)

1. Projects for GoF Review
    a. Li (R01) – SARS-like bat coronaviruses – Erik
    b. Kawaoka (CEIRS) – influenza fidelity variants – Diane
2. DURC Reach-Through Provision – Andrew
3. Updates
    a. NSABB WG – Dennis/Diane/Teresa
    b. DURC iDOWG – Andrew
    c. GOFROC Strawman – Linda
    d. Erasmus RMP – Ken/Tricia/Diane
4. Round Robin/Other Items

Teresa M. Hauguel, Ph.D.
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Getting ready to publish? Share the good news with your Program Officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

Please consider the environment before printing this e-mail 

Hauguel, Teresa (NIH/NIAID) [E]

From: Ford, Andrew (NIH/NIAID) [E]
Sent: Thursday, April 21, 2016 12:09 PM
To: Hauguel, Teresa (NIH/NIAID) [E]; Glowinski, Irene (NIH/NIAID) [E]; Dixon, Dennis M. (NIH/NIAID) [E]; Lambert, Linda (NIH/NIAID) [E]; Spiro, David (NIH/NIAID) [E]; Post, Diane (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Dugan, Vivien (NIH/NIAID) [E]; Mulach, Barbara (NIH/NIAID) [E]; Strickler-Dinglasan, Patricia (NIH/NIAID) [E]; Hanson, Christopher (NIH/NIAID) [E]; Delarosa, Patricia (NIH/NIAID) [E]; Santora, Kenneth (NIH/NIAID) [E]
Cc: Ford, Andrew (NIH/NIAID) [E]
Subject: RE: Reminder - no DURC/GoF meeting this week
Attachments: Review of Non-Fed-funded-DURC-SOPs 41816.docx; DURC and GOF, for thought

Dear All,

As mentioned at the April 15 DURC/GoF meeting, Trish, Barbara and I discussed with Chris V. and his group, the review of institutional DURC assessments about non-federally funded research received in accordance with the iDURC policy. The objective, from our perspective, was to discuss lessons learned and to get an idea as to how OSP was using the feedback they receive when responding to institutions. Thus far, 19 institutional assessments have been received; of these, additional information was requested for 3, while the other 16 should not have been sent (e.g. they did not include one of the 7 effects). Considering the topic of discussion, prior to the call OSP shared the attached draft SOP regarding review of non-federally funded research subject to the iDURC policy. Based on the draft SOP the agency/IC assigned to review the institutional assessment would assume the responsibility of corresponding with the institution, including sending the final disposition about the assessment. In addition, in instances of DURC the assigned agency/IC is to work with the institution to finalize the risk mitigation plan. We reiterated our recommendation that the activities associated with reviewing and finalizing the risk mitigation plans (RMP) be assigned to CDC/USDA due to their expertise in biosafety and biosecurity. He did provide some push back, but by the end of the call he seemed to understand that we view the science/research and biosecurity/biosafety to be separate issues resulting in our involvement in reviewing assessments and our recommendation regarding RMP review. There was also discussion that most likely no agency/IC would want to take, what would be perceived to be, ownership of the review of non-federally funded research and RMPs.

After the call, Chris V. followed-up with the attached email in which he discusses an idea explored in 2012 about creating a group – Federal Experts Panel on Dual Use Research (FEPDUR) – and the possibility of having such a group play a role in reviewing non-federally funded DURC research, proposed GOFROC research, and DURC/GoF manuscripts. He does mention a few pros and cons regarding the group.

Please note, Chris V. shared the draft SOP and FEPDUR idea for internal discussion by our DURC/GoF group; therefore, please do not distribute these items any further.

Should you have any questions please let us know.

Thanks
Andrew

Andrew Q. Ford, Ph.D.
Office of Scientific Coordination and Program Operations
Division of Microbiology and Infectious Diseases

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From: Hauguel, Teresa (NIH/NIAID) [E]
Sent: Wednesday, April 20, 2016 10:51 AM
To: Glowinski, Irene (NIH/NIAID) [E] (b)(6) Dixon, Dennis M. (NIH/NIAID) [E] (b)(6) Lambert, Linda (NIH/NIAID) [E] (b)(6) Spiro, David (NIH/NIAID) [E] (b)(6) Hauguel, Teresa (NIH/NIAID) [E] (b)(6) Post, Diane (NIH/NIAID) [E] (b)(6) Stemmy, Erik (NIH/NIAID) [E] (b)(6) Dugan, Vivien (NIH/NIAID) [E] (b)(6) Mulach, Barbara (NIH/NIAID) [E] (b)(6) Ford, Andrew (NIH/NIAID) [E] (b)(6) Strickler-Dinglasan, Patricia (NIH/NIAID) [E] (b)(6) Hanson, Christopher (NIH/NIAID) [E] (b)(6) Delarosa, Patricia (NIH/NIAID) [E] (b)(6) Santora, Kenneth (NIH/NIAID) [E] (b)(6)
Subject: Reminder - no DURC/GoF meeting this week

Hi Everyone,

Just a quick reminder that there is no DURC/GoF meeting this week. Our next meeting is scheduled for Friday, April 29th at 3pm.

Hope you all get a chance to get outside and enjoy this beautiful weather today!

Best,
Teresa

Teresa M. Hauguel, Ph.D.
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Please consider the environment before printing this e-mail

Hauguel, Teresa (NIH/NIAID) [E]

From: Viggiani, Christopher (NIH/OD) [E]
Sent: Wednesday, April 20, 2016 11:09 AM
To: Ford, Andrew (NIH/NIAID) [E]; Strickler-Dinglasan, Patricia (NIH/NIAID) [E]; Mulach, Barbara (NIH/NIAID) [E]
Cc: Harris, Kathryn (NIH/OD) [C]; Ramkissoon, Kevin (NIH/OD) [C]
Subject: DURC and GOF, for thought

Hi all,

Interesting call this morning, thanks. After the call we started talking internally and came back to an idea that was kicked around years ago. This potential solution has its pros and cons, which we can discuss sometime. It also has the potential to solve the GOF issue, and potentially other related issues. There are pros and cons.

In 2012 you might remember there was an idea to have a group called the FEPDUR—the Federal Experts Panel on Dual Use Research. This would be an interagency group of Federal Experts, kind of like FESAP. Originally, it was envisioned to be the USG group that would review any DURC manuscripts that came in (this was just in the wake of the H5 manuscripts and there was a feeling that an internal Federal group could be quicker and have more expertise than a FACA committee). Do you remember when the group reviewed the Arnon bot tox paper? That was kind of an ad hoc FEPDUR. For whatever reason, the FEPDUR died. But it could be a useful here.

What would you think about establishing an interagency group that could, for instance:

- Review non-Federally funded reports of DURC and advise on risk mitigation
- Review proposed GOF research of concern, as described by NSABB, and advise the funding agency
- Review DURC manuscripts that come in from journal editors or funding agencies

We would want to think carefully about this, there are real pros and cons. Some pros are that it would provide broad expertise and gives individual agencies some cover/assurance in their actions. Cons would be mission creep (e.g., what if this group wanted to review ALL DURC, even if that DURC is federally funded? Would we be OK with that?) and overly-zealous DAs (e.g., think of the recent ISATTAC debacle where security has overridden science). It would be important that it is clear that any new group provides recommendations only and that funding agencies retain authority over final decisions.

Just wanted to float this with you internally before we develop it further. We should talk more. Despite some of the concerns I have, I think this idea could have promise if we did it right.

cv

Christopher Viggiani, Ph.D.
Office of Science Policy
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(b)(6)

NIH National Institutes of Health
Turning Discovery Into Health

From:	Viggiani, Christopher (NIH/OD) [E]
To:	Betty Lee; Christine Grant; Christopher Park; Clifford W. Houston; Craig Cameron; David Woodland; Dixon, Dennis M. (NIH/NIAID) [E]; Diane DiEuliis; Drew Endy; Francis Macrina; Gangadharan, Denise (CDC/OPHPR/DSAT); Gary Resnick; Gerald Epstein; Hauguel, Teresa (NIH/NIAID) [E]; Hu-Primmer, Jean (OS/ASPR/BARDA); James LeDuc; Patterson, Jean (Texas Biomedical Research Institute); Joseph Kanabrocki; Joseph McDade; Kenneth I. Berns; Kimberly Orr; Lawrence, Theresa (OS/ASPR/OPP); Marcelle Layton; Margie Lee; Marie-Louise Hammarskjöld; Meg Flanagan; Post, Diane (NIH/NIAID) [E]; Resnik, David (NIH/NIEHS) [E]; Jaffe, Richard (OS/ASPR/OPP); Robert Miceli ; Phillips, Sally (HHS/ASPR/OPP); Sharlene Weatherwax; Stephen Morse; Susan Wolf; Theresa Koehler; Todd Anderson; Wendy Hall
Cc:	Alex Wadley; Alicia Simmons; Ashley Connally; Caroline Brendel; Christine Dorosin; Eileen Prainum; Eileen Rodriguez; Imelda Mendoza; Jane Lalich; Jeannette Gagnon; Jessica Petrillo; Lyz Morrison; Bull, Melbourne (NIH/NIAID) [E]; Sherry Coven; Beckham, Shayla (NIH/OD) [E]; Fennington, Kelly (NIH/OD) [E]; Harris, Kathryn (NIH/OD) [C]; Nightingale, Stuart (NIH/OD) [C]; O"Reilly, Marina (NIH/OD) [E]; Ramkissoon, Kevin (NIH/OD) [C]; Rona Hirschberg; Viggiani, Christopher (NIH/OD) [E]
Subject:	MATERIALS: NSABB WG call
Date:	Monday, April 18, 2016 11:55:16 AM
Attachments:	image001.png 3-NSABB Draft Report 4-15-2016 cv CLEAN.docx 0-Agenda 4-19-2016 WG Teleconfernce.docx 1-Summary of April 7 NSABB WG Discussions (002).docx 2-NSABB Draft Report 4-17-2016 CLEAN.pdf

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Dear NSABB WG,

Thanks to everyone who has submitted comments on the draft report over the last few weeks. We have assembled a new draft, see attached. We have also re-attached the summary from the previous WG call, which turned out to be an important call and also informed the new edits to the draft report.

Upcoming WG telecon: Tuesday, 4/19/2016 (10am – 12pm ET)
Call-in number: (b)(6)
Participant Code: (b)(6)

On the call we will:

- Briefly summarize the last WG meeting and the edits to the draft report
- Discuss/revise the principles for guiding funding decisions for GOFROC (PDF version, p45 – 46)
- Discuss any other areas of the draft report

Thanks all, talk to you tomorrow.

Chris

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OSP Blog: Under the Poliscope
Twitter: @CWolinetzNIH

DELIBERATIVE DRAFT

Policy Recommendations for the Evaluation of Proposed Gain-of-Function Research

A Draft Report of the NSABB Working Group on Evaluating the Potential Risks and Benefits of Gain-of-Function Studies

Version: April 15, 2016

Preface for NSABB Meeting on May 24, 2016

This draft report was developed by the NSABB working group tasked with evaluating the risks and benefits associated with gain-of-function studies and developing draft recommendations on a conceptual approach for the evaluation of proposed gain-of-function studies. The first version of this document was discussed at the NSABB meeting on January 7 & 8, 2016 and again at the symposium hosted by the National Academies on March 10 & 11, 2016. This version represents an updated draft of that initial working paper. This document is still pre-decisional and intended as a deliberative document to be discussed at the meeting of the full NSABB on May 24, 2016. This is document is not a formal NSABB work product and should not be considered to be official NSABB findings or recommendations to the U.S. government. This document does not represent official policy of the U.S. government.

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Executive Summary

NOTE: Executive Summary will be updated to reflect any changes in the rest of the document.

Research involving pathogens is essential to global health and security. Such research provides insight into the fundamental nature of human-pathogen interactions, enables the assessment of the pandemic potential of emerging infectious agents, and informs public health and preparedness efforts, including the development of medical countermeasures. Several policies are in place to help ensure that pathogen research is conducted safely and in ways to minimize the risks of laboratory accidents and security risks. Recently, and in the wake of a number of biosafety incidents at Federal facilities, concerns have been raised about certain "gain-of-function" (GOF) studies with the potential to generate pathogens with enhanced pathogenicity or transmissibility in mammals. The concerns center on whether a pathogen with enhanced characteristics could be accidentally or intentionally released from a laboratory, potentially exposing surrounding populations to a pathogen with pandemic potential.

The U.S. Government (USG), as part of its continued focus on biosafety and biosecurity, has undertaken a deliberative process to carefully examine the risks and benefits associated with certain GOF studies. The deliberative process involves the National Science Advisory Board for Biosecurity (NSABB), which has been tasked with making recommendations to the USG on this topic, and the National Academy of Sciences (NAS), which was tasked to convene two public symposia to generate broad discussion on the relevant issues. To further inform NSABB deliberations, the National Institutes of Health (NIH) commissioned an independent assessment of the risks and benefits associated with GOF studies and an ethical analysis of the issues related to funding and conducting such studies.

The NSABB was charged with 1) advising on the design, development, and conduct of the risk and benefit assessments for GOF studies, and 2) providing recommendations to the USG on a conceptual approach to the evaluation of proposed GOF studies. The NSABB established two working groups to address its tasks and the full Board convened publically five times between October 2014 and January 2016. In May 2015 the NSABB issued its *Framework for Guiding the Conduct of Risk and Benefit Assessments of Gain-of-Function Research*, which guided NIH in overseeing the contractor conducting the risk and benefit assessments.

The working group tasked with issuing recommendations on an approach to evaluating proposed GOF studies considered four major areas: the current policy landscape as it pertains to pathogen research, the results of the risk and benefit assessments, the analysis of relevant ethical issues, and broad stakeholder perspectives on the issues at hand. This working paper describes the working group's process, analysis, preliminary findings, and draft recommendations to date. This paper is not a final NSABB work product and does not represent NSABB recommendations to the U.S. government. This interim report is offered by the working group to the full NSABB, and the broader stakeholder community, to serve as a springboard for discussion at the NSABB meeting in May, 2016.

The working group has developed four key findings:

Key Finding 1: There are many types of GOF research and not all of them have the same level of risks. Only a small subset of GOF research—GOF research of concern (GOFROC)—entail risks that are potentially significant enough to warrant additional oversight.

Key Finding 2. The U.S. government has several policy frameworks in place for identifying and managing risks associated with life sciences research. There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOFROC could be applied.

Key Finding 3. Oversight policies vary in scope and applicability, and are not sufficiently harmonized; therefore, current oversight is not sufficient for all GOFROC.

Key Finding 4. An adaptive policy approach is a desirable way to ensure that oversight and risk mitigation measures remain commensurate with the risks associated with the research and the benefits of the research are being fully realized.

Key Finding 5. There are life sciences research studies, including possibly some GOFROC, that should not be conducted on ethical or public health grounds if the potential risks associated with the study are not justified by the potential benefits. Decisions about whether GOFROC should be permitted will entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of proposed studies but other considerations, including legal, ethical, and societal values are also important.

Key Finding 6. Managing risks associated with GOFROC, like all life sciences research, requires Federal-level and institutional oversight, awareness and compliance, and a commitment by all stakeholders to safety and security.

Key Finding 7. Consideration of the international dimensions associated with funding and conducting GOF research of concern is important.

Based on its analyses thus far, the NSABB working group has formulated the following draft recommendations for discussion:

Recommendation 1. Research proposals involving GOFROC entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the Federal and institutional levels.

As part of this recommendation, the NSABB working group has proposed a conceptual approach for guiding funding decisions about GOFROC. First, the working group identified the attributes of GOFROC, which is research that could generate a pathogen that is: highly transmissible and likely capable of wide and uncontrollable spread in human populations; and highly virulent and likely to cause significant morbidity and/or mortality in humans. Next, the working group identified a set of principles that should guide funding decisions for GOFROC. Only research that is determined to be

in line with these principles should be funded. Additional risk mitigation measures may be required for certain research studies to be deemed acceptable for funding.

Recommendation 2. In general, oversight mechanisms for GOFROC should be incorporated into existing policy frameworks when possible. The risks associated with some GOFROC can be identified and adequately managed by existing policy frameworks if those policies are implemented properly. However, the level of oversight provided by existing frameworks varies by pathogen. For some pathogens, existing oversight frameworks are robust and additional oversight mechanisms should generally not be required. For other pathogens, existing oversight frameworks are less robust and may require supplementation. All relevant policies should be implemented appropriately and enhanced when necessary to effectively manage risks.

Recommendation 3. The U.S. government should pursue an adaptive policy approach to help ensure that oversight remains commensurate with the risks associated with the GOFROC.

Recommendation 3.1. The U.S. government should consider developing a system to collect and analyze data associated with laboratory safety to inform policy development over time for GOFROC.

Recommendation 3.2. An external advisory body that is designed for transparency and public engagement should be utilized as part of the U.S. government's ongoing evaluation of oversight policies for GOFROC.

Recommendation 4. The U.S. government should pursue ways to ensure that all GOFROC conducted within the U.S. or by U.S. companies be subject to oversight, regardless of funding source.

Recommendation 5. The U.S. government should undertake broad efforts to strengthen biosafety and biosecurity and, as part of these efforts, seek to raise awareness about the specific issues associated with GOFROC.

Recommendation 5.1. The U.S. government should specifically develop a "Points to Consider" document to provide guidance to investigators and institutions when preparing research proposals that may involve GOFROC.

Recommendation 6. The U.S. government should engage the international community in a dialogue about the oversight and responsible conduct of GOFROC.

1. Introduction

A robust life sciences research enterprise is necessary to counter the continually evolving threats to public health and national security posed by endemic and emerging pathogens, as well as malicious biological threats. By helping to define the nature of human-pathogen interactions, life sciences research promotes public health and national security not only by enhancing our understanding of pathogen biology and disease pathogenesis, but also by informing biosurveillance and medical countermeasure development. Such research can also aid in the assessment of the pandemic potential of emerging infectious agents, thereby underpinning health policy decisions and preparedness and response efforts.

While the ultimate goal of life sciences research involving pathogens is the protection and promotion of public health, there are inherent associated biosafety and biosecurity risks. Potential risks might arise from laboratory accidents or security breaches that result in laboratory acquired infections or the accidental or deliberate release of a pathogen from containment. Life sciences research has "dual use" potential. That is, legitimate research may generate information, products or technologies that could be misused to threaten public health or national security. To mitigate such dual use concerns, as well as potential biosafety and biosecurity risks, research involving pathogens is subject to multiple layers of Federal and institutional oversight.

The Gain-of-Function Debate and the USG Response

Experimental techniques and approaches that modify the genome of microorganisms are routinely employed in pathogen research to ascertain the roles of genes and their functional products. Such studies are fundamental to the field of microbial genetics and facilitate correlation of genetic and phenotypic characteristics – a critical step in deciphering the complex nature of host-pathogen interactions that underlie transmission, infection, and pathogenesis. Such genetic manipulations can result in either diminished (loss-of-function) or enhanced (gain-of-function) biological phenotypes.

Studies that result in the generation of pathogens with enhanced, or gain-of-function (GOF), phenotypes are conducted for a number of valid scientific purposes. Such studies provide information that adds to the scientific knowledge base and can inform biosurveillance, medical countermeasure development, and public policy decision-making related to public health and preparedness. The vast majority of such GOF studies do not raise significant safety or security concerns. However, certain GOF studies involving pathogens have raised significant concerns about whether a laboratory-generated pathogen with pandemic potential could be accidentally or intentionally released, resulting in significant consequences to public, or perhaps, global health. Concerns have also been raised about whether certain GOF studies could generate information that could enable individuals with malevolent intent to generate a pathogen with pandemic potential (see Box 1).

The controversy over certain GOF studies arose after two groups demonstrated that highly pathogenic avian influenza H5N1 viruses with a small number of engineered mutations became transmissible between mammals by respiratory droplets.[1,2] In 2012, in response to the controversy associated with publishing the manuscripts describing these findings, the influenza community initiated a voluntary suspension of certain GOF studies involving highly pathogenic avian influenza H5N1 viruses. During that time, policymakers considered whether certain GOF studies should be conducted using Federal funds, and if so, how those studies could be safely conducted. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) issued new biosafety guidelines for working with highly pathogenic avian influenza strains.[3,4] The U.S. Department of Health and Human Services (HHS) developed a framework for guiding its funding decisions about GOF projects that may generate H5N1 or H7N9 avian influenza viruses that are transmissible between mammals by respiratory droplets.[5]

Box 1. Gain-of-Function Research

Recently, the phrase "gain-of-function research" has become synonymous with certain studies that enhance the ability of pathogens to cause disease. However, gain-of-function studies, as well as loss-of-function studies, are common in molecular and microbiology and form the foundation of microbial genetics. Changes to the genome of an organism, whether naturally occurring or directed through experimental manipulations in the laboratory, can result in altered phenotypes as biological functions are lost or gained. Investigators routinely conduct loss- and gain-of-function experiments to understand the complex nature of host-pathogen interactions that underlie transmission, infection, and pathogenesis.

The term "gain-of-function" is generally used to refer to changes resulting in the acquisition of new, or an enhancement of existing, biological phenotypes. This report further defines "gain-of-function research of concern" to describe the subset of studies that have been the subject of recent debate regarding potential biosafety and biosecurity implications -- that is, gain-of-function studies with the potential to generate pathogens with pandemic potential in humans by exhibiting high transmissibility and high virulence. See Section 6 for a more rigorous description of GOF research of concern (GOFROC).

Concerns regarding laboratory safety and biosecurity associated with GOF studies were renewed following a number of biosafety incidents at U.S. Federal laboratories during the summer of 2014. The incidents did not involve GOF studies *per se* but raised broader concerns about laboratory safety and security as it applies to pathogen research.

As one component of comprehensive efforts to review and enhance laboratory biosafety and biosecurity, the U.S. government (USG) embarked on a deliberative process to re-evaluate the risks and benefits of certain GOF research with a goal of developing policy governing the funding and conduct of

[1] Imai et al. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 486, 21 June 2012

[2] Herfst et al. Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets. *Science* 336, 22 June 2012

[3] Gangadharan D, Smith J, and Weyant R. Biosafety Recommendations for Work with Influenza Viruses Containing a Hemagglutinin from the A/goose/Guangdong/1/96 Lineage, Morbidity and Mortality Weekly Report 62(RR06); 1-7. http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6206a1.htm

[4] *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

[5] Framework for Guiding Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets, February 21, 2013. http://www.phe.gov/s3/dualuse/Documents/funding-hpai-h5n1.pdf

such research.[6] The deliberative process involves the National Science Advisory Board for Biosecurity (NSABB), which serves as the official Federal advisory body for providing advice in this area, and the National Academy of Sciences (NAS), which is to foster broader scientific and public discussions on the topics. To inform NSABB deliberations, NIH commissioned formal risk and benefit assessments (RBA) of GOF research involving pathogens with pandemic potential and an analysis of ethical issues surrounding the conduct of such studies. Stakeholder input is also essential to the process and has been received throughout NSABB's deliberative process.

The deliberative process is accompanied by a pause in the provision of new federal funds for certain GOF research involving influenza, Middle East Respiratory Syndrome (MERS) or Severe Acute Respiratory Syndrome (SARS) viruses—pathogens determined to have pandemic potential. Specifically:

> *New USG funding will not be released for gain-of-function research projects that may be reasonably anticipated to confer attributes to influenza, MERS, or SARS viruses such that the virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route. This restriction would not apply to characterization or testing of naturally occurring influenza, MERS, and SARS viruses, unless the tests are reasonably anticipated to increase transmissibility and/or pathogenicity.*[7]

In parallel, the USG has encouraged the research community (both those who receive USG funding and those who do not) to join in adopting a voluntary pause on any ongoing research that involves the types of studies that are subject to the funding restriction above.

NSABB recommendations will inform the USG as it develops policies about whether certain types of GOF studies on pathogens with pandemic potential should be supported and, if so, how such research proposals should be evaluated to inform funding and oversight decisions. It is expected that the temporary funding pause will be lifted and/or replaced by a decision or policy that addresses GOF research involving the generation of pathogens with pandemic potential.

2. NSABB Charge

On October 22, 2014, as part of the USG's deliberative process for GOF studies, the NSABB was issued its charge to:

1. Advise on the design, development, and conduct of risk and benefit assessments for GOF studies, and
2. Provide recommendations to the U.S. government on a conceptual approach to the evaluation of proposed GOF studies

In developing its recommendations the NSABB was asked to consider: the results of the risk and benefit assessments; the discussions hosted by the National Academies; the spectrum of potential risks and

[6] U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses, U.S. Government, October 17, 2014. http://www.phe.gov/s3/dualuse/documents/gain-of-function.pdf

[7] Ibid.

benefits associated with GOF studies; and any alternative methods that may be employed to yield similar scientific insights or benefits, while reducing potential risks.

3. NSABB Deliberative Approach

The deliberative process (Figure 1) initiated by the USG to evaluate the risks and benefits of GOF studies involves the NSABB and the National Academies. To address its charge, NSABB formed two working groups to develop draft recommendations, which were discussed by the full Board **[REF to meetings]**. The National Academies convened public forums to generate broad discussions and receive additional stakeholder input on the topic. The first forum was held early in the deliberative process and a second was held in March 2016; both were designed to inform NSABB deliberations.

To inform the deliberative process further, NIH commissioned two additional analyses: 1) qualitative and quantitative risk and benefit assessments, conducted by Gryphon Scientific, and 2) a review of the ethical considerations associated with the GOF issue and an analysis of relevant ethical decision-making frameworks, conducted by Dr. Michael Selgelid.

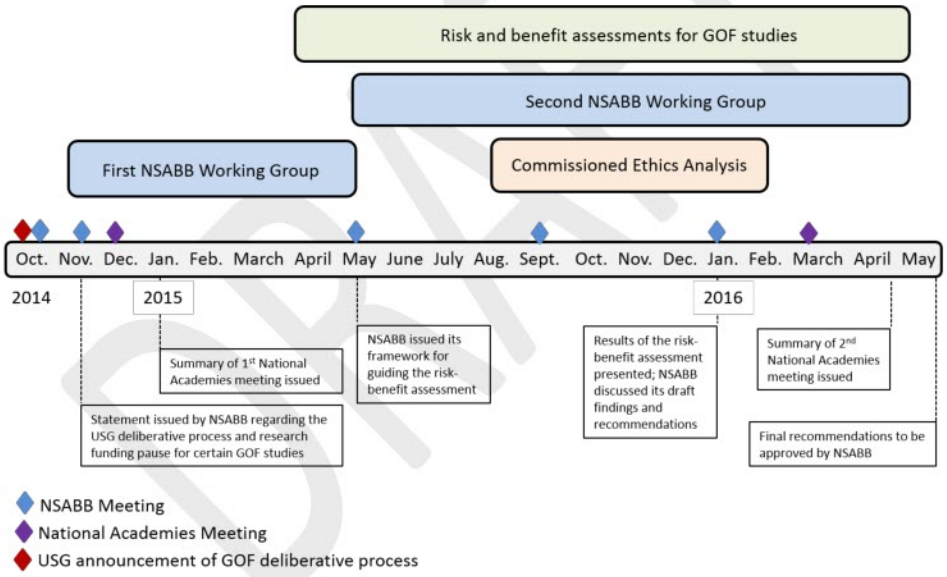

Figure 1. Timeline and major events of the GOF deliberative process.

The NIH Office of Science Policy, which administers the NSABB, managed the NSABB's overall deliberative process. NIH oversaw the work of its contractors, Gryphon Scientific and Dr. Michael Selgelid, and interfaced between the NSABB and contracted entities.

See Appendices A, B, C, and E for the NSABB and working group rosters, a detailed description of the NSABB's deliberative approach, an overview of stakeholder views that were considered, and a list of the experts and sources consulted, including those who submitted public comments.

Guiding Principles for NSABB Deliberations

Early in its deliberations the NSABB developed the principles below to guide its deliberations and underpin its analysis of the risk and benefit assessments.

1. The NSABB deliberations should focus on defining the GOF problem then include broad consideration of possible solutions. A range of approaches and decision-making frameworks will be considered, and the NSABB will take into account these various approaches when developing its recommendations.

2. NSABB will consider the potential risks and benefits of a broad range of GOF studies involving influenza, SARS, and MERS viruses in order to identify those that may raise significant concerns that should be addressed. However, the NSABB will aim to develop recommendations that are grounded in broadly-applicable concepts and principles that could, if necessary, apply to GOF studies involving other pathogens that may require evaluation in the future.

3. Similarly, NSABB will consider the risks and benefits associated with alternative research approaches to GOF research to understand whether or not these may substitute for or complement GOF studies.

4. NSABB recommendations will be informed by data and information about potential risks and benefits as well as values that will guide the evaluation and comparison of these risks and benefits. Ethical, societal, and legal considerations will also contribute to the development of recommendations and these inputs should be explicitly identified, discussed, and prioritized.

5. NSABB recognizes that not all analyses relevant to its task are quantitative and that uncertainties inherent in any quantitative analysis may remain. NSABB will seek to document important areas of uncertainty in any data or analysis when necessary.

6. NSABB should publicly debate its draft recommendations and describe in its report any dissenting views that may vary substantially from the Board's recommendations.

7. NSABB should consider current USG policies and guidelines, determine whether they adequately address risks associated with GOF research (in light of potential benefits), and make recommendations that are consistent with that determination. Current policies may be adequate or require only minor changes; alternatively, significant enhancements may be needed. The adequacy of current policy to cover GOF studies may vary by pathogen. Recognizing the paramount importance of ensuring safety, security, and public health, policies should also minimize the burdens placed upon the conduct of science.

8. NSABB recommendations will inform the development of U.S. government policy, which will apply to research funded, conducted, or overseen by the U.S. government either domestically or internationally. NSABB will be mindful in its deliberations of the likelihood that the Board's recommendations and U.S. policy decisions will also influence other governments and non-USG funders of life sciences research.

9. The NSABB will also consider whether there are certain studies that should not be conducted under any circumstances, and if so, articulate the critical characteristics of such studies.

10. Maintaining public trust and confidence in life sciences research is critical and must be taken into account as recommendations are formulated.

4. Analysis

The NSABB working group tasked with developing recommendations on a conceptual approach for evaluating GOF proposals examined three major areas: the current policy landscape for overseeing research involving pathogens, ethical issues associated with funding and conducting GOF studies, and the results of Gryphon's risk and benefit assessments. In addition, the NSABB considered broad stakeholder perspectives through presentations from domestic and international experts at Working Group and full NSABB meetings, expert consultations, individual NSABB member participation in and review of the National Academies workshops and proceedings, analysis of published articles, and comments from attendees at NSABB meetings and public comments submitted to the Board.

4.1. Analysis and Interpretation of the Risk and Benefit Assessment

The NSABB working group has reviewed the risk and benefit assessments (RBA) conducted by Gryphon Scientific, which were designed to evaluate the risks and benefits of GOF research in a manner that encompassed both benign and worrisome aspects of a broader range of GOF studies than those that have raised concern. The RBA analyzed biosafety and biosecurity risks as well as possible benefits. Overall, the RBA includes a commendable amount of sophisticated work and analysis, is generally well-done, and largely achieves the goals it was intended to address. Gryphon's draft RBA report was made publically available in December 2015 and key results were presented and discussed at NSABB and NAS meetings. The final report is available on Gryphon's website.[8]

Strengths of the Risk and Benefit Assessments

The RBA has numerous significant strengths. It is a thorough and extensive analysis of the risks and benefits of GOF work in the context of the guidance provided in the NSABB *Framework for Conducting Risk and Benefits Assessments of Gain-of-Function Research* (May 2015)[9]. It takes into account the principles articulated in the framework and includes the agents, categories of possible risks, types of possible benefits, and possibly concerning scenarios and phenotypes that were laid out in the *Framework*. A few items from the *Framework* were eliminated from consideration at the meeting of the NSABB where the framework was voted on[10], so that the most probable issues of concern could be thoroughly addressed within the available time and resources.

[8] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

[9] Framework for Conducting Risk and Benefits Assessments of Gain-of-Function Research, May 2015. http://osp.od.nih.gov/sites/default/files/resources/NSABB_Framework_for_Risk_and_Benefit_Assessments_of_GOF_Research-APPROVED.pdf

[10] National Science Advisory Board for Biosecurity Meeting, May 5, 2015. http://osp.od.nih.gov/office-biotechnology-activities/event/2015-05-05-120000-2015-05-05-200000/national-science-advisory-board-biosecurity-nsabb-meeting

The biosafety risk assessment does a credible job of defining the relative risks associated with potential laboratory accidents involving GOF manipulations of pathogens with enhanced characteristics as compared to wild-type pathogens. This analysis is performed in a semi-quantitative way; it uses appropriate, established, peer-reviewed methods to the extent available. The parametric approach employed is powerful and allows consideration of many situations of interest.

The report effectively illustrates that the harmful events being modeled are low probability (see Figures 6.2 and 6.4 in Gryphon's report). Only a small fraction of laboratory accidents would result in a loss of containment; of those, only a small fraction would result in a laboratory acquired infection, and of those, only a fraction would spread throughout the surrounding community (or to the global population). The working group recognizes the challenge of analyzing low-probability, high-consequence events for which little data exists and appreciates attempts to make this point clear in the RBA.

The biosecurity risk assessment is primarily qualitative, and highlights analysis of previous malevolent events and evasions of security systems, likely capabilities and motivations of various possible actors, and an evaluation of the systems in place to prevent biosecurity breaches. Information was obtained from a survey of literature and discussions with biosecurity, intelligence, and law enforcement professionals. It is an extensive gathering of a wide range of information that has not been presented before in one place.

The information risk assessment (an element of the biosecurity risk assessment) is a qualitative analysis of risks that may result from the misuse of information derived from certain GOF studies that might be published in the future. It identifies information that might be attractive to malicious actors and compares it to other sources of information they might find attractive.

The benefits assessment uses a novel approach to assess benefits of GOF studies, a difficult task without much prior methodology to draw upon. The results are not quantitative, and attempts to quantify would have been appreciated. However, as is, the assessment may be the best that can be done with the available information and analytic tools. The benefits assessment effectively analyzed the possible benefits of alternatives to GOF studies and identified areas where GOF research appears to provide unique benefits (i.e., benefits that are not attainable without the use of GOF), either currently or in the near future.

The RBA contains a number of other useful analyses as well, including background and contextual information on the biology of influenza and coronavirus, historical analysis of naturally-occurring seasonal and pandemic influenza and coronavirus outbreaks, an examination of the potential proliferation of GOF research, and analysis of the potential loss of public trust in science that could result if a laboratory incident involving GOF research were to occur. Significantly, the historical analysis notes that each year, influenza infects 5 – 10% of the world's population, resulting in significant morbidity and mortality (up to 500,000 deaths per year). This description of naturally-occurring influenza (and coronavirus) infections helps to establish the extant risks associated with these infectious diseases to which the risks associated with GOF studies might be compared.

Overall, the RBA is comprehensive, objective, reasonable, and generally extensively documented.

Limitations of the Risk and Benefit Assessments

The RBA also has some weaknesses and limitations that should be noted. First, the RBA was limited to the types of labs traditionally funded by the Federal government, which may not be representative of other settings where GOF research may be conducted. Every attempt was made to base the analyses in the RBA on scientific information and data. Nevertheless, data on the properties of the various pathogens being examined, events such as laboratory accidents or security breaches, or possible future acts of terrorism are limited in some cases and unavailable in principle in others. Therefore, assumptions and estimations were necessary. For this reason, the biosafety risk assessment is not fully quantitative, primarily because absolute, quantitative baselines for the risk of work with wild-type pathogens could not be estimated with any certainty. Thus, the data presented are primarily comparative, and provide relative, not absolute values, for the risks associated with laboratory accidents involving GOF studies. Gryphon compared the risks associated with potential lab accidents involving a GOF strain with the risks associated with the same accident involving a wild-type strain. This comparative approach is adequate for some instances but inadequate for others. For instance, an increased risk associated with a GOF study that is relatively large (5-10-fold or greater) may appear significant, but if this increase is in comparison to a very small risk baseline, the overall risk associated with the GOF study may not be significant or concerning. Similarly, small increases in risk over a higher risk baseline, in fact, may be concerning. Additionally, differences in risk that are relatively small (~2-fold) are difficult to interpret because such changes may fall within the limits of uncertainty for the analysis. Attempts to include some absolute baseline estimates of risk (an admittedly difficult task) were included in Section 6.8 of Gryphon's report. However, the lack of comprehensive estimates of baseline risk make interpreting the biosafety risks a challenge.

Given the comparative approach undertaken for the biosafety risk assessment, implications of the results of this analysis depend a great deal on the wild-type comparator strains that were selected for the analysis. For instance, for pandemic influenza Gryphon initially selected the 1918 influenza strain as the comparator. Gryphon regarded this strain as embodying the maximum risk for influenza, yet a level of risk that is also deemed as acceptable given that research with this strain is permitted. However, using 1918 influenza as the comparator for the analysis compares GOF risks to a relatively high level of baseline risk, making the changes in risk associated with GOF manipulations comparatively small. Utilizing different comparator strains alters the relative risks associated with GOF manipulations; using a high-risk baseline strain may obscure significant risks associated with GOF studies whereas using a low-risk baseline strain may inflate the potential risks associated with GOF studies. **Note to WG: Please review, the previous para was adapted significantly based on Gryphon's new analysis and subsequent discussions.**

Little data exists about the probabilities of the accidents that initiate the chain of events that may lead to a pandemic and therefore, the quantitative probability of these accidents could not be incorporated into the biosafety risk assessment. The modeling of secondary spread of a pathogen through populations once it is released from a laboratory allows for some estimation of the consequences of an

event but without a better understanding of the likelihood that an accident would result in loss of containment or a laboratory acquired infection, it is difficult to make judgments about the overall risk. Gryphon's analysis accounts for this by presenting relative, actuarial risk. However, this approach results in the challenges associated with comparing relative risks described above. There are large uncertainties in most of the input parameters that are the basis for the biosafety risk calculations. Uncertainties about inferring absolute risk from these relative risks exist and should be kept in mind as any conclusions are reached.

The biosecurity risk assessment attempts to examine how GOF studies add to the risk of malevolent acts. Portions of the biosecurity risk assessment focus on GOF studies but others describe the type of threats that could occur against any high-containment laboratory. The semi-quantitative portion of the biosecurity risk assessment estimates the number of infections that could occur if a pathogen with various enhanced characteristics were intentionally released. However, this analysis (see section 7.4.2 and Table 7.7 in Gryphon's report) assumes that 1 or 10 individuals are initially infected as a result of a malicious act with no indication of how likely such an event would be, since there is no way to make such an estimate.

While exhaustively documented, the RBA is not always transparent about data reliability. In particular, interviews were used to gather much critical information, and this was not always well documented in a way that reflects how robust the resulting information may be. For peer-reviewed publications, this is less of a concern.

While evaluation of the benefits of alternatives to GOF studies was extensive, evaluation of risks of alternative approaches was not as thorough. In addition, risks and benefits have not been presented in comparable terms, making it a challenge to determine whether certain risks are justified by potential benefits. Significantly, the benefit assessment is not quantitative and there is no probability analysis or attempt to estimate the likelihood that a certain benefit would be realized or what its impact might be.

Key Results of the Risk and Benefit Assessments

While NSABB has examined all of the analyses in the RBA, some results are important to highlight. In general, the RBA examined risks and benefits associated with the major GOF phenotypes with the intention of identifying types of studies that would be most and least concerning, based particularly on their risk profile.

With regard to biosafety risks, only some potential GOF phenotypes represent substantially increased (5- to 10-fold or more) risks over the starting strain. Two-fold changes most likely fall within the uncertainty of the data, and while small differences might be important if it could be shown that they are significant, this demonstration is probably difficult. For coronaviruses, GOF studies that would create strains with increased transmissibility among mammals may entail significant risks if they also increase human transmission. The risks, were this combination to occur, would include increased probability of an outbreak escaping local control and increased likelihood of global consequences. In addition, experiments that enhance coronavirus growth in culture would likely increase the possibility of laboratory acquired infections.

For seasonal influenza, the GOF-generated phenotypes entailing the greatest risks include enhanced transmission in mammals (assuming this increases transmission in humans), enhanced virulence, and evasion of immunity. Enhanced pathogenicity might significantly increase the global consequences of an outbreak. For pandemic influenza, no GOF-generated phenotypes led to greatly increased risk, but that is based on using 1918 influenza as the comparator; because the risk associated with the wild-type 1918 strain is already so great it is difficult to increase risk substantially. If less transmissible and/or less virulent wild-type strains were used as the basis of comparison, the risks of GOF studies with pandemic strains might appear higher. For avian influenza, the GOF experiments that lead to enhanced transmissibility in mammals (and presumably humans) would likely lead to an increased probability of local and widespread outbreaks, as well as increased global consequences. More subtle aspects of these very general conclusions may be found in the biosafety risk section of the Executive Summary of Gryphon's RBA report.

In general, GOF studies that were not considered by the working group to entail significant risks were those that would: adapt human pathogens to mammals to generate animal models; enhance the growth of attenuated vaccine strains; and antigenic drift or immune evasion studies that are commonly used to guide vaccine selection.

The biosecurity risk assessment shows that the most probable threats involve insiders who have direct access to dangerous pathogens or outsiders who collaborate with or subvert insiders. If currently mandated biosecurity systems are effective, outsiders have little chance of causing harm on their own. The RBA report also concludes that the risks associated with information from future GOF studies with influenza, SARS and MERS appear small; this is because most of the information of interest is already published, or non-GOF information relating to pathogens that are more attractive agents of harm is readily available. However, future scientific advancements could alter this assessment.

Most GOF studies provide benefits in the form of new scientific knowledge, and some of these benefits are unique (i.e., unable to be achieved by alternative, non-GOF approaches). While some GOF studies are likely to provide unique near-term benefits, these are associated with specific agents and phenotypes. With regard to more applied benefits, such as countermeasure development and biosurveillance, the most clear-cut situation is experiments that increase growth of seasonal influenza vaccine candidates in culture; these studies provide unique benefits to current production of seasonal influenza vaccines, and likely will in the future. Another reasonably clear unique benefit is derived from experiments that enhance mammalian pathogenicity for coronavirus as a means of developing animal models for studying disease and developing countermeasures. GOF studies that yield phenotypes that provide unique benefits to countermeasure development include enhanced pathogenicity, evasion of vaccines, and evasion of therapeutics. For several other potential benefits with seasonal influenza, either the potential benefit is long term, or alternative approaches may yield the same or similar benefits. Interestingly, few unique benefits pertaining to GOF studies involving pandemic influenza were identified. There are several types of GOF studies that entail generating avian influenza strains with phenotypes that may be valuable for surveillance and preparedness efforts, although other advances are needed to fully realize such benefits. This point is controversial, with strong proponents and critics. Additionally, a variety of benefits were identified that may also be provided to some extent

by alternative approaches. It should be noted that no attempt was made to provide a probability assessment based on historical data for potential benefits; hence no direct comparison of risk to benefit for a proposed research project is possible.

4.2. Consideration of Ethical Values

The risk and benefit assessments provide information about the potential risks and benefits associated with conducting GOF research. However, determinations about whether such studies should be undertaken will involve value judgments when weighing the risks and benefits. The NSABB identified a number of values (that are applicable to the decisions about whether to fund certain GOF studies and how to oversee them. Sources of these values include the Belmont Report,[11] the literature on public health ethics,[12] and the literature on oversight of emerging technologies,[13] as well as the literature specifically debating appropriate approaches to overseeing DURC and GOF research that has raised concern.[14,15,16,17,18] The commissioned ethics analysis conducted by Dr. Michael Selgelid also describes additional decision-making frameworks and values to be considered.[19]

Note to WG: The decision was made to leave this section here rather than shift to appendix

Substantive values

The following values are important to consider when considering funding of a research proposal involving GOF studies that might entail significant risks.

Non-maleficence: not causing harm. Harm might include: losing lives; causing disease; damage to the economy, national or international security, or agriculture; or loss of public trust in science or governance structures. There are inherent risks associated with research involving pathogens that could result in harm. Approaches aimed at preventing harm and mitigating potential risks should be

[11] The Belmont Report. Office of the Secretary, U.S. Department of Health and Human Services. Ethical Principles and Guidelines for the Protection of Human Subjects Research. The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, April 18, 1979. http://www.hhs.gov/ohrp/humansubjects/guidance/belmont.html
[12] Kass NE. An Ethics Framework for Public Health. *American Journal of Public Health*. 2001;91(11):1776-1782. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1446875/
[13] New Directions. The Ethics of Synthetic Biology and Emerging Technologies. Presidential Commission for the Study of Bioethical Issues, December 2010. http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf
[14] Resnik DB. H5N1 Avian flu research and the ethics of knowledge. Hastings Center Report 2013; 43, 2: 22-33.
[15] Kelle A. Beyond patchwork precaution in the dual-use governance of synthetic biology. Sci Eng Ethics. 2013 Sep;19(3):1121-39.
[16] Kuhlau F, Höglund AT, Evers K, Eriksson S. A precautionary principle for dual use research in the life sciences. Bioethics. 2011 Jan;25(1):1-8.
[17] Biotechnology Research in the Age of Terrorism. The National Academies, 2004. http://www.nap.edu/catalog/10827/biotechnology-research-in-an-age-of-terrorism
[18] Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information. National Science Advisory Board for Biosecurity, June, 2007. http://osp.od.nih.gov/sites/default/files/resources/Framework%20for%20transmittal%20duplex%209-10-07.pdf
[19] Selgelid, Michael. Gain-of-Function Research: Ethical Analysis. December 7, 2015. http://osp.od.nih.gov/sites/default/files/GOF%20%20White%20Paper%20by%20Michael%20Selgelid_0.pdf

considered and applied to the design, conduct, and communication of research involving pathogens in GOF studies.

Beneficence: promoting beneficial outcomes while preventing harmful outcomes; appropriately balancing benefits and risks; formulating policy that maximizes public benefit while minimizing public harm. Benefits might include: saving lives, preventing disease, improving public health; enhancing the economy, national and international security, or public trust in science and governance structures. When the ultimate goals of the research are to improve public health, public health ethics would ask how effective the research is likely to be in achieving those goals, what are the known or potential burdens of the research, can those burdens be minimized, whether there are alternative approaches that are less risky or burdensome, and how can the potential benefits and burdens of the research be fairly balanced. The work of the Presidential Commission for the Study of Bioethical Issues suggests that those formulating and effectuating government policy on scientific research and emerging technologies have a duty of public beneficence – a duty "to promote individual activities and institutional practices…that have great potential to improve the public's well-being," while being "vigilant about risks and harms, [and] standing ready to revise policies that pursue potential benefits with insufficient caution."[20] Both risks and benefits have associated probabilities, magnitudes, and uncertainties. In some instances, it may be justifiable to pursue benefits despite the potential risks; in others, the potential benefits may be foregone due to possible risks.

Social justice: distributing potential benefits and harms fairly (distributive justice) and selecting participants in research fairly, as well as those who may potentially be exposed to risk. There are many different approaches to social justice, such as egalitarianism, utilitarianism, and libertarianism,[21] to name but a few. Decisions about whether to fund research that entails some risk should consider how the risks and benefits associated with conducting that research will be distributed, with an effort to distribute risks and benefits as fairly as possible. When considering pandemic potential, fair distribution of risks and benefits must be considered on a global scale. Those who will potentially be exposed to risk, through participation in research or other avenues of exposure, should be selected equitably.

Respect for persons: allowing competent individuals to make informed choices, and ensuring that the representatives of individuals lacking capacity to choose can make choices in keeping with the wishes, values, or interests of those represented. Autonomy generally requires informing human research participants, laboratory workers, and the public about the risks of research and eliciting their free and uncoerced decision about whether to subject themselves to those risks. In the case of the public, mechanisms for representative decision-making and publicly accountable governance may be needed, as getting consent directly from the members of the public may be impracticable.

[20] New Directions. The Ethics of Synthetic Biology and Emerging Technologies. Presidential Commission for the Study of Bioethical Issues, December 2010. http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf
[21] Nozick R. Anarchy, State, and Utopia. New York: Basic Books, 1974.

Scientific Freedom: avoiding unnecessary interference with scientific research, debate, or publication. Scientific freedom includes an entitlement to avoid interference unless necessary (negative freedom), but not the affirmative right to receive funding or other forms of support for a particular project (positive freedom). Scientific freedom is compatible with norms and regulation to promote the responsible conduct of research and protect participants in research and the public. As a corollary to the principle of scientific or intellectual freedom, the Presidential Commission endorses a principle of regulatory parsimony, requiring "only as much oversight as is truly necessary to ensure justice, fairness, security, and safety while pursuing the public good."[22]

Responsible Stewardship: acting in a way that shows concern for children, future generations, and the environment. The Presidential Commission emphasizes that this is both a domestic and global responsibility that requires "prudent vigilance, establishing processes for assessing likely benefits along with assessing safety and security risks both before and after projects are undertaken."[23]

Procedural Values

The following values apply to the process of decision-making about GOF research and are important to consider when establishing mechanisms to review and/or approve the funding of research proposals involving gain-of-function studies that may entail significant risks.

Public participation & democratic deliberation: making decisions with participation from the public, utilizing respectful debate and inclusive deliberation. Life sciences research is largely a publicly-supported endeavor; therefore, those who allocate funds and conduct life sciences have a responsibility to be good stewards of public funds and to respond to the interests and concerns of the public. Many, if not all, members of society have a stake in the life sciences enterprise and will be affected directly or indirectly by the benefits and risks stemming from such research. This stakeholder community has diverse values and tolerances for risk, which are important to consider when making decisions about funding and overseeing life sciences research. Some forms of public participation include: oversight by the legislative or executive branches of government, public membership and input on government science advisory committees, other mechanisms of public governance, surveys of public opinion on science policy issues, research models such as community-based participatory research, and efforts by scientists and government officials to share information with the public and better understand the public's interests and concerns. The Presidential Commission urges the importance of democratic deliberation, as "[a]n inclusive process of deliberation, informed by relevant facts and sensitive to ethical concerns, promotes an atmosphere for debate and decision making that looks for common ground wherever possible and seeks to cultivate mutual respect where irreconcilable differences remain."[24]

[22] New Directions. The Ethics of Synthetic Biology and Emerging Technologies. Presidential Commission for the Study of Bioethical Issues, December 2010. http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf, p5.
[23] Ibid., p5.
[24] Ibid., p5.

Accountability: taking responsibility for one's actions and being prepared to justify or explain them to others. It is important that decisions to fund research are justifiable to the public and others. Decisions should be justified in terms of substantive and procedural values.

Transparency: sharing with the public the information and assumptions used to make a decision, including uncertainties, controversies, and limitations of analyses. Transparency is an important part of accountability and public participation. It allows review and reconsideration of policy over time as new facts emerge and analysis evolves.

4.3. Decision-Making Strategies and Frameworks for Evaluating and Managing Risks and Developing Policy

NOTE TO WG: The policy approaches and decision-making frameworks were combined and left here, rather than moving to an appendix

The field of decision-making theory is concerned with identifying reasons for and issues relevant in making decisions and is aimed at finding approaches to help people make better decisions. Experts in this area have identified a number of approaches or frameworks that may be used to guide making complex decisions with ethical implications in the face of uncertainty. These may also be used in developing policies such as that for managing GOF research. Different strategies reflect different attitudes toward risk-taking. Some may be more appropriate in some situations than others. The NSABB examined a number of such strategies as it attempted to determine the best option as relates to GOF research that has raised concerns. These options are not mutually exclusive, and elements from more than one may be used together to develop a path forward. The following are decision-making frameworks that were considered:

Maximax: This involves choosing the option with the best possible outcome. Maximax is a relatively simple strategy that focuses on choosing the option with the best possible outcomes While maximax may be appropriate for making some types of personal choices (e.g. playing games with nothing of value to lose), it may not be appropriate for making science and technology policy decisions because most people would want to take appropriate steps to prevent or mitigate risks regardless of benefits. For GOF studies, use of maximax would involve identifying research with the best possible benefits, generally regardless of risks.

Maximin: This involves choosing the option with best outcome among the worst possible outcomes. Maximin is a risk-averse approach because it aims to avoid the worst possible outcomes. Maximin is another relatively simple approach, but may present difficulties in making science and technology policy decisions, because it would recommend not developing a new technology if this decision could lead to the worst possible outcome. Since all technologies (and scientific ideas) can conceivably lead to good and bad outcomes, strict adherence to maximin would imply a very

cautious approach to science and technology development. For GOF studies, use of maximin would involve identifying studies with risks, and choosing the least risky regardless of benefits.

Expected Utility Theory: This involves choosing the option that maximizes expected utility, where expected utility for a possible outcome = probability x utility. Expected utility theory involves a quantitative balancing of risks and benefits and is inherently a more complex process. Cost-benefit analysis in economics is a form of expected utility theory. A problem with expected utility theory is that sufficient evidence may not always be available to confidently estimate the probabilities involved in the utility calculus. When this is the case, other approaches may be appropriate. For GOF studies, use of expected utility theory would require determining quantitatively the likelihood of risks and benefits and calculating the resulting utility.

Precautionary approach: This approach involves taking reasonable measures to prevent, minimize, or mitigate risks that are significant and plausible. A measure is "reasonable" if it: 1) appropriately balances the values at stake in the risk management; 2) is proportional to nature of the risk (i.e. greater risks require stronger measures); and 3) is likely to be effective. A risk is "plausible" if there is some scientific evidence that it could occur even if the probability of the risk cannot be confidently estimated. There are many versions of the precautionary principle, including ones that are more or less risk-averse.[25,26] A precautionary approach, in general, would limit an activity unless the environment, health, or security, are clearly protected. This approach can recognize a potential problem early and prevent harm from occurring but may lead to regulatory burdens or unnecessarily limit activities. This approach might restrict potential GOF research unless the studies are demonstrated to be safe.

Permissive approach: This approach, in general, would allow an activity unless the environment, health, or security, are clearly compromised. This approach may reduce unnecessary regulatory burdens but can result in after-the-fact reaction to harms. This approach might allow certain GOF studies to proceed until they are demonstrated to entail significant risk.

Planned adaptation or risk-based approach: This approach provides a systematic way to deal with managing risks in the face of uncertainty. It involves: 1) preparation to identify the risks and regulatory gaps, including getting input from a broad range of perspectives; 2) putting measures in place to control risk based on the best information available at the time; 3) systematically gathering data and observing effects of policies; and 4) updating and revising policy as needed. An example of an adaptive approach is the life cycle approach taken by the Food and Drug Administration when making decisions about whether to approve drugs, when that includes post-market surveillance.[27] For GOF studies, this approach might entail allowing GOF studies of potential concern—or certain GOF studies—to proceed under defined conditions, then evaluating the risk-benefit landscape

[25] Resnik DB. Environmental Health Ethics, New York: Oxford University Press, 2013.
[26] Munthe C. The Price of Precaution and the Ethics of Risks. Dordrecht: Springer, 2011.
[27] FDA determinations about whether a new drug is safe and effective are complex, address uncertainty, and involve ongoing monitoring to assess risks and benefits and take appropriate post-marketing actions as necessary. See: *Structured Approach to Benefit-Risk Assessment in Drug Regulatory Decision-Making*, 2013
http://www.fda.gov/downloads/ForIndustry/UserFees/PrescriptionDrugUserFee/UCM329758.pdf

periodically to determine whether the GOF studies that are permitted should continue, be expanded, or be restricted.

Threshold approach: This approach would entail identifying a risk threshold beyond which, certain studies are given special attention or subject to additional scrutiny or oversight and might preclude certain studies. Implementation would involve defining or describing the studies that would require additional oversight as well as a description of what that oversight would entail. This approach would allow for the identification of studies of concern but might need to be reevaluated if the risk landscape changes and the threshold that was identified is no longer appropriate. For GOFROC, this would entail identifying the characteristics of studies involving significant risks that may not be adequately managed and then stipulating further oversight or determining that they should not be conducted.

Point-source approach: This approach would involve controlling where certain studies are conducted and under what conditions. This approach would centralize certain research activities, restricting them to designated locations or facilities. For GOFROC this might involve requiring that certain studies only be conducted in facilities with certain biocontainment conditions, biosafety practices, and security measures.

The working group used ideas from a number of frameworks to inform its findings and deliberations (Sections 5 and 6). An adaptive approach was considered particularly attractive and appropriate for GOF research, and the Board incorporated it into its recommendations. Expected utility theory encompasses elements of risk-benefit analysis which the Board also deems important, although a strict quantitative calculation is probably not possible. The criteria for identifying GOFROC and principles for its evaluation reflect a threshold approach. Finally, recommended mitigation requirements incorporate some elements of point-source and precautionary approaches.

4.4. Examination of the Current Policy Landscape

Many Federal agencies fund life sciences research in furtherance of their specific missions. In general, research supported by the USG is founded on the principle of scientific merit and goals of the funding agency. Multiple complementary layers of oversight are in place to manage laboratory and other risks associated with Federally-funded life sciences research. These policies are intended to provide oversight at various points throughout the research life cycle, from research conception to its publication and translation into practice. These policies include a foundation of occupational health and medicine (for laboratory and clinical workers), laboratory biosafety practices, and policies that address biosecurity risks. Below is a description of the oversight policies in place for Federally-funded life sciences research involving pathogens, with discussion of whether and how such policies apply to GOF studies. This analysis is illustrated in Figures 2 and 3 and summarized in Appendix D.

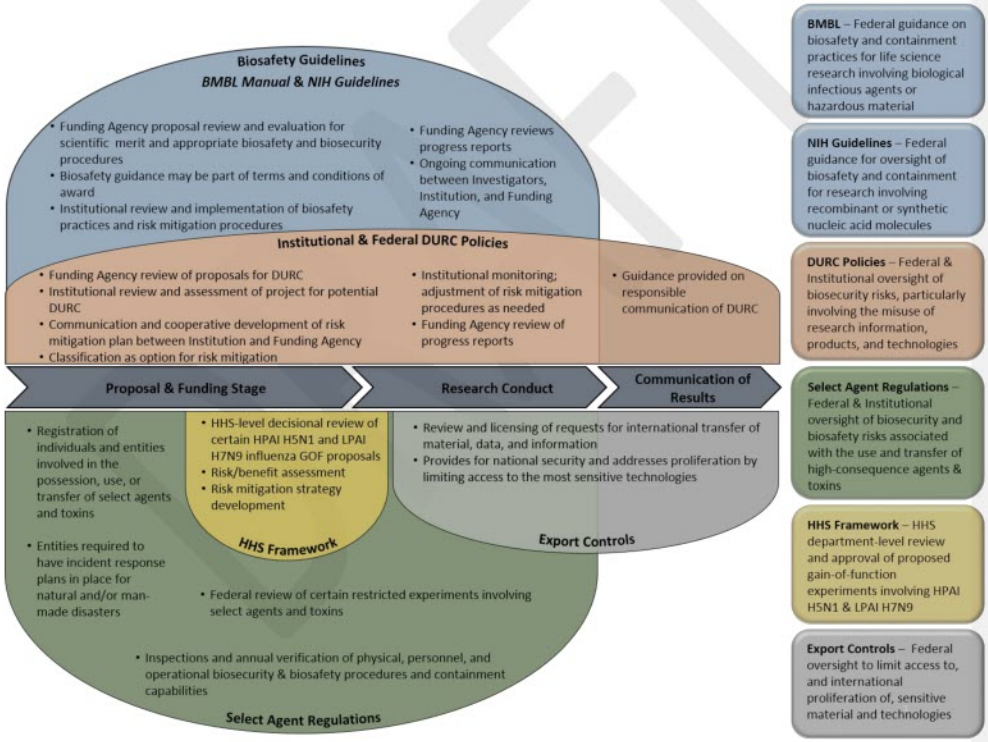

Figure 2. U.S. government oversight of life sciences research involving pathogens. Oversight policies apply at different stages and occur at different levels throughout the research life cycle. See text and Appendix D for descriptions of each policy. The policies depicted in this figure are defined by different applicability and scope requirements and therefore do not apply to all life sciences (or GOF) research projects.

Scientific Merit Review

Departments and agencies within the U.S. government fund diverse portfolios of life sciences research. Funding decisions are based on the scientific merit of a given proposal and the ability of a project to advance the agency's strategic mission. The U.S. government funds life sciences research through a variety of mechanisms including grants, contracts, and cooperative agreements. Each funding agency has its own processes for evaluating research proposals and awarding funds but, in general, proposals are subject to rigorous scientific review by Federal agency staff and often, scientific peers. NIH grant proposals, for example, undergo two levels of review. The first evaluation is by a panel of scientific peer reviewers who score proposals based on scientific merit and other criteria. The second round of review includes discussion of meritorious proposals at public meetings of advisory councils, specific to individual funding institutes and centers within NIH, to determine how proposals fit within their broader strategic objectives.

Biosafety Oversight

Oversight of pathogen research focuses first on ensuring the safe handling of biological agents through appropriate biosafety practices and containment measures, which are addressed by the *Biosafety in Microbiological and Biomedical Laboratories* (*BMBL*)[28], the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*)[29], and other documents. The BMBL and the *NIH Guidelines* provide for Federal and institutional biosafety oversight and guidance involving biosafety practices and containment features that are based on risk assessments for specific projects. Such determinations are typically made at the institutional level and are guided by Federal guidelines and policies, which are updated as necessary to provide additional guidance for research involving emerging pathogens or technologies. Biosafety is achieved by conducting research under appropriate physical and biological containment levels and employing practices that help to ensure a safe working laboratory environment.

The BMBL is a CDC-NIH guidance document that is generally considered the authoritative reference for laboratory biosafety. The BMBL provides summary statements for many bacterial, fungal, parasitic, rickettsial, viral, and other agents. These statements describe the characteristics of the pathogen, its natural mode of infection, potential occupational hazards with the agent, and recommendations for laboratory safety and containment. It also describes the fundamentals of biological containment, which includes descriptions of proper microbiological practices, safety equipment, and facility safeguards that protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory. It describes the process of biological risk

[28] Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition. http://www.cdc.gov/biosafety/publications/bmbl5/

[29] The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), November 2013. http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html

assessment, which enables the appropriate selection of microbiological practices, safety equipment, and facility safeguards that can prevent laboratory-associated infections. It also describes occupational health, immunoprophylaxis, and principles for laboratory biosecurity. The BMBL is updated periodically to refine guidance based on new knowledge and experiences and to address contemporary issues that present new risks that confront laboratory workers and the public health.

Analysis: The BMBL does not address GOF studies *per se* but does include summary statements and biocontainment guidance for research involving various influenza strains (including contemporary and non-contemporary human, high and low pathogenic avian, swine, the 1918 influenza strain, and reassortant viruses) and SARS-CoV. MERS-CoV had not emerged at the time of the last BMBL update, but interim laboratory biosafety guidance was issued by CDC.[30]

The BMBL is not a regulatory document. U.S. funding agencies may require it be followed as a term and condition of awards but in general, compliance with the BMBL is voluntary. In addition, the BMBL provides general biosafety guidance but does not describe detailed procedures or experiment-specific containment protocols.

The *NIH Guidelines* specify the practices for safely constructing and handling recombinant nucleic acid molecules; synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules; and cells, organisms, and viruses containing such molecules. The *NIH Guidelines* apply to basic and clinical recombinant or synthetic nucleic acid research conducted at or sponsored by institutions that receive NIH funding for any such research. Compliance with the *NIH Guidelines* is typically required as a term and condition of award of funding. Other Federal agencies may also require compliance with the *NIH Guidelines*.

The *NIH Guidelines* focus on the concepts of risk assessment, risk group classification of agents based on their ability to cause disease in humans and the availability of medical countermeasures, physical and biological containment levels, practices, personal protective equipment, and occupational health. To help ensure the safe conduct of this research, the *NIH Guidelines* specifies roles and responsibilities of investigators and institutions. Institutions subject to the *NIH Guidelines* must establish Institutional Biosafety Committees (IBCs) composed of members with appropriate expertise, to review and approve such research. IBCs provide local oversight and ensure compliance with the *NIH Guidelines*. Certain higher risk experiments require review by the Recombinant DNA Advisory Committee (RAC)[31] and specific approval by the NIH Director as Major Actions. These experiments involve the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if

[30] Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Middle East Respiratory Syndrome Coronavirus (MERS-CoV) – Version 2. http://www.cdc.gov/coronavirus/mers/guidelines-lab-biosafety.html [last updated June 18, 2015]

[31] The Recombinant DNA Advisory Committee (RAC) is a federal advisory committee that provides recommendations to the NIH Director related to basic and clinical research involving recombinant or synthetic nucleic acid molecules. See: http://osp.od.nih.gov/office-biotechnology-activities/biomedical-technology-assessment/hgt/rac

such acquisition could compromise the ability to control disease agents in humans, veterinary medicine or agriculture.

In order to continue to provide appropriate guidance for emerging pathogens or experimental approaches, the *NIH Guidelines* are updated periodically. The *NIH Guidelines* have been amended to include additional guidance for work with Risk Group 3 influenza viruses (1918 H1N1, H2N2, highly pathogenic avian influenza (HPAI) H5N1), to specify enhancements to biosafety level 3 containment, practices, and to incorporate occupational health requirements. In 2012, the *NIH Guidelines* were amended again to require further enhancements to facilities, biosafety equipment and practices, including occupational health practices, for research involving HPAI H5N1 strains transmissible among mammals by respiratory droplets.

Analysis:

The *NIH Guidelines* provide guidance on risk assessment and appropriate containment and practices for conducting research involving recombinant or synthetic nucleic acids, which would apply to most government-funded GOF research. Some IBCs also review and approve non-recombinant pathogen research; however, not all institutions require their IBCs to do so. While the *NIH Guidelines* are often used as a model of biosafety guidance by the broader scientific community, compliance is required only by institutions receiving such funding from the NIH. Therefore, some GOF studies may not be subject to the *NIH Guidelines* depending on whether the institution where the research is being conducted is subject to the *NIH Guidelines*.

The Federal Select Agent Program

Subtitle A and B of the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 requires the U.S. Departments of Health and Human Services (HHS) and Agriculture (USDA) to establish and regulate a list of select agents, biological agents and toxins that have the potential to pose a severe threat to public health and safety or animal or plant health or animal or plant products. The Select Agent Program (SAP) is administered jointly by the HHS Centers for Disease Control and Prevention and USDA Animal and Plant Inspection Service. The SAP oversees the possession, use and transfer of biological select agents and toxins. The Select Agents and Toxins List is reviewed and updated biennially. Under the select agents regulations, individuals and institutions that possess, use, or transfer any select agent are required to be registered, follow appropriate biosafety procedures, and undergo periodic inspections. Individuals must be registered with the SAP to have access to select agents or toxins, which requires that they undergo a security risk assessment performed by the Federal Bureau of Investigation (FBI). There are legal penalties for failing to comply with the select agent regulations.

In addition to the agents and toxins on the list, the select agent regulations apply to some genetic elements, including nucleic acids that are immediate precursors to infectious forms of any select agent viruses (i.e., complete positive strand RNA viral genomes), as well as some nucleic acids that encode select toxins. Select agent regulations also apply to genetically modified select agents and toxins.

Restricted experiments are described in the regulations and involve the deliberate transfer of or selection for a drug resistance trait to select agents that are not known to acquire the trait naturally. If the acquisition of resistance is to a first-line drug that could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, the restricted experiment requires special review and approval by the SAP. Some attenuated strains of select agents may be excluded from the regulations based upon a determination that the attenuated strain or modified toxin does not pose a severe threat to public, plant, or animal health or safety. The Intragovernmental Select Agent and Toxin Technical Advisory Committee serves as an advisory group to the SAP. In the wake of the recent laboratory incidents at Federal facilities involving select agents, two advisory committees have issued recommendations for ways to strengthen the Select Agent Program.[32] [33] Plans to implement these recommendations are also in place.[34]

Analysis: Studies that could be considered GOF studies are subject to oversight by the SAP if they involve pathogens on the select agent list. Researchers and institutions performing such studies must receive favorable security risk assessments by the FBI, register with the SAP, receive training on the proper procedures and practices for handling such agents, and abide by other aspects of the regulations. SARS-CoV, HPAI H5N1 influenza, and 1918 influenza viruses are select agents and GOF studies involving these pathogens are subject to oversight by the SAP. Restricted experiments that would entail conferring antiviral resistance to these viruses would require additional review and approval prior to being conducted. However, MERS-CoV is not a select agent. GOF experiments involving MERS, and other agents not included on the select agent list, would not be subject to oversight by the SAP (though they could be subject to Federal and institutional biosafety oversight). The SAP is underpinned by a regulatory requirement that applies to non-USG funded (i.e., private sector funded) pathogen research.

Federal and Institutional Oversight of Life Science Dual Use Research of Concern

The U.S. government has issued two Federal policies for the oversight of life sciences DURC. These policies focus oversight on research involving 15 high-consequence pathogens and toxins[35] that involve seven categories of experimental activity, which are projects that can be reasonably anticipated to:

1. Enhance the harmful consequences of the agent or toxin;
2. Disrupt immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification;

[32] Report of the Federal Experts Security Advisory Panel, U.S. Government, December 2014.
[33] Fast Track Action Committee Report: Recommendations on the Select Agent Regulations Based on Broad Stakeholder Engagement, U.S. Government, October 2015.
[34] Lisa Monaco and John Holdren White House Memorandum, October 29, 2015, Next Steps to Enhance Biosafety and Biosecurity in the United States. https://www.whitehouse.gov/sites/default/files/docs/10-2015_biosafety_and_biosecurity_memo.pdf
[35] The agents within the scope of the USG DURC policies are the 13 Tier 1 select agents plus HPAI H5N1 and 1918 influenza virus.

3. Confer to the agent or toxin resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies;
4. Increase the stability, transmissibility, or the ability to disseminate the agent or toxin;
5. Alter the host range or tropism of the agent or toxin;
6. Enhance the susceptibility of a host population to the agent or toxin; or
7. Generate or reconstitute an eradicated or extinct agent or toxin listed above.

Projects involving any of the 15 agents and that could be anticipated to involve any of these seven experimental effects are then determined to be DURC if they then meet the definition of DURC listed in the policy.[36]

The DURC policies outline a coordinated approach to oversight involving the Federal funding agencies and institutions that conduct such research. The policy for Federal oversight, issued in March 2012, requires Federal agencies to review proposed and ongoing research projects to identify any that constitute DURC. The policy for institutional oversight, issued in September 2014, articulates responsibilities of research institutions in identifying and managing DURC. Research institutions are to establish an Institutional Review Entity (IRE) to review research subject to the policy to determine whether any such research involves any of the seven experimental effects, and if so, whether the research constitutes DURC. IREs may review projects not specifically covered under the DURC policies but such additional reviews are voluntary.

When DURC is identified—either by a funding agency or a research institution—the funder and institution are to work collaboratively to develop a risk mitigation plan to help ensure that the research is conducted and communicated in a responsible manner. DURC risk mitigation plans are approved by the Federal funding agency and are reviewed on an annual basis by the funder and the institution. Specific risk mitigation measures may be incorporated into a term of award. Risk mitigation may involve modifying the design or conduct of the research in order to address the same scientific question in a manner that poses fewer biosafety or biosecurity risks. Other measures may involve applying enhanced biosafety or biosecurity measures, evaluating the effectiveness of extant medical countermeasures prior to proceeding with particular studies, or establishing a more frequent schedule of DURC reviews to more closely monitor the research as it evolves. It is also expected that a communication plan is established to ensure that DURC is communicated in a responsible manner. Federal funding agencies can provide advice and guidance on responsible communication, but recommendations on how to communicate research typically are not binding; ultimately, investigators and journal editors decide on how to communicate the research.

[36] The definition of dual use research of concern listed in the USG Policy for Oversight of Life Science DURC (USG, March 2012) and the USG Policy for Institutional Oversight of Life Sciences DURC (USG, September 2014) is "Life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security."

Analysis: Some of the seven experimental effects within the scope of the DURC policies could be considered GOF studies. However, GOF projects that might involve these effects are only subject to DURC oversight if the study involves one of the 15 agents listed in the policy. Only two influenza viruses are listed within the scope of these policies; SARS and MERS coronaviruses are not listed.[37] The DURC policies are also inherently subjective. While the list-based approach clearly delineates projects that are subject to oversight, the definition of DURC, and to a lesser extent, the seven experimental effects, all require significant judgment and interpretation.

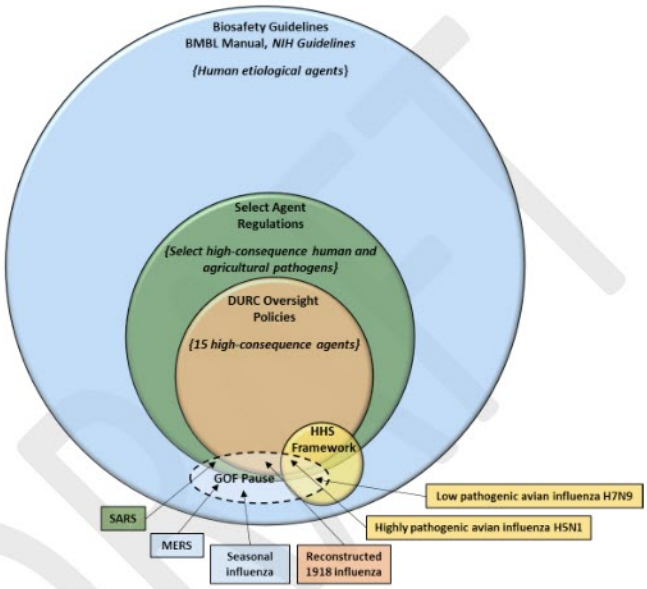

Figure 3. Comparison of the scope of different policies for the oversight of life sciences research involving pathogens. Oversight policies apply to research involving specified agents or procedures. GOF studies involving pathogens or manipulations covered under a given policy would be subject to oversight described by that policy.

Federal-Level Review of Certain Gain-of-Function Studies

The only U.S. Federal policy that specifically addresses GOF studies is the *Framework for Guiding U.S. Department of Health and Human Services Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets (HHS Framework)*, issued by the U.S. Department of Health and

[37] The policy for Federal DURC oversight requires Federal funding agencies to compile biannual inventories of projects identified as being subject to DURC oversight. As part of this process, Federal agencies have been identifying projects involving MERS and LPAI H7N9 influenza and proactively managing risks associated with those projects, as necessary.

Human Services in February, 2013. Under the *HHS Framework*[38,39] certain proposals with the potential for generating highly pathogenic avian influenza H5N1 viruses that are transmissible among mammals by respiratory droplets receive special review and approval before being funded by HHS. This policy was subsequently expanded to include review of similar proposals involving low pathogenic avian influenza H7N9 virus.[40]

Funding agencies within HHS (including NIH, CDC, and FDA) review relevant proposals for risks and benefits, and refer relevant studies to a Department-level review group, the HHS HPAI H5N1 Gain-of-Function Review Group, for advice prior to funding the proposal. The review group includes a wide range of interdisciplinary expertise from across HHS and the Federal government, if necessary. HHS reviews GOF research proposals that are subject to the *HHS Framework* and makes recommendations to HHS funding agencies about whether the study is acceptable for funding and whether additional measures may be needed to mitigate risks. HHS considers a number of factors including the following criteria, which must be met in order for a GOF study to be acceptable to receive HHS funding:

1. The virus anticipated to be generated could be produced through a natural evolutionary process;
2. The research addresses a scientific question with high significance to public health;
3. There are no feasible alternative methods to address the same scientific question in a manner that poses less risk than does the proposed approach;
4. Biosafety risks to laboratory workers and the public can be sufficiently mitigated and managed;
5. Biosecurity risks can be sufficiently mitigated and managed;
6. The research information is anticipated to be broadly shared in order to realize its potential benefits to global health; and
7. The research will be supported through funding mechanisms that facilitate appropriate oversight of the conduct and communication of the research

Analysis: The *HHS Framework* requires an explicit consideration of the risks and benefits associated with certain GOF studies prior to making a funding decision. This allows HHS to identify potential risks up front and make recommendations about risk mitigation—including consideration of alternative approaches or modifying the experimental design—at the outset. This review process also involves broader expertise including, ethical, legal, security, intelligence, and more. The criteria that must be met in order to receive funding are subject to judgment and interpretation. The scope of the *HHS Framework* is quite narrow and currently covers only projects involving two influenza viruses and that involve one specific experimental outcome (mammalian transmission by respiratory droplets); other GOF studies do not receive this pre-funding review.

[38] *A Framework for Guiding U.S. Department of Health and Human Services Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets*, U.S. Department of Health and Human Services, February, 2013. http://www.phe.gov/s3/dualuse/Documents/funding-hpai-h5n1.pdf

[39] Patterson, AP, et. al. A Framework for Decisions about Research with HPAI H5N1 Viruses. Science. 2013 Mar 1: 339(6123): 1036-1037.

[40] Jaffe H., et. al. Extra Oversight for H7N9 Experiments. Science. 2013 August 16: 341(6147):713-714.

Reviews under this framework are conducted by a group internal to the USG. Reviewing GOF studies in a confidential setting allows for the examination of potentially sensitive scientific, proprietary, and personal information, and allows discussions that may be sensitive from a national security or public health preparedness perspective. However, such reviews do not achieve the level of transparency desired by some stakeholders and also make it difficult to independently assess the effectiveness of the review process. Finally, the *HHS Framework* was in place for less than two years when the October 2014 funding pause was enacted and only a handful of GOF projects have been reviewed to date, making it difficult to fully evaluate this policy's strengths and limitations.

In response to the funding pause[41], the National Institute for Allergy and Infectious Diseases (NIAID), within the NIH, developed a process for considering on a case-by-case basis studies that might be subject to the GOF pause. Reviews by NIAID include a detailed consideration of the science, often including a specific examination of the viral strains in question and specific experiments being proposed. NIAID begins by consulting the investigators and an internal NIAID group determines whether the projects are subject to the pause. When identifying projects subject to the funding pause, NIAID has used a fairly broad interpretation of the language set forth in the pause statement and paused, at least initially, more projects than were ultimately determined to meet the scope of the pause policy. NIAID also sought exceptions (using a mechanism provided for in the USG's moratorium statement) for projects that were deemed critical to public health or national security. In determining whether an exception to the pause might be warranted, NIAID considers the intent of the research, the availability of countermeasures, potential alternative approaches, the risks of not conducting the research, and the available mechanisms for ongoing oversight. Exceptions may only granted by the NIH Director.

Analysis: NIAID's process for identifying GOF projects that are subject to the funding pause is rigorous and serves as an example of Federal-level identification and review of GOF studies of potential concern. It includes extensive scientific review and is performed by individuals with experience reviewing projects for DURC potential. It does not involve the same expertise that is provided under *HHS Framework* reviews such as national security, ethics, or legal. Given the limited number of projects that have been examined by NIAID it is difficult to fully evaluate how effective this approach is.

Sharing and Communicating Scientific Findings and Research Products

The majority of life sciences research is conducted in academic settings and the results are communicated openly in scientific journals and public forums. For a small subset of research with national security implications, there are policies in place to restrict access to scientific information or products. Under National Security Decision Directive (NSDD) 189, dissemination of fundamental research is to remain unrestricted to the maximum extent possible and in instances where restriction is

[41] U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses, U.S. Government, October 17, 2014. http://www.phe.gov/s3/dualuse/documents/gain-of-function.pdf

necessary for national security, classification is to be the appropriate mechanism for restricting access.[42] Life sciences research that requires classification is classified at its outset and conducted in designated facilities that are equipped with the infrastructure and personnel with appropriate level national security clearances to perform the research. Retroactively classifying research that was conducted in an unclassified setting is immensely challenging and may be unfeasible.

Export controls are Federal regulations that restrict exports that have national security or foreign policy implications. Certain materials and information related to biological agents and genetic elements, vaccines, equipment, and related technologies are covered by export control regulations. Furthermore, the transfer of controlled information to a foreign national within the United States is considered to be an export to that foreign national's country. The regulations are complex but, in general, they specify which items, when shipped to which destinations, will require export licenses. Life sciences research that is openly published is not subject to export controls, but information that is withheld from publication by the investigator or research institution based on security concerns may become subject to export control regulations, and an export license may be required before that information can be shared with foreign nationals.

Most biological research activities that are subject to export controls fall under the Department of Commerce's Export Administration Regulations, which control items that have both military and civilian applications.[43] However, some might fall under the jurisdiction of the State Department's International Traffic in Arms Regulations.[44]

A number of scientific journals and families of journals have policies for identifying and reviewing manuscripts that raise biosecurity and biosafety concerns. These efforts are commendable but some have noted the challenges associated with trying to identify DURC or implement risk mitigation measures at the publication stage.[45,46] NSABB has previously developed strategies and a risk assessment tool to assist in the development of a responsible communication plan for DURC, which might include altering the content, distribution, or timing of a publication.[47] The U.S. government, in most cases, has no authority to mandate redaction, restriction, or classification of a scientific publication that it does not

[42] NSDD 189 (September 21, 1985) defines fundamental research as "basic and applied research in science and engineering, the results of which ordinarily are published and shared broadly within the scientific community, as distinguished from proprietary research and from industrial development, design, production, and product utilization, the results of which ordinarily are restricted for proprietary or national security reasons." https://research.archives.gov/id/6879779

[43] Export Administration Regulations, 15 CFR Parts 730, 734, 736, 742, 744, and 745. https://www.bis.doc.gov/index.php/regulations/export-administration-regulations-ear

[44] International Traffic and Arms Regulations, 22 U.S.C. 2778 https://www.pmddtc.state.gov/regulations_laws/itar.html

[45] Casadevall A et al. Dual-Use Research of Concern Review at American Society for Microbiology Journals. mBio 6(4):e01236-15. 2015.

[46] Atlas et. al. Journal editors and authors group statement on scientific publication and security. Science, 299:1149. 2003.

[47] Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information. NSABB, June, 2007. http://osp.od.nih.gov/sites/default/files/resources/Framework%20for%20transmittal%20duplex%209-10-07.pdf

own or control, and the development of a mechanism for restricting communication of unclassified information to only those who require access, remain challenging and to date unsuccessful.[48]

Analysis: Once a study has been completed, it is difficult to limit the distribution of or access to the findings, particularly if the study was conducted in an open, academic environment. Oversight of DURC, and in particular GOF studies involving pathogens with pandemic potential, may be most feasible and effective if it occurs 1) upstream (i.e., during the review of proposed studies and before experiments are initiated) and 2) in an ongoing manner while the research is being conducted.

Classification may be an option for certain GOF studies, but this would entail that these studies be conducted in significantly different settings than they are conducted currently. Further, although certain GOF studies have raised concerns about whether they should be published, it is unlikely that such manuscripts would meet the criteria for classification under U.S. government classification authorities. It is conceivable that certain studies should not be undertaken at all or not published because of unanticipated findings. However, it may be very difficult to predict at the proposal stage whether findings of concern might arise during the experiment, and unanticipated findings that raise concern may be unavoidable. Individual investigators or journal editors have, on security grounds, decided to redact certain material from publication, possibly triggering export controls on the redacted material, but in general such a redaction could not be mandated by the U.S. government.

Broader U.S. Biosafety and Biosecurity Efforts

In parallel to the GOF deliberations, the USG has also initiated additional, broader reviews of biosafety and biosecurity policies and procedures following a series of laboratory incidents occurring at federal institutions in 2014 **[REF needed]**. The Holdren-Monoco memorandum[49] called for Federal and non-Federal reviews to provide recommendations to strengthen the biosafety and biosecurity practices and oversight system for USG funded research. The memo outlined three immediate actions for Federal Agencies:

1. Conduct a comprehensive review of current biosafety and biosecurity protocols to ensure adequacy and appropriateness for today's infectious disease research
2. Inventory and document culture collections
3. Increase attentiveness throughout research community to ensure the safety of laboratory workers and the American public.

In September 2015, The White House National Security Council tasked the Federal Experts Security Advisory Panel (FESAP) to 1) identify needs and gaps and make recommendations to optimize biosafety, biosecurity, oversight, and inventory management and control for biological select agents and toxins (BSAT); 2) identify actions and any regulatory changes to improve biosafety and biosecurity; and 3) identify an approach to determine the appropriate number of high-containment U.S. laboratories

[48] Research information produced under a U.S. government grant is not considered to be owned or controlled by the Federal Government. However, under the Invention Secrecy Act, the U.S. government can nevertheless impose secrecy orders on patent applications if the publication or disclosure of the ensuing patent would be detrimental to national security.
[49] https://www.whitehouse.gov/sites/default/files/microsites/ostp/enhancing_biosafety_and_biosecurity_19aug2014_final.pdf

required to possess, use, or transfer BSAT. To obtain broad stakeholder recommendations, the National Science and Technology Council established the Fast Track Action Committee on Select Agent Regulations (FTAC-SAR). In October 2015, USG released the FESAP and FTAC-SAR recommendations[50] that address the culture of responsibility, oversight, outreach and education; applied biosafety research; incident reporting; material accountability; inspection processes; and regulatory changes and guidance to improve biosafety and biosecurity. The USG has developed a plan to implement these recommendations in order to improve biosafety and biosecurity practices along with oversight.[51]

[50] http://www.phe.gov/s3/Documents/fesap.pdf; http://www.phe.gov/s3/Documents/ftac-sar.pdf.
[51] Implementation of Recommendations of the Federal Experts Security Advisory Panel and the Fast Track Action Committee on Select Agent Regulations, October 2015. http://www.phe.gov/s3/Documents/fesap-ftac-ip.pdf

5. Findings

In developing the findings below (Box 2), the NSABB working group considered the results of (i) the risk and benefit assessments, (ii) policy analysis and decision-making frameworks, (iii) discussions of ethics, and (iv) perspectives of domestic and international stakeholders.

NOTE: Box to be updated as Findings are finalized.

Box 2. Summary of Key Findings

Key Finding 1: There are many types of GOF studies and not all of them have the same level of risks. Only a small subset of GOF research—GOF research of concern (GOFROC)—entail risks that are potentially significant enough to warrant additional oversight.

Key Finding 2. The U.S. government has several policy frameworks in place for identifying and managing risks associated with life sciences research. There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOFROC could be applied.

Key Finding 3. Oversight policies vary in scope and applicability, and are not sufficiently harmonized; therefore, current oversight is not sufficient for all GOF studies that raise concern.

Key Finding 4. An adaptive policy approach is a desirable way to ensure that oversight and risk mitigation measures remain commensurate with the risks associated with the research and the benefits of the research are being fully realized.

Key Finding 5. There are life sciences research studies, including possibly some GOFROC, that should not be conducted on ethical or public health grounds if the potential risks associated with the study are not justified by the potential benefits. Decisions about whether GOFROC should be permitted will entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of proposed studies but other considerations, including legal, ethical, and societal values are also important.

Key Finding 6. Managing risks associated with GOFROC, like all life sciences research, requires Federal-level and institutional oversight, awareness and compliance, and a commitment by all stakeholders to safety and security.

Key Finding 1: There are many types of GOF studies and not all of them have the same level of risks. Only a small subset of GOF research—GOF research of concern—entail risks that are potentially significant enough to warrant additional oversight.

As with all life sciences research involving pathogens, GOF studies entail inherent biosafety and biosecurity risks. GOF research involving the generation of pathogens with pandemic potential involves the greatest risks. A laboratory accident involving such a pathogen could potentially release a pathogen that could spread rapidly and efficiently through the human population. A laboratory pathogen with enhanced characteristics could also, if malevolently used, pose a greater threat to national security or public health than similar misuse involving a wild type pathogen. The probability that such events would occur is low but non-zero and the potential consequences are uncertain but potentially significant.

Gryphon's biosafety risk assessment identified studies involving enhanced transmissibility, enhanced pathogenicity, and evasion of immunity as entailing the highest risks for coronaviruses, seasonal influenza, and avian influenza.[52] Manipulations that increase transmissibility, increase pathogenicity, and enable a pathogen to more readily spread through the population have the greatest potential to increase risk; in some strains even a moderate increase might be a concern.

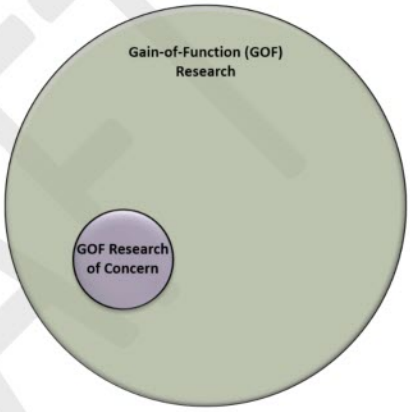

Figure 4. Conceptual categorization of GOF studies involving human or animal pathogens. GOF studies include a broad range of experimental approaches, most of which do not raise significant concerns. GOF studies of concern represent a small subset of all GOF research that can be reasonably anticipated to result in generation of a pathogen with pandemic potential, as described as a pathogen that is likely both highly transmissible and highly virulent in humans.

To help categorize studies based on the level of concern stemming from their associated risks, the working group has designated studies as: GOF research and GOF research of concern (GOFROC) (Figure 4). The term "GOF research" would encompass all studies involving human or animal pathogens whereby some characteristic of the pathogen is enhanced. The vast majority of GOF research does not raise any significant concerns; these studies do not entail novel or significant risks and are subject to layers of oversight to manage risks. GOF research of concern, or GOFROC, represents the small subset of studies that result in the generation of a pathogen with pandemic potential—that is, a pathogen that is highly virulent and highly transmissible, as judged by its likely ability to spread among human populations (see Recommendation 1 for more thorough description of these attributes).

[52] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

Key Finding 2. The U.S. government has several policy frameworks in place for identifying and managing risks associated with life sciences research. There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOF research of concern could be implemented.

Federally-funded life sciences research in the U.S. is conducted in accordance with occupational health and safety laws and regulations, the *NIH Guidelines*, the BMBL, policies for the Federal and institutional oversight of DURC, the Select Agent Regulations, export control regulations, international treaties and agreements, and other relevant policies. HHS has also developed a framework for guiding funding decisions for certain GOF studies involving H5N1 and H7N9 influenza viruses. Together, these policies aim to mitigate biosafety risks, biosecurity risks, and other risks associated with life sciences research, including many of the GOF studies that have raised concerns.

U.S. policies apply oversight and help manage risks at several points throughout the research life cycle including the proposal review, the funding decision, the time during which the research is being conducted, and at the time the research is being communicated. There are also numerous entities that are responsible for providing oversight, managing risks or issuing guidance, including funding agencies, institutional review and compliance committees, individual investigators, federal advisory committees, and journal editors.

While effective implementation of these policy frameworks can manage much of the risk associated with life sciences research, including the risks of some GOFROC, there remains variability in how policies are applied and coverage is incomplete (e.g., GOF research funded and conducted by/within the private sector may not be covered). Institutional oversight also varies. For example, IBCs differ in capabilities and expertise, and institutional resources and cultures vary. In addition, there is limited data describing the rate and extent of laboratory accidents, near-misses, and security breaches. Little comprehensive data about these critical issues exist, and no entity is currently authorized to collect all of what would be desirable.

Key Finding 3. Oversight policies vary in scope and applicability, and are not sufficiently harmonized; therefore, current oversight is not sufficient for all GOF research of concern.

U.S. policies are applicable to some but not all GOFROC. Risks associated with GOFROC that do not involve select agents or pathogens subject to oversight under the USG DURC policies or the *HHS Framework*, would largely be managed at the institutional level, in accordance with guidance in the *NIH Guidelines* and BMBL. In general, GOFROC that is not conducted with U.S. government funds is not subject to oversight by a Federal funding agency.[53] Other countries also fund and conduct life sciences research, including GOF studies, which are beyond the purview of the U.S. government as well.

[53] Research involving a select agent, whose oversight is articulated in Federal statute and requires compliance from all researchers and institutions, would be subject to Federal oversight, regardless of the funding source. Some privately-funded

Further, the U.S. government's oversight policies are not sufficiently harmonized. Different policies are aimed at managing different risks, and each is implemented by various Federal Departments and Agencies. This can result in redundancies as well as gaps in oversight.

In addition, full compliance with policies is essential to their effectiveness. The effectiveness of policies can be enhanced by a commitment to proper implementation and enforcement at the Federal, institutional, and individual investigator levels. This can include training, education, codes of conduct, and other mechanisms that are valuable tools for continuing to build a culture of responsibility among researchers.

Key Finding 4. An adaptive policy approach is a desirable way to ensure that oversight and risk mitigation measures remain commensurate with the risks associated with the research and the benefits of the research are being fully realized.

Many, but not all, of the policies that apply to GOF studies are adaptive in nature. The BMBL is updated periodically. The *NIH Guidelines* and the select agent programs are updated or revised periodically as well and both have processes for seeking external advice for informing policy development. The DURC policies and the *HHS Framework* do not have articulated mechanisms for seeking input on policy development, reviewing, or updating the policies, though both state an intention to be updated as necessary. Great uncertainty was identified with several key parameters effecting GOF risk and benefit assessment, and thereby risk management. An adaptive approach will facilitate refinement of GOF risk management as knowledge and experience is acquired.

Commented [RK([1]: **NOTE** LeDuc – add recommendation/suggestion that DURC and HHS Framework be evaluated and revised periodically somewhere in report

Key Finding 5. There are life sciences research studies, including possibly some GOFROC, that should not be conducted if the potential risks associated with the study are not justified by the potential benefits. Decisions about whether GOFROC should be permitted will entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of proposed studies but other considerations, including legal, ethical, public health, and societal values are also important.

Examples of studies that should not be conducted for ethical reasons include those that: involve human subjects who have not provided consent; are anticipated to cause undue harm to a human subject; or that entail benefits that are unjustifiable in the light of the risks. For example, the development of biological weapons is unethical and has been banned by international treaty.[54]

research being conducted at institutions that receive Federal funding for that research may also be subject to oversight under the *NIH Guidelines*, USG DURC policies, or other policies.

[54] Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction. Signed at London, Moscow and Washington on 10 April 1972; entered into force on 26

There may be GOFROC that should not be funded on ethical grounds but it is difficult to identify or describe such studies based on general or hypothetical descriptions. An ethical evaluation of a research study would entail an evaluation of the risks and benefits, which requires a thorough understanding of the scientific details of the proposal, including its aims and any adverse consequences that could be foreseen. In addition, the scientific, public health, and national security landscape is dynamic. Public health needs change as new diseases emerge. Risks may arise or diminish based on the availability (or lack) of effective countermeasures. Benefits may become more or less likely to be realized based on other enabling factors, such as new scientific findings or technologies. Decisions to fund GOF studies must take into account this anticipated variability in the risk-benefit landscape.

The NSABB did not seek to develop a list of studies that should not be conducted but rather sought to develop general principles that describe what is acceptable and not acceptable for funding. A principle-based approach to guiding funding decisions is adaptable and likely more effective than a list of specific studies that should not be funded.

However, one example of a scientific study that should not be conducted might be the insertion of a virulence gene from an unrelated organism into the genome of a virus transmissible through the respiratory route, which would be highly unlikely to occur by natural recombination. This study, and others that involve the transfer of virulence genes between disparate microbes would appear to lack public health benefit, since the novel, laboratory-generated pathogen is unlikely to arise naturally and would therefore entail potentially significant and unnecessary risks.

Key Finding 6. Managing risks associated with GOFROC, like all life sciences research, requires Federal-level and institutional oversight, awareness and compliance, and a commitment by all stakeholders to safety and security.

Biosafety and biosecurity risks associated with life sciences research are managed through engineering controls, laboratory practices, medical surveillance and support, appropriate training, and other controls. However, GOFROC has the potential to generate strains with significant risks that may require additional oversight and containment mechanisms. Managing the risks associated with GOFROC in particular requires a commitment to safety and security at the Federal and institutional level that includes a strong foundation of training and a commitment to compliance by the research institution, and the individual investigators at the local level..

Key Finding 7. Consideration of the international dimensions associated with funding and conducting GOF research of concern is important. The potential risks and benefits associated with GOFROC are

March 1975. Depositaries: UK, US and Soviet governments. http://www.opbw.org/

international in nature; laboratory accidents or intentional misuse could have global consequences. The relevant benefits for vaccine and other countermeasure development or disease surveillance could also have important international implications. In addition, the research enterprise is international in nature and GOFROC is being conducted in a number of countries already. While U.S. government policy regarding GOFROC will likely only directly affect domestic and international research within the purview of the U.S. government, decisions made by the United States in this area may influence oversight policies globally. Notably, several countries and international scientific organizations have been considering issues related to biosafety, biosecurity, dual use research, and GOFROC **[REFS, or reference section in this paper]**. International perspectives are important to the development of U.S. policy in this area; global engagement and active dialogue is necessary to foster responsible oversight mechanisms and an international culture of responsibility around research involving pathogens. The U.S. government, often in concert with the NSABB, has been engaged with the international community over the years and continues to work with those governments and organizations now actively considering GOFROC-related issues.

6. Recommendations of the NSABB

Based on its analyses, the NSABB has formulated the following recommendations.

NOTE: Box to be updated as Recs finalized

Box 3. Summary of Recommendations of the NSABB

Recommendation 1. Research proposals involving GOFROC entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the Federal and institutional levels.

Recommendation 2. In general, oversight mechanisms for GOF studies of concern should be incorporated into existing policy frameworks. The risks associated with some GOF studies of concern can be identified and adequately managed by existing policy frameworks if those policies are implemented properly. However, the level of oversight provided by existing frameworks varies by pathogen. For some pathogens, existing oversight frameworks are robust and additional oversight mechanisms should generally not be required. For other pathogens, existing oversight frameworks are less robust and may require supplementation. All relevant policies should be implemented appropriately and enhanced when necessary to effectively manage risks.

Recommendation 3. The risk-benefit profile for GOF studies of concern may change over time and should be re-evaluated periodically to ensure that the risks associated with such research is adequately managed and the benefits are being realized.

Recommendation 4. The U.S. government should continue efforts to strengthen biosafety and biosecurity, which will foster a culture of responsibility that will support not only the safe conduct of GOF studies of concern but of all research involving pathogens.

Recommendation 1. Research proposals involving GOFROC entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the Federal and institutional levels.

GOFROC entails the generation of pathogens—perhaps novel pathogens—with anticipated pandemic potential. The risks associated with generating pathogens with pandemic potential are uncertain but potentially significant. It is possible that generating a laboratory pathogen with pandemic potential introduces a risk of a pandemic, albeit a low probability risk, that did not exist before that pathogen was generated. Therefore, a new, pre-funding review and approval mechanism is warranted before such studies should be undertaken. The NSABB working group proposes a conceptual approach for guiding funding decisions about GOFROC. This conceptual approach entails identifying GOFROC and subjecting such studies to an additional pre-funding review and approval process. The attributes describing GOFROC, the principles that should guide funding decisions for GOFROC, and the features of the proposed review process are described below.

Identifying GOF research of concern

Note: The 2 attributes and accompanying language was discussed and approved by WG on 4/7. Minor additional edits are included.

GOFROC is research that can be reasonably anticipated to generate a pathogen with pandemic potential. Determining whether a proposed research project is likely to generate a pathogen with pandemic potential, as described by the attributes below, will entail uncertainty and will require scientific and other expert judgment.

To be considered GOFROC, the research must, in a single step or over the course of manipulations, be reasonably anticipated to generate a pathogen with both of the following attributes:

i. **The pathogen generated is likely highly transmissible and likely capable of wide and uncontrollable spread in human populations.** To be considered "highly transmissible" the pathogen must be judged to have the capacity for sustained secondary transmission among humans, particularly but not exclusively by the respiratory route. Such a determination might be informed by data describing human infections by naturally-circulating isolates of the pathogen or studies in relevant experimental mammalian models that serve as a proxy for human infections. To be considered "capable of wide and uncontrollable spread in human populations" it must be judged that there would be limited options for controlling the spread of the pathogen other than patient isolation or quarantine. Such a determination might be made, for instance, if humans lack population immunity to the resulting pathogen, if the pathogen would evade or suppress the human immune response, if the pathogen would be resistant to

medical countermeasures, or if existing countermeasures would be unavailable globally in sufficient quantities.

AND

ii. **The pathogen generated is likely highly virulent and likely to cause significant morbidity and/or mortality in humans.** To be considered "highly virulent" the pathogen must be judged to have the capacity for causing significant consequences in humans, such as severe disease and/or a high case fatality rate. Such a determination might be informed by data describing human infections by naturally-circulating isolates of the pathogen or studies in relevant experimental mammalian models that serve as a proxy for human disease.

Any study involving the generation of a pathogen exhibiting the two attributes above would be considered GOFROC. However, it is generally anticipated that the following types of activities would not be considered GOFROC:

- Studies to characterize the virulence and transmission properties of circulating pathogens
- Surveillance activities, including sampling and sequencing
- Activities associated with developing and producing vaccines, such as generation of high-growth strains

Importantly, a proposed experiment need not involve the simultaneous enhancement of both phenotypes. For instance, research involving a naturally-occurring pathogen that exhibits one of the above attributes would be considered GOFROC if a study were anticipated to confer the second attribute to the agent (while retaining the first attribute). Other studies may generate a pathogen with the above attributes after a series of manipulations that enhance the phenotypes separately but ultimately result in a pathogen with both attributes. Any route of experimentation that is anticipated to ultimately generate a pathogen that exhibits both of the characteristics above would be considered GOFROC and should be reviewed carefully before it can be funded.

Appendix B describes examples of studies that would and would not be considered GOFROC. These examples are provided as guidance and are described in general terms. A more detailed consideration of the specific pathogen in question as well as the proposed experimental manipulations would be required to determine whether a research proposal is likely to entail GOFROC. The specific nature of a given pathogen or manipulation could alter the determination about whether or not a study constitutes GOFROC.

Pre-funding review and approval of GOF research of concern

Proposals anticipated to involve GOFROC should be subject to additional review prior to making a funding decision and a higher degree of federal oversight throughout the course of the research, if funded. The working group has developed principles that should guide the review and funding of these proposals. There should be a high degree of confidence that a study will be conducted in accordance with these principles before determining whether the proposal is suitable for funding. Studies that cannot be or are not anticipated to a high degree of confidence to be conducted in accordance with the principles below should not be funded.

Principles for guiding review and funding decisions

NOTE: These principles are to be reviewed and finalized by WG on 4/19.

NSABB has developed the principles below to guide funding decisions regarding GOFROC. Only projects that are in line with all of the following principles should be considered acceptable for funding. The principles below are intended to embody the substantive ethical values described in section 4.3 and the process of applying these principles would involve scientific, security, ethical, and other considerations.

i. **The research proposal has been evaluated by a peer-review process, determined to be scientifically meritorious, and has been assessed to be likely to exert a sustained, powerful influence on the research field(s) involved.** If GOFROC is to be funded and conducted it must first and foremost address a valuable scientific question or public health need.

ii. **The pathogen(s) that is anticipated to be generated must be judged, based on scientific evidence, to be able to arise by natural processes.** It is difficult to predict the types of pathogens that can or will emerge in nature. Nevertheless, before a pathogen with pandemic potential is generated through laboratory manipulations it is essential to consider whether such a pathogen could arise in nature. GOFROC may be permissible if the study were to generate a pathogen that is anticipated to arise in nature or if the study were to provide insight into natural evolutionary processes. GOFROC would not be permissible if were to generate a laboratory pathogen that is highly unlikely to arise in nature (e.g., combining virulence factors of two viruses that are highly unlikely to recombine in nature). **NOTE: This is a NEW principle. Are there comments?**

iii. **An assessment of the overall potential risks and benefits associated with the project determines that the potential risks compared to the potential benefits are justified**. Prior to funding GOFROC, the anticipated risks and potential benefits must be carefully considered. In general, the potential benefits associated with a research project should be commensurate with or exceed the presumed risks. Projects involving significant risks and few anticipated benefits are ethically unacceptable and should not be funded. If the potential risks appear high, the possible benefits should also appear high. Risks should be mitigated and managed whenever possible.

Commented [RK([2]: ML suggested – ***A clear, realistic plan for mitigating and managing risks should be included and reviewed as part of the research review process*** (I think this blurs general principles and specific recommendations so did not insert)

iv. **There are no feasible, equally efficacious alternative methods to address the same scientific question in a manner that poses less risk than does the proposed approach.** Alternative approaches must be explored and critically examined before funding GOFROC. It is possible that the proposed experimental approach that raises concern is the only feasible approach for addressing the scientific question at hand. In other cases, modifications of the experimental design, selection of attenuated or other strains that pose fewer risks in humans, or different approaches that may provide the same or very similar information may be feasible. Lines of experimentation that entail less risk should be pursued whenever possible.

v. **The investigator and institution proposing the research have the demonstrated capacity to carry it out safely and securely and the ability to respond rapidly and adequately to laboratory accidents or security breaches.** Prior to funding, the risks associated with proposed GOFROC must be identified and assessed, and clear, realistic plans for managing risks should be developed. In order to manage risks associated with GOFROC, an institution must have adequate resources, security, trained personnel, administrative structures, occupational health and safety procedures, relationships with local public health authorities, and the ability to adapt to unanticipated results by increasing containment or adding safety or security features. In addition to adhering to standards of compliance, an institution (and the investigators proposing the study) should have a demonstrated commitment to laboratory safety and security, scientific integrity, and the responsible conduct of research. The researchers and institution should embody the culture of responsibility as it pertains to safety and security, perhaps demonstrated through adherence to a code of conduct or other voluntary measures.

vi. **The benefits of the research are anticipated to be broadly and legally shared in order to realize its potential benefits to global health.** Prior to funding GOFROC, consideration should be given to the type of research information and products that are likely to be generated. The research information and products are expected to be shared openly and a responsible communication plan should be developed at the outset, if necessary.

vii. **The research will be supported through funding mechanisms that allow for appropriate management of risks and ongoing oversight of all aspects of the research.** GOFROC should be funded through mechanisms that help to ensure that appropriate biocontainment conditions are utilized, adequate biosecurity precautions are in place, and that the data and materials generated will be shared appropriately. The funding mechanism should be flexible to allow for additional risk mitigation measures be required, if needed.

viii. **The proposed research is ethically justifiable.** Determinations about whether proposed GOFROC should be undertaken will involve value judgments to assess the potential risks and benefits and determine whether any potential risks are justified. Non-maleficence, beneficence, justice, respect for persons, scientific freedom, and responsible stewardship are among the values that should be considered when ultimately making decisions about whether to fund GOFROC.

Description of the Review Process for Proposals Involving GOF Research of Concern

NOTE: This section describing the additional review process was discussed on 4/7 and generally supported by the WG; see also new Recommendation 3.2 for proposed role of a FACA or other advisory committee.

The NSABB proposes the following conceptual approach for guiding funding decisions about GOFROC (Figure 5). Review of research projects that may involve GOFROC would involve four steps:

1. Investigators, institutions, and funding agencies identify proposed GOFROC, as described by the two attributes for identifying GOFROC.
2. A Department-level Federal panel with diverse expertise reviews proposals involving GOFROC to determine whether it meets the 8 principles for guiding funding decisions.
3. Funding agencies make a funding decision and establish risk mitigation plans and other conditions if the GOFROC is determined suitable for funding.
4. Investigators and institutions conduct the research in accordance with applicable Federal and local oversight policies and employ any additional mitigation strategies. Federal agencies provide oversight to ensure adherence to established risk mitigation plans and funding terms.

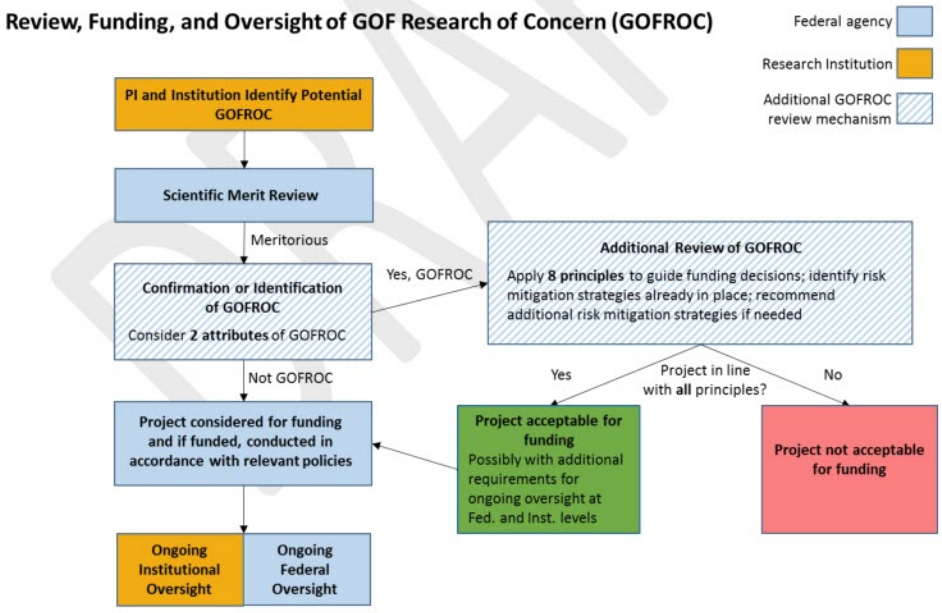

Figure 5. Proposed conceptual approach for guiding funding decisions for GOF research of concern.

Investigators and institutions identify GOFROC. Prior to submission of an application for funds, investigators and research institutions should identify possible GOFROC and submit with the research proposal any relevant information such as biosafety, biosecurity, or local public health response plans for the research, descriptions of facilities available, and a discussion of the value and potential benefits of the proposed research. Identification of possible GOFRC should not affect a subsequent scientific merit review either positively or negatively.

Department-level review of GOFROC. After the standard agency scientific merit review process, proposals that are determined to be scientifically meritorious and likely to be funded would be also reviewed by the funding agency to determine if they constitute GOFROC, as defined by whether the proposal can be anticipated to generate a pathogen with both of the attributes. Prior to being determined acceptable for funding, proposals identified by a funding agency as involving GOFROC would require an additional, higher level, Departmental review. If a proposal does not involve GOFROC, it would proceed along the normal pathway for further evaluation and funding decisions.

The additional review of proposals involving GOFROC would be to determine whether the proposed research aligns with the 8 principles to guide funding decisions. Applying these principles will help to ensure that the GOFROC is scientifically and ethically acceptable, that the risk-benefit balance is favorable, that alternative approaches are explicitly considered, and that the research can be performed safely and securely. It is envisioned that the additional review of proposals involving GOFROC would involve diverse, multidisciplinary expertise including scientific, public health, biosafety, national security and intelligence, legal and bioethics, and other perspectives. To the extent possible, the review process should be efficient, transparent, well-documented, and adaptive. In addition, the process should be structured to avoid real or apparent conflicts of interest and to provide consistency across Federal agencies that might fund GOFROC. It is also envisioned that research institutions proposing the GOFROC would have an opportunity to provide information that would be necessary for a thorough and substantive review of the research proposal.

Funding decision and risk mitigation. During the course of the Department-level review the relevant risk management plans should be critically evaluated and additional risk mitigation measures may be deemed necessary in order for GOFROC to be funded. A satisfactory risk management plan would entail appropriate biocontainment facilities and biosafety practices, appropriate standard operating procedures and administrative controls, occupational health and safety programs and security features aimed at protecting laboratory strains and reagents and promoting personal reliability. Some or all of the additional risk mitigation measures listed in Box 4 may also be required. A variety of additional measures could be required as a condition of funding such as more frequent institutional and Federal reviews of progress, site inspections, prohibition of adding new GOFROC experiments without approval, requirements to report unanticipated results, and/or Federal review of communication plans.

Ongoing oversight. Finally, throughout the course of the funding, both Federal and institutional oversight are critically important and the project should be carefully monitored to ensure that required conditions are met, that the principles guiding the decision to fund are still satisfied, and that any changes, significant developments, and publication/communication plans are discussed and addressed

in a timely manner. Additional ongoing oversight at the Federal and institutional level may be required and should be stipulated at the time of funding.

Recommendation 2. In general, oversight mechanisms for GOF research of concern should be incorporated into existing policy frameworks when possible.

All life science research involving pathogens entails risks; laboratory workers could be infected by a pathogen during the course of their work or a laboratory pathogen could be accidentally or intentionally released into the surrounding environment. There are numerous practices and procedures that are required of researchers and institutions conducting such work to manage these risks. The vast majority of studies do not entail generating pathogens with pandemic potential and as such, the risks associated with most studies are not novel or significantly concerning. Importantly, for risks to be adequately managed, policies must be implemented effectively at the Federal and institutional levels.

Any additional oversight of GOFROC should be built into existing mechanisms rather than having the U.S. government develop a novel regime specific to GOFROC. Adapting or harmonizing current policies is preferable to developing entirely new oversight frameworks or wholly new approaches to manage the risks associated with these studies. There are precedents for additional Federal-level pre-funding review of certain GOF studies (i.e. *HHS Framework*) as well as mechanisms for higher-level review and approval of certain studies (i.e., Major Actions, under the *NIH Guidelines*; restricted experiments, under the Select Agent Program). There are also mechanisms for continual Federal-level monitoring of biosafety and biosecurity risks for individual projects (i.e., USG Policy for Federal Oversight of DURC, select agent programs) and established mechanisms for ongoing institutional oversight (i.e., IREs under the USG Policy for Institutional Oversight of Life Sciences DURC; IBCs under the *NIH Guidelines*). Wherever possible, these mechanisms should be employed to ensure the initial and ongoing oversight of GOFROC.

Importantly, not all GOFROC would necessarily be subject to the entire suite of U.S. oversight policies. For instance, experimental manipulations with pathogens not included in the USG policies for DURC oversight or on the select agent list could still conceivably generate a pathogen with pandemic potential. Additional oversight measures may need to be stipulated at the time of funding for proposals involving potential GOFROC that are not subject to a particular policy that is deemed necessary. For instance, specific, enhanced containment practices may be required or a project may require ongoing monitoring for DURC potential at the Federal and institutional level. Box 4 describes a number of potential risk mitigation measures that may be required for GOFROC that could potentially be implemented by leveraging existing policy frameworks.

Box 4. Potential additional risk mitigation measures to be considered for GOF research of concern.

Potential risk mitigation features that should be considered prior to funding GOFROC might include requirements to:

- Provide additional training to researchers
- Enhance biosafety practices or features, as dictated by the specific strains and proposed manipulations
- Enhance security measures around strains, reagents, notebooks, and personnel
- Treat the research as if subject to the USG DURC policies, if it is not already
- Conduct more frequent institutional biosafety and biosecurity reviews of the research
- Conduct more frequent progress reports and discussions with Federal funding agency staff
- Conduct periodic site inspections/evaluations if not already required
- Identify certain experimental outcomes that would trigger a re-evaluation of the risks and benefits prior to proceeding with a study
- Develop a responsible communication plan, specifically, including a description of biosafety and biosecurity practices
- The institution to be in regular communication with local law enforcement and public health officials
- Conduct bioethics consultations at the local and Federal level throughout the lifecycle of the research
- The investigators to develop and/or adhere to an appropriate code of conduct

Recommendation 3. The U.S. government should pursue an adaptive policy approach to help ensure that oversight remains commensurate with the risks associated with the GOFROC. The risk/benefit profile for GOFROC may change over time and should be re-evaluated periodically to ensure that the risks associated with such research is adequately managed and the benefits are being realized. An adaptive approach to the oversight of GOFROC would entail the continual evaluation of the risks and benefits associated with the research as well as the burdens and effectiveness of the additional proposal review process and ongoing oversight measures. An adaptive approach would allow policymakers to learn from experience and update policies accordingly as the risk/benefit landscape changes. For instance, the risks associated with a study may change if newly developed countermeasures become available or if new information emerges to clarify certain risks or enable certain benefits.

Recommendation 3.1. The U.S. government should consider developing a system to collect and analyze data associated with laboratory safety to inform policy development over time for GOF research of concern. Examining such data would provide a better understanding of the risks, inform future risk assessments, and allow for the refinement of oversight policies over time.

New rec 3.2 was proposed on 4/7 WG call.

Recommendation 3.2. An external advisory body that is designed for transparency and public engagement should be utilized as part of the U.S. government's ongoing evaluation of oversight policies for GOF research of concern. An external advisory mechanism, such as a Federal advisory committee, would allow for an independent examination of the U.S. government's policies for reviewing, funding, and conducting GOFROC. Such a mechanism could review GOFROC funding decisions to understand how such decisions were made, identify challenges to implementing the policy, and recommend changes, if needed, that may improve the process. Importantly, this mechanism would also provide transparency and promote public engagement, and would facilitate continued dialogue about GOFROC. The NSABB is one such body that is well-suited to address this task.

Recommendation 4. The U.S. government should pursue ways to ensure that all GOF research of concern conducted within the U.S. or by U.S. companies be subject to oversight, regardless of funding source. GOFROC that is funded by the U.S. government or through private funding sources should be subject to equivalent oversight to ensure that the associated risks are adequately managed. The U.S. government should consider providing oversight not only as a term and condition of a funding award but also via other mechanisms that would enable oversight of all relevant research activities, regardless of the funding source.

Recommendation 5. The U.S. government should undertake broad efforts to strengthen laboratory biosafety and biosecurity and, as part of these efforts, seek to raise awareness about the specific issues associated with GOF research of concern. Current discussions about GOFROC are related to broader domestic and international discussions about laboratory safety and security. A "Top Down" approach to managing the risks associated with GOFROC through Federal policies and oversight is appropriate. However, top-down approaches alone, in the form of Federal and/or institutional leadership, will likely not be sufficient to fully address the associated risks. It is also critical to have adequately trained personnel that values safe and secure laboratory environments for conducting GOFROC. Therefore, it will also be important to facilitate a "Bottom Up" approach whereby scientific and institutional leaders, as well as research staff involved in the design and conduct of GOFROC, are educated about biosafety, biosecurity, and the responsible conduct of their research. The U.S. government should engage the research community with the goal of promoting a culture of responsibility, or "citizenship," whereby all participants in the research enterprise have a sense of shared responsibility for its continued beneficial contribution. Such a culture would value safety,

security, and compliance, and work to promote public trust in the scientific enterprise. For GOFROC a combination of voluntary and mandated oversight and risk mitigation measures would be beneficial.

Recommendation 5.1. The U.S. government should specifically develop a "Points to Consider" document to provide guidance to investigators and institutions when preparing research proposals that may involve GOFROC. Such a document would describe to investigators any new requirements for proposals involving GOFROC and provide guidance on the type of information that should be included in a proposal to facilitate its review. This document should be reviewed and updated as necessary.

Recommendation 6. The U.S. government should engage the international community in a dialogue about the oversight and responsible conduct of GOFROC. Life sciences research is a global endeavor that continues to grow as more countries invest in their research capacities and as scientists move and collaborate across national boundaries. Life sciences research enables biomedical breakthroughs, pandemic preparedness, public health response efforts for emerging infectious diseases, and also provides an important economic driver. As more investigators undertake research involving pathogens, however, the associated risks become more likely to have international implications. The risks associated with GOFROC are especially international in nature since laboratory accidents or the deliberate misuse of pathogens with pandemic potential could have global consequences. Laboratories anywhere can undertake GOFROC and publications in the open scientific literature can enable others to generate pathogens with pandemic potential.

In addition, the U.S. government should engage the international community on biosafety and biosecurity issues, including those related to dual use research and issues specifically associated with GOFROC. The U.S. government should share information on its relevant policy efforts, particularly as they relate to GOFROC. International engagement efforts should seek to promote a global scientific culture of responsibility and enhance the quality, legitimacy, and effectiveness of oversight processes.

The U.S. government should build these efforts on the substantial international engagement activities that it and the NSABB have carried out since the NSABB was established. Such efforts have included three international roundtable meetings on dual use research issues, a series of webinars focusing on different global regions, and an international consultative workshop on GOF issues[55]. In addition, the U.S. National Academy of Sciences and the European Academies Science Advisory Council have been engaged in the recent policy debates involving GOF studies and may be well positioned to continue the international dialogue on the issue in coordination with national governments and relevant international organizations.

[55] Information about these meetings and activities, including agendas, summaries, and archived videocasts, can be found on the NSABB website at: http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/nsabb/nsabb-meetings-and-conferences/international-engagement

7. Appendices

Note: All appendices are being reviewed and updated.

Appendix A. Detailed Description of NSABB Deliberations

NSABB Deliberations

The NSABB established two working groups to accomplish the two portions of its charge, which were to result in discrete work products.

- **Deliverable 1.** A report conveying NSABB's advice on the design, development, and conduct of the risk and benefit assessments.
- **Deliverable 2.** A report conveying NSABB's formal recommendations on the conceptual approach to the evaluation of proposed GOF studies.

DELIVERABLE 1: ADVISING ON THE RISK AND BENEFIT ASSESSMENTS

The first NSABB working group was tasked with advising on the design and conduct of the risk and benefit assessments. The group met between December 2014 and April 2015 and consisted of 13 NSABB voting members as well as non-voting *ex officio* members and other *ad hoc* members from Federal agencies. (Appendix A). The group convened by telephone conference calls and held a one-day in-person meeting.

The working group developed a draft *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*, which was presented to the full NSABB, which was developed further based on input from all Board members, and ultimately approved by the full Board on May 5, 2015. The recommendations in this framework were intended to inform the NIH as it guided the work of Gryphon Scientific in its risk and benefit assessments. The aim of the NSABB's framework was to help generate risk and benefit assessments that would provide information that would allow the NSABB to make sound, evidence-based recommendations.

The NSABB's framework describes: principles that should underpin the risk and benefit assessments; pathogens, pathogen characteristics, and types of GOF experiments and phenotypes that should be examined; the types of risks and benefits that should be analyzed; scenarios, conditions, and events to be examined; and approaches and methods that should be considered when analyzing risks and benefits. In order for the risk and benefit assessments to be grounded in scientific data and evidence, the assessments needed to focus on specific pathogens, experimental manipulations, and scenarios whose risks and benefits could be modeled and analyzed. The NSABB recommended that the risk and benefit assessments focus on studies involving influenza viruses (seasonal strains, as well as high and low pathogenic avian strains) and SARS and MERS coronaviruses. Given that most pandemics are associated with respiratory transmission, pathogens capable of airborne transmission were considered

to be of most acute concern. NSABB recognized that the risk and benefit assessments would provide information specific to the pathogens and scenarios that were examined, but intended that the assessment would generate information that could be more broadly interpreted and applied. Thus, NSABB's recommended approach to the risk and benefit assessments was intended to align with the USG's October 2014 statement, which states that while "gain-of-function studies that fall within the scope of research subject to the funding pause will be a starting point for deliberations, the suitability of other types of gain-of-function studies will be discussed."

DELIVERABLE 2: RECOMMENDATIONS ON A CONCEPTUAL APPROACH FOR EVALUATING PROPOSED GOF STUDIES

The second NSABB working group was tasked with developing draft recommendations on the conceptual approach for the evaluation of proposed GOF studies. The group met beginning in June 2015 and remains active the time of this writing. The working group consists of 18 NSABB voting members as well as non-voting *ex officio* members and other *ad hoc* members from Federal agencies. (Appendix A). The group convened by telephone conference calls and met twice in person.

In addition to the working group's primary task of developing draft recommendations, it continued to provide input on the conduct of the risk and benefit assessments. The working group also received periodic status updates on the risk and benefit assessments from NIH and Gryphon, as well as reports on the commissioned ethics analysis by Dr. Michael Selgelid, examined draft work products, and reported back to the full NSABB.

In developing draft recommendations on a conceptual framework for evaluating proposed GOF studies, the working group structured its deliberations into three phases.

Phase I. Policy examination, research, and information gathering
Phase II. Interpretation, analysis, and synthesis of information and results
Phase III. Development of recommendations

In Phase I the working group sought to 1) identify and examine the information necessary to inform development of recommendations and 2) begin to identify principles that should guide the development of NSABB recommendations. The working group began its deliberations by considering the topic areas discussed at the NSABB meeting in May 2015, which included examination of relevant U.S. and international policy and consideration of broader perspectives such as those from funding agencies, national security experts, journal editors and scientific publishers, ethicists, and others. The working group held an in-person meeting to consult with experts on many of these topics. The working group also examined a number of published GOF studies and discussed how current policies might apply to such studies to provide oversight and risk mitigation.

During Phase II the working group focused on translating information about risks and benefits as well as ethics into decisions and recommendations. It examined how current policies apply to GOF studies and began to develop preliminary observations and findings. The working group discussed the ethical issues associated with funding and conducting GOF studies, particularly noting the values and ethical decision-frameworks that might be applied to policy decisions about GOF studies. The working group also developed analytic tools to assist it in systematically analyzing the results of the risk and benefit assessments. In November 2015, the working group began receiving briefings from Gryphon Scientific conveying the results of the risk and benefit assessments, as well as reports on ethics from Dr. Selgelid. The group sought to identify GOF studies that might raise particular concerns and may require additional oversight or consideration prior to being funded.

In Phase III, the working group developed its draft recommendations, based on its analysis of the risk and benefit assessments and the ethics report and consideration of all other information and perspectives that were examined.

Deliberations by the Full NSABB

The full NSABB convened times 5 times between October 2014 and January 2016. At these meetings the NSABB working groups provided progress updates and the full Board deliberated the issues further, consulted with various experts, and sought public feedback. Public comments made at NSABB meetings and delivered to the NSABB in writing were carefully considered by the Board during its deliberations. The articles, resources, and stakeholders consulted by the NSABB and its working groups throughout this process are listed in Appendix D.

On November 25, 2014, NSABB voted to approve a statement conveying to the USG concerns it heard regarding the implementation of the funding pause for certain GOF studies.[56] On May 5, 2015, NSABB voted to approve its *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*.[57] This working paper was shared for discussion by the full NSABB on January 7 & 8, 2016.

Role of the National Academies in the Deliberative Process

The National Academies play a critical role in the ongoing deliberative process. The National Research Council and the Institute of Medicine (now National Academy of Medicine) have been asked to convene two forums to engage the life sciences community and to solicit feedback from scientists, the public, and

[56] Statement of the National Science Advisory Board for Biosecurity Regarding the USG Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses. National Science Advisory Board for Biosecurity, November 25, 2014. http://osp.od.nih.gov/sites/default/files/resources/Final%20NSABB%20Funding%20Pause%20Statement_12-12-14_0.pdf

[57] http://osp.od.nih.gov/sites/default/files/resources/NSABB_Framework_for_Risk_and_Benefit_Assessments_of_GOF_Research-APPROVED.pdf

other stakeholders. These forums are to involve discussion of principles important for the design of risk and benefit assessments of GOF research and of NSABB draft recommendations.

The first National Academies workshop was held on December 15 & 16, 2014 and focused on the potential risks and benefits associated with GOF studies, ways to assess risks and benefits, strengths and limitations of risk-benefit analyses, and the ethical and policy implications associated with funding and conducting GOF studies that have raised concerns.[58] The discussions at this meeting directly informed the development of NSABB recommendations for conducting the risk and benefit assessments and its subsequent deliberations. In particular, the discussions about the potential risks and benefits associated with GOF studies informed NSABB's recommendations for the types of risks and benefits that should be analyzed by Gryphon Scientific. A common theme at this National Academies meeting was also that the term "gain-of-function" is too broad and that in fact, only a subset of GOF studies truly raise concerns. NSABB applied this insight in its subsequent analysis of the risk and benefit assessments by seeking to identify the subset of GOF studies that raised significant or unique concerns. Finally, the legal and policy discussions that were initiated at this meeting prompted to the NSABB to explore these topics, as well as ethical issues, further.

The second National Academies meeting was held on March 10 & 11, 2016 and included a discussion of the completed risk and benefit assessments and NSABB's preliminary findings and draft recommendations. **NOTE: This is being expanded slightly to reflect discussion from NAS.**

The Risk and Benefit Assessments of GOF Studies

NIH commissioned Gryphon Scientific to perform a formal risk and benefit assessments to provide the NSABB with qualitative and quantitative information about the risks and benefits associated with conducting certain GOF studies. Dr. Rocco Casagrande, the principal investigator for the study, presented to the NSABB on May 5, 2015 an overview of Gryphon's approach to conducting the risk and benefit assessments, which included a quantitative biosafety risk assessment, a semi-quantitative biosecurity risk assessment, and a qualitative benefit assessment. Prior to voting to finalize its *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*, NSABB discussed with Dr. Casagrande its draft recommendations and how Gryphon's proposed approach aligned with NSABB's proposed recommendations. In June 2015, Dr. Casagrande presented and discussed a more detailed work plan with the NSABB working group. Over the course of the study, the NSABB working group received occasional progress reports from Gryphon and NIH staff, and were provided draft sections of the risk and benefit assessments. In November 2015 the NSABB working group began receiving the results of the completed risk and benefit assessments. Gryphon's final draft report was posted in advance of the NSABB meeting in January, 2016.[59]

[58] Potential Risks and Benefits of Gain-of-Function Research: Summary of a Workshop. National Research Council and the Institute of Medicine of the National Academies. The National Academies Press, Washington D.C., 2015. www.nap.edu.
[59] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

The NIH Office of Science Policy managed the contract with Gryphon Scientific. NIH staff met weekly with Gryphon to accomplish the goals of the Statement of Work and to ensure the recommendations provided in the NSABB's *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research* continued to inform the conduct of the risk and benefit assessments, as appropriate. NIH staff also consulted with NSABB *Ex officio* members to get broader expertise and advice, and to help ensure that the risk and benefit assessments would yield information that would inform subsequent policy deliberations by the U.S. government.

Considering Ethical Issues Associated with GOF Studies

To guide the NSABB's evaluation of the risks and benefits associated with GOF studies and its development of recommendations, the Board sought additional ethical input and analysis. NIH commissioned Dr. Michael Selgelid, Monash University, to examine the literature regarding the ethical issues associated with funding and conducting GOF research and to explore different ethical frameworks that might be utilized when considering how to evaluate the potential risk and benefits associated with GOF studies. Dr. Selgelid was also asked to provide an ethical decision-making framework that NSABB could consider using when analyzing the information provided in the risk and benefit assessments of GOF studies. The decision framework was to identify and consider ethical values that may not be fully captured by a risk-benefit analysis. Dr. Selgelid's analysis was to be accomplished in a neutral, objective manner, without making any definitive recommendations on whether and how to fund or conduct certain GOF studies or what policy course might be the most appropriate. Dr. Selegelid presented his initial work to the NSABB in September 2015 and delivered to the NIH a draft paper in December 2015, which was conveyed to the NSABB working group and posted in advance of the NSABB meeting in January, 2016. [60]

[60] Selgelid, Michael. Gain-of-Function Research: Ethical Analysis. December 7, 2015. http://osp.od.nih.gov/sites/default/files/GOF%20%20White%20Paper%20by%20Michael%20Selgelid_0.pdf

Appendix B. Examples of Studies that would and would not be expected to entail GOFROC

THIS TABLE IS BEING UPDATED TO USE CONSISTENT LANGUAGE WITH THE LANGUAGE LISTED IN THE GOFROC ATTRIBUTES.

Examples of studies that would and would not be expected to entail GOFROC	
Experiment that is anticipated to entail GOFROC and therefore require additional pre-funding review and approval	**Rationale**
An experiment that is anticipated to generate avian influenza viruses that are transmissible by the respiratory route in mammals if the starting virus is virulent in humans.	**Attribute 1.** The experiment is anticipated to increase transmissibility by the respiratory route in a relevant experimental mammalian model. Further, altering the host range from birds to mammals could generate a virus for which there is no existing population immunity in humans, therefore resulting in a virus capable of wide and potentially uncontrollable spread among humans. **Attribute 2.** Since the starting virus is highly virulent in humans it can be reasonably anticipated that the resulting virus will remain virulent in humans
Reassortant studies involving avian and human influenza virus strains to identify reassortants with pandemic potential that could arise naturally.	**Attribute 1 and attribute 2.** One goal of the experiment is to identify/select for reassortants that are potentially highly transmissible and highly virulent in mammals **Attribute 3.** Since the resulting viruses are reassortants between bird and human influenza viruses, it can be anticipated that the antigenicity of at least some resulting viruses will remain avian-specific such that human populations would not be expected to have been exposed to such a strain or have pre-existing immunity. Therefore resulting in a virus that could spread more efficiently among humans than the initial virus.
Studies utilizing a strain of SARS-CoV, or some other emerging human respiratory pathogen, which will be modified in ways that can be anticipated to render humans more susceptible to infection by for instance, introducing resistance to a countermeasure (were countermeasures available). [NOTE: this example will be replace with bacterial resp. pathogen]	**Attribute 1 and attribute 2.** The starting virus is both highly transmissible and highly virulent in human **Attribute 3.** Introducing resistance to a countermeasure in a respiratory virus that is highly transmissible and highly virulent could generate a virus that could spread more efficiently among humans than the initial virus with limited options for control.

NOT anticipated to entail GOFROC and therefore not require additional pre-funding review and approval	**Rationale**
Studies aimed at generating a mouse-adapted MERS-CoV or other emerging human respiratory pathogen,	**Attribute 1.** The starting virus is transmissible by the respiratory route in humans **Attribute 2.** The experiment will increase the virulence of the human pathogen in mice, resulting in a potentially highly virulent virus in mammals **Not attribute 3.** The experiment is not expected to generate a pathogen with this attribute. The starting virus is already transmissible and pathogenic in humans and adapting it to mice would not be expected to result in a virus to which humans are more susceptible than the naturally-circulating virus. In fact, the mouse-adapted strain is likely to be less virulent in humans.
Studies enhancing the growth of seasonal influenza viruses, which may be performed during vaccine production	**Attribute 1.** The starting seasonal influenza virus is highly transmissible by the respiratory route in humans **Possibly attribute 2.** Increasing the virus's ability to replicate could potentially result in its increased ability to cause disease, therefore, could result in highly virulent strains. **Note:** If this experiment were to involve an attenuated strain, as is often the case when involving vaccine production, it would be unlikely to result in a virus with this attribute **Not attribute 3.** The experiment is not expected to generate a pathogen with this attribute. The starting virus is already transmissible and the study does not propose introducing resistance to countermeasures or other manipulations that would render humans more susceptible than the naturally-circulating seasonal strains
Antigenic drift studies whereby seasonal influenza viruses that are no longer neutralized by vaccine-induced immunity are generated and selected for in the laboratory.	**Attribute 1.** The starting seasonal or pandemic influenza virus is highly transmissible by the respiratory route in humans **Not attribute 2.** While it would depend on the specific initial strain in use, it is unlikely that the starting virus would be highly virulent in humans nor would the experimental manipulation be anticipated to increase the virulence **Not attribute 3.** Antigenic drift studies generate influenza viruses with some resistance to a specific immunization but they do not change the antigenic character of the virus such that the virus would be unrecognizable by the human immune system. Given that the starting virus is a human virus—not a virus that naturally infects birds or other non-human hosts—humans would likely have some pre-existing immunity to the resulting strains.

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Appendix C. Summaries of Stakeholder Perspectives

The NSABB consulted a wide range of experts and stakeholder groups including not only scientists and institutions that fund and conduct life sciences research, but a much larger and diverse array of groups including public health officials, medical practitioners, emergency responders, vaccine developers, scientific journals, as well as the general public, non-governmental organizations, individuals with international perspectives and others. To accomplish this, NSABB provided a variety of opportunities for interested groups and individuals to express their views and contribute throughout the deliberative process in ways that have informed the NSABB deliberations. These include: several public full NSABB advisory committee meetings with sessions dedicated to obtaining public comment, two public symposia hosted by the National Academies that obtained comments from the public at the meetings and online, as well as comments submitted to the NIH/OSP and NSABB by email, and discussions with subject matter experts during NSABB WG conference calls and in-person meetings. Also included below are views expressed in some of the articles that have been published on this topic. A complete list of the individuals consulted and articles examined by NSABB are listed in Appendix D. Note that Gryphon Scientific also conducted extensive consultations with experts as part of their risk and benefit assessments. Those experts are not listed here but a listing is available in Gryphon's report. [61]

The following is a synthesis of stakeholder ideas and opinions expressed during the deliberative process. Many of these points were conveyed in more than one venue and by more than one person or group.

Scientists and Others Favoring GOF Research

A variety of influenza and coronavirus researchers who conduct GOF research, and other life sciences researchers have stated that GOF studies are widely used and fundamental for understanding viruses, and therefore are crucial to undertake. This group generally favors conducting such research because it aims to benefit society. In their view, such research can be safely conducted under current oversight frameworks and further restrictions will impede valuable work that will lead to important scientific information about these viruses, leading to better drugs and vaccines, as well as to improving the specificity of surveillance, particularly for influenza. In addition, some GOF studies are viewed as essential, specifically those that alter host range or enhance pathogenicity in order to develop animal models of disease (for example, with SARS-CoV) or GOF studies that generate drug or countermeasure resistance, which are important in satisfying various FDA requirements for marketing approval. Those who support GOF studies also point out that such studies are needed for predicting what amino acid changes are important for human transmission and therefore are important for the selection of candidate vaccine viruses. They also argue that GOF studies are important for prioritizing viruses for risk management (surveillance) and that further work will make these applications more robust. The risks

[61] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

associated with not doing GOF research (generally due to a lack of preparedness for natural public health threats) must also be considered.

While acknowledging there are risks associated with GOF research, proponents believe those risks are manageable and have been overstated by some, as evidenced by the fact that laboratory acquired infections are rare and infections in the community as a result of releases from a laboratory are almost unknown. While risk cannot be zero, the work can be conducted safely and securely with appropriate risk mitigation including containment along with good training and with the implementation of robust occupational medicine programs. Alternatives to GOF do not always provide the full answer to key questions and may yield misinformation. Supporters of GOF studies have also expressed concerns about the effects of the current funding pause and possible additional oversight on the field of virology and young researchers, and feel that there are costs of not undertaking the work in question. A major need is for better definition of what is meant by GOF with a clear distinction between GOF studies and GOF studies of concern. Some have suggested that only viruses with increased transmissibility and pathogenicity represent risks that exceed those of other infectious diseases research. They have also noted that SARS and MERS viruses are different from influenza, and require a different risk assessment approach since they are already virulent human pathogens; GOF research is needed to develop animal models that will benefit development of countermeasures for coronaviruses. Some supporters have acknowledged that there may be some experiments that should not be done. Finally, proponents of GOF research have stated that the risks from naturally occurring influenza viruses, which they argue could be reduced through GOF work, are greater than risks from performing GOF studies.

Scientists and Others Critical of GOF Studies

Opponents and critics of GOF research have generally focused their concern on a subset of GOF studies—those that involve enhancing the pathogenicity and/or transmissibility in mammals (particularly by the respiratory route), which may result in the generation of novel pathogens with pandemic potential. Critics have argued that the generation of novel laboratory pathogens with pandemic potential poses major public health risks and some have argued such studies should not be conducted. They have presented and published calculations that suggest a high probability of global outbreaks of influenza that might kill hundreds of millions of people, as a result of the release from a laboratory of a novel GOF virus. There is some disagreement about these estimates and how likely a pandemic might be, but opponents generally argue that even a relatively low probability of a potentially massive outbreak with major consequences is unacceptable. Some critics of GOF studies have acknowledged that there are a number of GOF studies that can and should be conducted.

Opponents of certain GOF studies have also argued that the benefits of GOF studies have been overstated, or are questionable, and that the benefits generally do not outweigh the biosafety risks. They also question claims about the effectiveness of risk mitigation strategies, since human factors and human error are unavoidable and hard to control, and institutional compliance and competence may vary. Critics have disputed the value of GOF studies to surveillance stating that it is not possible to predict phenotype from genotype; therefore predicting the pandemic risk of newly emergent strains is

not achievable given the current state of knowledge. Also, in their view, controlling outbreaks doesn't require GOF research.

Opponents of GOF research tend to favor alternative types of research that, in their view, can provide the same public health benefits without the large risks. It was suggested that the approach should be on reducing the risk by reducing the hazard, as opposed to focusing on mitigation of the risk. For example, if a universal influenza vaccine was developed, the need for many GOF experiments would be eliminated. Critics want to see funds currently used for GOF work provided to other types of research, which would be a better use of scarce resources in their view. Overall, they view preventing major public health problems as paramount, and see a need to define a critical set of experiments that should not be done, or only be done with additional strong oversight. Opponents are also concerned about proliferation and other factors that may lead to misuse and biosecurity threats. Finally, opponents have pointed out a moral issue if risks and benefits of certain GOF studies are not fairly distributed globally.

Funding Agencies

Public and private funding agencies support GOF research that has raised concerns with the goal of improving public health and well-being. These organizations in the US and abroad are aware of the issues surrounding DURC/GOF studies and are working diligently to implement and comply with existing policies in their countries. Most funders have requirements and procedures in place as they apply policies and guidance to evaluate proposed work and to oversee funded work. Current approaches involve education and awareness campaigns, project risk evaluation, ethics reviews, development of risk mitigation plans, and post-award monitoring. Funders believe they can contribute to the GOF deliberative process as a result of their practical, on-the-ground experience with DURC and GOF. They are concerned that interpreting policy can be very challenging, since it requires considerable expertise and judgment. They would welcome workable policies with clear guidance and have noted some unintended consequences of the funding pause, which affected some GOF projects that had not raised particular concerns. Some foreign government funders view government funding as a poor control point because this does not cover privately funded research and research funded by other entities. National regulations, compliance, training, awareness-raising, and self-monitoring have been noted as important.

Biosecurity Experts and Others Concerned about National Security

The ultimate goal of national security professionals, as it pertains to life sciences research, is to protect public health from natural or man-made health threats. Those concerned with national security aim to prevent terrorists and others with malicious intent or misguided motives from using products or information from GOF research to cause harm. This may include deliberate release of pathogens into the community, targeting of researchers or research facilities, or interference with on-going research activities. GOF research represents biosecurity risks in addition to biosafety risks; these overlap but are different with regard to important legal, policy and regulatory issues. Managing biosafety risks may or may not also manage biosecurity risks; GOF policy must take both types of risk into account.

When trying to assess biosecurity threats, security professionals have noted the importance of avoiding assumptions and predictions about the motives and capabilities of those who might be planning biosecurity actions. Those in the security field gather a large variety of data, but often their information is imprecise and may require consideration of what is feasible and plausible. Because of the paucity of biosecurity events, it is very difficult to evaluate and predict the likelihood and consequences of a deliberate release or determine how to prevent and/or mitigate one, and different experts view this issue very differently. It was stated that research policy in itself is not be the appropriate solution to prevent specific biological threats but specific research policies could help raise awareness of security issues among researchers, which would be important.

Security and intelligence professionals have described the challenges associated with using classification as a potential risk mitigation strategy. Classification would effectively restrict access to sensitive research information and research products and would limit the number of laboratories able to perform the studies. This could be described as both a strength and a limitation, depending on one's perspective. Life sciences research that requires classification is typically classified at the outset; the retroactive classification of research that had been conducted in an open, academic setting is exceedingly difficult.

Scientific and Medical Journals

Scientific and medical journals have been at the forefront of the GOF issue. While several have in place procedures in place for identifying DURC, including GOF and other biosecurity concerns in submitted manuscripts, many journal editors are not entirely comfortable with their role. Their mission is to transmit scientific information, not control it, and they may not have the security expertise or the access to such expertise to make the necessary judgments and decisions about risks associated with communicating certain research findings. Rejection and redaction are the major tools journals have to control dissemination of dual use information, and neither may actually address the concerns; they are also impractical to implement effectively. One suggestion voiced was to require that a description of the steps that were taken during conduct of the research to ensure safety be included in all manuscripts. Some journal editors and staff expressed a desire to get help in evaluating risks and mitigation strategies from an independent national group such as the NSABB and to involve them earlier in the overall process. Most think the publication stage is not the best point to exercise control or prevent misuse of data from GOF studies but realize they are the final gatekeepers. Earlier identification of DURC/GOF along with risk mitigation earlier in the research life cycle would reduce the burden on them. Also, new technology and novel publication venues make controlling information increasingly difficult, and, as noted above, not all journals are able to or choose to impose a rigorous review of manuscripts.

Countermeasure Developers

Companies and others that are attempting to develop vaccines and drugs against pathogens were represented in several discussions. Medical countermeasure (MCM) developers expressed quite divergent views and opinions. Those favoring GOF research argued that such work is absolutely necessary for antiviral drug development because GOF experiments to select for drug resistant mutants

as well as to develop animal models are part of the critical path to marketing approval. In their view, GOF studies also have had a major influence on developing influenza vaccines, both seasonal and pandemic, and are likely to result in improved ways to make even better vaccines in the future. GOF experiments are required for selection of strains with better growth properties, with key mutations that alter important phenotypes needed in the vaccine strain, and with incorporating characteristics of strains that are likely to emerge into proven backbones. It was noted that GOF studies that enhance virulence can help inform vaccine designers about which mutations to avoid incorporating into vaccine strains. This group is concerned that their efforts to improve public health may be limited or impeded by new policies and urge careful consideration of their needs as decisions are made.

Conversely, other MCM developers expressed the view that vaccine production now is little dependent on GOF research and that any possible benefits will be far into the future, although some feel long-term potential is there. Those who criticize GOF studies on these grounds have argued that vaccines are developed in response to strains that emerge as threats, rather than preemptively based on strains that might be predicted as threats. Rather than supporting GOF studies to enhance vaccine production and drug development, it has been suggested that the other constraints that impede MCM development be addressed, such as streamlining FDA approval procedures and improving manufacturing processes, which would have a much greater impact. These critics suggest limiting current GOF-related efforts and focusing attention and resources in other directions. Overall, they believe that impact of GOF research on vaccine and drug development has been overstated, and that the benefits articulated are more theoretical than practical.

The General Public and Those who Represent their Views.

A number of stakeholders stressed the importance of having meaningful public engagement with input and participation as part of the deliberative process. It is important that communities that might be affected by accidents or the misuse of research have a say in the research that is being conducted, however, but this may not generally be the case in their view. Real transparency, with the public good as the foremost consideration, must be part of a truly independent decision-making process. They note that it is important to maintain public trust in the scientific enterprise by involving non-scientists at stages when their views can still have an impact on policy-making. Public opinion of science is harmed when decisions that influence public health and safety are made without such input or the input has no real impact. Conversely, effective community engagement can convert sceptics to supporters. More than one participant raised the concern that if risks and benefits are not equitably distributed, it is a serious ethical issue[62].

Other issues that were mentioned include: how harms will be compensated if a laboratory incident were to affect the surrounding community; the need for enough resources to conduct research safely; and the opportunity to learn from other industries such as nuclear industry.

[62] The ethical issues are discussed in more depth elsewhere, notably, Dr. Michael Selgelid's ethical analysis and the section of this report on Ethical Values and Decision-Making Frameworks.

Research Institutions

Representatives of universities and other research institutions generally noted that there is already significant oversight of DURC and GOF at both the Federal and institutional levels. Biosafety professionals noted that potentially high risk projects would receive thorough scientific review and risk assessment, resulting in the development of risk mitigation plans, and on-going monitoring as a result of policies and requirements that are already in place. They cited concerns over any increase in compliance that would impose burdens on their already-limited resources or impede researchers from doing valuable work. They have difficulty, at times, deciding what is DURC when reviewing specific projects and would welcome more specificity and guidance. Many emphasized the need for policies that are unambiguous and straightforward to implement.

Public Health Officials

Public health officials feel they have lot to contribute to the GOF debate, but some feel they are often left out or brought in too late. Some believe that GOF research has and can continue to improve surveillance efforts, as well as vaccine and therapeutic development. Others expressed concerns that an accident involving a laboratory pathogen for which there are no countermeasures would be very concerning and difficult to respond to. At the local level it is important to have public health involvement in the decision-making process, because they will be incident responders. Any policy developed must be flexible enough to allow for emergency response. Strong connections with state and local laboratories should be established for sharing information and might include involving them in the review process. It should be noted that GOF and related policies may impact sample sharing and impede international relations relating to public health efforts. In general, public health laboratories welcome community involvement.

International Perspectives

Several participants noted that there is much interest in the GOF/DURC issue internationally, and the international community is looking to see what the USG will do as a result of the deliberative process. It was noted that U.S. policy often influences policies globally and the international ramifications should be considered. Recent biosafety incidents in U.S. Federal labs have raised concerns among many in other countries about the ability of the U.S. to adequately manage risks. A number of countries have well-developed systems of policy and regulation that would address some GOF and DURC issues, though international policy approaches are generally somewhat different from those in the U.S. International experiences, activities, and perspectives were cited as important to consider in the deliberative process. A collaborative approach and active attempts to engage the international community was viewed as the most effective way to benefit all. Many favored launching international dialog soon, with development of broad concepts and points of agreement that could be shared by all, while still respecting national differences. In addition, it was suggested that academies of science and multi-national organizations such as WHO can play an important role in such interactions. Those with a particular interest in the international aspects of GOF research also cited ethical issues associated with the unequal distribution

of risks and benefits across rich and poor countries. It was noted that the European Commission uses a comprehensive ethics process for screening and monitoring DURC/GOF in research projects.[63]

Those with an Interest in the Deliberative Process Itself

A broad group of individuals offered comments on the deliberative process itself. This included: federal government personnel, ethicists, decision-making experts, policy experts, other scientists, and includes people who are also members of the previously-mentioned groups. Those concerned with the deliberative process generally called for a well-planned and executed, thorough, scientifically rigorous, and impartial RBA that is technically sound and socially acceptable. They favored a democratic deliberative process and a policy that incorporates decisions made by neutral parties. Policy should be created using risk-based and value-based approaches to achieve desired outcomes. They want the final policy resulting from the deliberative process to be capable of reasonably identifying and mitigating risks related to GOF while protecting scientific autonomy, research progress, discovery and innovation, public health, national security, and other critical interests.

Many see an adaptive process as desirable, and recommend collecting appropriate data about laboratory accidents and mitigation effectiveness. It was noted that risks and benefits will change as science advances. The funding decision-making process should be accountable and limit inherent conflicts of interest; the individuals or entities that make decisions is critical. Most favor using existing policies as the basis of policy for GOF, while acknowledging that current frameworks are not entirely adequate. The question of how to incorporate non-USG funded research into an acceptable framework was raised several times. Deciding how to decide is a key point.

Both proponents and critics of GOF studies criticized the term "gain-of-function" as being too broad and not descriptive enough. There was much discussion about the appropriate definition of GOF research of concern; many strong, often conflicting, views were expressed. Unfortunately while it is important to have a working definition and criteria for what is GOF of concern as opposed to GOF, a binary distinction needed for deciding what requires extra scrutiny, GOF experiments are actually a continuum of increasing risk.

The funding pause was criticized for being too broad, and some described it as disruptive to scientific process. Finally, some feel that a definitive quantitative risk assessment is not possible because of the very large uncertainties and lack of critical information associated with doing such studies, and they question the value of any studies that are done.

[63] The EU Framework Programme for Research and Innovation, Horizon 2020. How to complete your ethics self-assessment, version 1.0, 11 July 2014. http://ec.europa.eu/research/participants/data/ref/h2020/call_ptef/pt/h2020-call-pt-ria-ia_en.pdf#page=27

Appendix D. Consultations, Comments, and Sources Consulted During NSABB Deliberations

Table 1. Experts consulted by NSABB or the NSABB working groups. Individuals listed here addressed the NSABB or NSABB working group in their individual or professional capacities. Members of the NSABB or an NSABB working group are listed if they presented as a subject matter expert on a specific topic.

Speaker/Commenter	Affiliation/Location	Venue
Regine Aalders, M.Sc.	Embassy of the Netherlands, Washington, D.C.	Public Comment
Richard Adams		Public Comment
Ronald Atlas, Ph.D.	University of Louisville	National Academies Workshop (December 15, 2014)
Ralph Baric, Ph.D.	University of North Carolina	National Academies Workshop (December 15, 2014), Public Comment
Kavita Berger, Ph.D.	Gryphon Scientific	NSABB Full Board Meeting (September 28, 2015)
Kenneth W. Bernard, M.D.	US Public Health Service (ret.)	Public Comment
Thomas Briese, Ph.D.	Columbia University	National Academies Workshop (December 15, 2014)
Arturo Casadevall, M.D., Ph.D.	Albert Einstein College of Medicine, mBio	NSABB Full Board Meeting (October 22, 2014), In-person WG Meeting (July 23, 2015), Public Comment
Rocco Casagrande, Ph.D.	Gryphon Scientific	NSABB Full Board Meeting (September 28, 2015)
R. Alta Charo, J.D.	University of Wisconsin–Madison	National Academies Workshop (December 15, 2014)
Susan Coller-Monarez, Ph.D.	Office of Science and Technology Policy	In-person WG Meeting (July 23, 2015)
Derrin Culp	White Plains, New York	Public Comment
Mark Denison, M.D.	Vanderbilt University	National Academies Workshop (December 15, 2014), Public Comment
Dennis Dixon, Ph.D.	HHS/National Institutes of Health	NSABB Full Board Meeting (November 25, 2014)
Marianne Donker, Ph.D.	Ministry of Health, Welfare and Sport; Netherlands	In-person WG Meeting (July 23, 2015)
Philip Dormitzer, M.D., Ph.D.	Novartis Vaccines	National Academies Workshop (December 15, 2014)
Ruxandra Draghia-Akli, M.D., Ph.D.	European Commission	In-person WG Meeting (July 23, 2015)
Rebecca Dresser, J.D.	Washington University in St. Louis	NSABB Full Board Meeting (September 28, 2015)
Paul Duprex, Ph.D.	Boston University, NEIDL Institute	NSABB Full Board Meeting (October 22, 2015)
Gerald Epstein, Ph.D.	Department of Homeland Security	In-person WG Meeting (July 23, 2015)
Stephen Eubank, Ph.D.	Virginia Polytechnic Institute and State University	NSABB Full Board Meeting (October 22, 2014)
Nicholas Evans, Ph.D.	University of Pennsylvania	Public Comment

David S. Fedson, M.D.	Sergy Haut, France	Public Comment
Scott Ferson, Ph.D.	Applied Biomathematics	NSABB Full Board Meeting (October 22, 2014), Public Comment
Harvey Fineberg M.D, Ph.D.	University of California, San Francisco	National Academies Workshop (December 15, 2014)
Baruch Fischhoff, Ph.D.	Carnegie Mellon University	NSABB Full Board Meeting (October 22, 2014); National Academies Workshop (December 15, 2014)
Ron Fouchier, Ph.D.	Erasmus Medical Center	National Academies Workshop (December 15, 2014), Public Comment
Gregory Frank, Ph.D.	Infectious Diseases Society of America	Public Comment
David Franz, D.V.M., Ph.D.	Former Commander, United States Army Medical Research Institute for Infectious Diseases	In-person WG Meeting (July 23, 2015)
Christophe Fraser, Ph.D.	Imperial College	National Academies Workshop (December 15, 2014)
Matt Frieman, Ph.D.	University of Maryland	Public Comment
Gigi Kwik Gronvall, Ph.D.	University of Pittsburgh Medical Center (UPMC) Center for Health Security	National Academies Workshop (December 15, 2014)
Charles Haas, Ph.D.	Drexel University	National Academies Workshop (December 15, 2014)
Peter Hale	Foundation for Vaccine Research	Public Comment
Elizabeth Hart	Adelaide, South Australia	Public Comment
Andrew M. Hebbeler, Ph.D.	White House Office of Science and Technology Policy	NSABB Full Board Meeting (October 22, 2014), National Academies Workshop (December 15, 2014)
Denise Hein		Public Comment
Gavin Huntley-Fenner, Ph.D.	Huntley-Fenner Advisors	National Academies Workshop (December 15, 2014)
Jo Husbands, Ph.D.	Board on Life Sciences of the US National Academy of Sciences	In-person WG Meeting (July 23, 2015)
Michael Imperiale, Ph.D.	University of Michigan	National Academies Workshop (December 15, 2014), Public Comment
Tom Inglesby M.D.	University of Pittsburgh	NSABB Full Board Meeting (October 22, 2014), Public Comment
Barbara Jasny, Ph.D.	Science	In-person WG Meeting (July 23, 2015)
Barbara Johnson, Ph.D., R.B.P.	Biosafety Biosecurity International	National Academies Workshop (December 15, 2014)
Laura Kahn, M.D., M.P.H., M.P.P.	Woodrow Wilson School of Public and International Affairs, Princeton University	Public Comment
Joseph Kanabrocki, Ph.D., C.B.S.P.	University of Chicago	In-person WG Meeting (January 22, 2015), In-person WG Meeting (July 23, 2015)
Yoshihiro Kawaoka, D.V.M., Ph.D.	University of Wisconsin, Madison	NSABB Full Board Meeting (October 22, 2014), National Academies Workshop (December 15, 2014), Public Comment
George Kemble, Ph.D.	3-V Biosciences	National Academies Workshop (December 15, 2014)
Larry Kerr, Ph.D.	National Security Council Staff	WG Meeting (November 5, 2015)
Andy Kilianski, Ph.D.	National Research Council Fellow at US Army	Public Comment

Lynn Klotz, Ph.D.	Center for Arms Control and Non-proliferation	Public Comment
Gregory Koblentz, Ph.D., M.P.P.	George Mason University	National Academies Workshop (December 15, 2014)
Todd Kuiken, Ph.D.	The Wilson Center	In-person Meeting (July 23, 2015)
Robert Lamb, Ph.D., Sc.D.	Northwestern University; Howard Hughes Medical Institute	National Academies Workshop (December 15, 2014)
Linda Lambert, Ph.D.	HHS/National Institutes of Health	In-person WG Meeting (July 23, 2015)
Carol Linden, Ph.D.	HHS/Biomedical Advanced Research and Development Authority	National Academies Workshop (December 15, 2014)
W. Ian Lipkin, M.D.	Columbia University	NSABB Full Board Meeting (October 22, 2014)
Marc Lipsitch, Ph.D.	Harvard School of Public Health	NSABB Full Board Meeting (October 22, 2014), National Academies Workshop (December 15, 2014), Public Comment
Patricia Long, J.D., LL.M.	HHS/Office of Security and Strategic Information	In-person WG Meeting (July 24, 2015)
Nicole Lurie, M.D., M.S.P.H.	HHS/Assistant Secretary for Preparedness and Response	NSABB Full Board Meeting (October 22, 2014); In-person WG Meeting (July 23, 2015)
Eric Meslin, Ph.D.	Indiana University School of Medicine	NSABB Full Board Meeting (September 28, 2015)
Corey Meyer, Ph.D.	Gryphon Scientific	NSABB Full Board Meeting (September 28, 2015)
Rebecca Moritz, M.S., C.B.S.P., S.M.(NRCM)	University of Wisconsin–Madison	National Academies Workshop (December 15, 2014)
Peter Murakami	Baltimore, Maryland	Public Comment
Kalyani Narasimhan, Ph.D.	Nature Publishing Group	In-person WG Meeting (July 23, 2015)
Daniel O'Connell	Albany, Oregon	Public Comment
Kimberly Orr, Ph.D.	US Department of Commerce	In-person WG Meeting (July 23, 2015)
Michael Osterholm, Ph.D., M.P.H.	University of Minnesota	NSABB Full Board Meeting (October 22, 2015)
Kenneth Oye, Ph.D.	Massachusetts Institute of Technology	In-person WG Meeting (July 23, 2015)
Megan Palmer, Ph.D.	Center for International Security and Cooperation, Stanford University	Public Comment
Christopher Park	U.S. Department of State	In-person WG Meeting (July 23, 2015)
Jean Patterson, Ph.D.	Texas Biomedical Research institute	In-person WG Meeting (January 22, 2015)
Daniel Perez, Ph.D.	University of Maryland	NSABB Full Board Meeting (October 22, 2014)
Janet Peterson, C.B.S.P.	University of Maryland	NSABB Full Board Meeting (October 22, 2014)
Dustin Phillips	Louisville, Kentucky	Public Comment
Stanley Plotkin, M.D.	University of Pennsylvania	Public Comment
David Relman, M.D.	Stanford University	National Academies Workshop (December 15, 2014)
David B. Resnik, J.D., Ph.D.	HHS/National Institutes of Health	NSABB Full Board Meeting (October 22, 2014)

Colin Russell, Ph.D.	University of Cambridge	National Academies Workshop (December 15, 2014)
Steven L. Salzberg, Ph.D.	Johns Hopkins University School of Medicine	Public Comment
Monica Schoch-Spana, Ph.D.	University of Pittsburgh Medical Center (UPMC) Center for Health Security	National Academies Workshop (December 15, 2014)
Stacey Schultz-Cherry, Ph.D.	St. Jude Children's Research Hospital	NSABB Full Board Meeting (October 22, 2014), National Academies Workshop (December 15, 2014)
Shannon Scott		Public Comment
Michael Selgelid, Ph.D.	Monash University	NSABB Full Board Meeting (September 28, 2015)
Billie Sellers		Public Comment
Richard Sever, Ph.D.	Cold Spring Harbor Laboratories Press bioRxiv	In-person WG Meeting (July 23, 2015)
Michael Shaw, Ph.D.	Centers for Disease Control and Prevention	In-person WG Meeting (July 23, 2015)
Bill Sheridan, M.B., B.S.	BioCryst Pharmaceuticals Inc.	NSABB Full Board Meeting (October 22, 2014)
Lone Simonsen, Ph.D.	George Washington University	Public Comment
Andrew Snyder-Beattie	Future of Humanity Institute, University of Oxford	Public Comment
Charles Stack, M.P.H.	University of Illinois at Chicago	Public Comment
John Steel, Ph.D.	Emory University	Public Comment
Kanta Subbarao, M.B.B.S., M.P.H.	HHS/National Institutes of Health	National Academies Workshop (December 15, 2014), Public Comment
Robert Temple, M.D.	Food and Drug Administration	In-person WG Meeting (July 23, 2015)
Eileen Thacker, D.V.M., Ph.D., DACVM	Department of Agriculture	In-person WG Meeting (July 23, 2015)
Kimball Ward		Public Comment
Robert Webster, Ph.D.	St. Jude Children's Research Hospital	National Academies Workshop (December 15, 2014)
Jerry Weir, Ph.D.	Food and Drug Administration	National Academies Workshop (December 15, 2014)
Robbin Weyant, Ph.D., R.B.P. (ABSA)	Center for Disease Control and Prevention	National Academies Workshop (December 15, 2014), In-person WG Meeting (July 23,2015)
Gary Whittaker, Ph.D.	Cornell University	Public Comment
Carrie Wolinetz, Ph.D.	HHS/National Institutes of Health	NSABB Full Board Meeting (May 5, 2015 and January 7-8, 2016)
Infectious Diseases Society of America	Infectious Diseases Society of America	Public Comment

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2024 **Table 2. Sources consulted by NSABB and NSABB working groups include but are not limited to the following**

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USG (September 2014)	Companion Guide to the USG Policies for Oversight of Life Sciences Dual Use Research of Concern
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USG (July 2012)	Final Supplementary Risk Assessment for the Boston University National Emerging Infectious Diseases Laboratories (NEIDL)
USG (August 2013)	HHS Funding Framework for HPAI H5N1 Studies
USG (February 2013)	NIH Guidelines for Research Involving Recombinant DNA Molecules - Amendment Notice. February 21, 2013
USG (November 2013)	NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
USG (October 2014)	USG Gain-of-function GOF Deliberative Process and Funding Pause Statement
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Appendix E. Policy Analysis Summary Table

Oversight Measures	Risks Addressed	Description of Oversight	Analysis/Applicability to GOF Studies
Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition (December 2009) http://www.cdc.gov/biosafety/publications/bmbl5/index.htm	Biosafety risks	**Applies to:** Life sciences research involving infectious microorganisms or hazardous biological materials **Description:** General biosafety practices and biological containment for various classifications (risk groups) of microorganisms and etiological agents	BMBL does not describe GOF studies per se but does include summary statements and biocontainment guidance for research involving various influenza strains (including contemporary and non-contemporary human, high and low pathogenic avian, swine, the 1918 influenza strain, and reassortant viruses) and SARS-CoV. MERS-CoV had not emerged at the time of the last BMBL update but interim laboratory biosafety guidance was issued by CDC and is referenced by BMBL. BMBL is a guidance document and generally considered the authoritative reference for laboratory biosafety but it is not a regulatory document; compliance is voluntary.
NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (November 2013) http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines	Biosafety risks	**Applies to:** Basic or clinical life sciences research that involves recombinant or synthetic nucleic acid molecules and is conducted at an institution receiving NIH funding for any such research **Description:** Describes roles and responsibilities of institutions and investigators in safely conducting research. Requires institutional review with a focus on the concepts of risk assessment, risk group classification of agents, physical and biological containment levels, practices, personal protective equipment, and occupational health. **Advised by:** NIH Recombinant DNA Advisory Committee (RAC)	The NIH Guidelines have been amended to include additional guidance for work with Risk Group 3 influenza viruses (1918 H1N1, H2N2, highly pathogenic avian influenza (HPAI) H5N1) to specify enhancements to biosafety level 3 containment, practices, and occupational health requirements. NIH Guidelines were amended again to require further enhancements to facilities, biosafety equipment and practices, including occupational health practices, for research involving HPAI H5N1 strains transmissible among mammals by respiratory droplets. NIH Guidelines are often used as a model of biosafety guidance by the broader scientific community but compliance is required only by institutions receiving such funding from the NIH. The scope is also limited to research involving recombinant or synthetic nucleic acids. Some IBCs also review and approve non-recombinant pathogen research; however, not all institutions require their IBCs to do so.
HHS and USDA Select Agent Program (as of July 2014) http://www.selectagents.gov/regulations.html	Biosecurity (physical and personnel) and biosafety risks	**Applies to:** Biological agents and toxins that have the potential to pose a severe threat to public health and safety, based on a set of criteria. **Description:** Regulates the possession, use, and transfer of select agents and toxins. Overseen by the Federal Select Agent Program. Requires registration of individuals and entities; federal background investigations; federal review of restricted experiments; training; institutional compliance; etc. **Advised by:** Intragovernmental Select Agents and Toxins Technical Advisory Committee (ISATTAC)	Studies that could be considered GOF studies, which involve pathogens on the select agent list, are subject to oversight by the SAP. Researchers and institutions performing such studies must receive favorable security risk assessments by the FBI, register with the SAP, receive training on the proper procedures and practices for handling such agents, and abide by other aspects of the regulations. SARS-CoV, HPAI H5N1 influenza, and 1918 influenza viruses are select agents and GOF studies involving these pathogens are subject to oversight by the SAP. Restricted experiments that would entail conferring antiviral resistance to these viruses would require additional review and approval prior to being conducted. GOF experiments involving MERS, and other agents not included on the select agent list, would not be subject to oversight by the SAP.

USG Policy for Federal Oversight of DURC (March 2012) http://www.phe.gov/s3/dualuse/Pages/USGOversightPolicy.aspx	Biosecurity risks, particularly involving misuse of research information, products, and technologies (DURC)	**Applies to:** Life sciences research conducted at an institution receiving USG funding that involves any of 15 agents that pose the greatest risk of deliberate misuse with most significant potential for mass casualties or devastating effects.	The federal DURC policy requires identification and oversight of certain pathogen research involving 7 experimental types, some of which can be described as GOF experiments (i.e., enhancing the harmful consequences of an agent; increase transmissibility; alter host range; etc.) by Federal funding agencies. DURC policies only apply to research involving 15 pathogens. Institutions may review other studies for DURC potential but are not required to do so. Certain GOF studies that involve other agents would not be subject to DURC oversight under the policies.
USG Policy for Institutional Oversight of DURC (September 2014) http://www.phe.gov/s3/dualuse/Pages/InstitutionalOversight.aspx	Biosecurity risks, particularly involving misuse of research information, products, and technologies (DURC)	**Applies to:** Life sciences research conducted at an institution receiving USG funding that involves any of 15 agents that pose the greatest risk of deliberate misuse with most significant potential for mass casualties or devastating effects.	The institutional DURC policy requires federally-funded institutions to establish a system for the identification and oversight of certain pathogen research involving 7 experimental types, some of which can be described as GOF experiments (i.e., enhancing the harmful consequences of an agent; increase transmissibility; alter host range; etc.) DURC policies only apply to research involving 15 pathogens. Institutions may review other studies for DURC potential but are not required to do so. Certain GOF studies that involve other agents would not be subject to DURC oversight under the policies.
HHS Funding Framework for GOF studies (August 2013) http://www.phe.gov/s3/dualuse/Pages/HHSh5n1Framework.aspx	Biosafety and biosecurity risks associated with certain GOF experiments involving agents with pandemic potential	**Applies to:** Gain-of-function studies that are reasonably anticipated to generate HPAI H5N1 viruses that are transmissible, and LPAI H7N9 viruses that have increased transmissibility, between mammals by respiratory droplets **Description:** Describes an HHS Department-level review pre-funding review and approval process for certain GOF studies, which can result in funding, not funding, or funding with certain conditions and ongoing oversight.	The only policy focused specifically on funding decisions related to the types of GOF studies that have raised concern. Narrowly focused only on specific GOF studies (enhancing mammalian transmissibility) on two avian influenza viruses; other GOF studies may raise concern and would not be reviewed under this framework.
USG Export Controls (as of July 2014) http://www.bis.doc.gov/index.php/regulations/export-administration-regulations-ear		**Applies to:** Export or release of equipment, software and technology, chemicals, microorganisms, toxins, and other materials and information deemed dual use or strategically important to U.S. national security, economic, and/or foreign policy interests	Comprehensive set of federal regulations that control and restrict the export and release of sensitive equipment, software and technology; chemical, biological, and other materials and information as a means to promote national security interests and foreign policy objectives.

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Appendix F. National Science Advisory Board for Biosecurity Roster

National Science Advisory Board for Biosecurity Roster

[Ŧ] NSABB Working Group Co-chair
[†] NSABB Working Group on the Design and Conduct of Risk and Benefit Assessments of Gain-of-Function Studies
[‡] NSABB Working Group on Evaluating the Risks and Benefits of Gain-of-Function Studies
[*] NSABB Member, Retired

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NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY

Working Group on Evaluating Risks and Benefits of GOF Studies

April 19, 2016
10:00 am – 12:00 pm Eastern
Call-in number: (b)(6)
Participant Code: (b)(6)

- **WG Roll Call** (5 min)
  Christopher Viggiani

- **Opening Remarks, Agenda Review, Meeting Goals** (5 min)
  Joe Kanabrocki, co-chair
  Ken Berns, co-chair

- **Summary of last meeting and overview of edits to draft report** (10 min)
  Kevin Ramkissoon

- **Discussion of Draft Report: Principles to Guide Funding Decisions for GOFROC**
  - Draft Report (PDF version; pgs. 45 – 46)

- **Discussion of Any Other Areas in Draft Report, Time Permitting**
  - Any recommendations needing revision (p43 – 52)
  - Key findings (p37 – 41)
  - Any other sections

- **Next Steps** (5 min)
  - Tue, May 3; 2:00PM – 4:00PM EDT
  - **NSABB Meeting** — May 24th from 10:30AM – 4:00PM EDT; Note new start time.

Meeting Materials

- Agenda
- Summary of 4-7-2016 WG Teleconference
- NSABB Draft Report, v4-18-2016—PDF Version (clean, significant edits in red)
- NSABB Draft Report—Word Version, v4-18-2016 (all edits shown in track changes)

Summary of Discussions by Agenda Items

- **Finalization of the attributes of GOFROC**
    - WG members generally approved of the edits made to the attributes for defining GOFROC.
- **Discussion of and Support for Proposed Review Process**
    - The WG discussed the pros, cons, and challenges of different review bodies (federal vs. FACA) towards achieving the three identified procedural values of (i) public participation & democratic deliberation, (ii) transparency, and (iii) accountability. The WG also discussed a number of practical issues such as the efficiency and expertise needed for the reviews, how to maintain necessary confidentiality regarding proprietary/sensitive information associated with unpublished proposals, and issues considering a national security and public health vulnerabilities.
    - There was some hesitation about being overly prescriptive with a desire instead to focus on what a review process should achieve.
    - The WG generally favored having a higher-level USG group perform reviews of GOFROC proposals; such a review mechanism was viewed as efficient, accountable, could be constituted of appropriate expertise, could protect sensitive information in proposals, and would demonstrate high-level USG commitment to reviewing GOFROC. To address real or perceived conflicts of interest, one member suggested that the review of individual proposals by the USG could occur at the inter-departmental level.
    - However, there was also broad agreement that an independent, non-USG (perhaps FACA) entity would substantially bolster public participation and transparency.
    - The WG coalesced around a suggestion to 1) utilize a high level USG group for individual GOFROC proposal reviews and 2) have an independent, perhaps FACA body periodically review and assess the overall USG review process. This approach would enable efficient and effective GOFROC proposal reviews, facilitate the desired adaptive policy approach, and promote transparency and public discussions on the process.
- **Discussion of and Support for New Recommendations**
    - **Recommendation 3 (adaptive approach)**
        - The WG generally approved of the edits made—including the addition of **Recommendation 3.1 (new)** which calls for consideration of a system to collect and analyze data associated with laboratory safety but some expressed concern about the burden that such a recommendation might entail.
        - WG agreed to **add a new Recommendation 3.2** that articulates the role and objectives of an independent, possibly FACA-like, body to periodically review the USG GOFROC review process and facilitate an adaptive policy approach.
    - **Recommendation 4 (oversight of non-USG funded GOFROC)**
        - WG members approved of **Recommendation 4 (new)** but also noted the challenges with implementing such oversight.

- **Recommendation 5 (outreach and education)**
  - WG members approved of the language in **Recommendation 5** which calls for broad USG efforts to strengthen laboratory biosafety and biosecurity and raising awareness about GOFROC.
  - The group agreed that **Recommendation 5.1 (new)** calling for the development of guidance for investigators and institutions is beneficial and may help streamline the submission and review of GOFROC proposals
- **Recommendation 6 (international engagement)**
  - The WG approved of the addition on the **Recommendation 6 (new)** which calls for international engagement on the issue but suggested edits to the language to encompass broader aspects of biosafety and biosecurity beyond GOFROC and dual use.

Action Items

- NIH will circulate a new Word version of draft report based on WG discussions. In general NIH will make final non-controversial edits to early sections (1 – 4) and appendices of the draft report that describe the NSABB's process.
- NSABB WG to review and provide to NIH by COB 4-13-2016:
  - Specific edits and comments to any section of the report
  - Comments on embedded, highlighted notes and questions in the report; no objections will be taken as concurrence
  - Requests about specific areas to discuss as a WG during the last two WG meetings
  - Any major, deal-breaker objections that need reconciling

Policy Recommendations for the Evaluation of Proposed Gain-of-Function Research

A Draft Report of the NSABB Working Group on Evaluating the Potential Risks and Benefits of Gain-of-Function Studies

Version: April 18, 2016

Preface for NSABB Meeting on May 24, 2016

This draft report was developed by the NSABB working group tasked with evaluating the risks and benefits associated with gain-of-function studies and developing draft recommendations on a conceptual approach for the evaluation of proposed gain-of-function studies. The first version of this document was discussed at the NSABB meeting on January 7 & 8, 2016 and again at the symposium hosted by the National Academies on March 10 & 11, 2016. This version represents an updated draft of that initial working paper. This document is still pre-decisional and intended as a deliberative document to be discussed at the meeting of the full NSABB on May 24, 2016. This is document is not a formal NSABB work product and should not be considered to be official NSABB findings or recommendations to the U.S. government. This document does not represent official policy of the U.S. government.

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Executive Summary

NOTE: Executive Summary will be updated to reflect any changes in the rest of the document.

Research involving pathogens is essential to global health and security. Such research provides insight into the fundamental nature of human-pathogen interactions, enables the assessment of the pandemic potential of emerging infectious agents, and informs public health and preparedness efforts, including the development of medical countermeasures. Several policies are in place to help ensure that pathogen research is conducted safely and in ways to minimize the risks of laboratory accidents and security risks. Recently, and in the wake of a number of biosafety incidents at Federal facilities, concerns have been raised about certain "gain-of-function" (GOF) studies with the potential to generate pathogens with enhanced pathogenicity or transmissibility in mammals. The concerns center on whether a pathogen with enhanced characteristics could be accidentally or intentionally released from a laboratory, potentially exposing surrounding populations to a pathogen with pandemic potential.

The U.S. Government (USG), as part of its continued focus on biosafety and biosecurity, has undertaken a deliberative process to carefully examine the risks and benefits associated with certain GOF studies. The deliberative process involves the National Science Advisory Board for Biosecurity (NSABB), which has been tasked with making recommendations to the USG on this topic, and the National Academy of Sciences (NAS), which was tasked to convene two public symposia to generate broad discussion on the relevant issues. To further inform NSABB deliberations, the National Institutes of Health (NIH) commissioned an independent assessment of the risks and benefits associated with GOF studies and an ethical analysis of the issues related to funding and conducting such studies.

The NSABB was charged with 1) advising on the design, development, and conduct of the risk and benefit assessments for GOF studies, and 2) providing recommendations to the USG on a conceptual approach to the evaluation of proposed GOF studies. The NSABB established two working groups to address its tasks and the full Board convened publically five times between October 2014 and January 2016. In May 2015 the NSABB issued its *Framework for Guiding the Conduct of Risk and Benefit Assessments of Gain-of-Function Research*, which guided NIH in overseeing the contractor conducting the risk and benefit assessments.

The working group tasked with issuing recommendations on an approach to evaluating proposed GOF studies considered four major areas: the current policy landscape as it pertains to pathogen research, the results of the risk and benefit assessments, the analysis of relevant ethical issues, and broad stakeholder perspectives on the issues at hand. This working paper describes the working group's process, analysis, preliminary findings, and draft recommendations to date. This paper is not a final NSABB work product and does not represent NSABB recommendations to the U.S. government. This interim report is offered by the working group to the full NSABB, and the broader stakeholder community, to serve as a springboard for discussion at the NSABB meeting in May, 2016.

The working group has developed four key findings:

Key Finding 1: There are many types of GOF research and not all of them have the same level of risks. Only a small subset of GOF research—GOF research of concern (GOFROC)—entail risks that are potentially significant enough to warrant additional oversight.

Key Finding 2. The U.S. government has several policy frameworks in place for identifying and managing risks associated with life sciences research. There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOFROC could be applied.

Key Finding 3. Oversight policies vary in scope and applicability, and are not sufficiently harmonized; therefore, current oversight is not sufficient for all GOFROC.

Key Finding 4. An adaptive policy approach is a desirable way to ensure that oversight and risk mitigation measures remain commensurate with the risks associated with the research and the benefits of the research are being fully realized**.**

Key Finding 5. There are life sciences research studies, including possibly some GOFROC, that should not be conducted on ethical or public health grounds if the potential risks associated with the study are not justified by the potential benefits. Decisions about whether GOFROC should be permitted will entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of proposed studies but other considerations, including legal, ethical, and societal values are also important.

Key Finding 6. Managing risks associated with GOFROC, like all life sciences research, requires Federal-level and institutional oversight, awareness and compliance, and a commitment by all stakeholders to safety and security.

Key Finding 7. Consideration of the international dimensions associated with funding and conducting GOF research of concern is important.

Based on its analyses thus far, the NSABB working group has formulated the following draft recommendations for discussion:

Recommendation 1. Research proposals involving GOFROC entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the Federal and institutional levels.

As part of this recommendation, the NSABB working group has proposed a conceptual approach for guiding funding decisions about GOFROC. First, the working group identified the attributes of GOFROC, which is research that could generate a pathogen that is: highly transmissible and likely capable of wide and uncontrollable spread in human populations; and highly virulent and likely to cause significant morbidity and/or mortality in humans. Next, the working group identified a set of principles that should guide funding decisions for GOFROC. Only research that is determined to be

in line with these principles should be funded. Additional risk mitigation measures may be required for certain research studies to be deemed acceptable for funding.

Recommendation 2. In general, oversight mechanisms for GOFROC should be incorporated into existing policy frameworks when possible. The risks associated with some GOFROC can be identified and adequately managed by existing policy frameworks if those policies are implemented properly. However, the level of oversight provided by existing frameworks varies by pathogen. For some pathogens, existing oversight frameworks are robust and additional oversight mechanisms should generally not be required. For other pathogens, existing oversight frameworks are less robust and may require supplementation. All relevant policies should be implemented appropriately and enhanced when necessary to effectively manage risks.

Recommendation 3. The U.S. government should pursue an adaptive policy approach to help ensure that oversight remains commensurate with the risks associated with the GOFROC.

> **Recommendation 3.1.** The U.S. government should consider developing a system to collect and analyze data associated with laboratory safety to inform policy development over time for GOFROC.

> **Recommendation 3.2.** An external advisory body that is designed for transparency and public engagement should be utilized as part of the U.S. government's ongoing evaluation of oversight policies for GOFROC.

Recommendation 4. The U.S. government should pursue ways to ensure that all GOFROC conducted within the U.S. or by U.S. companies be subject to oversight, regardless of funding source.

Recommendation 5. The U.S. government should undertake broad efforts to strengthen biosafety and biosecurity and, as part of these efforts, seek to raise awareness about the specific issues associated with GOFROC.

> **Recommendation 5.1.** The U.S. government should specifically develop a "Points to Consider" document to provide guidance to investigators and institutions when preparing research proposals that may involve GOFROC.

Recommendation 6. The U.S. government should engage the international community in a dialogue about the oversight and responsible conduct of GOFROC.

1. Introduction

A robust life sciences research enterprise is necessary to counter the continually evolving threats to public health and national security posed by endemic and emerging pathogens, as well as malicious biological threats. By helping to define the nature of human-pathogen interactions, life sciences research promotes public health and national security not only by enhancing our understanding of pathogen biology and disease pathogenesis, but also by informing biosurveillance and medical countermeasure development. Such research can also aid in the assessment of the pandemic potential of emerging infectious agents, thereby underpinning health policy decisions and preparedness and response efforts.

While the ultimate goal of life sciences research involving pathogens is the protection and promotion of public health, there are inherent associated biosafety and biosecurity risks. Potential risks might arise from laboratory accidents or security breaches that result in laboratory acquired infections or the accidental or deliberate release of a pathogen from containment. Life sciences research has "dual use" potential. That is, legitimate research may generate information, products or technologies that could be misused to threaten public health or national security. To mitigate such dual use concerns, as well as potential biosafety and biosecurity risks, research involving pathogens is subject to multiple layers of Federal and institutional oversight.

The Gain-of-Function Debate and the USG Response

Experimental techniques and approaches that modify the genome of microorganisms are routinely employed in pathogen research to ascertain the roles of genes and their functional products. Such studies are fundamental to the field of microbial genetics and facilitate correlation of genetic and phenotypic characteristics – a critical step in deciphering the complex nature of host-pathogen interactions that underlie transmission, infection, and pathogenesis. Such genetic manipulations can result in either diminished (loss-of-function) or enhanced (gain-of-function) biological phenotypes.

Studies that result in the generation of pathogens with enhanced, or gain-of-function (GOF), phenotypes are conducted for a number of valid scientific purposes. Such studies provide information that adds to the scientific knowledge base and can inform biosurveillance, medical countermeasure development, and public policy decision-making related to public health and preparedness. The vast majority of such GOF studies do not raise significant safety or security concerns. However, certain GOF studies involving pathogens have raised significant concerns about whether a laboratory-generated pathogen with pandemic potential could be accidentally or intentionally released, resulting in significant consequences to public, or perhaps, global health. Concerns have also been raised about whether certain GOF studies could generate information that could enable individuals with malevolent intent to generate a pathogen with pandemic potential (see Box 1).

The controversy over certain GOF studies arose after two groups demonstrated that highly pathogenic avian influenza H5N1 viruses with a small number of engineered mutations became transmissible between mammals by respiratory droplets.[1,2] In 2012, in response to the controversy associated with publishing the manuscripts describing these findings, the influenza community initiated a voluntary suspension of certain GOF studies involving highly pathogenic avian influenza H5N1 viruses. During that time, policymakers considered whether certain GOF studies should be conducted using Federal funds, and if so, how those studies could be safely conducted. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) issued new biosafety guidelines for working with highly pathogenic avian influenza strains.[3,4] The U.S. Department of Health and Human Services (HHS) developed a framework for guiding its funding decisions about GOF projects that may generate H5N1 or H7N9 avian influenza viruses that are transmissible between mammals by respiratory droplets.[5]

Concerns regarding laboratory safety and biosecurity associated with GOF studies were renewed following a number of biosafety incidents at U.S. Federal laboratories during the summer of 2014. The incidents did not involve GOF studies *per se* but raised broader concerns about laboratory safety and security as it applies to pathogen research.

Box 1. Gain-of-Function Research

Recently, the phrase "gain-of-function research" has become synonymous with certain studies that enhance the ability of pathogens to cause disease. However, gain-of-function studies, as well as loss-of-function studies, are common in molecular and microbiology and form the foundation of microbial genetics. Changes to the genome of an organism, whether naturally occurring or directed through experimental manipulations in the laboratory, can result in altered phenotypes as biological functions are lost or gained. Investigators routinely conduct loss- and gain-of-function experiments to understand the complex nature of host-pathogen interactions that underlie transmission, infection, and pathogenesis.

The term "gain-of-function" is generally used to refer to changes resulting in the acquisition of new, or an enhancement of existing, biological phenotypes. This report further defines "gain-of-function research of concern" to describe the subset of studies that have been the subject of recent debate regarding potential biosafety and biosecurity implications -- that is, gain-of-function studies with the potential to generate pathogens with pandemic potential in humans by exhibiting high transmissibility and high virulence. See Section 6 for a more rigorous description of GOF research of concern (GOFROC).

As one component of comprehensive efforts to review and enhance laboratory biosafety and biosecurity, the U.S. government (USG) embarked on a deliberative process to re-evaluate the risks and benefits of certain GOF research with a goal of developing policy governing the funding and conduct of

[1] Imai et al. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 486, 21 June 2012

[2] Herfst et al. Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets. *Science* 336, 22 June 2012

[3] Gangadharan D, Smith J, and Weyant R. Biosafety Recommendations for Work with Influenza Viruses Containing a Hemagglutinin from the A/goose/Guangdong/1/96 Lineage, Morbidity and Mortality Weekly Report 62(RR06); 1-7. http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6206a1.htm

[4] *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

[5] Framework for Guiding Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets, February 21, 2013. http://www.phe.gov/s3/dualuse/Documents/funding-hpai-h5n1.pdf

such research.[6] The deliberative process involves the National Science Advisory Board for Biosecurity (NSABB), which serves as the official Federal advisory body for providing advice in this area, and the National Academy of Sciences (NAS), which is to foster broader scientific and public discussions on the topics. To inform NSABB deliberations, NIH commissioned formal risk and benefit assessments (RBA) of GOF research involving pathogens with pandemic potential and an analysis of ethical issues surrounding the conduct of such studies. Stakeholder input is also essential to the process and has been received throughout NSABB's deliberative process.

The deliberative process is accompanied by a pause in the provision of new federal funds for certain GOF research involving influenza, Middle East Respiratory Syndrome (MERS) or Severe Acute Respiratory Syndrome (SARS) viruses—pathogens determined to have pandemic potential. Specifically:

> *New USG funding will not be released for gain-of-function research projects that may be reasonably anticipated to confer attributes to influenza, MERS, or SARS viruses such that the virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route. This restriction would not apply to characterization or testing of naturally occurring influenza, MERS, and SARS viruses, unless the tests are reasonably anticipated to increase transmissibility and/or pathogenicity.*[7]

In parallel, the USG has encouraged the research community (both those who receive USG funding and those who do not) to join in adopting a voluntary pause on any ongoing research that involves the types of studies that are subject to the funding restriction above.

NSABB recommendations will inform the USG as it develops policies about whether certain types of GOF studies on pathogens with pandemic potential should be supported and, if so, how such research proposals should be evaluated to inform funding and oversight decisions. It is expected that the temporary funding pause will be lifted and/or replaced by a decision or policy that addresses GOF research involving the generation of pathogens with pandemic potential.

2. NSABB Charge

On October 22, 2014, as part of the USG's deliberative process for GOF studies, the NSABB was issued its charge to:

1. Advise on the design, development, and conduct of risk and benefit assessments for GOF studies, and
2. Provide recommendations to the U.S. government on a conceptual approach to the evaluation of proposed GOF studies

In developing its recommendations the NSABB was asked to consider: the results of the risk and benefit assessments; the discussions hosted by the National Academies; the spectrum of potential risks and

[6] U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses, U.S. Government, October 17, 2014. http://www.phe.gov/s3/dualuse/documents/gain-of-function.pdf

[7] Ibid.

benefits associated with GOF studies; and any alternative methods that may be employed to yield similar scientific insights or benefits, while reducing potential risks.

3. NSABB Deliberative Approach

The deliberative process (Figure 1) initiated by the USG to evaluate the risks and benefits of GOF studies involves the NSABB and the National Academies. To address its charge, NSABB formed two working groups to develop draft recommendations, which were discussed by the full Board **[REF to meetings]**. The National Academies convened public forums to generate broad discussions and receive additional stakeholder input on the topic. The first forum was held early in the deliberative process and a second was held in March 2016; both were designed to inform NSABB deliberations.

To inform the deliberative process further, NIH commissioned two additional analyses: 1) qualitative and quantitative risk and benefit assessments, conducted by Gryphon Scientific, and 2) a review of the ethical considerations associated with the GOF issue and an analysis of relevant ethical decision-making frameworks, conducted by Dr. Michael Selgelid.

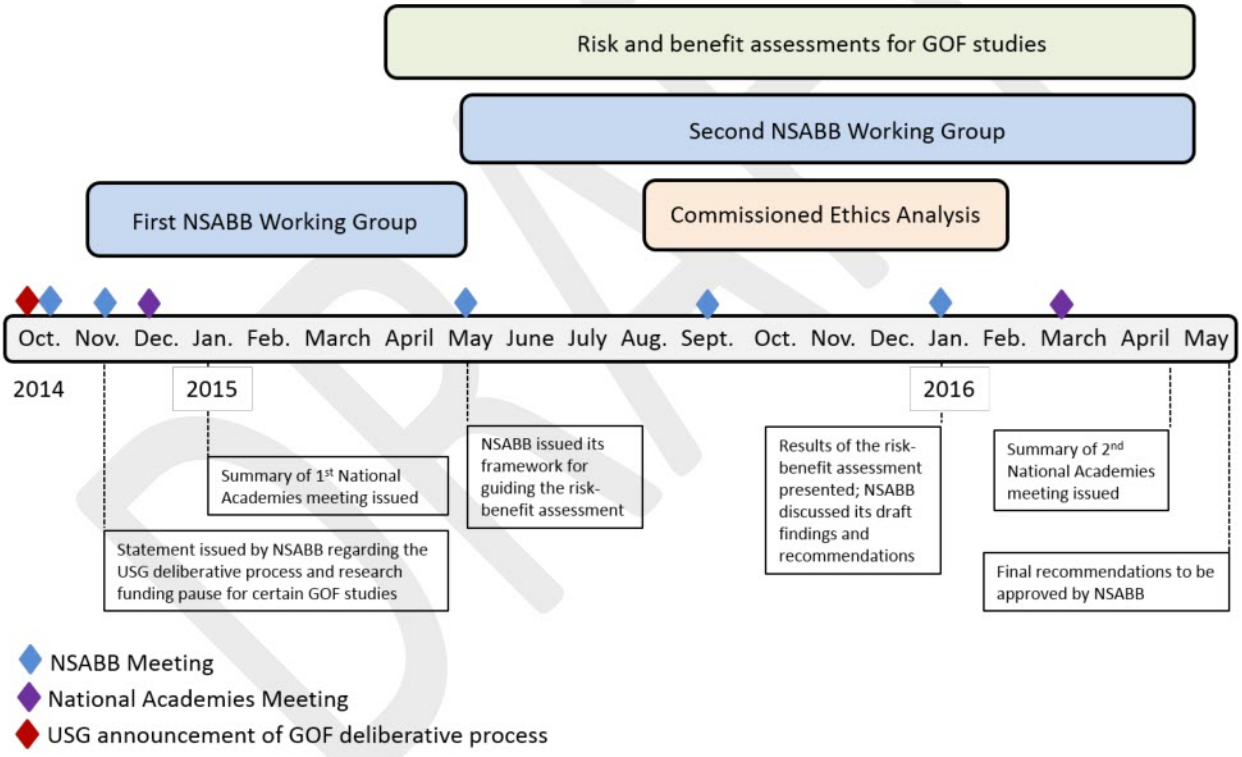

Figure 1. Timeline and major events of the GOF deliberative process.

The NIH Office of Science Policy, which administers the NSABB, managed the NSABB's overall deliberative process. NIH oversaw the work of its contractors, Gryphon Scientific and Dr. Michael Selgelid, and interfaced between the NSABB and contracted entities.

See Appendices A, B, C, and E for the NSABB and working group rosters, a detailed description of the NSABB's deliberative approach, an overview of stakeholder views that were considered, and a list of the experts and sources consulted, including those who submitted public comments.

Guiding Principles for NSABB Deliberations

Early in its deliberations the NSABB developed the principles below to guide its deliberations and underpin its analysis of the risk and benefit assessments.

1. The NSABB deliberations should focus on defining the GOF problem then include broad consideration of possible solutions. A range of approaches and decision-making frameworks will be considered, and the NSABB will take into account these various approaches when developing its recommendations.

2. NSABB will consider the potential risks and benefits of a broad range of GOF studies involving influenza, SARS, and MERS viruses in order to identify those that may raise significant concerns that should be addressed. However, the NSABB will aim to develop recommendations that are grounded in broadly-applicable concepts and principles that could, if necessary, apply to GOF studies involving other pathogens that may require evaluation in the future.

3. Similarly, NSABB will consider the risks and benefits associated with alternative research approaches to GOF research to understand whether or not these may substitute for or complement GOF studies.

4. NSABB recommendations will be informed by data and information about potential risks and benefits as well as values that will guide the evaluation and comparison of these risks and benefits. Ethical, societal, and legal considerations will also contribute to the development of recommendations and these inputs should be explicitly identified, discussed, and prioritized.

5. NSABB recognizes that not all analyses relevant to its task are quantitative and that uncertainties inherent in any quantitative analysis may remain. NSABB will seek to document important areas of uncertainty in any data or analysis when necessary.

6. NSABB should publicly debate its draft recommendations and describe in its report any dissenting views that may vary substantially from the Board's recommendations.

7. NSABB should consider current USG policies and guidelines, determine whether they adequately address risks associated with GOF research (in light of potential benefits), and make recommendations that are consistent with that determination. Current policies may be adequate or require only minor changes; alternatively, significant enhancements may be needed. The adequacy of current policy to cover GOF studies may vary by pathogen. Recognizing the paramount importance of ensuring safety, security, and public health, policies should also minimize the burdens placed upon the conduct of science.

8. NSABB recommendations will inform the development of U.S. government policy, which will apply to research funded, conducted, or overseen by the U.S. government either domestically or internationally. NSABB will be mindful in its deliberations of the likelihood that the Board's recommendations and U.S. policy decisions will also influence other governments and non-USG funders of life sciences research.

9. The NSABB will also consider whether there are certain studies that should not be conducted under any circumstances, and if so, articulate the critical characteristics of such studies.

10. Maintaining public trust and confidence in life sciences research is critical and must be taken into account as recommendations are formulated.

4. Analysis

The NSABB working group tasked with developing recommendations on a conceptual approach for evaluating GOF proposals examined three major areas: the current policy landscape for overseeing research involving pathogens, ethical issues associated with funding and conducting GOF studies, and the results of Gryphon's risk and benefit assessments. In addition, the NSABB considered broad stakeholder perspectives through presentations from domestic and international experts at Working Group and full NSABB meetings, expert consultations, individual NSABB member participation in and review of the National Academies workshops and proceedings, analysis of published articles, and comments from attendees at NSABB meetings and public comments submitted to the Board.

4.1. Analysis and Interpretation of the Risk and Benefit Assessment

The NSABB working group has reviewed the risk and benefit assessments (RBA) conducted by Gryphon Scientific, which were designed to evaluate the risks and benefits of GOF research in a manner that encompassed both benign and worrisome aspects of a broader range of GOF studies than those that have raised concern. The RBA analyzed biosafety and biosecurity risks as well as possible benefits. Overall, the RBA includes a commendable amount of sophisticated work and analysis, is generally well-done, and largely achieves the goals it was intended to address. Gryphon's draft RBA report was made publically available in December 2015 and key results were presented and discussed at NSABB and NAS meetings. The final report is available on Gryphon's website.[8]

Strengths of the Risk and Benefit Assessments

The RBA has numerous significant strengths. It is a thorough and extensive analysis of the risks and benefits of GOF work in the context of the guidance provided in the NSABB *Framework for Conducting Risk and Benefits Assessments of Gain-of-Function Research* (May 2015)[9]. It takes into account the principles articulated in the framework and includes the agents, categories of possible risks, types of possible benefits, and possibly concerning scenarios and phenotypes that were laid out in the *Framework*. A few items from the *Framework* were eliminated from consideration at the meeting of the NSABB where the framework was voted on[10], so that the most probable issues of concern could be thoroughly addressed within the available time and resources.

The biosafety risk assessment does a credible job of defining the relative risks associated with potential laboratory accidents involving GOF manipulations of pathogens with enhanced characteristics as

[8] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

[9] Framework for Conducting Risk and Benefits Assessments of Gain-of-Function Research, May 2015. http://osp.od.nih.gov/sites/default/files/resources/NSABB_Framework_for_Risk_and_Benefit_Assessments_of_GOF_Research-APPROVED.pdf

[10] National Science Advisory Board for Biosecurity Meeting, May 5, 2015. http://osp.od.nih.gov/office-biotechnology-activities/event/2015-05-05-120000-2015-05-05-200000/national-science-advisory-board-biosecurity-nsabb-meeting

compared to wild-type pathogens. This analysis is performed in a semi-quantitative way; it uses appropriate, established, peer-reviewed methods to the extent available. The parametric approach employed is powerful and allows consideration of many situations of interest.

The report effectively illustrates that the harmful events being modeled are low probability (see Figures 6.2 and 6.4 in Gryphon's report). Only a small fraction of laboratory accidents would result in a loss of containment; of those, only a small fraction would result in a laboratory acquired infection, and of those, only a fraction would spread throughout the surrounding community (or to the global population). The working group recognizes the challenge of analyzing low-probability, high-consequence events for which little data exists and appreciates attempts to make this point clear in the RBA.

The biosecurity risk assessment is primarily qualitative, and highlights analysis of previous malevolent events and evasions of security systems, likely capabilities and motivations of various possible actors, and an evaluation of the systems in place to prevent biosecurity breaches. Information was obtained from a survey of literature and discussions with biosecurity, intelligence, and law enforcement professionals. It is an extensive gathering of a wide range of information that has not been presented before in one place.

The information risk assessment (an element of the biosecurity risk assessment) is a qualitative analysis of risks that may result from the misuse of information derived from certain GOF studies that might be published in the future. It identifies information that might be attractive to malicious actors and compares it to other sources of information they might find attractive.

The benefits assessment uses a novel approach to assess benefits of GOF studies, a difficult task without much prior methodology to draw upon. The results are not quantitative, and attempts to quantify would have been appreciated. However, as is, the assessment may be the best that can be done with the available information and analytic tools. The benefits assessment effectively analyzed the possible benefits of alternatives to GOF studies and identified areas where GOF research appears to provide unique benefits (i.e., benefits that are not attainable without the use of GOF), either currently or in the near future.

The RBA contains a number of other useful analyses as well, including background and contextual information on the biology of influenza and coronavirus, historical analysis of naturally-occurring seasonal and pandemic influenza and coronavirus outbreaks, an examination of the potential proliferation of GOF research, and analysis of the potential loss of public trust in science that could result if a laboratory incident involving GOF research were to occur. Significantly, the historical analysis notes that each year, influenza infects 5 – 10% of the world's population, resulting in significant morbidity and mortality (up to 500,000 deaths per year). This description of naturally-occurring influenza (and coronavirus) infections helps to establish the extant risks associated with these infectious diseases to which the risks associated with GOF studies might be compared.

Overall, the RBA is comprehensive, objective, reasonable, and generally extensively documented.

Limitations of the Risk and Benefit Assessments

The RBA also has some weaknesses and limitations that should be noted. First, the RBA was limited to the types of labs traditionally funded by the Federal government, which may not be representative of other settings where GOF research may be conducted. Every attempt was made to base the analyses in the RBA on scientific information and data. Nevertheless, data on the properties of the various pathogens being examined, events such as laboratory accidents or security breaches, or possible future acts of terrorism are limited in some cases and unavailable in principle in others. Therefore, assumptions and estimations were necessary. For this reason, the biosafety risk assessment is not fully quantitative, primarily because absolute, quantitative baselines for the risk of work with wild-type pathogens could not be estimated with any certainty. Thus, the data presented are primarily comparative, and provide relative, not absolute values, for the risks associated with laboratory accidents involving GOF studies. Gryphon compared the risks associated with potential lab accidents involving a GOF strain with the risks associated with the same accident involving a wild-type strain. This comparative approach is adequate for some instances but inadequate for others. For instance, an increased risk associated with a GOF study that is relatively large (5-10-fold or greater) may appear significant, but if this increase is in comparison to a very small risk baseline, the overall risk associated with the GOF study may not be significant or concerning. Similarly, small increases in risk over a higher risk baseline, in fact, may be concerning. Additionally, differences in risk that are relatively small (~2-fold) are difficult to interpret because such changes may fall within the limits of uncertainty for the analysis. Attempts to include some absolute baseline estimates of risk (an admittedly difficult task) were included in Section 6.8 of Gryphon's report. However, the lack of comprehensive estimates of baseline risk make interpreting the biosafety risks a challenge.

Given the comparative approach undertaken for the biosafety risk assessment, the implications of the results of this analysis depend a great deal on the wild-type comparator strains that were selected for the analysis. For instance, for pandemic influenza Gryphon initially selected the 1918 influenza strain as the comparator. Gryphon regarded this strain as embodying the maximum risk for influenza, yet a level of risk that is also deemed as acceptable given that research with this strain is permitted. However, using 1918 influenza as the comparator for the analysis compares GOF risks to a relatively high level of baseline risk, making the changes in risk associated with GOF manipulations comparatively small. Utilizing different comparator strains alters the relative risks associated with GOF manipulations; using a high-risk baseline strain may obscure significant risks associated with GOF studies whereas using a low-risk baseline strain may inflate the potential risks associated with GOF studies. **Note to WG: Please review, the previous para was adapted significantly based on Gryphon's new analysis and subsequent discussions.**

Little data exists about the probabilities of the accidents that initiate the chain of events that may lead to a pandemic and therefore, the quantitative probability of these accidents could not be incorporated into the biosafety risk assessment. The modeling of secondary spread of a pathogen through populations once it is released from a laboratory allows for some estimation of the consequences of an event but without a better understanding of the likelihood that an accident would result in loss of containment or a laboratory acquired infection, it is difficult to make judgments about the overall risk.

Gryphon's analysis accounts for this by presenting relative, actuarial risk. However, this approach results in the challenges associated with comparing relative risks described above. There are large uncertainties in most of the input parameters that are the basis for the biosafety risk calculations. Uncertainties about inferring absolute risk from these relative risks exist and should be kept in mind as any conclusions are reached.

The biosecurity risk assessment attempts to examine how GOF studies add to the risk of malevolent acts. Portions of the biosecurity risk assessment focus on GOF studies but others describe the type of threats that could occur against any high-containment laboratory. The semi-quantitative portion of the biosecurity risk assessment estimates the number of infections that could occur if a pathogen with various enhanced characteristics were intentionally released. However, this analysis (see section 7.4.2 and Table 7.7 in Gryphon's report) assumes that 1 or 10 individuals are initially infected as a result of a malicious act with no indication of how likely such an event would be, since there is no way to make such an estimate.

While exhaustively documented, the RBA is not always transparent about data reliability. In particular, interviews were used to gather much critical information, and this was not always well documented in a way that reflects how robust the resulting information may be. For peer-reviewed publications, this is less of a concern.

While evaluation of the benefits of alternatives to GOF studies was extensive, evaluation of risks of alternative approaches was not as thorough. In addition, risks and benefits have not been presented in comparable terms, making it a challenge to determine whether certain risks are justified by potential benefits. Significantly, the benefit assessment is not quantitative and there is no probability analysis or attempt to estimate the likelihood that a certain benefit would be realized or what its impact might be.

Key Results of the Risk and Benefit Assessments

While NSABB has examined all of the analyses in the RBA, some results are important to highlight. In general, the RBA examined risks and benefits associated with the major GOF phenotypes with the intention of identifying types of studies that would be most and least concerning, based particularly on their risk profile.

With regard to biosafety risks, only some potential GOF phenotypes represent substantially increased (5- to 10-fold or more) risks over the starting strain. Two-fold changes most likely fall within the uncertainty of the data, and while small differences might be important if it could be shown that they are significant, this demonstration is probably difficult. For coronaviruses, GOF studies that would create strains with increased transmissibility among mammals may entail significant risks if they also increase human transmission. The risks, were this combination to occur, would include increased probability of an outbreak escaping local control and increased likelihood of global consequences. In addition, experiments that enhance coronavirus growth in culture would likely increase the possibility of laboratory acquired infections.

For seasonal influenza, the GOF-generated phenotypes entailing the greatest risks include enhanced transmission in mammals (assuming this increases transmission in humans), enhanced virulence, and evasion of immunity. Enhanced pathogenicity might significantly increase the global consequences of an outbreak. For pandemic influenza, no GOF-generated phenotypes led to greatly increased risk, but that is based on using 1918 influenza as the comparator; because the risk associated with the wild-type 1918 strain is already so great it is difficult to increase risk substantially. If less transmissible and/or less virulent wild-type strains were used as the basis of comparison, the risks of GOF studies with pandemic strains might appear higher. For avian influenza, the GOF experiments that lead to enhanced transmissibility in mammals (and presumably humans) would likely lead to an increased probability of local and widespread outbreaks, as well as increased global consequences. More subtle aspects of these very general conclusions may be found in the biosafety risk section of the Executive Summary of Gryphon's RBA report.

In general, GOF studies that were not considered by the working group to entail significant risks were those that would: adapt human pathogens to mammals to generate animal models; enhance the growth of attenuated vaccine strains; and antigenic drift or immune evasion studies that are commonly used to guide vaccine selection.

The biosecurity risk assessment shows that the most probable threats involve insiders who have direct access to dangerous pathogens or outsiders who collaborate with or subvert insiders. If currently mandated biosecurity systems are effective, outsiders have little chance of causing harm on their own. The RBA report also concludes that the risks associated with information from future GOF studies with influenza, SARS and MERS appear small; this is because most of the information of interest is already published, or non-GOF information relating to pathogens that are more attractive agents of harm is readily available. However, future scientific advancements could alter this assessment.

Most GOF studies provide benefits in the form of new scientific knowledge, and some of these benefits are unique (i.e., unable to be achieved by alternative, non-GOF approaches). While some GOF studies are likely to provide unique near-term benefits, these are associated with specific agents and phenotypes. With regard to more applied benefits, such as countermeasure development and biosurveillance, the most clear-cut situation is experiments that increase growth of seasonal influenza vaccine candidates in culture; these studies provide unique benefits to current production of seasonal influenza vaccines, and likely will in the future. Another reasonably clear unique benefit is derived from experiments that enhance mammalian pathogenicity for coronavirus as a means of developing animal models for studying disease and developing countermeasures. GOF studies that yield phenotypes that provide unique benefits to countermeasure development include enhanced pathogenicity, evasion of vaccines, and evasion of therapeutics. For several other potential benefits with seasonal influenza, either the potential benefit is long term, or alternative approaches may yield the same or similar benefits. Interestingly, few unique benefits pertaining to GOF studies involving pandemic influenza were identified. There are several types of GOF studies that entail generating avian influenza strains with phenotypes that may be valuable for surveillance and preparedness efforts, although other advances are needed to fully realize such benefits. This point is controversial, with strong proponents and critics. Additionally, a variety of benefits were identified that may also be provided to some extent

by alternative approaches. It should be noted that no attempt was made to provide a probability assessment based on historical data for potential benefits; hence no direct comparison of risk to benefit for a proposed research project is possible.

4.2. Consideration of Ethical Values

The risk and benefit assessments provide information about the potential risks and benefits associated with conducting GOF research. However, determinations about whether such studies should be undertaken will involve value judgments when weighing the risks and benefits. The NSABB identified a number of values (that are applicable to the decisions about whether to fund certain GOF studies and how to oversee them. Sources of these values include the Belmont Report,[11] the literature on public health ethics,[12] and the literature on oversight of emerging technologies,[13] as well as the literature specifically debating appropriate approaches to overseeing DURC and GOF research that has raised concern.[14,15,16,17,18] The commissioned ethics analysis conducted by Dr. Michael Selgelid also describes additional decision-making frameworks and values to be considered.[19]

Note to WG: The decision was made to leave this section here rather than shift to appendix

Substantive values

The following values are important to consider when considering funding of a research proposal involving GOF studies that might entail significant risks.

Non-maleficence: not causing harm. Harm might include: losing lives; causing disease; damage to the economy, national or international security, or agriculture; or loss of public trust in science or governance structures. There are inherent risks associated with research involving pathogens that could result in harm. Approaches aimed at preventing harm and mitigating potential risks should be

[11] The Belmont Report. Office of the Secretary, U.S. Department of Health and Human Services. Ethical Principles and Guidelines for the Protection of Human Subjects Research. The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, April 18, 1979. http://www.hhs.gov/ohrp/humansubjects/guidance/belmont.html
[12] Kass NE. An Ethics Framework for Public Health. *American Journal of Public Health*. 2001;91(11):1776-1782. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1446875/
[13] New Directions. The Ethics of Synthetic Biology and Emerging Technologies. Presidential Commission for the Study of Bioethical Issues, December 2010. http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf
[14] Resnik DB. H5N1 Avian flu research and the ethics of knowledge. Hastings Center Report 2013; 43, 2: 22-33.
[15] Kelle A. Beyond patchwork precaution in the dual-use governance of synthetic biology. Sci Eng Ethics. 2013 Sep;19(3):1121-39.
[16] Kuhlau F, Höglund AT, Evers K, Eriksson S. A precautionary principle for dual use research in the life sciences. Bioethics. 2011 Jan;25(1):1-8.
[17] Biotechnology Research in the Age of Terrorism. The National Academies, 2004. http://www.nap.edu/catalog/10827/biotechnology-research-in-an-age-of-terrorism
[18] Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information. National Science Advisory Board for Biosecurity, June, 2007. http://osp.od.nih.gov/sites/default/files/resources/Framework%20for%20transmittal%20duplex%209-10-07.pdf
[19] Selgelid, Michael. Gain-of-Function Research: Ethical Analysis. December 7, 2015. http://osp.od.nih.gov/sites/default/files/GOF%20%20White%20Paper%20by%20Michael%20Selgelid_0.pdf

considered and applied to the design, conduct, and communication of research involving pathogens in GOF studies.

Beneficence: promoting beneficial outcomes while preventing harmful outcomes; appropriately balancing benefits and risks; formulating policy that maximizes public benefit while minimizing public harm. Benefits might include: saving lives, preventing disease, improving public health; enhancing the economy, national and international security, or public trust in science and governance structures. When the ultimate goals of the research are to improve public health, public health ethics would ask how effective the research is likely to be in achieving those goals, what are the known or potential burdens of the research, can those burdens be minimized, whether there are alternative approaches that are less risky or burdensome, and how can the potential benefits and burdens of the research be fairly balanced. The work of the Presidential Commission for the Study of Bioethical Issues suggests that those formulating and effectuating government policy on scientific research and emerging technologies have a duty of public beneficence – a duty "to promote individual activities and institutional practices…that have great potential to improve the public's well-being," while being "vigilant about risks and harms, [and] standing ready to revise policies that pursue potential benefits with insufficient caution."[20] Both risks and benefits have associated probabilities, magnitudes, and uncertainties. In some instances, it may be justifiable to pursue benefits despite the potential risks; in others, the potential benefits may be foregone due to possible risks.

Social justice: distributing potential benefits and harms fairly (distributive justice) and selecting participants in research fairly, as well as those who may potentially be exposed to risk. There are many different approaches to social justice, such as egalitarianism, utilitarianism, and libertarianism,[21] to name but a few. Decisions about whether to fund research that entails some risk should consider how the risks and benefits associated with conducting that research will be distributed, with an effort to distribute risks and benefits as fairly as possible. When considering pandemic potential, fair distribution of risks and benefits must be considered on a global scale. Those who will potentially be exposed to risk, through participation in research or other avenues of exposure, should be selected equitably.

Respect for persons: allowing competent individuals to make informed choices, and ensuring that the representatives of individuals lacking capacity to choose can make choices in keeping with the wishes, values, or interests of those represented. Autonomy generally requires informing human research participants, laboratory workers, and the public about the risks of research and eliciting their free and uncoerced decision about whether to subject themselves to those risks. In the case of the public, mechanisms for representative decision-making and publicly accountable governance may be needed, as getting consent directly from the members of the public may be impracticable.

[20] New Directions. The Ethics of Synthetic Biology and Emerging Technologies. Presidential Commission for the Study of Bioethical Issues, December 2010. http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf
[21] Nozick R. Anarchy, State, and Utopia. New York: Basic Books, 1974.

Scientific Freedom: avoiding unnecessary interference with scientific research, debate, or publication. Scientific freedom includes an entitlement to avoid interference unless necessary (negative freedom), but not the affirmative right to receive funding or other forms of support for a particular project (positive freedom). Scientific freedom is compatible with norms and regulation to promote the responsible conduct of research and protect participants in research and the public. As a corollary to the principle of scientific or intellectual freedom, the Presidential Commission endorses a principle of regulatory parsimony, requiring "only as much oversight as is truly necessary to ensure justice, fairness, security, and safety while pursuing the public good."[22]

Responsible Stewardship: acting in a way that shows concern for children, future generations, and the environment. The Presidential Commission emphasizes that this is both a domestic and global responsibility that requires "prudent vigilance, establishing processes for assessing likely benefits along with assessing safety and security risks both before and after projects are undertaken."[23]

Procedural Values

The following values apply to the process of decision-making about GOF research and are important to consider when establishing mechanisms to review and/or approve the funding of research proposals involving gain-of-function studies that may entail significant risks.

Public participation & democratic deliberation: making decisions with participation from the public, utilizing respectful debate and inclusive deliberation. Life sciences research is largely a publicly-supported endeavor; therefore, those who allocate funds and conduct life sciences have a responsibility to be good stewards of public funds and to respond to the interests and concerns of the public. Many, if not all, members of society have a stake in the life sciences enterprise and will be affected directly or indirectly by the benefits and risks stemming from such research. This stakeholder community has diverse values and tolerances for risk, which are important to consider when making decisions about funding and overseeing life sciences research. Some forms of public participation include: oversight by the legislative or executive branches of government, public membership and input on government science advisory committees, other mechanisms of public governance, surveys of public opinion on science policy issues, research models such as community-based participatory research, and efforts by scientists and government officials to share information with the public and better understand the public's interests and concerns. The Presidential Commission urges the importance of democratic deliberation, as "[a]n inclusive process of deliberation, informed by relevant facts and sensitive to ethical concerns, promotes an atmosphere for debate and decision making that looks for common ground wherever possible and seeks to cultivate mutual respect where irreconcilable differences remain."[24]

[22] New Directions. The Ethics of Synthetic Biology and Emerging Technologies. Presidential Commission for the Study of Bioethical Issues, December 2010. http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf, p5.
[23] Ibid., p5.
[24] Ibid., p5.

Accountability: taking responsibility for one's actions and being prepared to justify or explain them to others. It is important that decisions to fund research are justifiable to the public and others. Decisions should be justified in terms of substantive and procedural values.

Transparency: sharing with the public the information and assumptions used to make a decision, including uncertainties, controversies, and limitations of analyses. Transparency is an important part of accountability and public participation. It allows review and reconsideration of policy over time as new facts emerge and analysis evolves.

4.3. Decision-Making Strategies and Frameworks for Evaluating and Managing Risks and Developing Policy

NOTE TO WG: The policy approaches and decision-making frameworks were combined and left here, rather than moving to an appendix

The NSABB working group identified a number of approaches or frameworks that may be used to guide the making of complex decisions with ethical implications, particularly in the face of uncertainty. These may also be used in developing policies such as that for managing GOF research. Different strategies reflect different attitudes toward risk-taking. Some may be more appropriate in some situations than others. The NSABB working group examined a number of such strategies as it attempted to determine the best option as relates to GOF research that has raised concerns. These options are not mutually exclusive, and elements from more than one may be used together to develop a path forward. The following are decision-making frameworks that were considered.

Maximax: This involves choosing the option with the best possible outcome. Maximax is a relatively simple strategy that focuses on choosing the option with the best possible outcomes While maximax may be appropriate for making some types of personal choices (e.g. playing games with nothing of value to lose), it may not be appropriate for making science and technology policy decisions because most people would want to take appropriate steps to prevent or mitigate risks regardless of benefits. For GOF studies, use of maximax would involve identifying research with the best possible benefits, generally regardless of risks.

Maximin: This involves choosing the option with best outcome among the worst possible outcomes. Maximin is a risk-averse approach because it aims to avoid the worst possible outcomes. Maximin is another relatively simple approach, but may present difficulties in making science and technology policy decisions, because it would recommend not developing a new technology if this decision could lead to the worst possible outcome. Since all technologies (and scientific ideas) can conceivably lead to good and bad outcomes, strict adherence to maximin would imply a very cautious approach to science and technology development. For GOF studies, use of maximin would involve identifying studies with risks, and choosing the least risky regardless of benefits.

Expected Utility Theory: This involves choosing the option that maximizes expected utility, where expected utility for a possible outcome = probability x utility. Expected utility theory involves a quantitative balancing of risks and benefits and is inherently a more complex process. Cost-benefit

analysis in economics is a form of expected utility theory. A problem with expected utility theory is that sufficient evidence may not always be available to confidently estimate the probabilities involved in the utility calculus. When this is the case, other approaches may be appropriate. For GOF studies, use of expected utility theory would require determining quantitatively the likelihood of risks and benefits and calculating the resulting utility.

Precautionary approach: This approach involves taking reasonable measures to prevent, minimize, or mitigate risks that are significant and plausible. A measure is "reasonable" if it: 1) appropriately balances the values at stake in the risk management; 2) is proportional to nature of the risk (i.e. greater risks require stronger measures); and 3) is likely to be effective. A risk is "plausible" if there is some scientific evidence that it could occur even if the probability of the risk cannot be confidently estimated. There are many versions of the precautionary principle, including ones that are more or less risk-averse.[25,26] A precautionary approach, in general, would limit an activity unless the environment, health, or security, are clearly protected. This approach can recognize a potential problem early and prevent harm from occurring but may lead to regulatory burdens or unnecessarily limit activities. This approach might restrict potential GOF research unless the studies are demonstrated to be safe.

Permissive approach: This approach, in general, would allow an activity unless the environment, health, or security, are clearly compromised. This approach may reduce unnecessary regulatory burdens but can result in after-the-fact reaction to harms. This approach might allow certain GOF studies to proceed until they are demonstrated to entail significant risk.

Planned adaptation or risk-based approach: This approach provides a systematic way to deal with managing risks in the face of uncertainty. It involves: 1) preparation to identify the risks and regulatory gaps, including getting input from a broad range of perspectives; 2) putting measures in place to control risk based on the best information available at the time; 3) systematically gathering data and observing effects of policies; and 4) updating and revising policy as needed. An example of an adaptive approach is the life cycle approach taken by the Food and Drug Administration when making decisions about whether to approve drugs, when that includes post-market surveillance.[27] For GOF studies, this approach might entail allowing GOF studies of potential concern—or certain GOF studies—to proceed under defined conditions, then evaluating the risk-benefit landscape periodically to determine whether the GOF studies that are permitted should continue, be expanded, or be restricted.

Threshold approach: This approach would entail identifying a risk threshold beyond which, certain studies are given special attention or subject to additional scrutiny or oversight and might preclude certain studies. Implementation would involve defining or describing the studies that would require

[25] Resnik DB. Environmental Health Ethics, New York: Oxford University Press, 2013.
[26] Munthe C. The Price of Precaution and the Ethics of Risks. Dordrecht: Springer, 2011.
[27] FDA determinations about whether a new drug is safe and effective are complex, address uncertainty, and involve ongoing monitoring to assess risks and benefits and take appropriate post-marketing actions as necessary. See: *Structured Approach to Benefit-Risk Assessment in Drug Regulatory Decision-Making*, 2013
http://www.fda.gov/downloads/ForIndustry/UserFees/PrescriptionDrugUserFee/UCM329758.pdf

additional oversight as well as a description of what that oversight would entail. This approach would allow for the identification of studies of concern but might need to be reevaluated if the risk landscape changes and the threshold that was identified is no longer appropriate. For GOFROC, this would entail identifying the characteristics of studies involving significant risks that may not be adequately managed and then stipulating further oversight or determining that they should not be conducted.

Point-source approach: This approach would involve controlling where certain studies are conducted and under what conditions. This approach would centralize certain research activities, restricting them to designated locations or facilities. For GOFROC this might involve requiring that certain studies only be conducted in facilities with certain biocontainment conditions, biosafety practices, and security measures.

The NSABB working group used ideas from a number of frameworks to inform its findings and deliberations (Sections 5 and 6). The criteria for identifying GOF research of concern (see Recommendation 1) reflect a threshold approach. The principles for guiding funding decisions for GOF research of concern entails elements from several of the decision frameworks above. For instance, an explicit call for a risk-benefit analysis (Recommendation 1, Guiding Principle 3) reflects expected utility theory, however, a strict quantitative calculation is probably not possible. The principles to guide funding decisions that call for risk mitigation and a restriction to laboratories with a demonstrated capacity to safely carry out the studies (Recommendation 1, Guiding Principles 4 and 5) incorporate elements of point-source and precautionary approaches. An adaptive approach was considered particularly attractive and appropriate for policies aimed at providing oversight of GOF research (see Recommendation 3).

4.4. Examination of the Current Policy Landscape

Many Federal agencies fund life sciences research in furtherance of their specific missions. In general, research supported by the USG is founded on the principle of scientific merit and goals of the funding agency. Multiple complementary layers of oversight are in place to manage laboratory and other risks associated with Federally-funded life sciences research. These policies are intended to provide oversight at various points throughout the research life cycle, from research conception to its publication and translation into practice. These policies include a foundation of occupational health and medicine (for laboratory and clinical workers), laboratory biosafety practices, and policies that address biosecurity risks. Below is a description of the oversight policies in place for Federally-funded life sciences research involving pathogens, with discussion of whether and how such policies apply to GOF studies. This analysis is illustrated in Figures 2 and 3 and summarized in Appendix D.

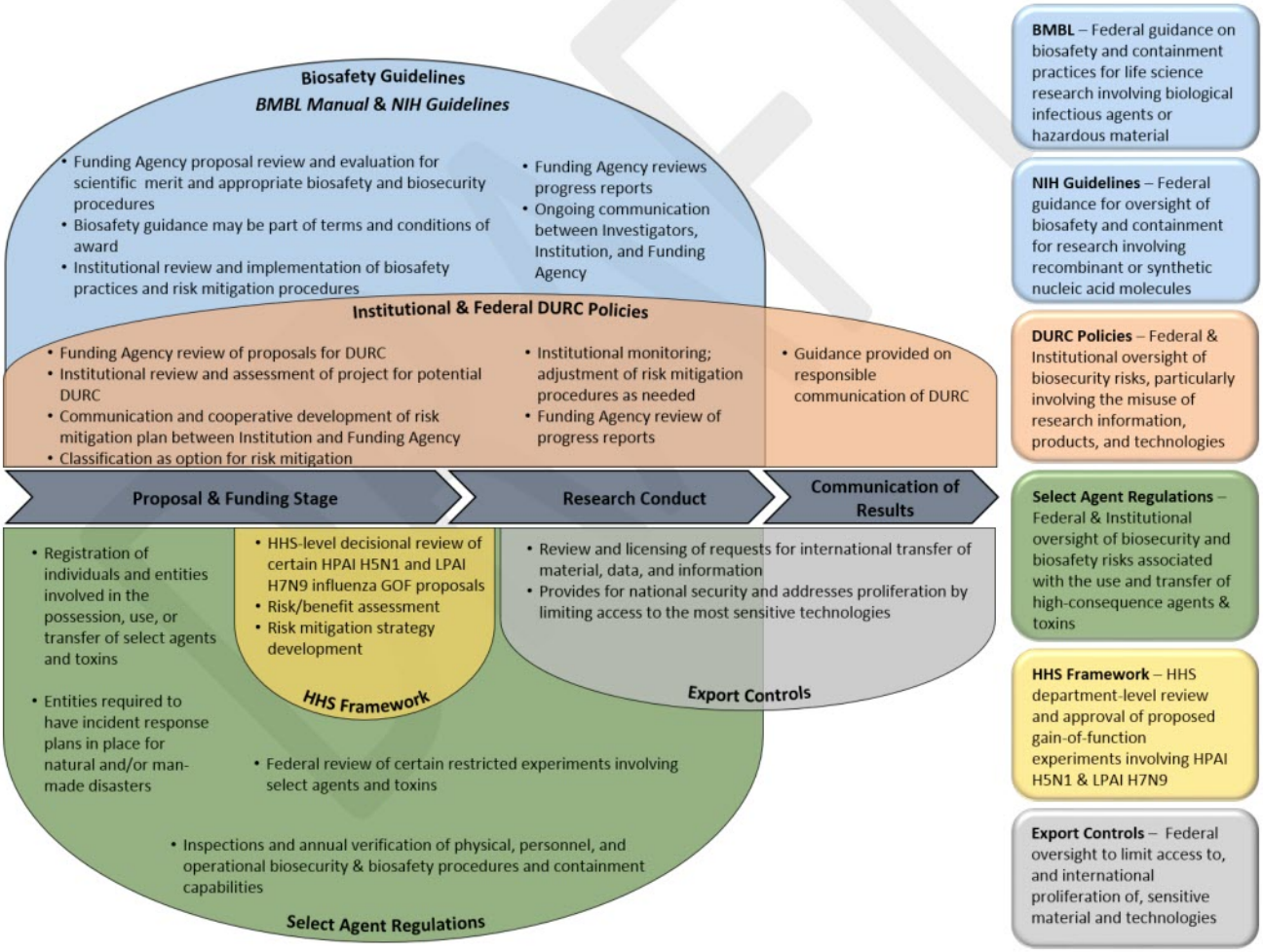

Figure 2. U.S. government oversight of life sciences research involving pathogens. Oversight policies apply at different stages and occur at different levels throughout the research life cycle. See text and Appendix D for descriptions of each policy. The policies depicted in this figure are defined by different applicability and scope requirements and therefore do not apply to all life sciences (or GOF) research projects.

Scientific Merit Review

Departments and agencies within the U.S. government fund diverse portfolios of life sciences research. Funding decisions are based on the scientific merit of a given proposal and the ability of a project to advance the agency's strategic mission. The U.S. government funds life sciences research through a variety of mechanisms including grants, contracts, and cooperative agreements. Each funding agency has its own processes for evaluating research proposals and awarding funds but, in general, proposals are subject to rigorous scientific review by Federal agency staff and often, scientific peers. NIH grant proposals, for example, undergo two levels of review. The first evaluation is by a panel of scientific peer reviewers who score proposals based on scientific merit and other criteria. The second round of review includes discussion of meritorious proposals at public meetings of advisory councils, specific to individual funding institutes and centers within NIH, to determine how proposals fit within their broader strategic objectives.

Biosafety Oversight

Oversight of pathogen research focuses first on ensuring the safe handling of biological agents through appropriate biosafety practices and containment measures, which are addressed by the *Biosafety in Microbiological and Biomedical Laboratories* (*BMBL*)[28], the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*)[29], and other documents. The BMBL and the *NIH Guidelines* provide for Federal and institutional biosafety oversight and guidance involving biosafety practices and containment features that are based on risk assessments for specific projects. Such determinations are typically made at the institutional level and are guided by Federal guidelines and policies, which are updated as necessary to provide additional guidance for research involving emerging pathogens or technologies. Biosafety is achieved by conducting research under appropriate physical and biological containment levels and employing practices that help to ensure a safe working laboratory environment.

The BMBL is a CDC-NIH guidance document that is generally considered the authoritative reference for laboratory biosafety. The BMBL provides summary statements for many bacterial, fungal, parasitic, rickettsial, viral, and other agents. These statements describe the characteristics of the pathogen, its natural mode of infection, potential occupational hazards with the agent, and recommendations for laboratory safety and containment. It also describes the fundamentals of biological containment, which includes descriptions of proper microbiological practices, safety equipment, and facility safeguards that protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory. It describes the process of biological risk

[28] Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition. http://www.cdc.gov/biosafety/publications/bmbl5/

[29] The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), November 2013. http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html

assessment, which enables the appropriate selection of microbiological practices, safety equipment, and facility safeguards that can prevent laboratory-associated infections. It also describes occupational health, immunoprophylaxis, and principles for laboratory biosecurity. The BMBL is updated periodically to refine guidance based on new knowledge and experiences and to address contemporary issues that present new risks that confront laboratory workers and the public health.

Analysis: The BMBL does not address GOF studies *per se* but does include summary statements and biocontainment guidance for research involving various influenza strains (including contemporary and non-contemporary human, high and low pathogenic avian, swine, the 1918 influenza strain, and reassortant viruses) and SARS-CoV. MERS-CoV had not emerged at the time of the last BMBL update, but interim laboratory biosafety guidance was issued by CDC.[30]

The BMBL is not a regulatory document. U.S. funding agencies may require it be followed as a term and condition of awards but in general, compliance with the BMBL is voluntary. In addition, the BMBL provides general biosafety guidance but does not describe detailed procedures or experiment-specific containment protocols.

The *NIH Guidelines* specify the practices for safely constructing and handling recombinant nucleic acid molecules; synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules; and cells, organisms, and viruses containing such molecules. The *NIH Guidelines* apply to basic and clinical recombinant or synthetic nucleic acid research conducted at or sponsored by institutions that receive NIH funding for any such research. Compliance with the *NIH Guidelines* is typically required as a term and condition of award of funding. Other Federal agencies may also require compliance with the *NIH Guidelines*.

The *NIH Guidelines* focus on the concepts of risk assessment, risk group classification of agents based on their ability to cause disease in humans and the availability of medical countermeasures, physical and biological containment levels, practices, personal protective equipment, and occupational health. To help ensure the safe conduct of this research, the *NIH Guidelines* specifies roles and responsibilities of investigators and institutions. Institutions subject to the *NIH Guidelines* must establish Institutional Biosafety Committees (IBCs) composed of members with appropriate expertise, to review and approve such research. IBCs provide local oversight and ensure compliance with the *NIH Guidelines*. Certain higher risk experiments require review by the Recombinant DNA Advisory Committee (RAC)[31] and specific approval by the NIH Director as Major Actions. These experiments involve the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if

[30] Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Middle East Respiratory Syndrome Coronavirus (MERS-CoV) – Version 2. http://www.cdc.gov/coronavirus/mers/guidelines-lab-biosafety.html [last updated June 18, 2015]

[31] The Recombinant DNA Advisory Committee (RAC) is a federal advisory committee that provides recommendations to the NIH Director related to basic and clinical research involving recombinant or synthetic nucleic acid molecules. See: http://osp.od.nih.gov/office-biotechnology-activities/biomedical-technology-assessment/hgt/rac

such acquisition could compromise the ability to control disease agents in humans, veterinary medicine or agriculture.

In order to continue to provide appropriate guidance for emerging pathogens or experimental approaches, the *NIH Guidelines* are updated periodically. The *NIH Guidelines* have been amended to include additional guidance for work with Risk Group 3 influenza viruses (1918 H1N1, H2N2, highly pathogenic avian influenza (HPAI) H5N1), to specify enhancements to biosafety level 3 containment, practices, and to incorporate occupational health requirements. In 2012, the *NIH Guidelines* were amended again to require further enhancements to facilities, biosafety equipment and practices, including occupational health practices, for research involving HPAI H5N1 strains transmissible among mammals by respiratory droplets.

Analysis:

The *NIH Guidelines* provide guidance on risk assessment and appropriate containment and practices for conducting research involving recombinant or synthetic nucleic acids, which would apply to most government-funded GOF research. Some IBCs also review and approve non-recombinant pathogen research; however, not all institutions require their IBCs to do so. While the *NIH Guidelines* are often used as a model of biosafety guidance by the broader scientific community, compliance is required only by institutions receiving such funding from the NIH. Therefore, some GOF studies may not be subject to the *NIH Guidelines* depending on whether the institution where the research is being conducted is subject to the *NIH Guidelines*.

The Federal Select Agent Program

Subtitle A and B of the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 requires the U.S. Departments of Health and Human Services (HHS) and Agriculture (USDA) to establish and regulate a list of select agents, biological agents and toxins that have the potential to pose a severe threat to public health and safety or animal or plant health or animal or plant products. The Select Agent Program (SAP) is administered jointly by the HHS Centers for Disease Control and Prevention and USDA Animal and Plant Inspection Service. The SAP oversees the possession, use and transfer of biological select agents and toxins. The Select Agents and Toxins List is reviewed and updated biennially. Under the select agents regulations, individuals and institutions that possess, use, or transfer any select agent are required to be registered, follow appropriate biosafety procedures, and undergo periodic inspections. Individuals must be registered with the SAP to have access to select agents or toxins, which requires that they undergo a security risk assessment performed by the Federal Bureau of Investigation (FBI). There are legal penalties for failing to comply with the select agent regulations.

In addition to the agents and toxins on the list, the select agent regulations apply to some genetic elements, including nucleic acids that are immediate precursors to infectious forms of any select agent viruses (i.e., complete positive strand RNA viral genomes), as well as some nucleic acids that encode select toxins. Select agent regulations also apply to genetically modified select agents and toxins.

Restricted experiments are described in the regulations and involve the deliberate transfer of or selection for a drug resistance trait to select agents that are not known to acquire the trait naturally. If the acquisition of resistance is to a first-line drug that could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, the restricted experiment requires special review and approval by the SAP. Some attenuated strains of select agents may be excluded from the regulations based upon a determination that the attenuated strain or modified toxin does not pose a severe threat to public, plant, or animal health or safety. The Intragovernmental Select Agent and Toxin Technical Advisory Committee serves as an advisory group to the SAP. In the wake of the recent laboratory incidents at Federal facilities involving select agents, two advisory committees have issued recommendations for ways to strengthen the Select Agent Program.[32] [33] Plans to implement these recommendations are also in place.[34]

Analysis: Studies that could be considered GOF studies are subject to oversight by the SAP if they involve pathogens on the select agent list. Researchers and institutions performing such studies must receive favorable security risk assessments by the FBI, register with the SAP, receive training on the proper procedures and practices for handling such agents, and abide by other aspects of the regulations. SARS-CoV, HPAI H5N1 influenza, and 1918 influenza viruses are select agents and GOF studies involving these pathogens are subject to oversight by the SAP. Restricted experiments that would entail conferring antiviral resistance to these viruses would require additional review and approval prior to being conducted. However, MERS-CoV is not a select agent. GOF experiments involving MERS, and other agents not included on the select agent list, would not be subject to oversight by the SAP (though they could be subject to Federal and institutional biosafety oversight). The SAP is underpinned by a regulatory requirement that applies to non-USG funded (i.e., private sector funded) pathogen research.

Federal and Institutional Oversight of Life Science Dual Use Research of Concern

The U.S. government has issued two Federal policies for the oversight of life sciences DURC. These policies focus oversight on research involving 15 high-consequence pathogens and toxins[35] that involve seven categories of experimental activity, which are projects that can be reasonably anticipated to:

1. Enhance the harmful consequences of the agent or toxin;
2. Disrupt immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification;

[32] Report of the Federal Experts Security Advisory Panel, U.S. Government, December 2014.
[33] Fast Track Action Committee Report: Recommendations on the Select Agent Regulations Based on Broad Stakeholder Engagement, U.S. Government, October 2015.
[34] Lisa Monaco and John Holdren White House Memorandum, October 29, 2015, Next Steps to Enhance Biosafety and Biosecurity in the United States. https://www.whitehouse.gov/sites/default/files/docs/10-2015_biosafety_and_biosecurity_memo.pdf
[35] The agents within the scope of the USG DURC policies are the 13 Tier 1 select agents plus HPAI H5N1 and 1918 influenza virus.

3. Confer to the agent or toxin resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies;
4. Increase the stability, transmissibility, or the ability to disseminate the agent or toxin;
5. Alter the host range or tropism of the agent or toxin;
6. Enhance the susceptibility of a host population to the agent or toxin; or
7. Generate or reconstitute an eradicated or extinct agent or toxin listed above.

Projects involving any of the 15 agents and that could be anticipated to involve any of these seven experimental effects are then determined to be DURC if they then meet the definition of DURC listed in the policy.[36]

The DURC policies outline a coordinated approach to oversight involving the Federal funding agencies and institutions that conduct such research. The policy for Federal oversight, issued in March 2012, requires Federal agencies to review proposed and ongoing research projects to identify any that constitute DURC. The policy for institutional oversight, issued in September 2014, articulates responsibilities of research institutions in identifying and managing DURC. Research institutions are to establish an Institutional Review Entity (IRE) to review research subject to the policy to determine whether any such research involves any of the seven experimental effects, and if so, whether the research constitutes DURC. IREs may review projects not specifically covered under the DURC policies but such additional reviews are voluntary.

When DURC is identified—either by a funding agency or a research institution—the funder and institution are to work collaboratively to develop a risk mitigation plan to help ensure that the research is conducted and communicated in a responsible manner. DURC risk mitigation plans are approved by the Federal funding agency and are reviewed on an annual basis by the funder and the institution. Specific risk mitigation measures may be incorporated into a term of award. Risk mitigation may involve modifying the design or conduct of the research in order to address the same scientific question in a manner that poses fewer biosafety or biosecurity risks. Other measures may involve applying enhanced biosafety or biosecurity measures, evaluating the effectiveness of extant medical countermeasures prior to proceeding with particular studies, or establishing a more frequent schedule of DURC reviews to more closely monitor the research as it evolves. It is also expected that a communication plan is established to ensure that DURC is communicated in a responsible manner. Federal funding agencies can provide advice and guidance on responsible communication, but recommendations on how to communicate research typically are not binding; ultimately, investigators and journal editors decide on how to communicate the research.

[36] The definition of dual use research of concern listed in the USG Policy for Oversight of Life Science DURC (USG, March 2012) and the USG Policy for Institutional Oversight of Life Sciences DURC (USG, September 2014) is "Life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security."

Analysis: Some of the seven experimental effects within the scope of the DURC policies could be considered GOF studies. However, GOF projects that might involve these effects are only subject to DURC oversight if the study involves one of the 15 agents listed in the policy. Only two influenza viruses are listed within the scope of these policies; SARS and MERS coronaviruses are not listed.[37] The DURC policies are also inherently subjective. While the list-based approach clearly delineates projects that are subject to oversight, the definition of DURC, and to a lesser extent, the seven experimental effects, all require significant judgment and interpretation.

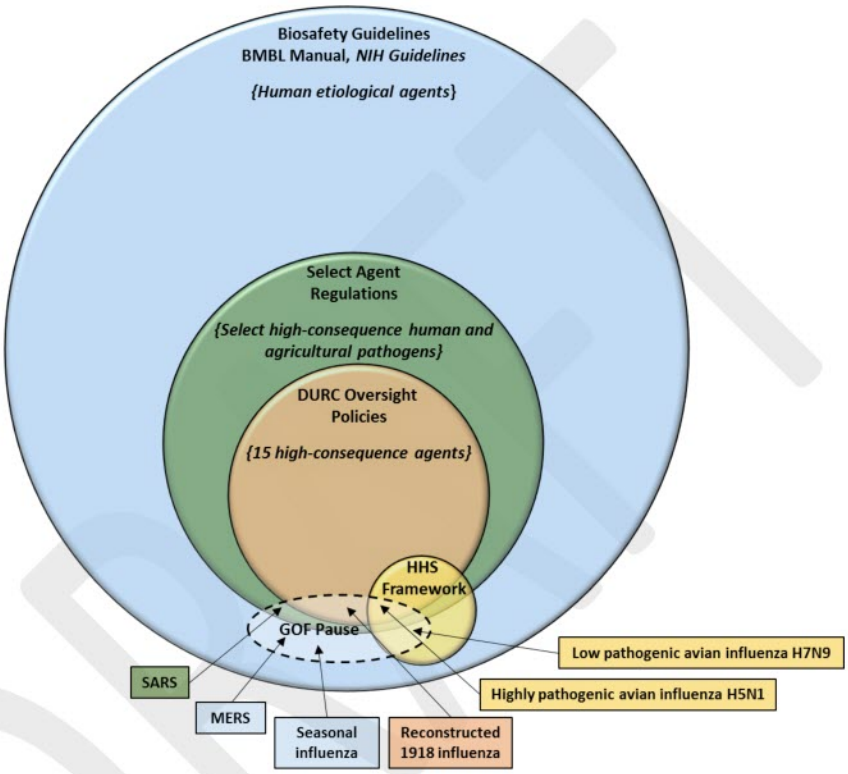

Figure 3. Comparison of the scope of different policies for the oversight of life sciences research involving pathogens. Oversight policies apply to research involving specified agents or procedures. GOF studies involving pathogens or manipulations covered under a given policy would be subject to oversight described by that policy.

Federal-Level Review of Certain Gain-of-Function Studies

The only U.S. Federal policy that specifically addresses GOF studies is the *Framework for Guiding U.S. Department of Health and Human Services Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets (HHS Framework)*, issued by the U.S. Department of Health and

[37] The policy for Federal DURC oversight requires Federal funding agencies to compile biannual inventories of projects identified as being subject to DURC oversight. As part of this process, Federal agencies have been identifying projects involving MERS and LPAI H7N9 influenza and proactively managing risks associated with those projects, as necessary.

Human Services in February, 2013. Under the *HHS Framework*[38,39] certain proposals with the potential for generating highly pathogenic avian influenza H5N1 viruses that are transmissible among mammals by respiratory droplets receive special review and approval before being funded by HHS. This policy was subsequently expanded to include review of similar proposals involving low pathogenic avian influenza H7N9 virus.[40]

Funding agencies within HHS (including NIH, CDC, and FDA) review relevant proposals for risks and benefits, and refer relevant studies to a Department-level review group, the HHS HPAI H5N1 Gain-of-Function Review Group, for advice prior to funding the proposal. The review group includes a wide range of interdisciplinary expertise from across HHS and the Federal government, if necessary. HHS reviews GOF research proposals that are subject to the *HHS Framework* and makes recommendations to HHS funding agencies about whether the study is acceptable for funding and whether additional measures may be needed to mitigate risks. HHS considers a number of factors including the following criteria, which must be met in order for a GOF study to be acceptable to receive HHS funding:

1. The virus anticipated to be generated could be produced through a natural evolutionary process;
2. The research addresses a scientific question with high significance to public health;
3. There are no feasible alternative methods to address the same scientific question in a manner that poses less risk than does the proposed approach;
4. Biosafety risks to laboratory workers and the public can be sufficiently mitigated and managed;
5. Biosecurity risks can be sufficiently mitigated and managed;
6. The research information is anticipated to be broadly shared in order to realize its potential benefits to global health; and
7. The research will be supported through funding mechanisms that facilitate appropriate oversight of the conduct and communication of the research

Analysis: The *HHS Framework* requires an explicit consideration of the risks and benefits associated with certain GOF studies prior to making a funding decision. This allows HHS to identify potential risks up front and make recommendations about risk mitigation—including consideration of alternative approaches or modifying the experimental design—at the outset. This review process also involves broader expertise including, ethical, legal, security, intelligence, and more. The criteria that must be met in order to receive funding are subject to judgment and interpretation. The scope of the *HHS Framework* is quite narrow and currently covers only projects involving two influenza viruses and that involve one specific experimental outcome (mammalian transmission by respiratory droplets); other GOF studies do not receive this pre-funding review.

[38] *A Framework for Guiding U.S. Department of Health and Human Services Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets*, U.S. Department of Health and Human Services, February, 2013. http://www.phe.gov/s3/dualuse/Documents/funding-hpai-h5n1.pdf

[39] Patterson, AP, et. al. A Framework for Decisions about Research with HPAI H5N1 Viruses. Science. 2013 Mar 1: 339(6123): 1036-1037.

[40] Jaffe H., et. al. Extra Oversight for H7N9 Experiments. Science. 2013 August 16: 341(6147):713-714.

Reviews under this framework are conducted by a group internal to the USG. Reviewing GOF studies in a confidential setting allows for the examination of potentially sensitive scientific, proprietary, and personal information, and allows discussions that may be sensitive from a national security or public health preparedness perspective. However, such reviews do not achieve the level of transparency desired by some stakeholders and also make it difficult to independently assess the effectiveness of the review process. Finally, the *HHS Framework* was in place for less than two years when the October 2014 funding pause was enacted and only a handful of GOF projects have been reviewed to date, making it difficult to fully evaluate this policy's strengths and limitations.

In response to the funding pause[41], the National Institute for Allergy and Infectious Diseases (NIAID), within the NIH, developed a process for considering on a case-by-case basis studies that might be subject to the GOF pause. Reviews by NIAID include a detailed consideration of the science, often including a specific examination of the viral strains in question and specific experiments being proposed. NIAID begins by consulting the investigators and an internal NIAID group determines whether the projects are subject to the pause. When identifying projects subject to the funding pause, NIAID has used a fairly broad interpretation of the language set forth in the pause statement and paused, at least initially, more projects than were ultimately determined to meet the scope of the pause policy. NIAID also sought exceptions (using a mechanism provided for in the USG's moratorium statement) for projects that were deemed critical to public health or national security. In determining whether an exception to the pause might be warranted, NIAID considers the intent of the research, the availability of countermeasures, potential alternative approaches, the risks of not conducting the research, and the available mechanisms for ongoing oversight. Exceptions may only granted by the NIH Director.

Analysis: NIAID's process for identifying GOF projects that are subject to the funding pause is rigorous and serves as an example of Federal-level identification and review of GOF studies of potential concern. It includes extensive scientific review and is performed by individuals with experience reviewing projects for DURC potential. It does not involve the same expertise that is provided under *HHS Framework* reviews such as national security, ethics, or legal. Given the limited number of projects that have been examined by NIAID it is difficult to fully evaluate how effective this approach is.

Sharing and Communicating Scientific Findings and Research Products

The majority of life sciences research is conducted in academic settings and the results are communicated openly in scientific journals and public forums. For a small subset of research with national security implications, there are policies in place to restrict access to scientific information or products. Under National Security Decision Directive (NSDD) 189, dissemination of fundamental research is to remain unrestricted to the maximum extent possible and in instances where restriction is

[41] U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses, U.S. Government, October 17, 2014. http://www.phe.gov/s3/dualuse/documents/gain-of-function.pdf

necessary for national security, classification is to be the appropriate mechanism for restricting access.[42] Life sciences research that requires classification is classified at its outset and conducted in designated facilities that are equipped with the infrastructure and personnel with appropriate level national security clearances to perform the research. Retroactively classifying research that was conducted in an unclassified setting is immensely challenging and may be unfeasible.

Export controls are Federal regulations that restrict exports that have national security or foreign policy implications. Certain materials and information related to biological agents and genetic elements, vaccines, equipment, and related technologies are covered by export control regulations. Furthermore, the transfer of controlled information to a foreign national within the United States is considered to be an export to that foreign national's country. The regulations are complex but, in general, they specify which items, when shipped to which destinations, will require export licenses. Life sciences research that is openly published is not subject to export controls, but information that is withheld from publication by the investigator or research institution based on security concerns may become subject to export control regulations, and an export license may be required before that information can be shared with foreign nationals.

Most biological research activities that are subject to export controls fall under the Department of Commerce's Export Administration Regulations, which control items that have both military and civilian applications.[43] However, some might fall under the jurisdiction of the State Department's International Traffic in Arms Regulations.[44]

A number of scientific journals and families of journals have policies for identifying and reviewing manuscripts that raise biosecurity and biosafety concerns. These efforts are commendable but some have noted the challenges associated with trying to identify DURC or implement risk mitigation measures at the publication stage.[45,46] NSABB has previously developed strategies and a risk assessment tool to assist in the development of a responsible communication plan for DURC, which might include altering the content, distribution, or timing of a publication.[47] The U.S. government, in most cases, has no authority to mandate redaction, restriction, or classification of a scientific publication that it does not

[42] NSDD 189 (September 21, 1985) defines fundamental research as "basic and applied research in science and engineering, the results of which ordinarily are published and shared broadly within the scientific community, as distinguished from proprietary research and from industrial development, design, production, and product utilization, the results of which ordinarily are restricted for proprietary or national security reasons." https://research.archives.gov/id/6879779

[43] Export Administration Regulations, 15 CFR Parts 730, 734, 736, 742, 744, and 745. https://www.bis.doc.gov/index.php/regulations/export-administration-regulations-ear

[44] International Traffic and Arms Regulations, 22 U.S.C. 2778 https://www.pmddtc.state.gov/regulations_laws/itar.html

[45] Casadevall A et al. Dual-Use Research of Concern Review at American Society for Microbiology Journals. mBio 6(4):e01236-15. 2015.

[46] Atlas et. al. Journal editors and authors group statement on scientific publication and security. Science, 299:1149. 2003.

[47] Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information. NSABB, June, 2007. http://osp.od.nih.gov/sites/default/files/resources/Framework%20for%20transmittal%20duplex%209-10-07.pdf

own or control, and the development of a mechanism for restricting communication of unclassified information to only those who require access, remain challenging and to date unsuccessful.[48]

Analysis: Once a study has been completed, it is difficult to limit the distribution of or access to the findings, particularly if the study was conducted in an open, academic environment. Oversight of DURC, and in particular GOF studies involving pathogens with pandemic potential, may be most feasible and effective if it occurs 1) upstream (i.e., during the review of proposed studies and before experiments are initiated) and 2) in an ongoing manner while the research is being conducted.

Classification may be an option for certain GOF studies, but this would entail that these studies be conducted in significantly different settings than they are conducted currently. Further, although certain GOF studies have raised concerns about whether they should be published, it is unlikely that such manuscripts would meet the criteria for classification under U.S. government classification authorities. It is conceivable that certain studies should not be undertaken at all or not published because of unanticipated findings. However, it may be very difficult to predict at the proposal stage whether findings of concern might arise during the experiment, and unanticipated findings that raise concern may be unavoidable. Individual investigators or journal editors have, on security grounds, decided to redact certain material from publication, possibly triggering export controls on the redacted material, but in general such a redaction could not be mandated by the U.S. government.

Broader U.S. Biosafety and Biosecurity Efforts

In parallel to the GOF deliberations, the USG has also initiated additional, broader reviews of biosafety and biosecurity policies and procedures following a series of laboratory incidents occurring at federal institutions in 2014 **[REF needed]**. The Holdren-Monoco memorandum[49] called for Federal and non-Federal reviews to provide recommendations to strengthen the biosafety and biosecurity practices and oversight system for USG funded research. The memo outlined three immediate actions for Federal Agencies:

1. Conduct a comprehensive review of current biosafety and biosecurity protocols to ensure adequacy and appropriateness for today's infectious disease research
2. Inventory and document culture collections
3. Increase attentiveness throughout research community to ensure the safety of laboratory workers and the American public.

In September 2015, The White House National Security Council tasked the Federal Experts Security Advisory Panel (FESAP) to 1) identify needs and gaps and make recommendations to optimize biosafety, biosecurity, oversight, and inventory management and control for biological select agents and toxins (BSAT); 2) identify actions and any regulatory changes to improve biosafety and biosecurity; and 3) identify an approach to determine the appropriate number of high-containment U.S. laboratories

[48] Research information produced under a U.S. government grant is not considered to be owned or controlled by the Federal Government. However, under the Invention Secrecy Act, the U.S. government can nevertheless impose secrecy orders on patent applications if the publication or disclosure of the ensuing patent would be detrimental to national security.
[49] https://www.whitehouse.gov/sites/default/files/microsites/ostp/enhancing_biosafety_and_biosecurity_19aug2014_final.pdf

1053 required to possess, use, or transfer BSAT. To obtain broad stakeholder recommendations, the National
1054 Science and Technology Council established the Fast Track Action Committee on Select Agent
1055 Regulations (FTAC-SAR). In October 2015, USG released the FESAP and FTAC-SAR recommendations[50]
1056 that address the culture of responsibility, oversight, outreach and education; applied biosafety research;
1057 incident reporting; material accountability; inspection processes; and regulatory changes and guidance
1058 to improve biosafety and biosecurity. The USG has developed a plan to implement these
1059 recommendations in order to improve biosafety and biosecurity practices along with oversight.[51]

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[50] http://www.phe.gov/s3/Documents/fesap.pdf; http://www.phe.gov/s3/Documents/ftac-sar.pdf.
[51] Implementation of Recommendations of the Federal Experts Security Advisory Panel and the Fast Track Action Committee on Select Agent Regulations, October 2015. http://www.phe.gov/s3/Documents/fesap-ftac-ip.pdf

5. Findings

In developing the findings below (Box 2), the NSABB working group considered the results of (i) the risk and benefit assessments, (ii) policy analysis and decision-making frameworks, (iii) discussions of ethics, and (iv) perspectives of domestic and international stakeholders.

NOTE: Box to be updated as Findings are finalized.

Box 2. Summary of Key Findings

Key Finding 1: There are many types of GOF studies and not all of them have the same level of risks. Only a small subset of GOF research—GOF research of concern (GOFROC)—entail risks that are potentially significant enough to warrant additional oversight.

Key Finding 2. The U.S. government has several policy frameworks in place for identifying and managing risks associated with life sciences research. There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOFROC could be applied.

Key Finding 3. Oversight policies vary in scope and applicability, and are not sufficiently harmonized; therefore, current oversight is not sufficient for all GOF studies that raise concern.

Key Finding 4. An adaptive policy approach is a desirable way to ensure that oversight and risk mitigation measures remain commensurate with the risks associated with the research and the benefits of the research are being fully realized.

Key Finding 5. There are life sciences research studies, including possibly some GOFROC, that should not be conducted on ethical or public health grounds if the potential risks associated with the study are not justified by the potential benefits. Decisions about whether GOFROC should be permitted will entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of proposed studies but other considerations, including legal, ethical, and societal values are also important.

Key Finding 6. Managing risks associated with GOFROC, like all life sciences research, requires Federal-level and institutional oversight, awareness and compliance, and a commitment by all stakeholders to safety and security.

<u>Key Finding 1</u>: There are many types of GOF studies and not all of them have the same level of risks. Only a small subset of GOF research—GOF research of concern—entail risks that are potentially significant enough to warrant additional oversight.

As with all life sciences research involving pathogens, GOF studies entail inherent biosafety and biosecurity risks. GOF research involving the generation of pathogens with pandemic potential involves the greatest risks. A laboratory accident involving such a pathogen could potentially release a pathogen that could spread rapidly and efficiently through the human population. A laboratory pathogen with enhanced characteristics could also, if malevolently used, pose a greater threat to national security or public health than similar misuse involving a wild type pathogen. The probability that such events would occur is low but non-zero and the potential consequences are uncertain but potentially significant.

Gryphon's biosafety risk assessment identified studies involving enhanced transmissibility, enhanced pathogenicity, and evasion of immunity as entailing the highest risks for coronaviruses, seasonal influenza, and avian influenza.[52] Manipulations that increase transmissibility, increase pathogenicity, and enable a pathogen to more readily spread through the population have the greatest potential to increase risk; in some strains even a moderate increase might be a concern.

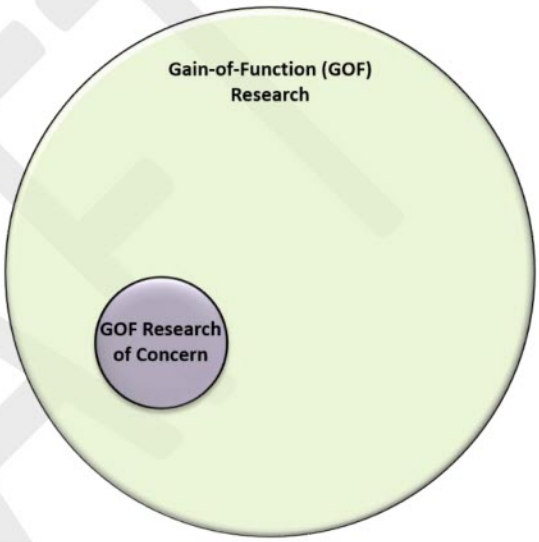

Figure 4. Conceptual categorization of GOF studies involving human or animal pathogens. GOF studies include a broad range of experimental approaches, most of which do not raise significant concerns. GOF studies of concern represent a small subset of all GOF research that can be reasonably anticipated to result in generation of a pathogen with pandemic potential, as described as a pathogen that is likely both highly transmissible and highly virulent in humans.

To help categorize studies based on the level of concern stemming from their associated risks, the working group has designated studies as: GOF research and GOF research of concern (GOFROC) (Figure 4). The term "GOF research" would encompass all studies involving human or animal pathogens whereby some characteristic of the pathogen is enhanced. The vast majority of GOF research does not raise any significant concerns; these studies do not entail novel or significant risks and are subject to layers of oversight to manage risks. GOF research of concern, or GOFROC, represents the small subset of studies that result in the generation of a pathogen with pandemic potential—that is, a pathogen that is highly virulent and highly transmissible, as judged by its likely ability to spread among human populations (see Recommendation 1 for more thorough description of these attributes).

[52] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

Key Finding 2. The U.S. government has several policy frameworks in place for identifying and managing risks associated with life sciences research. There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOF research of concern could be implemented.

Federally-funded life sciences research in the U.S. is conducted in accordance with occupational health and safety laws and regulations, the *NIH Guidelines*, the BMBL, policies for the Federal and institutional oversight of DURC, the Select Agent Regulations, export control regulations, international treaties and agreements, and other relevant policies. HHS has also developed a framework for guiding funding decisions for certain GOF studies involving H5N1 and H7N9 influenza viruses. Together, these policies aim to mitigate biosafety risks, biosecurity risks, and other risks associated with life sciences research, including many of the GOF studies that have raised concerns.

U.S. policies apply oversight and help manage risks at several points throughout the research life cycle including the proposal review, the funding decision, the time during which the research is being conducted, and at the time the research is being communicated. There are also numerous entities that are responsible for providing oversight, managing risks or issuing guidance, including funding agencies, institutional review and compliance committees, individual investigators, federal advisory committees, and journal editors.

While effective implementation of these policy frameworks can manage much of the risk associated with life sciences research, including the risks of some GOFROC, there remains variability in how policies are applied and coverage is incomplete (e.g., GOF research funded and conducted by/within the private sector may not be covered). Institutional oversight also varies. For example, IBCs differ in capabilities and expertise, and institutional resources and cultures vary. In addition, there is limited data describing the rate and extent of laboratory accidents, near-misses, and security breaches. Little comprehensive data about these critical issues exist, and no entity is currently authorized to collect all of what would be desirable.

Key Finding 3. Oversight policies vary in scope and applicability, and are not sufficiently harmonized; therefore, current oversight is not sufficient for all GOF research of concern.

U.S. policies are applicable to some but not all GOFROC. Risks associated with GOFROC that do not involve select agents or pathogens subject to oversight under the USG DURC policies or the *HHS Framework*, would largely be managed at the institutional level, in accordance with guidance in the *NIH Guidelines* and BMBL. In general, GOFROC that is not conducted with U.S. government funds is not subject to oversight by a Federal funding agency.[53] Other countries also fund and conduct life sciences research, including GOF studies, which are beyond the purview of the U.S. government as well.

[53] Research involving a select agent, whose oversight is articulated in Federal statute and requires compliance from all researchers and institutions, would be subject to Federal oversight, regardless of the funding source. Some privately-funded

Further, the U.S. government's oversight policies are not sufficiently harmonized. Different policies are aimed at managing different risks, and each is implemented by various Federal Departments and Agencies. This can result in redundancies as well as gaps in oversight.

In addition, full compliance with policies is essential to their effectiveness. The effectiveness of policies can be enhanced by a commitment to proper implementation and enforcement at the Federal, institutional, and individual investigator levels. This can include training, education, codes of conduct, and other mechanisms that are valuable tools for continuing to build a culture of responsibility among researchers.

Key Finding 4. An adaptive policy approach is a desirable way to ensure that oversight and risk mitigation measures remain commensurate with the risks associated with the research and the benefits of the research are being fully realized.

Many, but not all, of the policies that apply to GOF studies are adaptive in nature. The BMBL is updated periodically. The *NIH Guidelines* and the select agent programs are updated or revised periodically as well and both have processes for seeking external advice for informing policy development. The DURC policies and the *HHS Framework* do not have articulated mechanisms for seeking input on policy development, reviewing, or updating the policies, though both state an intention to be updated as necessary. Great uncertainty was identified with several key parameters effecting GOF risk and benefit assessment, and thereby risk management. An adaptive approach will facilitate refinement of GOF risk management as knowledge and experience is acquired.

Key Finding 5. There are life sciences research studies, including possibly some GOF research of concern, that should not be conducted if the potential risks associated with the study are not justified by the potential benefits. Decisions about whether GOFROC should be permitted will entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of proposed studies but other considerations, including legal, ethical, public health, and societal values are also important.

Examples of studies that should not be conducted for ethical reasons include those that: involve human subjects who have not provided consent; are anticipated to cause undue harm to a human subject; or that entail benefits that are unjustifiable in the light of the risks. For example, the development of biological weapons is unethical and has been banned by international treaty.[54]

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research being conducted at institutions that receive Federal funding for that research may also be subject to oversight under the *NIH Guidelines*, USG DURC policies, or other policies.

[54] Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction. Signed at London, Moscow and Washington on 10 April 1972; entered into force on 26

There may be GOFROC that should not be funded on ethical grounds but it is difficult to identify or describe such studies based on general or hypothetical descriptions. An ethical evaluation of a research study would entail an evaluation of the risks and benefits, which requires a thorough understanding of the scientific details of the proposal, including its aims and any adverse consequences that could be foreseen. In addition, the scientific, public health, and national security landscape is dynamic. Public health needs change as new diseases emerge. Risks may arise or diminish based on the availability (or lack) of effective countermeasures. Benefits may become more or less likely to be realized based on other enabling factors, such as new scientific findings or technologies. Decisions to fund GOF studies must take into account this anticipated variability in the risk-benefit landscape.

The NSABB did not seek to develop a list of studies that should not be conducted but rather sought to develop general principles that describe what is acceptable and not acceptable for funding. A principle-based approach to guiding funding decisions is adaptable and likely more effective than a list of specific studies that should not be funded.

However, one example of a scientific study that should not be conducted might be the insertion of a virulence gene from an unrelated organism into the genome of a virus transmissible through the respiratory route, which would be highly unlikely to occur by natural recombination. This study, and others that involve the transfer of virulence genes between disparate microbes would appear to lack public health benefit, since the novel, laboratory-generated pathogen is unlikely to arise naturally and would therefore entail potentially significant and unnecessary risks.

Key Finding 6. Managing risks associated with GOF research of concern, like all life sciences research, requires Federal-level and institutional oversight, awareness and compliance, and a commitment by all stakeholders to safety and security.

Biosafety and biosecurity risks associated with life sciences research are managed through engineering controls, laboratory practices, medical surveillance and support, appropriate training, and other controls. However, GOFROC has the potential to generate strains with significant risks that may require additional oversight and containment mechanisms. Managing the risks associated with GOFROC in particular requires a commitment to safety and security at the Federal and institutional level that includes a strong foundation of training and a commitment to compliance by the research institution, and the individual investigators at the local level.

March 1975. Depositaries: UK, US and Soviet governments. http://www.opbw.org/

Key Finding 7. Funding and conducting GOF research of concern involves biosafety, biosecurity, and policy issues that are international in nature. The potential risks and benefits associated with GOFROC are international in nature; laboratory accidents or intentional misuse could have global consequences. The relevant benefits for vaccine and other countermeasure development or disease surveillance could also have important international implications. In addition, the research enterprise is international in nature and GOFROC is being conducted in a number of countries already. While U.S. government policy regarding GOFROC will likely only directly affect domestic and international research within the purview of the U.S. government, decisions made by the United States in this area may influence oversight policies globally. Notably, several countries and international scientific organizations have been considering issues related to biosafety, biosecurity, dual use research, and GOFROC **[REFS, or reference section in this paper]**. International perspectives are important to the development of U.S. policy in this area and global engagement is necessary to foster effective oversight mechanisms and an international culture of responsibility around research involving pathogens. The U.S. government, often in concert with the NSABB, has been engaged with the international community over the years and continues to work with those governments and organizations now actively considering GOFROC-related issues.

6. Recommendations of the NSABB

Based on its analyses, the NSABB has formulated the following recommendations.

NOTE: Box to be updated as Recs finalized

Box 3. Summary of Recommendations of the NSABB

Recommendation 1. Research proposals involving GOFROC entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the Federal and institutional levels.

Recommendation 2. In general, oversight mechanisms for GOF studies of concern should be incorporated into existing policy frameworks. The risks associated with some GOF studies of concern can be identified and adequately managed by existing policy frameworks if those policies are implemented properly. However, the level of oversight provided by existing frameworks varies by pathogen. For some pathogens, existing oversight frameworks are robust and additional oversight mechanisms should generally not be required. For other pathogens, existing oversight frameworks are less robust and may require supplementation. All relevant policies should be implemented appropriately and enhanced when necessary to effectively manage risks.

Recommendation 3. The risk-benefit profile for GOF studies of concern may change over time and should be re-evaluated periodically to ensure that the risks associated with such research is adequately managed and the benefits are being realized.

Recommendation 4. The U.S. government should continue efforts to strengthen biosafety and biosecurity, which will foster a culture of responsibility that will support not only the safe conduct of GOF studies of concern but of all research involving pathogens.

Recommendation 1. Research proposals involving GOFROC entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the Federal and institutional levels.

GOFROC entails the generation of pathogens—perhaps novel pathogens—with anticipated pandemic potential. The risks associated with generating pathogens with pandemic potential are uncertain but potentially significant. It is possible that generating a laboratory pathogen with pandemic potential introduces a risk of a pandemic, albeit a low probability risk, that did not exist before that pathogen was generated. Therefore, a new, pre-funding review and approval mechanism is warranted before such studies should be undertaken. The NSABB working group proposes a conceptual approach for guiding funding decisions about GOFROC. This conceptual approach entails identifying GOFROC and subjecting such studies to an additional pre-funding review and approval process. The attributes describing GOFROC, the principles that should guide funding decisions for GOFROC, and the features of the proposed review process are described below.

Identifying GOF research of concern

Note: The 2 attributes and accompanying language was discussed and approved by WG on 4/7. Minor additional edits are included.

GOFROC is research that can be reasonably anticipated to generate a pathogen with pandemic potential. Determining whether a proposed research project is likely to generate a pathogen with pandemic potential, as described by the attributes below, will entail uncertainty and will require scientific and other expert judgment.

To be considered GOFROC, the research must, in a single step or over the course of manipulations, be reasonably anticipated to generate a pathogen with both of the following attributes:

i. **The pathogen generated is likely highly transmissible and likely capable of wide and uncontrollable spread in human populations.** To be considered "highly transmissible" the pathogen must be judged to have the capacity for sustained secondary transmission among humans, particularly but not exclusively by the respiratory route. Such a determination might be informed by data describing human infections by naturally-circulating isolates of the pathogen or studies in relevant experimental mammalian models that serve as a proxy for human infections. To be considered "capable of wide and uncontrollable spread in human populations" it must be judged that there would be limited options for controlling the spread of the pathogen other than patient isolation or quarantine. Such a determination might be made, for instance, if humans lack population immunity to the resulting pathogen, if the pathogen would evade or suppress the human immune response, if the pathogen would be resistant to

medical countermeasures, or if existing countermeasures would be unavailable globally in sufficient quantities.

AND

ii. **The pathogen generated is likely highly virulent and likely to cause significant morbidity and/or mortality in humans.** To be considered "highly virulent" the pathogen must be judged to have the capacity for causing significant consequences in humans, such as severe disease and/or a high case fatality rate. Such a determination might be informed by data describing human infections by naturally-circulating isolates of the pathogen or studies in relevant experimental mammalian models that serve as a proxy for human disease.

Any study involving the generation of a pathogen exhibiting the two attributes above would be considered GOFROC. However, it is generally anticipated that the following types of activities would not be considered GOFROC:

- Studies to characterize the virulence and transmission properties of circulating pathogens
- Surveillance activities, including sampling and sequencing
- Activities associated with developing and producing vaccines, such as generation of high-growth strains

Importantly, a proposed experiment need not involve the simultaneous enhancement of both phenotypes. For instance, research involving a naturally-occurring pathogen that exhibits one of the above attributes would be considered GOFROC if a study were anticipated to confer the second attribute to the agent (while retaining the first attribute). Other studies may generate a pathogen with the above attributes after a series of manipulations that enhance the phenotypes separately but ultimately result in a pathogen with both attributes. Any route of experimentation that is anticipated to ultimately generate a pathogen that exhibits both of the characteristics above would be considered GOFROC and should be reviewed carefully before it can be funded.

Appendix B describes examples of studies that would and would not be considered GOFROC. These examples are provided as guidance and are described in general terms. A more detailed consideration of the specific pathogen in question as well as the proposed experimental manipulations would be required to determine whether a research proposal is likely to entail GOFROC. The specific nature of a given pathogen or manipulation could alter the determination about whether or not a study constitutes GOFROC.

Pre-funding review and approval of GOF research of concern

Proposals anticipated to involve GOFROC should be subject to additional review prior to making a funding decision and a higher degree of Federal oversight throughout the course of the research, if funded. The working group has developed principles that should guide the review and funding of these proposals. There should be a high degree of confidence that a study will be conducted in accordance with these principles before determining whether the proposal is suitable for funding. Studies that cannot be or are not anticipated to be conducted in accordance with the principles below should not be funded.

Principles for guiding review and funding decisions

NOTE: These principles are to be reviewed and finalized by WG on 4/19.

The NSABB working group has developed the principles below to guide funding decisions regarding GOFROC. Only projects that are in line with all of the following principles should be considered acceptable for funding. The principles below are intended to embody the substantive ethical values described in section 4.2 and the process of applying these principles would involve scientific, security, ethical, and other considerations.

i. **The research proposal has been evaluated by a peer-review process, determined to be scientifically meritorious, and has been assessed to be likely to exert a sustained, powerful influence on the research field(s) involved.** If GOFROC is to be funded and conducted it must first and foremost address a valuable scientific question or public health need.

ii. **The pathogen(s) that is anticipated to be generated must be judged, based on scientific evidence, to be able to arise by natural processes.** It is difficult to predict the types of pathogens that can or will emerge in nature. Nevertheless, before a pathogen with pandemic potential is generated through laboratory manipulations it is essential to consider whether such a pathogen could arise in nature. GOFROC may be permissible if the study were to generate a pathogen that is anticipated to arise in nature or if the study were to provide insight into natural evolutionary processes. GOFROC would not be permissible if it were to generate a laboratory pathogen that is highly unlikely to arise in nature (e.g., combining virulence factors of two viruses that are highly unlikely to recombine in nature). **NOTE: This is a NEW principle. Are there comments?**

iii. **An assessment of the overall potential risks and benefits associated with the project determines that the potential risks compared to the potential benefits are justified**. Prior to funding GOFROC, the anticipated risks and potential benefits must be carefully considered. In general, the potential benefits associated with a research project should be commensurate with or exceed the presumed risks. Projects involving significant risks and few anticipated benefits are ethically unacceptable and should not be funded. If the potential risks appear high, the possible benefits should also appear high. Risks should be mitigated and managed whenever possible.

iv. **There are no feasible, equally efficacious alternative methods to address the same scientific question in a manner that poses less risk than does the proposed approach.** Alternative approaches must be explored and critically examined before funding GOFROC. It is possible that the proposed experimental approach that raises concern is the only feasible approach for addressing the scientific question at hand. In other cases, modifications of the experimental design, selection of attenuated or other strains that pose fewer risks in humans, or different approaches that may provide the same or very similar information may be feasible. Lines of experimentation that entail less risk should be pursued whenever possible.

v. **The investigator and institution proposing the research have the demonstrated capacity to carry it out safely and securely and the ability to respond rapidly and adequately to laboratory accidents or security breaches.** Prior to funding, the risks associated with proposed GOFROC must be identified and assessed, and clear, realistic plans for managing risks should be developed. In order to manage risks associated with GOFROC, an institution must have adequate resources, security, trained personnel, administrative structures, occupational health and safety procedures, relationships with local public health authorities, and the ability to adapt to unanticipated results by increasing containment or adding safety or security features. In addition to adhering to standards of compliance, an institution (and the investigators proposing the study) should have a demonstrated commitment to laboratory safety and security, scientific integrity, and the responsible conduct of research. The researchers and institution should embody the culture of responsibility as it pertains to safety and security, perhaps demonstrated through adherence to a code of conduct or other voluntary measures.

vi. **The benefits of the research are anticipated to be broadly and legally shared in order to realize its potential benefits to global health.** Prior to funding GOFROC, consideration should be given to the type of research information and products that are likely to be generated. The research information and products are expected to be shared openly and a responsible communication plan should be developed at the outset, if necessary.

vii. **The research will be supported through funding mechanisms that allow for appropriate management of risks and ongoing oversight of all aspects of the research.** GOFROC should be funded through mechanisms that help to ensure that appropriate biocontainment conditions are utilized, adequate biosecurity precautions are in place, and that the data and materials generated will be shared appropriately. The funding mechanism should allow for additional risk mitigation measures to be required during the course of the research, if needed.

viii. **The proposed research is ethically justifiable.** Determinations about whether proposed GOFROC should be undertaken will involve value judgments to assess the potential risks and benefits and determine whether any potential risks are justified. Non-maleficence, beneficence, justice, respect for persons, scientific freedom, and responsible stewardship are among the values that should be considered when ultimately making decisions about whether to fund GOFROC.

Description of the Review Process for Proposals Involving GOF Research of Concern

NOTE: This section describing the additional review process was discussed on 4/7 and generally supported by the WG; see also new Recommendation 3.2 for proposed role of a FACA or other advisory committee.

The NSABB proposes the following conceptual approach for guiding funding decisions about GOFROC (Figure 5). Review of research projects that may involve GOFROC would involve four steps:

1. Investigators, institutions, and funding agencies identify proposed GOFROC, as described by the two attributes for identifying GOFROC.
2. A Department-level Federal panel with diverse expertise reviews proposals involving GOFROC to determine whether it meets the 8 principles for guiding funding decisions.
3. Funding agencies make a funding decision and establish risk mitigation plans and other conditions if the GOFROC is determined suitable for funding.
4. Investigators and institutions conduct the research in accordance with applicable Federal and local oversight policies and employ any additional mitigation strategies. Federal agencies provide oversight to ensure adherence to established risk mitigation plans and funding terms.

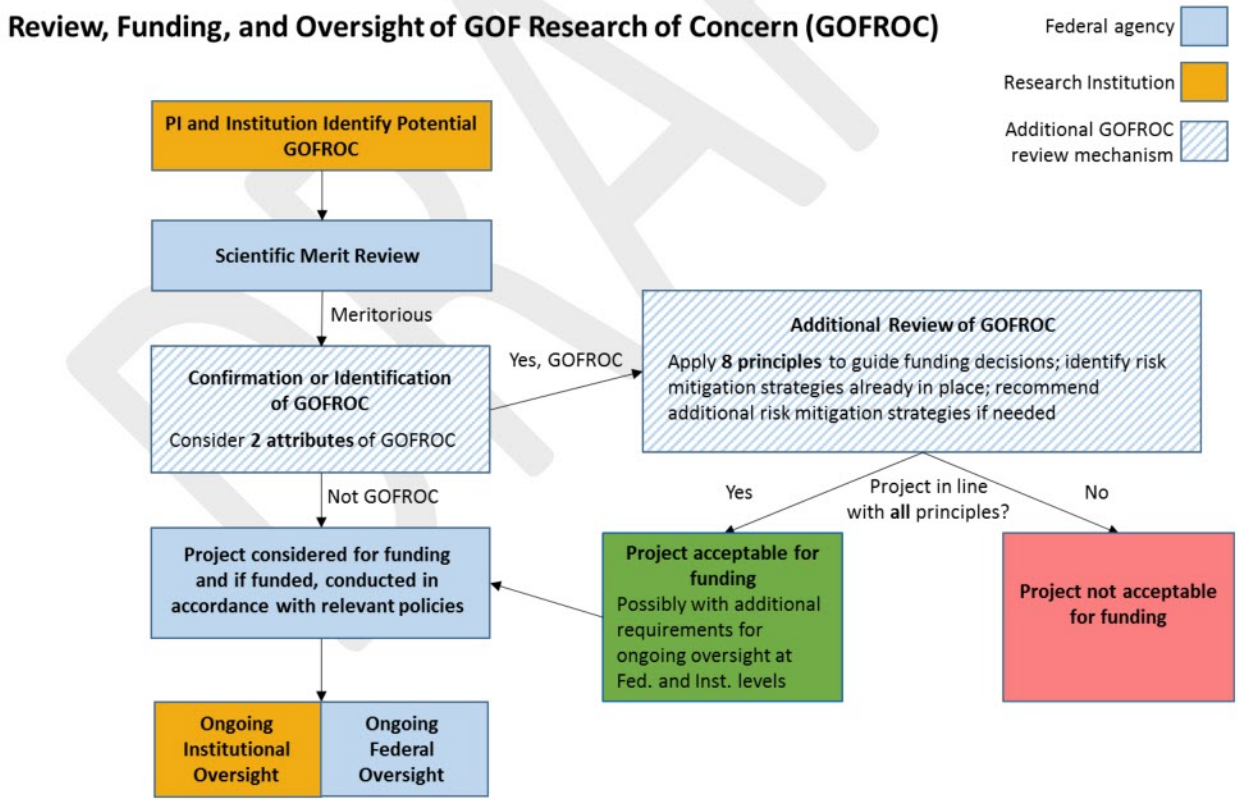

Figure 5. Proposed conceptual approach for guiding funding decisions for GOF research of concern.

Investigators and institutions identify GOFROC. Prior to submission of an application for funds, investigators and research institutions should identify possible GOFROC and submit with the research proposal any relevant information such as biosafety, biosecurity, or local public health response plans, descriptions of facilities available, and a discussion of the value and potential benefits of the proposed research. Identification of possible GOFROC should not affect a subsequent scientific merit review either positively or negatively.

Department-level review of GOFROC. After the standard agency scientific merit review process, proposals that are determined to be scientifically meritorious and likely to be funded would also be reviewed by the funding agency to determine if they constitute GOFROC, as defined by whether the proposal can be anticipated to generate a pathogen exhibiting the two attributes. Prior to being determined acceptable for funding, proposals identified by a funding agency as involving GOFROC would require an additional, higher level, Departmental review. If a proposal does not involve GOFROC, it would proceed along the normal pathway for further evaluation and funding decisions.

The additional review of proposals involving GOFROC would be to determine whether the proposed research aligns with the 8 principles to guide funding decisions. Applying these principles will help to ensure that the GOFROC is scientifically and ethically acceptable, that the risk-benefit balance is favorable, that alternative approaches are explicitly considered, and that the research can be performed safely and securely. It is envisioned that the additional review of proposals involving GOFROC would involve diverse, multidisciplinary expertise including scientific, public health, biosafety, national security and intelligence, legal and bioethics, and other perspectives. To the extent possible, the review process should be efficient, transparent, well-documented, and adaptive. In addition, the process should be structured to avoid real or apparent conflicts of interest and to provide consistency across Federal agencies that might fund GOFROC. It is also envisioned that research institutions proposing the GOFROC would have an opportunity to provide information that would be necessary for a thorough and substantive review of the research proposal.

Funding decision and risk mitigation. During the course of the Department-level review the relevant risk management plans should be critically evaluated and additional risk mitigation measures may be deemed necessary in order for GOFROC to be funded. A satisfactory risk management plan would entail appropriate biocontainment facilities and biosafety practices, appropriate standard operating procedures and administrative controls, occupational health and safety programs and security features aimed at protecting laboratory strains and reagents and promoting personal reliability. Some or all of the additional risk mitigation measures listed in Box 4 may also be required. A variety of additional measures could be required as a condition of funding such as more frequent institutional and Federal reviews of progress, site inspections, prohibition of adding new GOFROC experiments without approval, requirements to report unanticipated results, and/or Federal review of communication plans.

Ongoing oversight. Finally, throughout the course of the funding, both Federal and institutional oversight are critically important and the project should be carefully monitored to ensure that required conditions are met, that the principles guiding the decision to fund are still satisfied, and that any changes, significant developments, and publication/communication plans are discussed and addressed

in a timely manner. Additional ongoing oversight at the Federal and institutional level may be required and should be stipulated at the time of funding.

Recommendation 2. In general, oversight mechanisms for GOF research of concern should be incorporated into existing policy frameworks when possible.

Any additional oversight of GOFROC should be built into existing mechanisms rather than having the U.S. government develop a novel regime specific to GOFROC. Adapting or harmonizing current policies is preferable to developing entirely new oversight frameworks or wholly new approaches to manage the risks associated with these studies. There are precedents for additional Federal-level pre-funding review of certain GOF studies (i.e. *HHS Framework*) as well as mechanisms for higher-level review and approval of certain studies (i.e., Major Actions, under the *NIH Guidelines*; restricted experiments, under the Select Agent Program). There are also mechanisms for continual Federal-level monitoring of biosafety and biosecurity risks for individual projects (i.e., USG Policy for Federal Oversight of DURC, select agent programs) and established mechanisms for ongoing institutional oversight (i.e., IREs under the USG Policy for Institutional Oversight of Life Sciences DURC; IBCs under the *NIH Guidelines*). Wherever possible, these mechanisms should be employed to ensure the initial and ongoing oversight of GOFROC.

Importantly, not all GOFROC would necessarily be subject to the entire suite of U.S. oversight policies. For instance, experimental manipulations with pathogens not included in the USG policies for DURC oversight or on the select agent list could still conceivably generate a pathogen with pandemic potential. Additional oversight measures may need to be stipulated at the time of funding for proposals involving potential GOFROC that are not subject to a particular policy that is deemed necessary. For instance, specific, enhanced containment practices may be required or a project may require ongoing monitoring for DURC potential at the Federal and institutional level. Box 4 describes a number of potential risk mitigation measures that may be required for GOFROC that could potentially be implemented by leveraging existing policy frameworks.

Box 4. Potential additional risk mitigation measures to be considered for GOF research of concern.

Potential risk mitigation features that should be considered prior to funding GOFROC might include requirements to:

- Provide additional training to researchers
- Enhance biosafety practices or features, as dictated by the specific strains and proposed manipulations
- Enhance security measures around strains, reagents, notebooks, and personnel
- Treat the research as if subject to the USG DURC policies, if it is not already
- Conduct more frequent institutional biosafety and biosecurity reviews of the research
- Conduct more frequent progress reports and discussions with Federal funding agency staff
- Conduct periodic site inspections/evaluations if not already required
- Identify certain experimental outcomes that would trigger a re-evaluation of the risks and benefits prior to proceeding with a study
- Develop a responsible communication plan, specifically, including a description of biosafety and biosecurity practices
- The institution to be in regular communication with local law enforcement and public health officials
- Conduct bioethics consultations at the local and Federal level throughout the lifecycle of the research
- The investigators to develop and/or adhere to an appropriate code of conduct

Recommendation 3. The U.S. government should pursue an adaptive policy approach to help ensure that oversight remains commensurate with the risks associated with the GOFROC. The risk/benefit profile for GOFROC may change over time and should be re-evaluated periodically to ensure that the risks associated with such research is adequately managed and the benefits are being realized. An adaptive approach to the oversight of GOFROC would entail the continual evaluation of the risks and benefits associated with the research as well as the burdens and effectiveness of the additional proposal review process and ongoing oversight measures. An adaptive approach would allow policymakers to learn from experience and update policies accordingly as the risk/benefit landscape changes. For instance, the risks associated with a study may change if newly developed countermeasures become available or if new information emerges to clarify certain risks or enable certain benefits.

Recommendation 3.1. The U.S. government should consider developing a system to collect and analyze data associated with laboratory safety to inform policy development over time for GOF research of concern. Examining such data would provide a better understanding of the risks, inform future risk assessments, and allow for the refinement of oversight policies over time.

New rec 3.2 was proposed on 4/7 WG call.

Recommendation 3.2. An external advisory body that is designed for transparency and public engagement should be utilized as part of the U.S. government's ongoing evaluation of oversight policies for GOF research of concern. An external advisory mechanism, such as a Federal advisory committee, would allow for an independent examination of the U.S. government's policies for reviewing, funding, and conducting GOFROC. Such a mechanism could review GOFROC funding decisions to understand how such decisions were made, identify challenges to implementing the policy, and recommend changes, if needed, that may improve the process. Importantly, this mechanism would also provide transparency and promote public engagement, and would facilitate continued dialogue about GOFROC. The NSABB is one such body that is well-suited to address this task.

Recommendation 4. The U.S. government should pursue ways to ensure that all GOF research of concern conducted within the U.S. or by U.S. companies be subject to oversight, regardless of funding source. GOFROC that is funded by the U.S. government or through private funding sources should be subject to equivalent oversight to ensure that the associated risks are adequately managed. The U.S. government should consider providing oversight not only as a term and condition of a funding award but also via other mechanisms that would enable oversight of all relevant research activities, regardless of the funding source.

Recommendation 5. The U.S. government should undertake broad efforts to strengthen laboratory biosafety and biosecurity and, as part of these efforts, seek to raise awareness about the specific issues associated with GOF research of concern. Current discussions about GOFROC are related to broader domestic and international discussions about laboratory safety and security. A "Top Down" approach to managing the risks associated with GOFROC through Federal policies and oversight is appropriate. However, top-down approaches alone, in the form of Federal and/or institutional leadership, will likely not be sufficient to fully address the associated risks. It is also critical to have adequately trained personnel that values safe and secure laboratory environments for conducting GOFROC. Therefore, it will also be important to facilitate a "Bottom Up" approach whereby scientific and institutional leaders, as well as research staff involved in the design and conduct of GOFROC, are educated about biosafety, biosecurity, and the responsible conduct of their research. The U.S. government should engage the research community with the goal of promoting a culture of responsibility, or "citizenship," whereby all participants in the research enterprise have a sense of shared responsibility for its continued beneficial contribution. Such a culture would value safety,

security, and compliance, and work to promote public trust in the scientific enterprise. For GOFROC a combination of voluntary and mandated oversight and risk mitigation measures would be beneficial.

Recommendation 5.1. The U.S. government should specifically develop a "Points to Consider" document to provide guidance to investigators and institutions when preparing research proposals that may involve GOFROC. Such a document would describe to investigators any new requirements for proposals involving GOFROC and provide guidance on the type of information that should be included in a proposal to facilitate its review. This document should be reviewed and updated as necessary.

Recommendation 6. The U.S. government should engage the international community in a dialogue about the oversight and responsible conduct of GOFROC. Life sciences research is a global endeavor that continues to grow as more countries invest in their research capacities and as scientists move and collaborate across national boundaries. Life sciences research enables biomedical breakthroughs, pandemic preparedness, public health response efforts for emerging infectious diseases, and also provides an important economic driver. As more investigators undertake research involving pathogens, however, the associated risks become more likely to have international implications. The risks associated with GOFROC are especially international in nature since laboratory accidents or the deliberate misuse of pathogens with pandemic potential could have global consequences. Laboratories anywhere can undertake GOFROC and publications in the open scientific literature can enable others to generate pathogens with pandemic potential.

In addition, the U.S. government should engage the international community on biosafety and biosecurity issues, including those related to dual use research and issues specifically associated with GOFROC. The U.S. government should share information on its relevant policy efforts, particularly as they relate to GOFROC. International engagement efforts should seek to promote a global scientific culture of responsibility and enhance the quality, legitimacy, and effectiveness of oversight processes.

The U.S. government should build these efforts on the substantial international engagement activities that it and the NSABB have carried out since the NSABB was established. Such efforts have included three international roundtable meetings on dual use research issues, a series of webinars focusing on different global regions, and an international consultative workshop on GOF issues[55]. In addition, the U.S. National Academy of Sciences and the European Academies Science Advisory Council have been engaged in the recent policy debates involving GOF studies and may be well positioned to continue the international dialogue on the issue in coordination with national governments and relevant international organizations.

[55] Information about these meetings and activities, including agendas, summaries, and archived videocasts, can be found on the NSABB website at: http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/nsabb/nsabb-meetings-and-conferences/international-engagement

7. Appendices

Note: Appendices have been updated but are not complete.

Appendix A. Detailed Description of NSABB Deliberations

NSABB Deliberations

The NSABB established two working groups to accomplish the two portions of its charge, which were to result in discrete work products.

- **Deliverable 1.** A report conveying NSABB's advice on the design, development, and conduct of the risk and benefit assessments.
- **Deliverable 2.** A report conveying NSABB's formal recommendations on the conceptual approach to the evaluation of proposed GOF studies.

DELIVERABLE 1: ADVISING ON THE RISK AND BENEFIT ASSESSMENTS

The first NSABB working group was tasked with advising on the design and conduct of the risk and benefit assessments. The group met between December 2014 and April 2015 and consisted of 13 NSABB voting members as well as non-voting *ex officio* members and other *ad hoc* members from Federal agencies. (Appendix A). The group convened by telephone conference calls and held a one-day in-person meeting.

The working group developed a draft *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*, which was presented to the full NSABB, which was developed further based on input from all Board members, and ultimately approved by the full Board on May 5, 2015. The recommendations in this framework were intended to inform the NIH as it guided the work of Gryphon Scientific in its risk and benefit assessments. The aim of the NSABB's framework was to help generate risk and benefit assessments that would provide information that would allow the NSABB to make sound, evidence-based recommendations.

The NSABB's framework describes: principles that should underpin the risk and benefit assessments; pathogens, pathogen characteristics, and types of GOF experiments and phenotypes that should be examined; the types of risks and benefits that should be analyzed; scenarios, conditions, and events to be examined; and approaches and methods that should be considered when analyzing risks and benefits. In order for the risk and benefit assessments to be grounded in scientific data and evidence, the assessments needed to focus on specific pathogens, experimental manipulations, and scenarios whose risks and benefits could be modeled and analyzed. The NSABB recommended that the risk and benefit assessments focus on studies involving influenza viruses (seasonal strains, as well as high and low pathogenic avian strains) and SARS and MERS coronaviruses. Given that most pandemics are associated with respiratory transmission, pathogens capable of airborne transmission were considered

to be of most acute concern. NSABB recognized that the risk and benefit assessments would provide information specific to the pathogens and scenarios that were examined, but intended that the assessment would generate information that could be more broadly interpreted and applied. Thus, NSABB's recommended approach to the risk and benefit assessments was intended to align with the USG's October 2014 statement, which states that while "gain-of-function studies that fall within the scope of research subject to the funding pause will be a starting point for deliberations, the suitability of other types of gain-of-function studies will be discussed."

DELIVERABLE 2: RECOMMENDATIONS ON A CONCEPTUAL APPROACH FOR EVALUATING PROPOSED GOF STUDIES

The second NSABB working group was tasked with developing draft recommendations on the conceptual approach for the evaluation of proposed GOF studies. The group met beginning in June 2015 and remains active the time of this writing. The working group consists of 18 NSABB voting members as well as non-voting *ex officio* members and other *ad hoc* members from Federal agencies. (Appendix A). The group convened by telephone conference calls and met twice in person.

In addition to the working group's primary task of developing draft recommendations, it continued to provide input on the conduct of the risk and benefit assessments. The working group also received periodic status updates on the risk and benefit assessments from NIH and Gryphon, as well as reports on the commissioned ethics analysis by Dr. Michael Selgelid, examined draft work products, and reported back to the full NSABB.

In developing draft recommendations on a conceptual framework for evaluating proposed GOF studies, the working group structured its deliberations into three phases.

Phase I. Policy examination, research, and information gathering
Phase II. Interpretation, analysis, and synthesis of information and results
Phase III. Development of recommendations

In Phase I the working group sought to 1) identify and examine the information necessary to inform development of recommendations and 2) begin to identify principles that should guide the development of NSABB recommendations. The working group began its deliberations by considering the topic areas discussed at the NSABB meeting in May 2015, which included examination of relevant U.S. and international policy and consideration of broader perspectives such as those from funding agencies, national security experts, journal editors and scientific publishers, ethicists, and others. The working group held an in-person meeting to consult with experts on many of these topics. The working group also examined a number of published GOF studies and discussed how current policies might apply to such studies to provide oversight and risk mitigation.

During Phase II the working group focused on translating information about risks and benefits as well as ethics into decisions and recommendations. It examined how current policies apply to GOF studies and began to develop preliminary observations and findings. The working group discussed the ethical issues associated with funding and conducting GOF studies, particularly noting the values and ethical decision-frameworks that might be applied to policy decisions about GOF studies. The working group also developed analytic tools to assist it in systematically analyzing the results of the risk and benefit assessments. In November 2015, the working group began receiving briefings from Gryphon Scientific conveying the results of the risk and benefit assessments, as well as reports on ethics from Dr. Selgelid. The group sought to identify GOF studies that might raise particular concerns and may require additional oversight or consideration prior to being funded.

In Phase III, the working group developed its draft recommendations, based on its analysis of the risk and benefit assessments and the ethics report and consideration of all other information and perspectives that were examined.

Deliberations by the Full NSABB

The full NSABB convened times 5 times between October 2014 and January 2016. At these meetings the NSABB working groups provided progress updates and the full Board deliberated the issues further, consulted with various experts, and sought public feedback. Public comments made at NSABB meetings and delivered to the NSABB in writing were carefully considered by the Board during its deliberations. The articles, resources, and stakeholders consulted by the NSABB and its working groups throughout this process are listed in Appendix D.

On November 25, 2014, NSABB voted to approve a statement conveying to the USG concerns it heard regarding the implementation of the funding pause for certain GOF studies.[56] On May 5, 2015, NSABB voted to approve its *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*.[57] This working paper was shared for discussion by the full NSABB on January 7 & 8, 2016.

Role of the National Academies in the Deliberative Process

The National Academies play a critical role in the ongoing deliberative process. The National Research Council and the Institute of Medicine (now National Academy of Medicine) have been asked to convene two forums to engage the life sciences community and to solicit feedback from scientists, the public, and

[56] Statement of the National Science Advisory Board for Biosecurity Regarding the USG Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses. National Science Advisory Board for Biosecurity, November 25, 2014. http://osp.od.nih.gov/sites/default/files/resources/Final%20NSABB%20Funding%20Pause%20Statement_12-12-14_0.pdf

[57] http://osp.od.nih.gov/sites/default/files/resources/NSABB_Framework_for_Risk_and_Benefit_Assessments_of_GOF_Research-APPROVED.pdf

other stakeholders. These forums are to involve discussion of principles important for the design of risk and benefit assessments of GOF research and of NSABB draft recommendations.

The first National Academies workshop was held on December 15 & 16, 2014 and focused on the potential risks and benefits associated with GOF studies, ways to assess risks and benefits, strengths and limitations of risk-benefit analyses, and the ethical and policy implications associated with funding and conducting GOF studies that have raised concerns.[58] The discussions at this meeting directly informed the development of NSABB recommendations for conducting the risk and benefit assessments and its subsequent deliberations. In particular, the discussions about the potential risks and benefits associated with GOF studies informed NSABB's recommendations for the types of risks and benefits that should be analyzed by Gryphon Scientific. A common theme at this National Academies meeting was also that the term "gain-of-function" is too broad and that in fact, only a subset of GOF studies truly raise concerns. NSABB applied this insight in its subsequent analysis of the risk and benefit assessments by seeking to identify the subset of GOF studies that raised significant or unique concerns. Finally, the legal and policy discussions that were initiated at this meeting prompted to the NSABB to explore these topics, as well as ethical issues, further.

The second National Academies meeting was held on March 10 & 11, 2016 and included a discussion of the completed risk and benefit assessments and NSABB's preliminary findings and draft recommendations. **NOTE: This is being expanded slightly to reflect discussion from NAS.**

The Risk and Benefit Assessments of GOF Studies

NIH commissioned Gryphon Scientific to perform a formal risk and benefit assessments to provide the NSABB with qualitative and quantitative information about the risks and benefits associated with conducting certain GOF studies. Dr. Rocco Casagrande, the principal investigator for the study, presented to the NSABB on May 5, 2015 an overview of Gryphon's approach to conducting the risk and benefit assessments, which included a quantitative biosafety risk assessment, a semi-quantitative biosecurity risk assessment, and a qualitative benefit assessment. Prior to voting to finalize its *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*, NSABB discussed with Dr. Casagrande its draft recommendations and how Gryphon's proposed approach aligned with NSABB's proposed recommendations. In June 2015, Dr. Casagrande presented and discussed a more detailed work plan with the NSABB working group. Over the course of the study, the NSABB working group received occasional progress reports from Gryphon and NIH staff, and were provided draft sections of the risk and benefit assessments. In November 2015 the NSABB working group began receiving the results of the completed risk and benefit assessments. Gryphon's final draft report was posted in advance of the NSABB meeting in January, 2016.[59]

[58] Potential Risks and Benefits of Gain-of-Function Research: Summary of a Workshop. National Research Council and the Institute of Medicine of the National Academies. The National Academies Press, Washington D.C., 2015. www.nap.edu.
[59] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

The NIH Office of Science Policy managed the contract with Gryphon Scientific. NIH staff met weekly with Gryphon to accomplish the goals of the Statement of Work and to ensure the recommendations provided in the NSABB's *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research* continued to inform the conduct of the risk and benefit assessments, as appropriate. NIH staff also consulted with NSABB *Ex officio* members to get broader expertise and advice, and to help ensure that the risk and benefit assessments would yield information that would inform subsequent policy deliberations by the U.S. government.

Considering Ethical Issues Associated with GOF Studies

To guide the NSABB's evaluation of the risks and benefits associated with GOF studies and its development of recommendations, the Board sought additional ethical input and analysis. NIH commissioned Dr. Michael Selgelid, Monash University, to examine the literature regarding the ethical issues associated with funding and conducting GOF research and to explore different ethical frameworks that might be utilized when considering how to evaluate the potential risk and benefits associated with GOF studies. Dr. Selgelid was also asked to provide an ethical decision-making framework that NSABB could consider using when analyzing the information provided in the risk and benefit assessments of GOF studies. The decision framework was to identify and consider ethical values that may not be fully captured by a risk-benefit analysis. Dr. Selgelid's analysis was to be accomplished in a neutral, objective manner, without making any definitive recommendations on whether and how to fund or conduct certain GOF studies or what policy course might be the most appropriate. Dr. Selegelid presented his initial work to the NSABB in September 2015 and delivered to the NIH a draft paper in December 2015, which was conveyed to the NSABB working group and posted in advance of the NSABB meeting in January, 2016. [60]

[60] Selgelid, Michael. Gain-of-Function Research: Ethical Analysis. December 7, 2015. http://osp.od.nih.gov/sites/default/files/GOF%20%20White%20Paper%20by%20Michael%20Selgelid_0.pdf

Appendix B. Examples of Studies that would and would not be expected to entail GOFROC

THIS TABLE IS BEING UPDATED TO USE CONSISTENT LANGUAGE WITH THE LANGUAGE LISTED IN THE GOFROC ATTRIBUTES.

Examples of studies that would and would not be expected to entail GOFROC	
Experiment that is anticipated to entail GOFROC and therefore require additional pre-funding review and approval	**Rationale**
An experiment that is anticipated to generate avian influenza viruses that are transmissible by the respiratory route in mammals if the starting virus is virulent in humans.	**Attribute 1.** The experiment is anticipated to increase transmissibility by the respiratory route in a relevant experimental mammalian model. Further, altering the host range from birds to mammals could generate a virus for which there is no existing population immunity in humans, therefore resulting in a virus capable of wide and potentially uncontrollable spread among humans. **Attribute 2.** Since the starting virus is highly virulent in humans it can be reasonably anticipated that the resulting virus will remain virulent in humans
Reassortant studies involving avian and human influenza virus strains to identify reassortants with pandemic potential that could arise naturally.	**Attribute 1.** Given the starting viruses and the goal of the experiment to identify/select for reassortants that are potentially highly transmissible in mammals, it can be reasonably expected that the resulting pathogen could be highly transmissible in humans. Since the resulting viruses are reassortants between bird and human influenza viruses, it can be anticipated that the antigenicity of at least some resulting viruses will remain avian-specific such that human populations would not be expected to have been exposed to such a strain or have pre-existing immunity. Therefore resulting in a virus that could spread more efficiently among humans than the initial virus. **Attribute 2.** Given the starting viruses and the goal of the experiment to identify/select for reassortants that are potentially highly transmissible in mammals, it can be reasonably expected that the resulting pathogen could be highly virulent in humans.
Studies utilizing a strain of SARS-CoV, or some other emerging human respiratory pathogen, which will be modified in ways that can be anticipated to render humans more susceptible to infection by for instance, introducing resistance to a countermeasure (were countermeasures	

available). [NOTE: this example will be replace with bacterial resp. pathogen]	
NOT anticipated to entail GOFROC and therefore not require additional pre-funding review and approval	**Rationale**
Studies aimed at generating a mouse-adapted MERS-CoV or other emerging human respiratory pathogen	**Attribute 1.** The starting virus is transmissible by the respiratory route in humans **Attribute 2.** The experiment will increase the virulence of the human pathogen in mice, resulting in a potentially highly virulent virus in mammals **Not attribute 3.** The experiment is not expected to generate a pathogen with this attribute. The starting virus is already transmissible and pathogenic in humans and adapting it to mice would not be expected to result in a virus to which humans are more susceptible than the naturally-circulating virus. In fact, the mouse-adapted strain is likely to be less virulent in humans.
Studies enhancing the growth of seasonal influenza viruses, which may be performed during vaccine production	**Attribute 1.** The starting seasonal influenza virus is highly transmissible by the respiratory route in humans **Possibly attribute 2.** Increasing the virus's ability to replicate could potentially result in its increased ability to cause disease, therefore, could result in highly virulent strains. **Note:** If this experiment were to involve an attenuated strain, as is often the case when involving vaccine production, it would be unlikely to result in a virus with this attribute **Not attribute 3.** The experiment is not expected to generate a pathogen with this attribute. The starting virus is already transmissible and the study does not propose introducing resistance to countermeasures or other manipulations that would render humans more susceptible than the naturally-circulating seasonal strains
Antigenic drift studies whereby seasonal influenza viruses that are no longer neutralized by vaccine-induced immunity are generated and selected for in the laboratory.	**Attribute 1.** The starting seasonal or pandemic influenza virus is highly transmissible by the respiratory route in humans **Not attribute 2.** While it would depend on the specific initial strain in use, it is unlikely that the starting virus would be highly virulent in humans nor would the experimental manipulation be anticipated to increase the virulence **Not attribute 3.** Antigenic drift studies generate influenza viruses with some resistance to a specific immunization but they do not change the antigenic character of the virus such that the virus would be unrecognizable by the human immune system. Given that the starting virus is a human virus—not a virus

	that naturally infects birds or other non-human hosts—humans would likely have some pre-existing immunity to the resulting strains.

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Appendix C. Summaries of Stakeholder Perspectives

The NSABB consulted a wide range of experts and stakeholder groups including not only scientists and institutions that fund and conduct life sciences research, but a much larger and diverse array of groups including public health officials, medical practitioners, emergency responders, vaccine developers, scientific journals, as well as the general public, non-governmental organizations, individuals with international perspectives and others. To accomplish this, NSABB provided a variety of opportunities for interested groups and individuals to express their views and contribute throughout the deliberative process in ways that have informed the NSABB deliberations. These include: several public full NSABB advisory committee meetings with sessions dedicated to obtaining public comment, two public symposia hosted by the National Academies that obtained comments from the public at the meetings and online, as well as comments submitted to the NIH/OSP and NSABB by email, and discussions with subject matter experts during NSABB WG conference calls and in-person meetings. Also included below are views expressed in some of the articles that have been published on this topic. A complete list of the individuals consulted and articles examined by NSABB are listed in Appendix D. Note that Gryphon Scientific also conducted extensive consultations with experts as part of their risk and benefit assessments. Those experts are not listed here but a listing is available in Gryphon's report. [61]

The following is a synthesis of stakeholder ideas and opinions expressed during the deliberative process. Many of these points were conveyed in more than one venue and by more than one person or group.

Scientists and Others Favoring GOF Research

A variety of influenza and coronavirus researchers who conduct GOF research, and other life sciences researchers have stated that GOF studies are widely used and fundamental for understanding viruses, and therefore are crucial to undertake. This group generally favors conducting such research because it aims to benefit society. In their view, such research can be safely conducted under current oversight frameworks and further restrictions will impede valuable work that will lead to important scientific information about these viruses, leading to better drugs and vaccines, as well as to improving the specificity of surveillance, particularly for influenza. In addition, some GOF studies are viewed as essential, specifically those that alter host range or enhance pathogenicity in order to develop animal models of disease (for example, with SARS-CoV) or GOF studies that generate drug or countermeasure resistance, which are important in satisfying various FDA requirements for marketing approval. Those who support GOF studies also point out that such studies are needed for predicting what amino acid changes are important for human transmission and therefore are important for the selection of candidate vaccine viruses. They also argue that GOF studies are important for prioritizing viruses for risk management (surveillance) and that further work will make these applications more robust. The risks

[61] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

associated with not doing GOF research (generally due to a lack of preparedness for natural public health threats) must also be considered.

While acknowledging there are risks associated with GOF research, proponents believe those risks are manageable and have been overstated by some, as evidenced by the fact that laboratory acquired infections are rare and infections in the community as a result of releases from a laboratory are almost unknown. While risk cannot be zero, the work can be conducted safely and securely with appropriate risk mitigation including containment along with good training and with the implementation of robust occupational medicine programs. Alternatives to GOF do not always provide the full answer to key questions and may yield misinformation. Supporters of GOF studies have also expressed concerns about the effects of the current funding pause and possible additional oversight on the field of virology and young researchers, and feel that there are costs of not undertaking the work in question. A major need is for better definition of what is meant by GOF with a clear distinction between GOF studies and GOF studies of concern. Some have suggested that only viruses with increased transmissibility and pathogenicity represent risks that exceed those of other infectious diseases research. They have also noted that SARS and MERS viruses are different from influenza, and require a different risk assessment approach since they are already virulent human pathogens; GOF research is needed to develop animal models that will benefit development of countermeasures for coronaviruses. Some supporters have acknowledged that there may be some experiments that should not be done. Finally, proponents of GOF research have stated that the risks from naturally occurring influenza viruses, which they argue could be reduced through GOF work, are greater than risks from performing GOF studies.

Scientists and Others Critical of GOF Studies

Opponents and critics of GOF research have generally focused their concern on a subset of GOF studies—those that involve enhancing the pathogenicity and/or transmissibility in mammals (particularly by the respiratory route), which may result in the generation of novel pathogens with pandemic potential. Critics have argued that the generation of novel laboratory pathogens with pandemic potential poses major public health risks and some have argued such studies should not be conducted. They have presented and published calculations that suggest a high probability of global outbreaks of influenza that might kill hundreds of millions of people, as a result of the release from a laboratory of a novel GOF virus. There is some disagreement about these estimates and how likely a pandemic might be, but opponents generally argue that even a relatively low probability of a potentially massive outbreak with major consequences is unacceptable. Some critics of GOF studies have acknowledged that there are a number of GOF studies that can and should be conducted.

Opponents of certain GOF studies have also argued that the benefits of GOF studies have been overstated, or are questionable, and that the benefits generally do not outweigh the biosafety risks. They also question claims about the effectiveness of risk mitigation strategies, since human factors and human error are unavoidable and hard to control, and institutional compliance and competence may vary. Critics have disputed the value of GOF studies to surveillance stating that it is not possible to predict phenotype from genotype; therefore predicting the pandemic risk of newly emergent strains is

not achievable given the current state of knowledge. Also, in their view, controlling outbreaks doesn't require GOF research.

Opponents of GOF research tend to favor alternative types of research that, in their view, can provide the same public health benefits without the large risks. It was suggested that the approach should be on reducing the risk by reducing the hazard, as opposed to focusing on mitigation of the risk. For example, if a universal influenza vaccine was developed, the need for many GOF experiments would be eliminated. Critics want to see funds currently used for GOF work provided to other types of research, which would be a better use of scarce resources in their view. Overall, they view preventing major public health problems as paramount, and see a need to define a critical set of experiments that should not be done, or only be done with additional strong oversight. Opponents are also concerned about proliferation and other factors that may lead to misuse and biosecurity threats. Finally, opponents have pointed out a moral issue if risks and benefits of certain GOF studies are not fairly distributed globally.

Funding Agencies

Public and private funding agencies support GOF research that has raised concerns with the goal of improving public health and well-being. These organizations in the US and abroad are aware of the issues surrounding DURC/GOF studies and are working diligently to implement and comply with existing policies in their countries. Most funders have requirements and procedures in place as they apply policies and guidance to evaluate proposed work and to oversee funded work. Current approaches involve education and awareness campaigns, project risk evaluation, ethics reviews, development of risk mitigation plans, and post-award monitoring. Funders believe they can contribute to the GOF deliberative process as a result of their practical, on-the-ground experience with DURC and GOF. They are concerned that interpreting policy can be very challenging, since it requires considerable expertise and judgment. They would welcome workable policies with clear guidance and have noted some unintended consequences of the funding pause, which affected some GOF projects that had not raised particular concerns. Some foreign government funders view government funding as a poor control point because this does not cover privately funded research and research funded by other entities. National regulations, compliance, training, awareness-raising, and self-monitoring have been noted as important.

Biosecurity Experts and Others Concerned about National Security

The ultimate goal of national security professionals, as it pertains to life sciences research, is to protect public health from natural or man-made health threats. Those concerned with national security aim to prevent terrorists and others with malicious intent or misguided motives from using products or information from GOF research to cause harm. This may include deliberate release of pathogens into the community, targeting of researchers or research facilities, or interference with on-going research activities. GOF research represents biosecurity risks in addition to biosafety risks; these overlap but are different with regard to important legal, policy and regulatory issues. Managing biosafety risks may or may not also manage biosecurity risks; GOF policy must take both types of risk into account.

When trying to assess biosecurity threats, security professionals have noted the importance of avoiding assumptions and predictions about the motives and capabilities of those who might be planning biosecurity actions. Those in the security field gather a large variety of data, but often their information is imprecise and may require consideration of what is feasible and plausible. Because of the paucity of biosecurity events, it is very difficult to evaluate and predict the likelihood and consequences of a deliberate release or determine how to prevent and/or mitigate one, and different experts view this issue very differently. It was stated that research policy in itself is not be the appropriate solution to prevent specific biological threats but specific research policies could help raise awareness of security issues among researchers, which would be important.

Security and intelligence professionals have described the challenges associated with using classification as a potential risk mitigation strategy. Classification would effectively restrict access to sensitive research information and research products and would limit the number of laboratories able to perform the studies. This could be described as both a strength and a limitation, depending on one's perspective. Life sciences research that requires classification is typically classified at the outset; the retroactive classification of research that had been conducted in an open, academic setting is exceedingly difficult.

Scientific and Medical Journals

Scientific and medical journals have been at the forefront of the GOF issue. While several have in place procedures in place for identifying DURC, including GOF and other biosecurity concerns in submitted manuscripts, many journal editors are not entirely comfortable with their role. Their mission is to transmit scientific information, not control it, and they may not have the security expertise or the access to such expertise to make the necessary judgments and decisions about risks associated with communicating certain research findings. Rejection and redaction are the major tools journals have to control dissemination of dual use information, and neither may actually address the concerns; they are also impractical to implement effectively. One suggestion voiced was to require that a description of the steps that were taken during conduct of the research to ensure safety be included in all manuscripts. Some journal editors and staff expressed a desire to get help in evaluating risks and mitigation strategies from an independent national group such as the NSABB and to involve them earlier in the overall process. Most think the publication stage is not the best point to exercise control or prevent misuse of data from GOF studies but realize they are the final gatekeepers. Earlier identification of DURC/GOF along with risk mitigation earlier in the research life cycle would reduce the burden on them. Also, new technology and novel publication venues make controlling information increasingly difficult, and, as noted above, not all journals are able to or choose to impose a rigorous review of manuscripts.

Countermeasure Developers

Companies and others that are attempting to develop vaccines and drugs against pathogens were represented in several discussions. Medical countermeasure (MCM) developers expressed quite divergent views and opinions. Those favoring GOF research argued that such work is absolutely necessary for antiviral drug development because GOF experiments to select for drug resistant mutants

as well as to develop animal models are part of the critical path to marketing approval. In their view, GOF studies also have had a major influence on developing influenza vaccines, both seasonal and pandemic, and are likely to result in improved ways to make even better vaccines in the future. GOF experiments are required for selection of strains with better growth properties, with key mutations that alter important phenotypes needed in the vaccine strain, and with incorporating characteristics of strains that are likely to emerge into proven backbones. It was noted that GOF studies that enhance virulence can help inform vaccine designers about which mutations to avoid incorporating into vaccine strains. This group is concerned that their efforts to improve public health may be limited or impeded by new policies and urge careful consideration of their needs as decisions are made.

Conversely, other MCM developers expressed the view that vaccine production now is little dependent on GOF research and that any possible benefits will be far into the future, although some feel long-term potential is there. Those who criticize GOF studies on these grounds have argued that vaccines are developed in response to strains that emerge as threats, rather than preemptively based on strains that might be predicted as threats. Rather than supporting GOF studies to enhance vaccine production and drug development, it has been suggested that the other constraints that impede MCM development be addressed, such as streamlining FDA approval procedures and improving manufacturing processes, which would have a much greater impact. These critics suggest limiting current GOF-related efforts and focusing attention and resources in other directions. Overall, they believe that impact of GOF research on vaccine and drug development has been overstated, and that the benefits articulated are more theoretical than practical.

The General Public and Those who Represent their Views.

A number of stakeholders stressed the importance of having meaningful public engagement with input and participation as part of the deliberative process. It is important that communities that might be affected by accidents or the misuse of research have a say in the research that is being conducted, however, but this may not generally be the case in their view. Real transparency, with the public good as the foremost consideration, must be part of a truly independent decision-making process. They note that it is important to maintain public trust in the scientific enterprise by involving non-scientists at stages when their views can still have an impact on policy-making. Public opinion of science is harmed when decisions that influence public health and safety are made without such input or the input has no real impact. Conversely, effective community engagement can convert sceptics to supporters. More than one participant raised the concern that if risks and benefits are not equitably distributed, it is a serious ethical issue[62].

Other issues that were mentioned include: how harms will be compensated if a laboratory incident were to affect the surrounding community; the need for enough resources to conduct research safely; and the opportunity to learn from other industries such as nuclear industry.

[62] The ethical issues are discussed in more depth elsewhere, notably, Dr. Michael Selgelid's ethical analysis and the section of this report on Ethical Values and Decision-Making Frameworks.

Research Institutions

Representatives of universities and other research institutions generally noted that there is already significant oversight of DURC and GOF at both the Federal and institutional levels. Biosafety professionals noted that potentially high risk projects would receive thorough scientific review and risk assessment, resulting in the development of risk mitigation plans, and on-going monitoring as a result of policies and requirements that are already in place. They cited concerns over any increase in compliance that would impose burdens on their already-limited resources or impede researchers from doing valuable work. They have difficulty, at times, deciding what is DURC when reviewing specific projects and would welcome more specificity and guidance. Many emphasized the need for policies that are unambiguous and straightforward to implement.

Public Health Officials

Public health officials have expressed diverse opinions. Some believe that GOF research has and can continue to improve surveillance efforts, as well as vaccine and therapeutic development. Others expressed concerns that an accident involving a laboratory pathogen for which there are no countermeasures would be very concerning and difficult to respond to. At the local level it is important to have public health involvement in the decision-making process because they will be incident responders. Strong connections with state and local laboratories should be established for sharing information and might include involving them in the review process. It was also noted that GOF and related policies may impact sample sharing and impede international relations relating to public health efforts.

International Perspectives

Several participants noted that there is much interest in the GOF/DURC issue internationally, and the international community is looking to see what the USG will do as a result of the deliberative process. It was noted that U.S. policy often influences policies globally and the international ramifications should be considered. Recent biosafety incidents in U.S. Federal labs have raised concerns among many in other countries about the ability of the U.S. to adequately manage risks. A number of countries have well-developed systems of policy and regulation that would address some GOF and DURC issues, though international policy approaches are generally somewhat different from those in the U.S. International experiences, activities, and perspectives were cited as important to consider in the deliberative process. A collaborative approach and active attempts to engage the international community was viewed as the most effective way to benefit all. Many favored launching international dialog soon, with development of broad concepts and points of agreement that could be shared by all, while still respecting national differences. In addition, it was suggested that academies of science and multi-national organizations such as WHO can play an important role in such interactions. Those with a particular interest in the international aspects of GOF research also cited ethical issues associated with the unequal distribution

of risks and benefits across rich and poor countries. It was noted that the European Commission uses a comprehensive ethics process for screening and monitoring DURC/GOF in research projects.[63]

Those with an Interest in the Deliberative Process Itself

A broad group of individuals offered comments on the deliberative process itself. This included: federal government personnel, ethicists, decision-making experts, policy experts, other scientists, and includes people who are also members of the previously-mentioned groups. Those concerned with the deliberative process generally called for a well-planned and executed, thorough, scientifically rigorous, and impartial RBA that is technically sound and socially acceptable. They favored a democratic deliberative process and a policy that incorporates decisions made by neutral parties. Policy should be created using risk-based and value-based approaches to achieve desired outcomes. They want the final policy resulting from the deliberative process to be capable of reasonably identifying and mitigating risks related to GOF while protecting scientific autonomy, research progress, discovery and innovation, public health, national security, and other critical interests.

Many see an adaptive process as desirable, and recommend collecting appropriate data about laboratory accidents and mitigation effectiveness. It was noted that risks and benefits will change as science advances. The funding decision-making process should be accountable and limit inherent conflicts of interest; the individuals or entities that make decisions is critical. Most favor using existing policies as the basis of policy for GOF, while acknowledging that current frameworks are not entirely adequate. The question of how to incorporate non-USG funded research into an acceptable framework was raised several times. Deciding how to decide is a key point.

Both proponents and critics of GOF studies criticized the term "gain-of-function" as being too broad and not descriptive enough. There was much discussion about the appropriate definition of GOF research of concern; many strong, often conflicting, views were expressed. Unfortunately while it is important to have a working definition and criteria for what is GOF of concern as opposed to GOF, a binary distinction needed for deciding what requires extra scrutiny, GOF experiments are actually a continuum of increasing risk.

The funding pause was criticized for being too broad, and some described it as disruptive to scientific process. Finally, some feel that a definitive quantitative risk assessment is not possible because of the very large uncertainties and lack of critical information associated with doing such studies, and they question the value of any studies that are done.

[63] The EU Framework Programme for Research and Innovation, Horizon 2020. How to complete your ethics self-assessment, version 1.0, 11 July 2014. http://ec.europa.eu/research/participants/data/ref/h2020/call_ptef/pt/h2020-call-pt-ria-ia_en.pdf#page=27

Appendix D. Consultations, Comments, and Sources Consulted During NSABB Deliberations

Table 1. Experts consulted by NSABB or the NSABB working groups. Individuals listed here addressed the NSABB or NSABB working group in their individual or professional capacities. Members of the NSABB or an NSABB working group are listed if they presented as a subject matter expert on a specific topic.

Speaker/Commenter	Affiliation/Location	Venue
Regine Aalders, M.Sc.	Embassy of the Netherlands, Washington, D.C.	Public Comment
Richard Adams		Public Comment
Ronald Atlas, Ph.D.	University of Louisville	National Academies Workshop (December 15, 2014)
Ralph Baric, Ph.D.	University of North Carolina	National Academies Workshop (December 15, 2014), Public Comment
Kavita Berger, Ph.D.	Gryphon Scientific	NSABB Full Board Meeting (September 28, 2015)
Kenneth W. Bernard, M.D.	US Public Health Service (ret.)	Public Comment
Thomas Briese, Ph.D.	Columbia University	National Academies Workshop (December 15, 2014)
Arturo Casadevall, M.D., Ph.D.	Albert Einstein College of Medicine, mBio	NSABB Full Board Meeting (October 22, 2014), In-person WG Meeting (July 23, 2015), Public Comment
Rocco Casagrande, Ph.D.	Gryphon Scientific	NSABB Full Board Meeting (September 28, 2015)
R. Alta Charo, J.D.	University of Wisconsin–Madison	National Academies Workshop (December 15, 2014)
Susan Coller-Monarez, Ph.D.	Office of Science and Technology Policy	In-person WG Meeting (July 23, 2015)
Derrin Culp	White Plains, New York	Public Comment
Mark Denison, M.D.	Vanderbilt University	National Academies Workshop (December 15, 2014), Public Comment
Dennis Dixon, Ph.D.	HHS/National Institutes of Health	NSABB Full Board Meeting (November 25, 2014)
Marianne Donker, Ph.D.	Ministry of Health, Welfare and Sport; Netherlands	In-person WG Meeting (July 23, 2015)
Philip Dormitzer, M.D., Ph.D.	Novartis Vaccines	National Academies Workshop (December 15, 2014)
Ruxandra Draghia-Akli, M.D., Ph.D.	European Commission	In-person WG Meeting (July 23, 2015)
Rebecca Dresser, J.D.	Washington University in St. Louis	NSABB Full Board Meeting (September 28, 2015)
Paul Duprex, Ph.D.	Boston University, NEIDL Institute	NSABB Full Board Meeting (October 22, 2015)
Gerald Epstein, Ph.D.	Department of Homeland Security	In-person WG Meeting (July 23, 2015)
Stephen Eubank, Ph.D.	Virginia Polytechnic Institute and State University	NSABB Full Board Meeting (October 22, 2014)
Nicholas Evans, Ph.D.	University of Pennsylvania	Public Comment

David S. Fedson, M.D.	Sergy Haut, France	Public Comment
Scott Ferson, Ph.D.	Applied Biomathematics	NSABB Full Board Meeting (October 22, 2014), Public Comment
Harvey Fineberg M.D, Ph.D.	University of California, San Francisco	National Academies Workshop (December 15, 2014)
Baruch Fischhoff, Ph.D.	Carnegie Mellon University	NSABB Full Board Meeting (October 22, 2014); National Academies Workshop (December 15, 2014)
Ron Fouchier, Ph.D.	Erasmus Medical Center	National Academies Workshop (December 15, 2014), Public Comment
Gregory Frank, Ph.D.	Infectious Diseases Society of America	Public Comment
David Franz, D.V.M., Ph.D.	Former Commander, United States Army Medical Research Institute for Infectious Diseases	In-person WG Meeting (July 23, 2015)
Christophe Fraser, Ph.D.	Imperial College	National Academies Workshop (December 15, 2014)
Matt Frieman, Ph.D.	University of Maryland	Public Comment
Gigi Kwik Gronvall, Ph.D.	University of Pittsburgh Medical Center (UPMC) Center for Health Security	National Academies Workshop (December 15, 2014)
Charles Haas, Ph.D.	Drexel University	National Academies Workshop (December 15, 2014)
Peter Hale	Foundation for Vaccine Research	Public Comment
Elizabeth Hart	Adelaide, South Australia	Public Comment
Andrew M. Hebbeler, Ph.D.	White House Office of Science and Technology Policy	NSABB Full Board Meeting (October 22, 2014), National Academies Workshop (December 15, 2014)
Denise Hein		Public Comment
Gavin Huntley-Fenner, Ph.D.	Huntley-Fenner Advisors	National Academies Workshop (December 15, 2014)
Jo Husbands, Ph.D.	Board on Life Sciences of the US National Academy of Sciences	In-person WG Meeting (July 23, 2015)
Michael Imperiale, Ph.D.	University of Michigan	National Academies Workshop (December 15, 2014), Public Comment
Tom Inglesby M.D.	University of Pittsburgh	NSABB Full Board Meeting (October 22, 2014), Public Comment
Barbara Jasny, Ph.D.	Science	In-person WG Meeting (July 23, 2015)
Barbara Johnson, Ph.D., R.B.P.	Biosafety Biosecurity International	National Academies Workshop (December 15, 2014)
Laura Kahn, M.D., M.P.H., M.P.P.	Woodrow Wilson School of Public and International Affairs, Princeton University	Public Comment
Joseph Kanabrocki, Ph.D., C.B.S.P.	University of Chicago	In-person WG Meeting (January 22, 2015), In-person WG Meeting (July 23, 2015)
Yoshihiro Kawaoka, D.V.M., Ph.D.	University of Wisconsin, Madison	NSABB Full Board Meeting (October 22, 2014), National Academies Workshop (December 15, 2014), Public Comment
George Kemble, Ph.D.	3-V Biosciences	National Academies Workshop (December 15, 2014)
Larry Kerr, Ph.D.	National Security Council Staff	WG Meeting (November 5, 2015)
Andy Kilianski, Ph.D.	National Research Council Fellow at US Army	Public Comment

Lynn Klotz, Ph.D.	Center for Arms Control and Non-proliferation	Public Comment
Gregory Koblentz, Ph.D., M.P.P.	George Mason University	National Academies Workshop (December 15, 2014)
Todd Kuiken, Ph.D.	The Wilson Center	In-person Meeting (July 23, 2015)
Robert Lamb, Ph.D., Sc.D.	Northwestern University; Howard Hughes Medical Institute	National Academies Workshop (December 15, 2014)
Linda Lambert, Ph.D.	HHS/National Institutes of Health	In-person WG Meeting (July 23, 2015)
Carol Linden, Ph.D.	HHS/Biomedical Advanced Research and Development Authority	National Academies Workshop (December 15, 2014)
W. Ian Lipkin, M.D.	Columbia University	NSABB Full Board Meeting (October 22, 2014)
Marc Lipsitch, Ph.D.	Harvard School of Public Health	NSABB Full Board Meeting (October 22, 2014), National Academies Workshop (December 15, 2014), Public Comment
Patricia Long, J.D., LL.M.	HHS/Office of Security and Strategic Information	In-person WG Meeting (July 24, 2015)
Nicole Lurie, M.D., M.S.P.H.	HHS/Assistant Secretary for Preparedness and Response	NSABB Full Board Meeting (October 22, 2014); In-person WG Meeting (July 23, 2015)
Eric Meslin, Ph.D.	Indiana University School of Medicine	NSABB Full Board Meeting (September 28, 2015)
Corey Meyer, Ph.D.	Gryphon Scientific	NSABB Full Board Meeting (September 28, 2015)
Rebecca Moritz, M.S., C.B.S.P., S.M.(NRCM)	University of Wisconsin–Madison	National Academies Workshop (December 15, 2014)
Peter Murakami	Baltimore, Maryland	Public Comment
Kalyani Narasimhan, Ph.D.	Nature Publishing Group	In-person WG Meeting (July 23, 2015)
Daniel O'Connell	Albany, Oregon	Public Comment
Kimberly Orr, Ph.D.	US Department of Commerce	In-person WG Meeting (July 23, 2015)
Michael Osterholm, Ph.D., M.P.H.	University of Minnesota	NSABB Full Board Meeting (October 22, 2015)
Kenneth Oye, Ph.D.	Massachusetts Institute of Technology	In-person WG Meeting (July 23, 2015)
Megan Palmer, Ph.D.	Center for International Security and Cooperation, Stanford University	Public Comment
Christopher Park	U.S. Department of State	In-person WG Meeting (July 23, 2015)
Jean Patterson, Ph.D.	Texas Biomedical Research institute	In-person WG Meeting (January 22, 2015)
Daniel Perez, Ph.D.	University of Maryland	NSABB Full Board Meeting (October 22, 2014)
Janet Peterson, C.B.S.P.	University of Maryland	NSABB Full Board Meeting (October 22, 2014)
Dustin Phillips	Louisville, Kentucky	Public Comment
Stanley Plotkin, M.D.	University of Pennsylvania	Public Comment
David Relman, M.D.	Stanford University	National Academies Workshop (December 15, 2014)
David B. Resnik, J.D., Ph.D.	HHS/National Institutes of Health	NSABB Full Board Meeting (October 22, 2014)

Colin Russell, Ph.D.	University of Cambridge	National Academies Workshop (December 15, 2014)
Steven L. Salzberg, Ph.D.	Johns Hopkins University School of Medicine	Public Comment
Monica Schoch-Spana, Ph.D.	University of Pittsburgh Medical Center (UPMC) Center for Health Security	National Academies Workshop (December 15, 2014)
Stacey Schultz-Cherry, Ph.D.	St. Jude Children's Research Hospital	NSABB Full Board Meeting (October 22, 2014), National Academies Workshop (December 15, 2014)
Shannon Scott		Public Comment
Michael Selgelid, Ph.D.	Monash University	NSABB Full Board Meeting (September 28, 2015)
Billie Sellers		Public Comment
Richard Sever, Ph.D.	Cold Spring Harbor Laboratories Press bioRxiv	In-person WG Meeting (July 23, 2015)
Michael Shaw, Ph.D.	Centers for Disease Control and Prevention	In-person WG Meeting (July 23, 2015)
Bill Sheridan, M.B., B.S.	BioCryst Pharmaceuticals Inc.	NSABB Full Board Meeting (October 22, 2014)
Lone Simonsen, Ph.D.	George Washington University	Public Comment
Andrew Snyder-Beattie	Future of Humanity Institute, University of Oxford	Public Comment
Charles Stack, M.P.H.	University of Illinois at Chicago	Public Comment
John Steel, Ph.D.	Emory University	Public Comment
Kanta Subbarao, M.B.B.S., M.P.H.	HHS/National Institutes of Health	National Academies Workshop (December 15, 2014), Public Comment
Robert Temple, M.D.	Food and Drug Administration	In-person WG Meeting (July 23, 2015)
Eileen Thacker, D.V.M., Ph.D., DACVM	Department of Agriculture	In-person WG Meeting (July 23, 2015)
Kimball Ward		Public Comment
Robert Webster, Ph.D.	St. Jude Children's Research Hospital	National Academies Workshop (December 15, 2014)
Jerry Weir, Ph.D.	Food and Drug Administration	National Academies Workshop (December 15, 2014)
Robbin Weyant, Ph.D., R.B.P. (ABSA)	Center for Disease Control and Prevention	National Academies Workshop (December 15, 2014), In-person WG Meeting (July 23,2015)
Gary Whittaker, Ph.D.	Cornell University	Public Comment
Carrie Wolinetz, Ph.D.	HHS/National Institutes of Health	NSABB Full Board Meeting (May 5, 2015 and January 7-8, 2016)
Infectious Diseases Society of America	Infectious Diseases Society of America	Public Comment

2002

2003 **Table 2. Sources consulted by NSABB and NSABB working groups include but are not limited to the following**

Authors	Title
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Doshi, P., 2008	Trends in Recorded Influenza Mortality - United States 1900–2004
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Fedson, D.S., and Opal, S.M., 2013	The controversy over H5N1 transmissibility research
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Jaffe, H., et al., 2013	Extra oversight for H7N9 experiments
Linster, M., et al., 2014	Identification, characterization, and natural selection of mutations driving airborne transmission of A/H5N1 virus
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Miller, M., and Palese, P., 2014	Peering into the crystal ball: Influenza pandemics and vaccine efficacy
National Research Council/Institute of Medicine, 2015	Potential Risks and Benefits of GOF Research – NRC/IOM Workshop Summary (Full Report)
Nature Editorial, 2014	A ripe time for gaining ground
NIH Blue Ribbon Panel Slide Presentation, 2008	Blue Ribbon Panel Scientific Subcommittee Teleconference slide presentation (May 2008)
Osterholm, M., and Relman, D., 2012	Creating mammalian-transmissible A/H5N1 influenza virus: Social contracts, prudence, and alternative perspectives
Palmer, M.J., et al., 2015	A more systematic approach to biological risk
Pascua, P.N., et al., 2012	Virulence and transmissibility of H1N2 influenza virus in ferrets imply the continuing threat of triple-reassortant swine viruses
Patterson, A., et al., 2013	A framework for decisions about research with HPAI H5N1 viruses
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Stern, P.C., and Fineberg, H.V., 1996	Understanding Risk - Informing Decisions in a Democratic Society
Sullivan, M., et al., 2013 (RMS White Paper)	Influenza Pandemic Risk - The Contribution of Laboratory Pathogens to Excess Mortality Risk
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Trock, S., et al., 2015	Development of Framework for Assessing Influenza Virus Pandemic Risk
USG (June 2013)	Biological Safety Guidance for Research with Risk Group 3 Influenza Viruses - Human H2N2, 1918 H1N1, and HPAI H5N1
USG (December 2009)	Biosafety in Microbiological and Biomedical Laboratories BMBL (5th Edition)
USG (September 2014)	Companion Guide to the USG Policies for Oversight of Life Sciences Dual Use Research of Concern
USG (February 2005)	Environmental Impact Statement For the Galveston National Laboratory for Biodefense and Emerging Infectious Diseases
USG (as of July 2015)	Federal Select Agents and Toxins List
USG (July 2012)	Final Supplementary Risk Assessment for the Boston University National Emerging Infectious Diseases Laboratories (NEIDL)
USG (August 2013)	HHS Funding Framework for HPAI H5N1 Studies
USG (February 2013)	NIH Guidelines for Research Involving Recombinant DNA Molecules - Amendment Notice. February 21, 2013
USG (November 2013)	NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
USG (October 2014)	USG Gain-of-function GOF Deliberative Process and Funding Pause Statement
USG (September 2014)	USG Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern
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Volkswagen Foundation and Max Plank Society, 2014	Dual Use Research on Microbes - Biosafety, Biosecurity, Responsibility - Hanover Symposium Summary Report
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Zhang, Y., et al., 2013	H5N1 hybrid viruses bearing 2009/H1N1 virus genes transmit in guinea pigs by respiratory droplet

2004

2005 ## Appendix E. Policy Analysis Summary Table

2006

Oversight Measures	Risks Addressed	Description of Oversight	Analysis/Applicability to GOF Studies
Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition (December 2009) http://www.cdc.gov/biosafety/publications/bmbl5/index.htm	Biosafety risks	**Applies to:** Life sciences research involving infectious microorganisms or hazardous biological materials **Description:** General biosafety practices and biological containment for various classifications (risk groups) of microorganisms and etiological agents	BMBL does not describe GOF studies per se but does include summary statements and biocontainment guidance for research involving various influenza strains (including contemporary and non-contemporary human, high and low pathogenic avian, swine, the 1918 influenza strain, and reassortant viruses) and SARS-CoV. MERS-CoV had not emerged at the time of the last BMBL update but interim laboratory biosafety guidance was issued by CDC and is referenced by BMBL. BMBL is a guidance document and generally considered the authoritative reference for laboratory biosafety but it is not a regulatory document; compliance is voluntary.
NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (November 2013) http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines	Biosafety risks	**Applies to:** Basic or clinical life sciences research that involves recombinant or synthetic nucleic acid molecules and is conducted at an institution receiving NIH funding for any such research **Description:** Describes roles and responsibilities of institutions and investigators in safely conducting research. Requires institutional review with a focus on the concepts of risk assessment, risk group classification of agents, physical and biological containment levels, practices, personal protective equipment, and occupational health. **Advised by:** NIH Recombinant DNA Advisory Committee (RAC)	The NIH Guidelines have been amended to include additional guidance for work with Risk Group 3 influenza viruses (1918 H1N1, H2N2, highly pathogenic avian influenza (HPAI) H5N1) to specify enhancements to biosafety level 3 containment, practices, and occupational health requirements. NIH Guidelines were amended again to require further enhancements to facilities, biosafety equipment and practices, including occupational health practices, for research involving HPAI H5N1 strains transmissible among mammals by respiratory droplets. NIH Guidelines are often used as a model of biosafety guidance by the broader scientific community but compliance is required only by institutions receiving such funding from the NIH. The scope is also limited to research involving recombinant or synthetic nucleic acids. Some IBCs also review and approve non-recombinant pathogen research; however, not all institutions require their IBCs to do so.
HHS and USDA Select Agent Program (as of July 2014) http://www.selectagents.gov/regulations.html	Biosecurity (physical and personnel) and biosafety risks	**Applies to:** Biological agents and toxins that have the potential to pose a severe threat to public health and safety, based on a set of criteria. **Description:** Regulates the possession, use, and transfer of select agents and toxins. Overseen by the Federal Select Agent Program. Requires registration of individuals and entities; federal background investigations; federal review of restricted experiments; training; institutional compliance; etc. **Advised by:** Intragovernmental Select Agents and Toxins Technical Advisory Committee (ISATTAC)	Studies that could be considered GOF studies, which involve pathogens on the select agent list, are subject to oversight by the SAP. Researchers and institutions performing such studies must receive favorable security risk assessments by the FBI, register with the SAP, receive training on the proper procedures and practices for handling such agents, and abide by other aspects of the regulations. SARS-CoV, HPAI H5N1 influenza, and 1918 influenza viruses are select agents and GOF studies involving these pathogens are subject to oversight by the SAP. Restricted experiments that would entail conferring antiviral resistance to these viruses would require additional review and approval prior to being conducted.

			GOF experiments involving MERS, and other agents not included on the select agent list, would not be subject to oversight by the SAP.
USG Policy for Federal Oversight of DURC (March 2012) http://www.phe.gov/s3/dualuse/Pages/USGOversightPolicy.aspx	Biosecurity risks, particularly involving misuse of research information, products, and technologies (DURC)	**Applies to:** Life sciences research conducted at an institution receiving USG funding that involves any of 15 agents that pose the greatest risk of deliberate misuse with most significant potential for mass casualties or devastating effects.	The federal DURC policy requires identification and oversight of certain pathogen research involving 7 experimental types, some of which can be described as GOF experiments (i.e., enhancing the harmful consequences of an agent; increase transmissibility; alter host range; etc.) by Federal funding agencies. DURC policies only apply to research involving 15 pathogens. Institutions may review other studies for DURC potential but are not required to do so. Certain GOF studies that involve other agents would not be subject to DURC oversight under the policies.
USG Policy for Institutional Oversight of DURC (September 2014) http://www.phe.gov/s3/dualuse/Pages/InstitutionalOversight.aspx	Biosecurity risks, particularly involving misuse of research information, products, and technologies (DURC)	**Applies to:** Life sciences research conducted at an institution receiving USG funding that involves any of 15 agents that pose the greatest risk of deliberate misuse with most significant potential for mass casualties or devastating effects.	The institutional DURC policy requires federally-funded institutions to establish a system for the identification and oversight of certain pathogen research involving 7 experimental types, some of which can be described as GOF experiments (i.e., enhancing the harmful consequences of an agent; increase transmissibility; alter host range; etc.) DURC policies only apply to research involving 15 pathogens. Institutions may review other studies for DURC potential but are not required to do so. Certain GOF studies that involve other agents would not be subject to DURC oversight under the policies.
HHS Funding Framework for GOF studies (August 2013) http://www.phe.gov/s3/dualuse/Pages/HHSh5n1Framework.aspx	Biosafety and biosecurity risks associated with certain GOF experiments involving agents with pandemic potential	**Applies to:** Gain-of-function studies that are reasonably anticipated to generate HPAI H5N1 viruses that are transmissible, and LPAI H7N9 viruses that have increased transmissibility, between mammals by respiratory droplets **Description:** Describes an HHS Department-level review pre-funding review and approval process for certain GOF studies, which can result in funding, not funding, or funding with certain conditions and ongoing oversight.	The only policy focused specifically on funding decisions related to the types of GOF studies that have raised concern. Narrowly focused only on specific GOF studies (enhancing mammalian transmissibility) on two avian influenza viruses; other GOF studies may raise concern and would not be reviewed under this framework.
USG Export Controls (as of July 2014) http://www.bis.doc.gov/index.php/regulations/export-administration-regulations-ear		**Applies to:** Export or release of equipment, software and technology, chemicals, microorganisms, toxins, and other materials and information deemed dual use or strategically important to U.S. national security, economic, and/or foreign policy interests	Comprehensive set of federal regulations that control and restrict the export and release of sensitive equipment, software and technology; chemical, biological, and other materials and information as a means to promote national security interests and foreign policy objectives.

2007

2008

Appendix F. National Science Advisory Board for Biosecurity Roster

National Science Advisory Board for Biosecurity Roster

[Ŧ] NSABB Working Group Co-chair
[†] NSABB Working Group on the Design and Conduct of Risk and Benefit Assessments of Gain-of-Function Studies
[‡] NSABB Working Group on Evaluating the Risks and Benefits of Gain-of-Function Studies
[*] NSABB Member, Retired

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U.S. GOVERNMENT POLICY FOR INSTITUTIONAL OVERSIGHT OF LIFE SCIENCES DUAL USE RESEARCH OF CONCERN (iDURC Policy)

Institutional DURC Policy Implementation Metrics

Background

On September 24, 2014, the United States Government (USG) released the *United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern* (Institutional DURC Policy). The policy addresses institutional oversight of dual use research of concern (DURC), which includes policies, practices, and procedures to ensure DURC is identified and risk mitigation measures are implemented, where applicable. Institutional oversight of DURC is the critical component of a comprehensive oversight system because institutions are most familiar with the life sciences research conducted in their facilities and are in the best position to promote and strengthen the responsible conduct and communication.

According to the policy, the USG is required to periodically assess the impact of this Policy on life sciences research programs and institutions, and update this Policy and the *United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern* (March 2012 DURC Policy), as appropriate. This should be informed by national and international dialogue with interested communities, including scientists, research administrators, security experts, and public health officials. In support of this effort to assess the impact of this Policy, the USG is requesting voluntary information from institutions subject to the Policy.

1) **The USG is seeking information from institutions on any processes, methods or parameters used by the institution, Institutional Review Entity (IRE), or Principal Investigator (PI) to determine if research is subject to the Policy, meets the definition of DURC, or what risk mitigation measures are required.**
2) **The USG is seeking information on the burden of the Policy on institutions, in terms of time or personnel required or financial cost. (e.g., IRE decision-making, compliance certification, training, risk/benefit assessment and mitigation planning). It would also be helpful to know if your institution or PIs have opted out of research because it might be subject to the Policy.**
3) **The USG is seeking information on the communication of DURC research during the publication phase, including whether papers required modification or types of risk communication strategies that have been employed.**

Full List of Institutional Metrics

Parameters Used to Identify Research Subject to the Policy and that Meets the Definition of DURC, and to Identify Risks and Benefits and Complete a Risk/Benefit Assessment

- What are the parameters used by an institution to determine if the research would produce one or more of the 7 experimental effects?
- What are the parameters used by an institution to determine if the research meets the definition of DURC?
- What are the parameters used by an institution to identify risks and benefits and complete a risk/benefit assessment for the determination of DURC?

Burden on Institutions (in terms of time, personnel, or financial cost)

- # of person-hours and/or financial cost it takes the institution/IRE to :
    - Determine whether the research involves one of the 15 agents/toxins and also produces one or more of the 7 experimental effects
    - Certify that they are in compliance (have ICDUR, IRE and trained personnel with training records);
    - Train staff working on DURC projects and maintain their training records
    - Complete a risk/benefit assessment
    - Make a determination whether the research is DURC or not
    - Develop a draft initial risk mitigation plan
- Are there additional costs of implementing the institutional DURC policy? If so, what are those costs? (e.g., standing up DURC oversight program, designating an Institutional Contact for Dual Use Research (ICDUR), IRE, training personnel, maintaining training records, amending standard terms conditions for funding agreements, etc.)
- Number of times the iDURC policy was cited as a barrier to continuation or initiation of research that would be subject to the policy

Communication of DURC Research

- Number of instances in which an institution had to review or modify a research report pre-publication because of the Institutional DURC Policy requirements
- Types of risk communication strategies that are being employed by institutions when publishing DURC research and number of times used

U.S. GOVERNMENT POLICY FOR INSTITUTIONAL OVERSIGHT OF LIFE SCIENCES DUAL USE RESEARCH OF CONCERN (iDURC Policy)

DURC Federal Funding Agency iDURC Implementation Metrics

Identification of Research Subject to the Policy and the Determination of DURC

- Number of projects identified as using one or more of the 15 DURC agents/toxins but not producing one or more of the 7 experimental effects
- Number of projects identified as using one or more of the 15 agents/toxins and producing one or more of the 7 experimental effects that were defined as DURC
- Number of projects identified as using one or more of the 15 agents/toxins and producing one or more of the 7 experimental effects that were defined as non-DURC
- Number of institutions/projects that fall under the policy due to use of botulinum neurotoxin but are not defined to be DURC
- Number of times a project not initially evaluated as DURC was reevaluated and determined to meet the definition of DURC while underway/ Number of times status of an institution's DURC project changes from one reporting period to another [this data may help to make the case to reduce reporting under DURC policy from a semi-annual to an annual cycle].

Required Policy Notification Timeframes

- Number of days, relative to funding opportunity closing date (negative numbers allowed) , taken to notify the USG of research activity that the IRE assesses and determines to be DURC/non-DURC
- Number of days taken for the U.S. Government (USG) funding agency to concur or not-concur with the institution's initial IRE review
- In the event of non-concurrence with initial review, number of days to achieve either concurrence or a final USG determination.
- Number of days taken to achieve USG-Institutional Review Entity (IRE) concurrence on, and finalization of, risk mitigation plan

Adjudication of Differences in Identification of Research Subject to the Policy

- Number of projects in which the USG disagreed with the institutions in the determination of whether or not the project meets the definition of DURC
- Number of projects in which the USG does not come to agreement with the IRE concerning adequacy of the IRE-proposed risk mitigation plan
- Number of projects in which award offers or remaining funding was retracted or terminated

Totals by Number for Institutions under the Policy

- Number of times institutions consulted with the USG funding agency on advice for matters related to DURC and type of issue

- Number of non-USG funded projects that were submitted to NIH per the policy if the research was subject to the scope of the policy
- Number of international institutions that fell under the policy because they received USG funds
- Number of institutions that were determined to be non-compliant within the last year
- Number of multi-agency projects that fall under the policy [For example, we have U.S. Geological Survey scientists conducting research funded by another D/A. Is this a single occurrence? Are there other situations that may lead to double-counting/duplication in the reporting?]
- Number of projects/institutions that fall under the policy via the reach-through provision

From:	Viggiani, Christopher (NIH/OD) [E]
To:	Betty Lee; Christine Grant; Christopher Park; Clifford W. Houston; Craig Cameron; David Woodland; Dixon, Dennis M. (NIH/NIAID) [E]; Diane DiEuliis; Drew Endy; Francis Macrina; Gangadharan, Denise (CDC/OPHPR/DSAT); Gary Resnick; Gerald Epstein; Hauguel, Teresa (NIH/NIAID) [E]; Hu-Primmer, Jean (OS/ASPR/BARDA); James LeDuc; Patterson, Jean (Texas Biomedical Research Institute); Joseph Kanabrocki; Joseph McDade; Kenneth I. Berns; Kimberly Orr; Lawrence, Theresa (OS/ASPR/OPP); Marcelle Layton; Margie Lee; Marie-Louise Hammarskjöld; Meg Flanagan; Post, Diane (NIH/NIAID) [E]; Resnik, David (NIH/NIEHS) [E]; Jaffe, Richard (OS/ASPR/OPP); Robert Miceli ; Phillips, Sally (HHS/ASPR/OPP); Sharlene Weatherwax; Stephen Morse; Susan Wolf; Theresa Koehler; Todd Anderson; Wendy Hall
Cc:	Alex Wadley; Alicia Simmons; Ashley Connally; Caroline Brendel; Christine Dorosin; Eileen Prainum; Eileen Rodriguez; Imelda Mendoza; Jane Lalich; Jeannette Gagnon; Jessica Petrillo; Lyz Morrison; Bull, Melbourne (NIH/NIAID) [E]; Sherry Coven; Beckham, Shayla (NIH/OD) [E]; Fennington, Kelly (NIH/OD) [E]; Harris, Kathryn (NIH/OD) [C]; Nightingale, Stuart (NIH/OD) [C]; O"Reilly, Marina (NIH/OD) [E]; Ramkissoon, Kevin (NIH/OD) [C]; Rona Hirschberg; Viggiani, Christopher (NIH/OD) [E]
Subject:	MATERIALS: NSABB Working Group Teleconference
Date:	Friday, April 29, 2016 4:21:49 PM
Attachments:	image001.png 3-Draft Figures 4-29-2016.pdf 0-Agenda 5-3-2016 WG Teleconfernce.docx 1-NSABB Draft Report 4-29-2016 CLEAN.pdf 2-NSABB Draft Report 4-29-2016 Tracked.docx

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Dear NSABB working group,

Attached please find materials for the teleconference next week, **Tuesday May 3, from 2:00 pm – 4:00 pm Eastern.**

Call-in number: (b)(6)
Participant Code: (b)(6)

Based on the last call and in consultation with the WG co-chairs, NIH has updated the draft report. **The purpose of this call will be to finalize the draft report before the upcoming NSABB meeting.** After this call the draft report will be made public and is expected to be voted on by the full Board. On the attached agenda we have indicated specific edits for your review but please examine the entire report and come prepared to discuss any outstanding issues.

Attachments

Agenda

NSABB Draft Report, v4-29-2016—PDF Version (clean, significant edits in red)

NSABB Draft Report—Word Version, v4-29-2016 (all edits shown in track changes)

Draft Figures of proposed review process and FACA evaluation

NOTE: Please hold the date: May 9th from 2:00 pm – 4:00 pm Eastern. This is a "just in case" call if needed for the draft report or planning purposes.

Have a nice weekend all, look forward to talking with you next week.

Chris

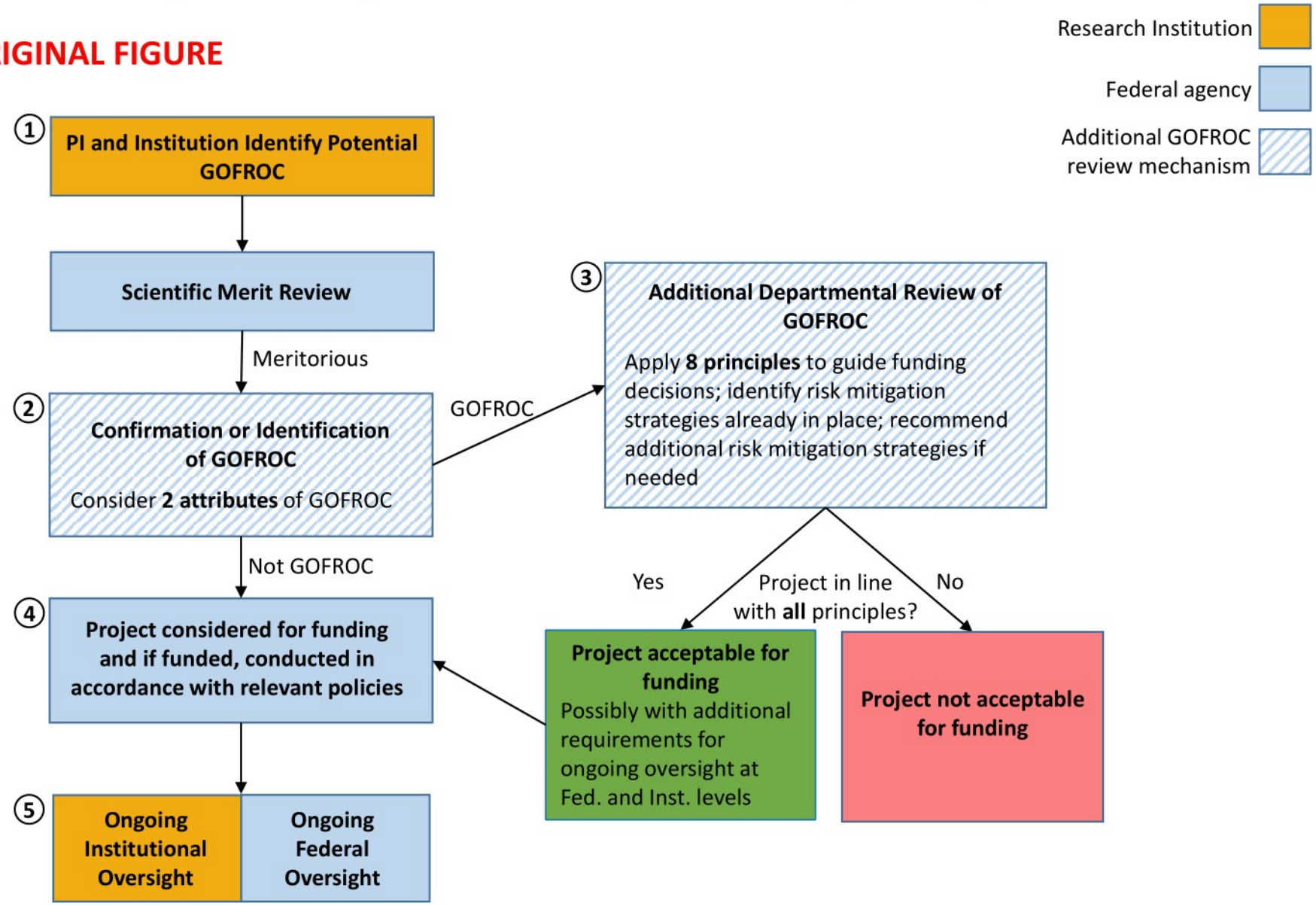
Christopher Viggiani, Ph.D.
Office of Science Policy
Office of the Director
National Institutes of Health
Office: (b)(6) || Mobile: (b)(6)
(b)(6)

NIH logo

OSP Blog: Under the Poliscope
Twitter: @CWolinetzNIH

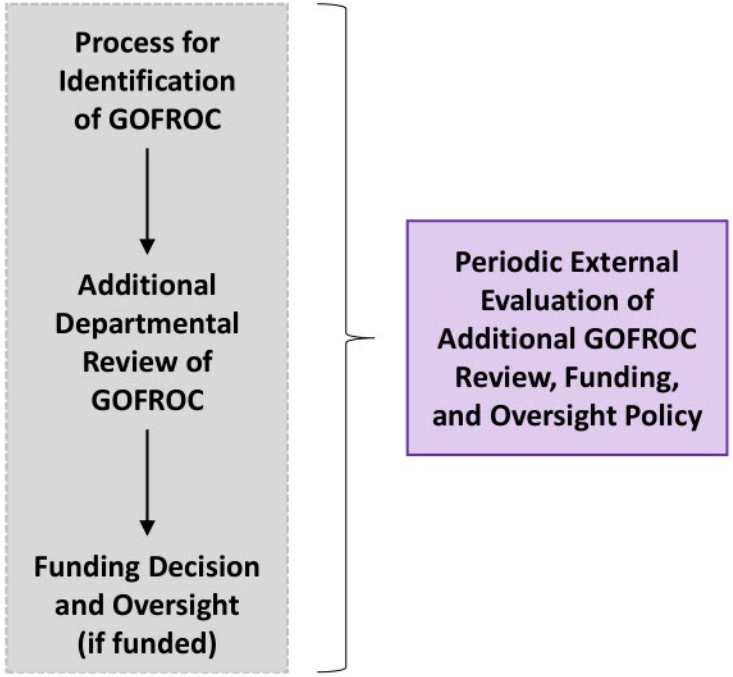
Review, Funding, and Oversight of GOF Research of Concern (GOFROC)

ORIGINAL FIGURE

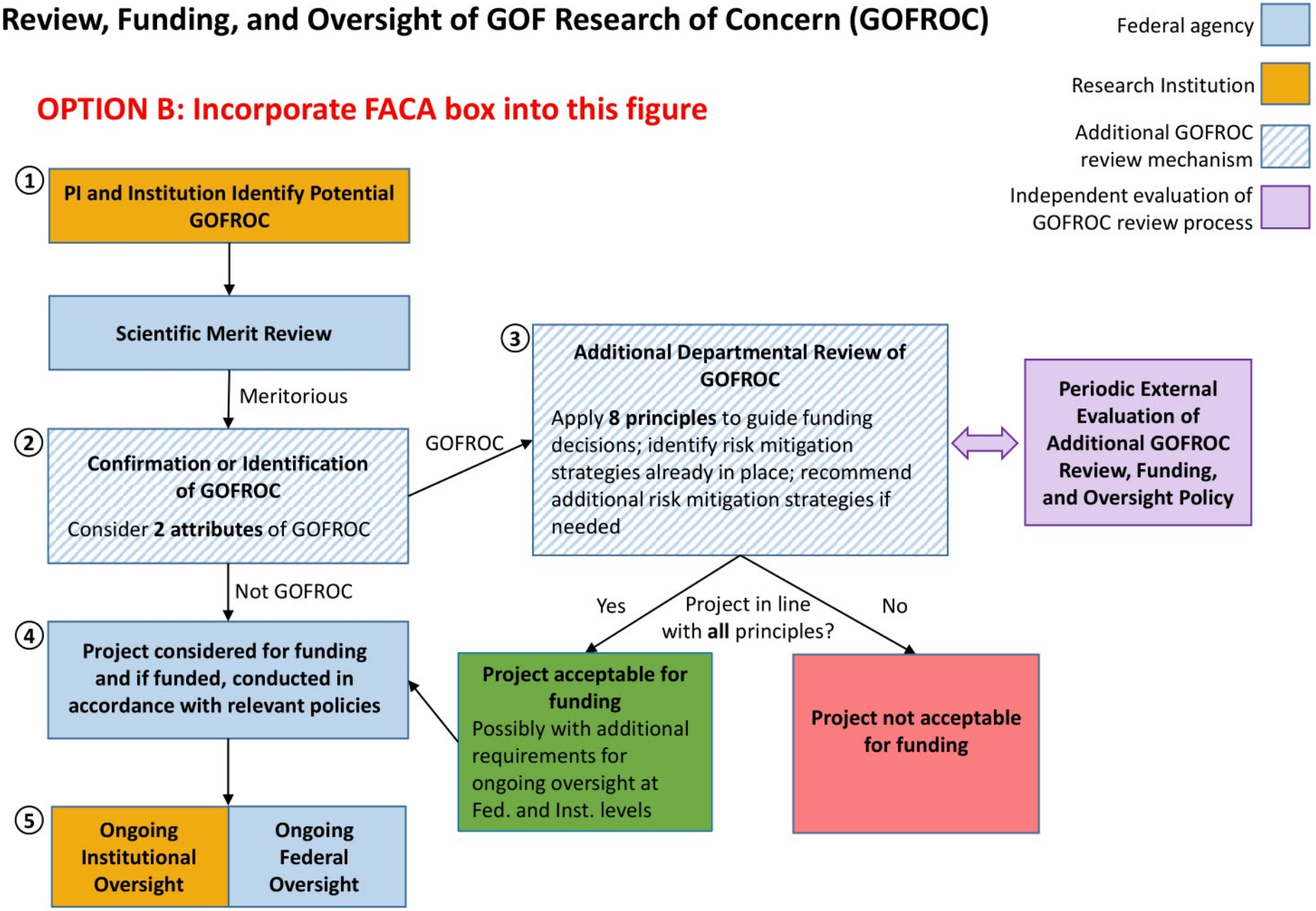


Evaluation of Additional GOF Research of Concern (GOFROC) Review, Funding, and Oversight Process

OPTION A: Illustrate FACA evaluation as its own figure



Review, Funding, and Oversight of GOF Research of Concern (GOFROC)



NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY

Working Group on Evaluating Risks and Benefits of GOF Studies

May 3, 2016
2:00 pm – 4:00 pm Eastern
Call-in number: (b)(6)
Participant Code: (b)(6)

- **WG Roll Call** (5 min)
  Christopher Viggiani

- **Opening Remarks, Agenda Review, Meeting Goals** (5 min)
  Joe Kanabrocki, co-chair
  Ken Berns, co-chair

- **Discussion of Major Edits to Draft Report**
  - Page 9, line 240: Added description of NSABB's interpretation of its charge
  - Page 43, line 1359: Discussion of principle 6 and whether intention is for "results" or "benefits" should be shared
  - Page 48, line 1413: The "points to consider" recommendation was moved here
  - Page 49, line 1449: Box 4 was updated and moved from Rec 3 to here
  - Page 50, line 1461: The FACA rec was moved from Rec 3.2 to here [See new Figure in text and separately, an alternative figure]

- **Discussion of Any Other Areas in Draft Report**

- **Next Steps**
  - Submit any specific edits to NIH as soon as possible
  - NIH will make any edits discussed today
  - NIH will circulate a final draft to WG for a quick clearance (~24 hours) and work with NSABB chair and WG co-chairs to finalize
  - NIH plans to post online in advance of the meeting by Friday May 6.
  - This draft report will be discussed and voted on at NSABB meeting
    - Edits and changes can be proposed at the meeting and a report can be voted on with specific edits or amendments to be included
    - Copyedits can be included after the NSABB meeting and will be reviewed/approved by NSABB chair
  - **Please hold the date: May 9th from 2:00 pm – 4:00 pm EDT.** This is a "just in case" call if needed for draft report or planning purposes.
  - **NSABB Meeting** — May 24th from 10:30AM – 4:00PM EDT; Note new start time.

Meeting Materials

- Agenda
- NSABB Draft Report, v4-29-2016—PDF Version (clean, significant edits in red)
- NSABB Draft Report—Word Version, v4-29-2016 (all edits shown in track changes)
- Draft Figures

Recommendations for the Evaluation and Oversight of Proposed Gain-of-Function Research

A Draft Report of the NSABB Working Group

Version: April 29, 2016

Preface for NSABB Meeting on May 24, 2016

This draft report was developed by the NSABB working group tasked with evaluating the risks and benefits associated with gain-of-function studies and developing draft recommendations on a conceptual approach for the evaluation of proposed gain-of-function studies. The first version of this document was discussed at the NSABB meeting on January 7 & 8, 2016 and again at the symposium hosted by the National Academies on March 10 & 11, 2016. This version represents an updated draft of that initial working paper. This document is still pre-decisional and intended as a deliberative document to be discussed at the meeting of the full NSABB on May 24, 2016. This is document is not a formal NSABB work product and should not be considered to be official NSABB findings or recommendations to the U.S. government. This document does not represent official policy of the U.S. government.

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Executive Summary

Research involving pathogens is essential to global health and security. Such research provides insight into the fundamental nature of human-pathogen interactions, enables the assessment of the pandemic potential of emerging infectious agents, and informs public health and preparedness efforts, including the development of medical countermeasures. Several policies are in place to help ensure that pathogen research is conducted safely and in ways to minimize the risks of laboratory accidents and security risks. Recently, and in the wake of a number of biosafety incidents at Federal facilities, concerns have been raised about certain "gain-of-function" (GOF) studies with the potential to generate pathogens with pandemic potential. The concerns center on whether a pathogen with enhanced transmissibility and/or virulence could be accidentally or intentionally released from a laboratory, potentially exposing surrounding populations to a pathogen with pandemic potential.

The U.S. Government (USG), as part of its continued focus on biosafety and biosecurity, has undertaken a deliberative process to carefully examine the risks and benefits associated with certain GOF studies. The deliberative process involves the National Science Advisory Board for Biosecurity (NSABB), which has been tasked with making recommendations to the USG on this topic, and the National Academy of Sciences (NAS), which was tasked to convene two public symposia to generate broad discussion on the relevant issues. To further inform NSABB deliberations, the National Institutes of Health (NIH) commissioned Gryphon Scientific to perform an independent assessment of the risks and benefits associated with GOF studies and an ethical analysis of the issues related to funding and conducting such studies.

The NSABB was charged with advising on the design of the risk and benefit assessments (RBA) for GOF studies and with providing recommendations to the USG on a conceptual approach for evaluating proposed GOF studies. In May 2015 the NSABB issued its *Framework for Guiding the Conduct of Risk and Benefit Assessments of Gain-of-Function Research*, which guided NIH in overseeing the contractor conducting the risk and benefit assessments. In May 2016, informed by the results of the RBA as well as its analysis of the current policy landscape, consideration of relevant ethical issues, and consultations with domestic and international stakeholders, the NSABB working group will present this draft report for consideration and finalization by the full NSABB.

The NSABB working group has developed 7 major findings:

Key Finding 1: There are many types of GOF research and not all of them have the same level of risks. Only a small subset of GOF research—GOF research of concern (GOFROC)—entail risks that are potentially significant enough to warrant additional oversight.

Key Finding 2. The U.S. government has several policy frameworks in place for identifying and managing risks associated with life sciences research. There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOFROC could be applied.

Key Finding 3. Oversight policies vary in scope and applicability, and are not sufficiently harmonized; therefore, current oversight is not sufficient for all GOFROC.

Key Finding 4. An adaptive policy approach is a desirable way to ensure that oversight and risk mitigation measures remain commensurate with the risks associated with the research and the benefits of the research are being fully realized**.**

Key Finding 5. There are life sciences research studies, including possibly some GOFROC, that should not be conducted on ethical or public health grounds if the potential risks associated with the study are not justified by the potential benefits. Decisions about whether GOFROC should be permitted will entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of proposed studies but other considerations, including legal, ethical, and societal values are also important.

Key Finding 6. Managing risks associated with GOFROC, like all life sciences research, requires Federal-level and institutional oversight, awareness and compliance, and a commitment by all stakeholders to safety and security.

Key Finding 7. Consideration of the international dimensions associated with funding and conducting GOF research of concern is important. It is important to engage with and to continue an active dialogue with the international community on GOFORC as well as on DUR/DURC.

The NSABB working group has developed 7 recommendations to the U.S. government:

Recommendation 1. Research proposals involving GOFROC entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the Federal and institutional levels.

As part of this recommendation, the NSABB working group has proposed a conceptual approach for guiding funding decisions about GOFROC. First, the working group identified the attributes of GOFROC, which is research that could generate a pathogen that is: 1) highly transmissible and likely capable of wide and uncontrollable spread in human populations; and 2) highly virulent and likely to cause significant morbidity and/or mortality in humans. Next, the working group identified a set of principles that should guide funding decisions for GOFROC. Only research that is determined to be in line with these principles should be funded. Additional risk mitigation measures may be required for certain research studies to be deemed acceptable for funding.

Recommendation 2. An external advisory body that is designed for transparency and public engagement should be utilized as part of the U.S. government's ongoing evaluation of oversight policies for GOFROC.

Recommendation 3. In general, oversight mechanisms for GOFROC should be incorporated into existing policy frameworks when possible.

Recommendation 4. The U.S. government should pursue an adaptive policy approach to help ensure that oversight remains commensurate with the risks associated with the GOFROC.

Recommendation 4.1. The U.S. government should consider developing a system to collect and analyze data about laboratory safety incidents to inform GOFROC policy development over time.

Recommendation 5. The U.S. government should consider ways to ensure that all GOFROC conducted within the U.S. or by U.S. companies be subject to oversight, regardless of funding source.

Recommendation 6. The U.S. government should undertake broad efforts to strengthen laboratory biosafety and biosecurity and, as part of these efforts, seek to raise awareness about the specific issues associated with GOFROC.

Recommendation 7. The U.S. government should engage the international community in a dialogue about the oversight and responsible conduct of GOFROC.

1. Introduction

A robust life sciences research enterprise is necessary to counter the continually evolving threats to public health and national security posed by endemic and emerging pathogens, as well as malicious biological threats. By helping to define the nature of human-pathogen interactions, life sciences research promotes public health and national security not only by enhancing our understanding of pathogen biology and disease pathogenesis, but also by informing biosurveillance and medical countermeasure development. Such research can also aid in the assessment of the pandemic potential of emerging infectious agents, thereby underpinning health policy decisions and preparedness and response efforts.

While the ultimate goal of life sciences research involving pathogens is the protection and promotion of public health, there are inherent associated biosafety and biosecurity risks. Potential risks might arise from laboratory accidents or security breaches that result in laboratory acquired infections or the accidental or deliberate release of a pathogen from containment. Life sciences research has "dual use" potential. That is, legitimate research may generate information, products or technologies that could be misused to threaten public health or national security. To mitigate such dual use concerns, as well as potential biosafety and biosecurity risks, research involving pathogens is subject to multiple layers of Federal and institutional oversight.

The Gain-of-Function Debate and the USG Response

Experimental techniques and approaches that modify the genome of microorganisms are routinely employed in pathogen research to ascertain the roles of genes and their functional products. Such studies are fundamental to the field of microbial genetics and facilitate correlation of genetic and phenotypic characteristics – a critical step in deciphering the complex nature of host-pathogen interactions that underlie transmission, infection, and pathogenesis. Such genetic manipulations can result in either diminished (loss-of-function) or enhanced (gain-of-function) biological phenotypes.

Studies that result in the generation of pathogens with enhanced, or gain-of-function (GOF), phenotypes are conducted for a number of valid scientific purposes. Such studies provide information that adds to the scientific knowledge base and can inform biosurveillance, medical countermeasure development, and public policy decision-making related to public health and preparedness. The vast majority of such GOF studies do not raise significant safety or security concerns. However, certain GOF studies involving pathogens have raised significant concerns about whether a laboratory-generated pathogen with pandemic potential could be accidentally or intentionally released, resulting in significant consequences to public, or perhaps, global health. Concerns have also been raised about whether certain GOF studies could generate information that could enable individuals with malevolent intent to generate a pathogen with pandemic potential (see Box 1).

The controversy over certain GOF studies arose after two groups demonstrated that highly pathogenic avian influenza H5N1 viruses with a small number of engineered mutations became transmissible between mammals by respiratory droplets.[1,2] In 2012, in response to the controversy associated with publishing the manuscripts describing these findings, the influenza community initiated a voluntary suspension of certain GOF studies involving highly pathogenic avian influenza H5N1 viruses. During that time, policymakers considered whether certain GOF studies should be conducted using Federal funds, and if so, how those studies could be safely conducted. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) issued new biosafety guidelines for working with highly pathogenic avian influenza strains.[3,4] The U.S. Department of Health and Human Services (HHS) developed a framework for guiding its funding decisions about GOF projects that may generate H5N1 or H7N9 avian influenza viruses that are transmissible between mammals by respiratory droplets.[5]

Concerns regarding laboratory safety and biosecurity associated with GOF studies were renewed following a number of biosafety incidents at U.S. Federal laboratories during the summer of 2014. The incidents did not involve GOF studies *per se* but raised broader concerns about laboratory safety and security as it applies to pathogen research.

Box 1. Gain-of-Function Research

Recently, the phrase "gain-of-function research" has become synonymous with certain studies that enhance the ability of pathogens to cause disease. However, gain-of-function studies, as well as loss-of-function studies, are common in molecular and microbiology and form the foundation of microbial genetics. Changes to the genome of an organism, whether naturally occurring or directed through experimental manipulations in the laboratory, can result in altered phenotypes as biological functions are lost or gained. Investigators routinely conduct loss- and gain-of-function experiments to understand the complex nature of host-pathogen interactions that underlie transmission, infection, and pathogenesis.

The term "gain-of-function" is generally used to refer to changes resulting in the acquisition of new, or an enhancement of existing, biological phenotypes. This report further defines "gain-of-function research of concern" to describe the subset of studies that have been the subject of recent debate regarding potential biosafety and biosecurity implications -- that is, gain-of-function studies with the potential to generate pathogens with pandemic potential in humans by exhibiting high transmissibility and high virulence. See Section 6 for a more rigorous description of GOF research of concern (GOFROC).

As one component of comprehensive efforts to review and enhance laboratory biosafety and biosecurity, the U.S. government (USG) embarked on a deliberative process to re-evaluate the risks and benefits of certain GOF research with a goal of developing policy governing the funding and conduct of

[1] Imai et al. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 486, 21 June 2012

[2] Herfst et al. Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets. *Science* 336, 22 June 2012

[3] Gangadharan D, Smith J, and Weyant R. Biosafety Recommendations for Work with Influenza Viruses Containing a Hemagglutinin from the A/goose/Guangdong/1/96 Lineage, Morbidity and Mortality Weekly Report 62(RR06); 1-7. http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6206a1.htm

[4] *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

[5] Framework for Guiding Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets, February 21, 2013. http://www.phe.gov/s3/dualuse/Documents/funding-hpai-h5n1.pdf

such research.[6] The deliberative process involves the National Science Advisory Board for Biosecurity (NSABB), which serves as the official Federal advisory body for providing advice in this area, and the National Academy of Sciences (NAS), which is to foster broader scientific and public discussions on the topics. To inform NSABB deliberations, NIH commissioned formal risk and benefit assessments (RBA) of GOF research involving pathogens with pandemic potential and an analysis of ethical issues surrounding the conduct of such studies. Stakeholder input is also essential to the process and has been received throughout NSABB's deliberative process.

The deliberative process is accompanied by a pause in the provision of new federal funds for certain GOF research involving influenza, Middle East Respiratory Syndrome (MERS) or Severe Acute Respiratory Syndrome (SARS) viruses—pathogens determined to have pandemic potential. Specifically:

> *New USG funding will not be released for gain-of-function research projects that may be reasonably anticipated to confer attributes to influenza, MERS, or SARS viruses such that the virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route. This restriction would not apply to characterization or testing of naturally occurring influenza, MERS, and SARS viruses, unless the tests are reasonably anticipated to increase transmissibility and/or pathogenicity.*[7]

In parallel, the USG has encouraged the research community (both those who receive USG funding and those who do not) to join in adopting a voluntary pause on any ongoing research that involves the types of studies that are subject to the funding restriction above.

NSABB recommendations will inform the USG as it develops policies about whether certain types of GOF studies on pathogens with pandemic potential should be supported and, if so, how such research proposals should be evaluated to inform funding and oversight decisions. It is expected that the temporary funding pause will be lifted and/or replaced by a decision or policy that addresses GOF research involving the generation of pathogens with pandemic potential.

2. NSABB Charge

On October 22, 2014, as part of the USG's deliberative process for GOF studies, the NSABB was issued its charge to:

1. Advise on the design, development, and conduct of risk and benefit assessments for GOF studies, and
2. Provide recommendations to the U.S. government on a conceptual approach to the evaluation of proposed GOF studies

In developing its recommendations the NSABB was asked to consider: the results of the risk and benefit assessments; the discussions hosted by the National Academies; the spectrum of potential risks and

[6] U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses, U.S. Government, October 17, 2014. http://www.phe.gov/s3/dualuse/documents/gain-of-function.pdf

[7] Ibid.

benefits associated with GOF studies; and any alternative methods that may be employed to yield similar scientific insights or benefits, while reducing potential risks.

Since gain-of-function studies encompass a broad spectrum of pathogens and experimental manipulations, the NSABB discussed its charge and sought to identify the appropriate scope of its deliberations. Since the experiments that initiated the controversy involved the generation of pathogens that were concerning from a human health perspective, NSABB deliberations and recommendations focus for pathogens that pose risks to human populations. NSABB deliberations also focused on guiding U.S. funding decisions but Board also considered issues associated with non-Federally funded research and international research.

3. NSABB Deliberative Approach

The deliberative process (Figure 1) initiated by the USG to evaluate the risks and benefits of GOF studies involves the NSABB and the National Academies. To address its charge, NSABB formed two working groups to develop draft recommendations, which were discussed by the full Board[8]. The National Academies convened public forums to generate broad discussions and receive additional stakeholder input. The first forum was held early in the deliberative process and a second was held in March 2016; both were designed to inform NSABB deliberations.

To inform the deliberative process further, NIH commissioned two additional analyses: 1) qualitative and quantitative risk and benefit assessments, conducted by Gryphon Scientific, and 2) a review of the ethical considerations associated with the GOF issue and an analysis of relevant ethical decision-making frameworks, conducted by Dr. Michael Selgelid.

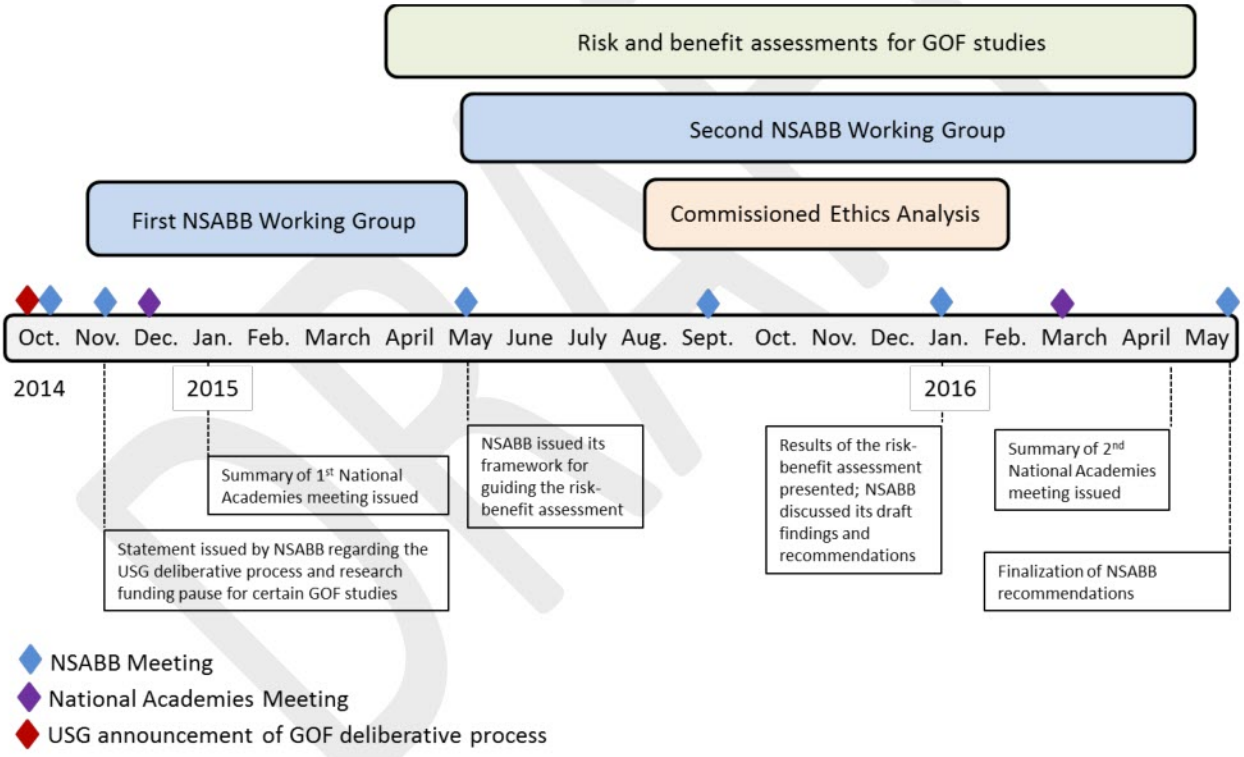

Figure 1. Timeline and major events of the GOF deliberative process.

[8] Information about these meetings and activities, including agendas, summaries, and archived videocasts, can be found on the NSABB website at: http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/nsabb/nsabb-meetings-and-conferences/past-meetings

The NIH Office of Science Policy, which administers the NSABB, managed the overall deliberative process. NIH oversaw the work of its contractors, Gryphon Scientific and Dr. Michael Selgelid, and interfaced between the NSABB and contracted entities.

See Appendices for more information. Appendix A provides a detailed description of the NSABB's deliberative approach. Appendix B describes examples of studies that would or would not be considered GOF research of concern. Appendix C provides an overview of the stakeholder views that were presented and considered by NSABB. Appendix D lists the experts and sources consulted by NSABB, including those who submitted public comments. Appendix E and F list the NSABB roster and charter.

Guiding Principles for NSABB Deliberations

Early in its deliberations the NSABB developed the principles below to guide its deliberations and underpin its analysis of the risk and benefit assessments.

1. The NSABB deliberations should focus on defining the GOF problem then include broad consideration of possible solutions. A range of approaches and decision-making frameworks will be considered, and the NSABB will take into account these various approaches when developing its recommendations.

2. NSABB will consider the potential risks and benefits of a broad range of GOF studies involving influenza, SARS, and MERS viruses in order to identify those that may raise significant concerns that should be addressed. However, the NSABB will aim to develop recommendations that are grounded in broadly-applicable concepts and principles that could, if necessary, apply to GOF studies involving other pathogens that may require evaluation in the future.

3. Similarly, NSABB will consider the risks and benefits associated with alternative research approaches to GOF research to understand whether or not these may substitute for or complement GOF studies.

4. NSABB recommendations will be informed by data and information about potential risks and benefits as well as values that will guide the evaluation and comparison of these risks and benefits. Ethical, societal, and legal considerations will also contribute to the development of recommendations and these inputs should be explicitly identified, discussed, and prioritized.

5. NSABB recognizes that not all analyses relevant to its task are quantitative and that uncertainties inherent in any quantitative analysis may remain. NSABB will seek to document important areas of uncertainty in any data or analysis when necessary.

6. NSABB should publicly debate its draft recommendations and describe in its report any dissenting views that may vary substantially from the Board's recommendations.

7. NSABB should consider current USG policies and guidelines, determine whether they adequately address risks associated with GOF research (in light of potential benefits), and make recommendations that are consistent with that determination. Current policies may be adequate or require only minor changes; alternatively, significant enhancements may be needed. The adequacy of current policy to cover GOF studies may vary by pathogen. Recognizing the paramount importance of ensuring safety, security, and public health, policies should also minimize the burdens placed upon the conduct of science.

8. NSABB recommendations will inform the development of U.S. government policy, which will apply to research funded, conducted, or overseen by the U.S. government either domestically or internationally. NSABB will be mindful in its deliberations of the likelihood that the Board's recommendations and U.S. policy decisions will also influence other governments and non-USG funders of life sciences research.

9. The NSABB will also consider whether there are certain studies that should not be conducted under any circumstances, and if so, articulate the critical characteristics of such studies.

10. Maintaining public trust and confidence in life sciences research is critical and must be taken into account as recommendations are formulated.

4. Analysis

In developing recommendations on a conceptual approach for evaluating GOF proposals, NSABB examined three major areas: the current policy landscape for overseeing research involving pathogens, ethical issues associated with funding and conducting GOF studies, and the results of Gryphon's risk and benefit assessments. In addition, the NSABB considered broad stakeholder perspectives through presentations from domestic and international experts at Working Group and full NSABB meetings, expert consultations, individual NSABB member participation in and ideas and views from the National Academies workshops and proceedings, analysis of published articles, and comments from attendees at NSABB meetings or public comments submitted to the Board.

4.1. Analysis and Interpretation of the Risk and Benefit Assessment

The NSABB working group has reviewed the risk and benefit assessments (RBA) conducted by Gryphon Scientific, which were designed to evaluate the risks and benefits of GOF research in a manner that encompassed both benign and worrisome aspects of a broader range of GOF studies than those that have raised concern. The RBA analyzed biosafety and biosecurity risks as well as possible benefits. Overall, the RBA includes a commendable amount of sophisticated work and analysis, is generally well-done, and largely achieves the goals it was intended to address. Gryphon's draft RBA report was made publically available in December 2015 and key results were presented and discussed at NSABB and NAS meetings. The final report is available on Gryphon's website.[9]

Strengths of the Risk and Benefit Assessments

The RBA has significant strengths. It is a thorough and extensive analysis of the risks and benefits of GOF work in the context of the guidance provided in the NSABB *Framework for Conducting Risk and Benefits Assessments of Gain-of-Function Research* (May 2015)[10]. It takes into account the principles articulated in the framework and includes the agents, categories of possible risks, types of possible benefits, and possibly concerning scenarios and phenotypes that were laid out in the *Framework*. A few items from the *Framework* were eliminated from consideration at the meeting of the NSABB where the framework was voted on[11], so that the most probable issues of concern could be thoroughly addressed within the available time and resources.

The biosafety risk assessment does a credible job of defining the relative risks associated with potential laboratory accidents involving GOF manipulations of pathogens with enhanced characteristics as compared to wild-type pathogens. This analysis is performed in a semi-quantitative way; it uses

[9] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

[10] Framework for Conducting Risk and Benefits Assessments of Gain-of-Function Research, May 2015. http://osp.od.nih.gov/sites/default/files/resources/NSABB_Framework_for_Risk_and_Benefit_Assessments_of_GOF_Research-APPROVED.pdf

[11] National Science Advisory Board for Biosecurity Meeting, May 5, 2015. http://osp.od.nih.gov/office-biotechnology-activities/event/2015-05-05-120000-2015-05-05-200000/national-science-advisory-board-biosecurity-nsabb-meeting

appropriate, established, peer-reviewed methods to the extent available. The parametric approach employed is powerful and allows consideration of many situations of interest.

The report effectively illustrates that the harmful events being modeled are low probability (see Figures 6.2 and 6.4 in Gryphon's report). Only a small fraction of laboratory accidents would result in a loss of containment; of those, only a small fraction would result in a laboratory acquired infection, and of those, only a fraction would spread throughout the surrounding community (or to the global population). The working group recognizes the challenge of analyzing low-probability, high-consequence events for which little data exists and appreciates attempts to make this point clear in the RBA.

The biosecurity risk assessment is primarily qualitative, and highlights analysis of previous malevolent events and evasions of security systems, likely capabilities and motivations of various possible actors, and an evaluation of the systems in place to prevent biosecurity breaches. Information was obtained from a survey of literature and discussions with biosecurity, intelligence, and law enforcement professionals. It is an extensive gathering of a wide range of information that has not been presented before in one place.

The information risk assessment (an element of the biosecurity risk assessment) is a qualitative analysis of risks that may result from the misuse of information derived from certain GOF studies that might be published in the future. It identifies information that might be attractive to malicious actors and compares it to other sources of information they might find attractive.

The benefits assessment uses a novel approach to assess benefits of GOF studies, a difficult task without much prior methodology to draw upon. The results are not quantitative, and attempts to quantify would have been appreciated. However, as is, the assessment may be the best that can be done with the available information and analytic tools. The benefits assessment thoroughly analyzed the possible benefits of alternatives to GOF studies and identified areas where GOF research appears to provide unique benefits (i.e., benefits that are not attainable without the use of GOF), either currently or in the near future.

The RBA contains a number of other useful analyses as well, including background and contextual information on the biology of influenza and coronavirus, historical analysis of naturally-occurring seasonal and pandemic influenza and coronavirus outbreaks, an examination of the potential proliferation of GOF research, and analysis of the potential loss of public trust in science that could result if a laboratory incident involving GOF research were to occur. Significantly, the historical analysis notes that each year, influenza infects 5 – 10% of the world's population, resulting in significant morbidity and mortality (up to 500,000 deaths per year). This description of naturally-occurring influenza (and coronavirus) infections helps to establish the extant risks associated with these infectious diseases to which the risks associated with GOF studies might be compared.

Overall, the RBA is comprehensive, objective, reasonable, and generally extensively documented.

Limitations of the Risk and Benefit Assessments

The RBA also has some weaknesses and limitations that should be noted. First, the RBA was limited to the types of labs traditionally funded by the Federal government, which may not be representative of other settings where GOF research may be conducted. Every attempt was made to base the analyses in the RBA on scientific information and data. Nevertheless, data on the properties of the various pathogens being examined, events such as laboratory accidents or security breaches, or possible future acts of terrorism are limited in some cases and unavailable in principle in others. Therefore, assumptions and estimations were necessary. For this reason, the biosafety risk assessment is not fully quantitative, primarily because absolute, quantitative baselines for the risk of work with wild-type pathogens could not be estimated with any certainty. Thus, the data presented are primarily comparative, and provide relative, not absolute values, for the risks associated with laboratory accidents involving GOF studies. Gryphon compared the risks associated with potential lab accidents involving a GOF strain with the risks associated with the same accident involving a wild-type strain. This comparative approach is adequate for some instances but inadequate for others. For instance, an increased risk associated with a GOF study that is relatively large (5-10-fold or greater) may appear significant, but if this increase is in comparison to a very small risk baseline, the overall risk associated with the GOF study may not be significant or concerning. Similarly, small increases in risk over a higher risk baseline, in fact, may be concerning. Additionally, differences in risk that are relatively small (~2-fold) are difficult to interpret because such changes may fall within the limits of uncertainty for the analysis. Attempts to include some absolute baseline estimates of risk (an admittedly difficult task) were included in Section 6.8 of Gryphon's report. However, the lack of comprehensive estimates of baseline risk make interpreting the biosafety risks a challenge.

Given the comparative approach undertaken for the biosafety risk assessment, the implications of the results of this analysis depend a great deal on the wild-type comparator strains that were selected for the analysis. For instance, for pandemic influenza Gryphon initially selected the 1918 influenza strain as the comparator. Gryphon regarded this strain as embodying the maximum risk for influenza, yet a level of risk that is also deemed as acceptable given that research with this strain is permitted. However, using 1918 influenza as the comparator for the analysis compares GOF risks to a relatively high level of baseline risk, making the changes in risk associated with GOF manipulations comparatively small. Utilizing different comparator strains alters the relative risks associated with GOF manipulations; using a high-risk baseline strain may obscure significant risks associated with GOF studies whereas using a low-risk baseline strain may inflate the potential risks associated with GOF studies.

Little data exists about the probabilities of the accidents that initiate the chain of events that may lead to a pandemic and therefore, the quantitative probability of these accidents could not be incorporated into the biosafety risk assessment. The modeling of secondary spread of a pathogen through populations once it is released from a laboratory allows for some estimation of the consequences of an event but without a better understanding of the likelihood that an accident would result in loss of containment or a laboratory acquired infection, it is difficult to make judgments about the overall risk. Gryphon's analysis accounts for this by presenting relative, actuarial risk. However, this approach results in the challenges associated with comparing relative risks described above. There are large

uncertainties in most of the input parameters that are the basis for the biosafety risk calculations. Uncertainties about inferring absolute risk from these relative risks exist and should be kept in mind as any conclusions are reached.

The biosecurity risk assessment attempts to examine how GOF studies add to the risk of malevolent acts. Portions of the biosecurity risk assessment focus on GOF studies but others describe the type of threats that could occur against any high-containment laboratory. The semi-quantitative portion of the biosecurity risk assessment estimates the number of infections that could occur if a pathogen with various enhanced characteristics were intentionally released. However, this analysis (see section 7.4.2 and Table 7.7 in Gryphon's report) assumes that 1 or 10 individuals are initially infected as a result of a malicious act with no indication of how likely such an event would be, since there is no way to make such an estimate.

While exhaustively documented, the RBA is not always transparent about data reliability. In particular, interviews were used to gather much critical information, and this was not always well documented in a way that reflects how robust the resulting information may be. For peer-reviewed publications, this is less of a concern.

While evaluation of the benefits of alternatives to GOF studies was extensive, evaluation of risks of alternative approaches was not as thorough. In addition, risks and benefits have not been presented in comparable terms, making it a challenge to determine whether certain risks are justified by potential benefits. Significantly, the benefit assessment is not quantitative and there is no probability analysis or attempt to estimate the likelihood that a certain benefit would be realized or what its impact might be.

Key Results of the Risk and Benefit Assessments

While NSABB has examined all of the analyses in the RBA, some results are important to highlight. In general, the RBA examined risks and benefits associated with the major GOF phenotypes with the intention of identifying types of studies that would be most and least concerning, based particularly on their risk profile.

With regard to biosafety risks, only some potential GOF phenotypes represent substantially increased (5- to 10-fold or more) risks over the starting strain. Two-fold changes most likely fall within the uncertainty of the data, and while small differences might be important if it could be shown that they are significant, this demonstration is probably difficult. For coronaviruses, GOF studies that would create strains with increased transmissibility among mammals may entail significant risks if they also increase human transmission. The risks, were this combination to occur, would include increased probability of an outbreak escaping local control and increased likelihood of global consequences. In addition, experiments that enhance coronavirus growth in culture would likely increase the possibility of laboratory acquired infections.

For seasonal influenza, the GOF-generated phenotypes entailing the greatest risks include enhanced transmission in mammals (assuming this increases transmission in humans), enhanced virulence, and evasion of immunity. Enhanced pathogenicity might significantly increase the global consequences of

an outbreak. For pandemic influenza, no GOF-generated phenotypes led to greatly increased risk, but that is based on using 1918 influenza as the comparator; because the risk associated with the wild-type 1918 strain is already so great it is difficult to increase risk substantially. If less transmissible and/or less virulent wild-type strains were used as the basis of comparison, the risks of GOF studies with pandemic strains might appear higher. For avian influenza, the GOF experiments that lead to enhanced transmissibility in mammals (and presumably humans) would likely lead to an increased probability of local and widespread outbreaks, as well as increased global consequences. More subtle aspects of these very general conclusions may be found in the biosafety risk section of the Executive Summary of Gryphon's RBA report.

In general, GOF studies that were not considered by the working group to entail significant risks were those that would: adapt human pathogens to mammals to generate animal models; enhance the growth of attenuated vaccine strains; and antigenic drift or immune evasion studies that are commonly used to guide vaccine selection.

The biosecurity risk assessment shows that the most probable threats involve insiders who have direct access to dangerous pathogens or outsiders who collaborate with or subvert insiders. If currently mandated biosecurity systems are effective, outsiders have little chance of causing harm on their own. The RBA report also concludes that the risks associated with information from future GOF studies with influenza, SARS and MERS appear small; this is because most of the information of interest is already published, or non-GOF information relating to pathogens that are more attractive agents of harm is readily available. However, future scientific advancements could alter this assessment.

Most GOF studies provide benefits in the form of new scientific knowledge, and some of these benefits are unique (i.e., unable to be achieved by alternative, non-GOF approaches). While some GOF studies are likely to provide unique near-term benefits, these are associated with specific agents and phenotypes. With regard to more applied benefits, such as countermeasure development and biosurveillance, the most clear-cut situation is experiments that increase growth of seasonal influenza vaccine candidates in culture; these studies provide unique benefits to current production of seasonal influenza vaccines, and likely will in the future. Another reasonably clear unique benefit is derived from experiments that enhance mammalian pathogenicity for coronavirus as a means of developing animal models for studying disease and developing countermeasures. GOF studies that yield phenotypes that provide unique benefits to countermeasure development include enhanced pathogenicity, evasion of vaccines, and evasion of therapeutics. For several other potential benefits with seasonal influenza, either the potential benefit is long term, or alternative approaches may yield the same or similar benefits. Interestingly, few unique benefits pertaining to GOF studies involving pandemic influenza were identified. There are several types of GOF studies that entail generating avian influenza strains with phenotypes that may be valuable for surveillance and preparedness efforts, although other advances are needed to fully realize such benefits. This point is controversial, with strong proponents and critics. Additionally, a variety of benefits were identified that may also be provided to some extent by alternative approaches. It should be noted that no attempt was made to provide a probability assessment based on historical data for potential benefits; hence no direct comparison of risk to benefit for a proposed research project is possible.

4.2. Consideration of Ethical Values

The risk and benefit assessments provide information about the potential risks and benefits associated with conducting GOF research. However, determinations about whether such studies should be undertaken will involve value judgments when weighing the risks and benefits. The NSABB identified a number of values (that are applicable to the decisions about whether to fund certain GOF studies and how to oversee them. Sources of these values include the Belmont Report,[12] the literature on public health ethics,[13] and the literature on oversight of emerging technologies,[14] as well as the literature specifically debating appropriate approaches to overseeing DURC and GOF research that has raised concern.[15,16,17,18,19] The commissioned ethics analysis conducted by Dr. Michael Selgelid also describes additional decision-making frameworks and values to be considered.[20]

Substantive values

The following values are important to consider when considering funding of a research proposal involving GOF studies that might entail significant risks.

Non-maleficence: not causing harm. Harm might include: losing lives; causing disease; damage to the economy, national or international security, or agriculture; or loss of public trust in science or governance structures. There are inherent risks associated with research involving pathogens that could result in harm. Approaches aimed at preventing harm and mitigating potential risks should be considered and applied to the design, conduct, and communication of research involving pathogens in GOF studies.

Beneficence: promoting beneficial outcomes while preventing harmful outcomes; appropriately balancing benefits and risks; formulating policy that maximizes public benefit while minimizing public harm. Benefits might include: saving lives, preventing disease, improving public health; enhancing the economy, national and international security, or public trust in science and

[12] The Belmont Report. Office of the Secretary, U.S. Department of Health and Human Services. Ethical Principles and Guidelines for the Protection of Human Subjects Research. The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, April 18, 1979. http://www.hhs.gov/ohrp/humansubjects/guidance/belmont.html
[13] Kass NE. An Ethics Framework for Public Health. *American Journal of Public Health*. 2001;91(11):1776-1782. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1446875/
[14] New Directions. The Ethics of Synthetic Biology and Emerging Technologies. Presidential Commission for the Study of Bioethical Issues, December 2010. http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf
[15] Resnik DB. H5N1 Avian flu research and the ethics of knowledge. Hastings Center Report 2013; 43, 2: 22-33.
[16] Kelle A. Beyond patchwork precaution in the dual-use governance of synthetic biology. Sci Eng Ethics. 2013 Sep;19(3):1121-39.
[17] Kuhlau F, Höglund AT, Evers K, Eriksson S. A precautionary principle for dual use research in the life sciences. Bioethics. 2011 Jan;25(1):1-8.
[18] Biotechnology Research in the Age of Terrorism. The National Academies, 2004. http://www.nap.edu/catalog/10827/biotechnology-research-in-an-age-of-terrorism
[19] Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information. National Science Advisory Board for Biosecurity, June, 2007. http://osp.od.nih.gov/sites/default/files/resources/Framework%20for%20transmittal%20duplex%209-10-07.pdf
[20] Selgelid, Michael. Gain-of-Function Research: Ethical Analysis. December 7, 2015. http://osp.od.nih.gov/sites/default/files/GOF%20%20White%20Paper%20by%20Michael%20Selgelid_0.pdf

governance structures. When the ultimate goals of the research are to improve public health, public health ethics would ask how effective the research is likely to be in achieving those goals, what are the known or potential burdens of the research, can those burdens be minimized, whether there are alternative approaches that are less risky or burdensome, and how can the potential benefits and burdens of the research be fairly balanced. The work of the Presidential Commission for the Study of Bioethical Issues suggests that those formulating and effectuating government policy on scientific research and emerging technologies have a duty of public beneficence – a duty "to promote individual activities and institutional practices…that have great potential to improve the public's well-being," while being "vigilant about risks and harms, [and] standing ready to revise policies that pursue potential benefits with insufficient caution."[21] Both risks and benefits have associated probabilities, magnitudes, and uncertainties. In some instances, it may be justifiable to pursue benefits despite the potential risks; in others, the potential benefits may be foregone due to possible risks.

Social justice: distributing potential benefits and harms fairly (distributive justice) and selecting participants in research fairly, as well as those who may potentially be exposed to risk. There are many different approaches to social justice, such as egalitarianism, utilitarianism, and libertarianism,[22] to name but a few. Decisions about whether to fund research that entails some risk should consider how the risks and benefits associated with conducting that research will be distributed, with an effort to distribute risks and benefits as fairly as possible. When considering pandemic potential, fair distribution of risks and benefits must be considered on a global scale. Those who will potentially be exposed to risk, through participation in research or other avenues of exposure, should be selected equitably.

Respect for persons: allowing competent individuals to make informed choices, and ensuring that the representatives of individuals lacking capacity to choose can make choices in keeping with the wishes, values, or interests of those represented. Autonomy generally requires informing human research participants, laboratory workers, and the public about the risks of research and eliciting their free and uncoerced decision about whether to subject themselves to those risks. In the case of the public, mechanisms for representative decision-making and publicly accountable governance may be needed, as getting consent directly from the members of the public may be impracticable.

Scientific Freedom: avoiding unnecessary interference with scientific research, debate, or publication. Scientific freedom includes an entitlement to avoid interference unless necessary (negative freedom), but not the affirmative right to receive funding or other forms of support for a particular project (positive freedom). Scientific freedom is compatible with norms and regulation to promote the responsible conduct of research and protect participants in research and the public. As a corollary to the principle of scientific or intellectual freedom, the Presidential Commission

[21] New Directions. The Ethics of Synthetic Biology and Emerging Technologies. Presidential Commission for the Study of Bioethical Issues, December 2010. http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf
[22] Nozick R. Anarchy, State, and Utopia. New York: Basic Books, 1974.

endorses a principle of regulatory parsimony, requiring "only as much oversight as is truly necessary to ensure justice, fairness, security, and safety while pursuing the public good."[23]

Responsible Stewardship: acting in a way that shows concern for children, future generations, and the environment. The Presidential Commission emphasizes that this is both a domestic and global responsibility that requires "prudent vigilance, establishing processes for assessing likely benefits along with assessing safety and security risks both before and after projects are undertaken."[24]

Procedural Values

The following values apply to the process of decision-making about GOF research and are important to consider when establishing mechanisms to review and/or approve the funding of research proposals involving gain-of-function studies that may entail significant risks.

Public participation & democratic deliberation: making decisions with participation from the public, utilizing respectful debate and inclusive deliberation. Life sciences research is largely a publicly-supported endeavor; therefore, those who allocate funds and conduct life sciences have a responsibility to be good stewards of public funds and to respond to the interests and concerns of the public. Many, if not all, members of society have a stake in the life sciences enterprise and will be affected directly or indirectly by the benefits and risks stemming from such research. This stakeholder community has diverse values and tolerances for risk, which are important to consider when making decisions about funding and overseeing life sciences research. Some forms of public participation include: oversight by the legislative or executive branches of government, public membership and input on government science advisory committees, other mechanisms of public governance, surveys of public opinion on science policy issues, research models such as community-based participatory research, and efforts by scientists and government officials to share information with the public and better understand the public's interests and concerns. The Presidential Commission urges the importance of democratic deliberation, as "[a]n inclusive process of deliberation, informed by relevant facts and sensitive to ethical concerns, promotes an atmosphere for debate and decision making that looks for common ground wherever possible and seeks to cultivate mutual respect where irreconcilable differences remain."[25]

Accountability: taking responsibility for one's actions and being prepared to justify or explain them to others. It is important that decisions to fund research are justifiable to the public and others. Decisions should be justified in terms of substantive and procedural values.

Transparency: sharing with the public the information and assumptions used to make a decision, including uncertainties, controversies, and limitations of analyses. Transparency is an important

[23] New Directions. The Ethics of Synthetic Biology and Emerging Technologies. Presidential Commission for the Study of Bioethical Issues, December 2010. http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf, p5.
[24] Ibid., p5.
[25] Ibid., p5.

part of accountability and public participation. It allows review and reconsideration of policy over time as new facts emerge and analysis evolves.

4.3. Decision-Making Strategies and Frameworks for Evaluating and Managing Risks and Developing Policy

The NSABB working group identified a number of approaches or frameworks that may be used to guide the making of complex decisions with ethical implications, particularly in the face of uncertainty. These may also be used in developing policies such as that for managing GOF research. Different strategies reflect different attitudes toward risk-taking. Some may be more appropriate in some situations than others. The NSABB working group examined a number of such strategies as it attempted to determine the best option as relates to GOF research that has raised concerns. These options are not mutually exclusive, and elements from more than one may be used together to develop a path forward. The following are decision-making frameworks that were considered.

Maximax: This involves choosing the option with the best possible outcome. Maximax is a relatively simple strategy that focuses on choosing the option with the best possible outcomes While maximax may be appropriate for making some types of personal choices (e.g. playing games with nothing of value to lose), it may not be appropriate for making science and technology policy decisions because most people would want to take appropriate steps to prevent or mitigate risks regardless of benefits. For GOF studies, use of maximax would involve identifying research with the best possible benefits, generally regardless of risks.

Maximin: This involves choosing the option with best outcome among the worst possible outcomes. Maximin is a risk-averse approach because it aims to avoid the worst possible outcomes. Maximin is another relatively simple approach, but may present difficulties in making science and technology policy decisions, because it would recommend not developing a new technology if this decision could lead to the worst possible outcome. Since all technologies (and scientific ideas) can conceivably lead to good and bad outcomes, strict adherence to maximin would imply a very cautious approach to science and technology development. For GOF studies, use of maximin would involve identifying studies with risks, and choosing the least risky regardless of benefits.

Expected Utility Theory: This involves choosing the option that maximizes expected utility, where expected utility for a possible outcome = probability x utility. Expected utility theory involves a quantitative balancing of risks and benefits and is inherently a more complex process. Cost-benefit analysis in economics is a form of expected utility theory. A problem with expected utility theory is that sufficient evidence may not always be available to confidently estimate the probabilities involved in the utility calculus. When this is the case, other approaches may be appropriate. For GOF studies, use of expected utility theory would require determining quantitatively the likelihood of risks and benefits and calculating the resulting utility.

Precautionary approach: This approach involves taking reasonable measures to prevent, minimize, or mitigate risks that are significant and plausible. A measure is "reasonable" if it: 1) appropriately balances the values at stake in the risk management; 2) is proportional to nature of the risk (i.e. greater risks require stronger measures); and 3) is likely to be effective. A risk is "plausible" if there is some scientific evidence that it could occur even if the probability of the risk cannot be confidently estimated. There are many versions of the precautionary principle, including ones that are more or less risk-averse.[26,27] A precautionary approach, in general, would limit an activity unless the environment, health, or security, are clearly protected. This approach can recognize a potential problem early and prevent harm from occurring but may lead to regulatory burdens or unnecessarily limit activities. This approach might restrict potential GOF research unless the studies are demonstrated to be safe.

Permissive approach: This approach, in general, would allow an activity unless the environment, health, or security, are clearly compromised. This approach may reduce unnecessary regulatory burdens but can result in after-the-fact reaction to harms. This approach might allow certain GOF studies to proceed until they are demonstrated to entail significant risk.

Planned adaptation or risk-based approach: This approach provides a systematic way to deal with managing risks in the face of uncertainty. It involves: 1) preparation to identify the risks and regulatory gaps, including getting input from a broad range of perspectives; 2) putting measures in place to control risk based on the best information available at the time; 3) systematically gathering data and observing effects of policies; and 4) updating and revising policy as needed. An example of an adaptive approach is the life cycle approach taken by the Food and Drug Administration when making decisions about whether to approve drugs, when that includes post-market surveillance.[28] For GOF studies, this approach might entail allowing GOF studies of potential concern—or certain GOF studies—to proceed under defined conditions, then evaluating the risk-benefit landscape periodically to determine whether the GOF studies that are permitted should continue, be expanded, or be restricted.

Threshold approach: This approach would entail identifying a risk threshold beyond which, certain studies are given special attention or subject to additional scrutiny or oversight and might preclude certain studies. Implementation would involve defining or describing the studies that would require additional oversight as well as a description of what that oversight would entail. This approach would allow for the identification of studies of concern but might need to be reevaluated if the risk landscape changes and the threshold that was identified is no longer appropriate. For GOFROC, this would entail identifying the characteristics of studies involving significant risks that may not be

[26] Resnik DB. Environmental Health Ethics, New York: Oxford University Press, 2013.
[27] Munthe C. The Price of Precaution and the Ethics of Risks. Dordrecht: Springer, 2011.
[28] FDA determinations about whether a new drug is safe and effective are complex, address uncertainty, and involve ongoing monitoring to assess risks and benefits and take appropriate post-marketing actions as necessary. See: *Structured Approach to Benefit-Risk Assessment in Drug Regulatory Decision-Making*, 2013
http://www.fda.gov/downloads/ForIndustry/UserFees/PrescriptionDrugUserFee/UCM329758.pdf

adequately managed and then stipulating further oversight or determining that they should not be conducted.

Point-source approach: This approach would involve controlling where certain studies are conducted and under what conditions. This approach would centralize certain research activities, restricting them to designated locations or facilities. For GOFROC this might involve requiring that certain studies only be conducted in facilities with certain biocontainment conditions, biosafety practices, and security measures.

The NSABB working group used ideas from a number of frameworks to inform its findings and deliberations (Sections 5 and 6). The criteria for identifying GOF research of concern (see Recommendation 1) reflect a threshold approach. The principles for guiding funding decisions for GOF research of concern entails elements from several of the decision frameworks above. For instance, an explicit call for a risk-benefit analysis (Recommendation 1, Guiding Principle 3) reflects expected utility theory, however, a strict quantitative calculation is probably not possible. The principles to guide funding decisions that call for risk mitigation and a restriction to laboratories with a demonstrated capacity to safely carry out the studies (Recommendation 1, Guiding Principles 4 and 5) incorporate elements of point-source and precautionary approaches. An adaptive approach was considered particularly attractive and appropriate for policies aimed at providing oversight of GOF research (see Recommendation 3).

4.4. Examination of the Current Policy Landscape

Many Federal agencies fund life sciences research in furtherance of their specific missions. In general, research supported by the USG is founded on the principle of scientific merit and goals of the funding agency. Multiple complementary layers of oversight are in place to manage laboratory and other risks associated with Federally-funded life sciences research. These policies are intended to provide oversight at various points throughout the research life cycle, from research conception to its publication and translation into practice. These policies include a foundation of occupational health and medicine (for laboratory and clinical workers), laboratory biosafety practices, and policies that address biosecurity risks. Below is a description of the oversight policies in place for Federally-funded life sciences research involving pathogens, with discussion of whether and how such policies apply to GOF studies. This analysis is illustrated in Figures 2 and 3 and summarized in Appendix D.

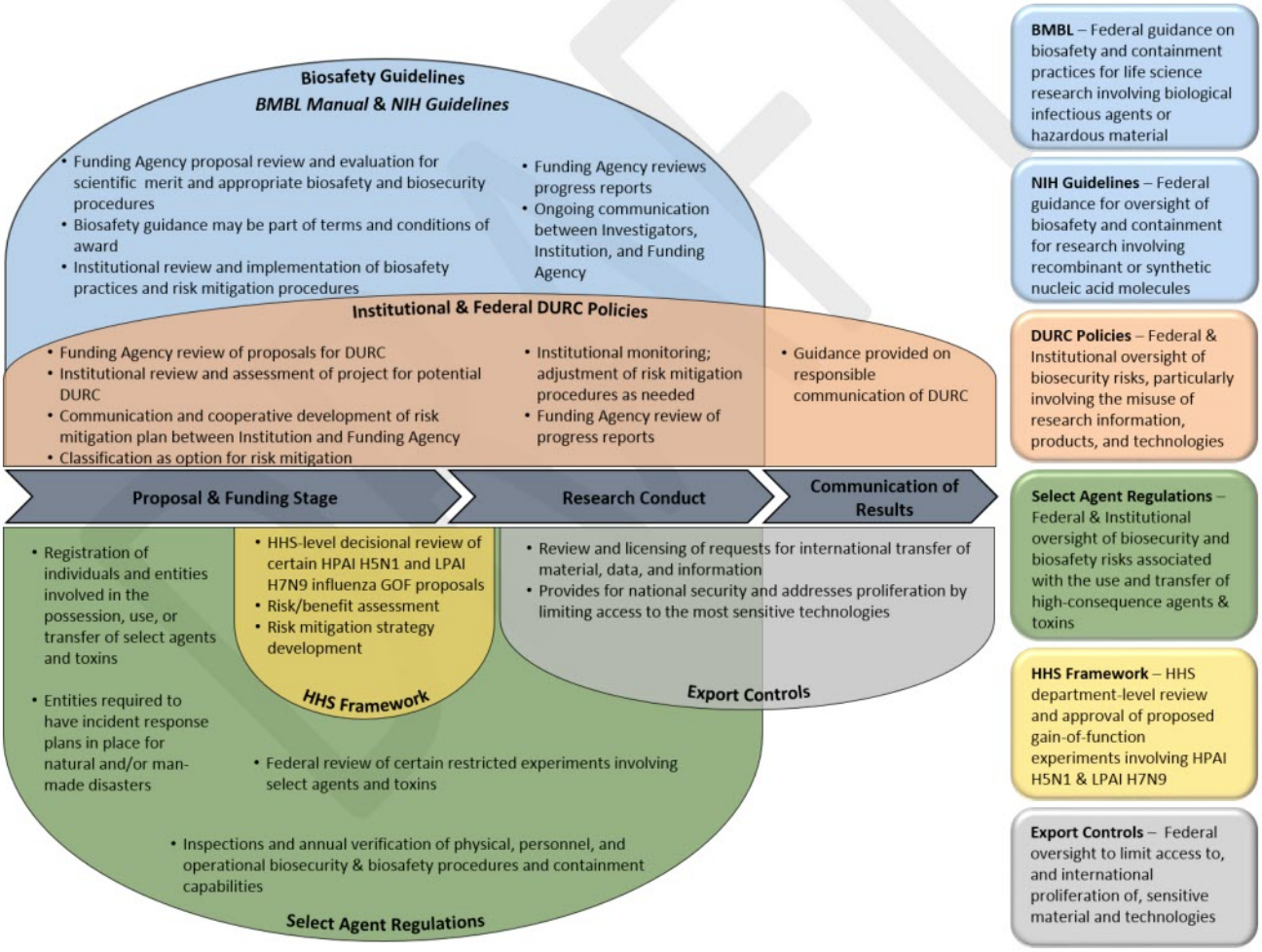

Figure 2. U.S. government oversight of life sciences research involving pathogens. Oversight policies apply at different stages and occur at different levels throughout the research life cycle. See text and Appendix D for descriptions of each policy. The policies depicted in this figure are defined by different applicability and scope requirements and therefore do not apply to all life sciences (or GOF) research projects.

Scientific Merit Review

Departments and agencies within the U.S. government fund diverse portfolios of life sciences research. Funding decisions are based on the scientific merit of a given proposal and the ability of a project to advance the agency's strategic mission. The U.S. government funds life sciences research through a variety of mechanisms including grants, contracts, and cooperative agreements. Each funding agency has its own processes for evaluating research proposals and awarding funds but, in general, proposals are subject to rigorous scientific review by Federal agency staff and often, scientific peers. NIH grant proposals, for example, undergo two levels of review. The first evaluation is by a panel of scientific peer reviewers who score proposals based on scientific merit and other criteria. The second round of review includes discussion of meritorious proposals at public meetings of advisory councils, specific to individual funding institutes and centers within NIH, to determine how proposals fit within their broader strategic objectives.

Biosafety Oversight

Oversight of pathogen research focuses first on ensuring the safe handling of biological agents through appropriate biosafety practices and containment measures, which are addressed by the *Biosafety in Microbiological and Biomedical Laboratories* (*BMBL*)[29], the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*)[30], and other documents. The BMBL and the *NIH Guidelines* provide for Federal and institutional biosafety oversight and guidance involving biosafety practices and containment features that are based on risk assessments for specific projects. Such determinations are typically made at the institutional level and are guided by Federal guidelines and policies, which are updated as necessary to provide additional guidance for research involving emerging pathogens or technologies. Biosafety is achieved by conducting research under appropriate physical and biological containment levels and employing practices that help to ensure a safe working laboratory environment.

The BMBL is a CDC-NIH guidance document that is generally considered the authoritative reference for laboratory biosafety. The BMBL provides summary statements for many bacterial, fungal, parasitic, rickettsial, viral, and other agents. These statements describe the characteristics of the pathogen, its natural mode of infection, potential occupational hazards with the agent, and recommendations for laboratory safety and containment. It also describes the fundamentals of biological containment, which includes descriptions of proper microbiological practices, safety equipment, and facility safeguards that protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory. It describes the process of biological risk

[29] Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition. http://www.cdc.gov/biosafety/publications/bmbl5/

[30] The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), November 2013. http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html

assessment, which enables the appropriate selection of microbiological practices, safety equipment, and facility safeguards that can prevent laboratory-associated infections. It also describes occupational health, immunoprophylaxis, and principles for laboratory biosecurity. The BMBL is updated periodically to refine guidance based on new knowledge and experiences and to address contemporary issues that present new risks that confront laboratory workers and the public health.

Analysis: The BMBL does not address GOF studies *per se* but does include summary statements and biocontainment guidance for research involving various influenza strains (including contemporary and non-contemporary human, high and low pathogenic avian, swine, the 1918 influenza strain, and reassortant viruses) and SARS-CoV. MERS-CoV had not emerged at the time of the last BMBL update, but interim laboratory biosafety guidance was issued by CDC.[31]

The BMBL is not a regulatory document. U.S. funding agencies may require it be followed as a term and condition of awards but in general, compliance with the BMBL is voluntary. In addition, the BMBL provides general biosafety guidance but does not describe detailed procedures or experiment-specific containment protocols.

The *NIH Guidelines* specify the practices for safely constructing and handling recombinant nucleic acid molecules; synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules; and cells, organisms, and viruses containing such molecules. The *NIH Guidelines* apply to basic and clinical recombinant or synthetic nucleic acid research conducted at or sponsored by institutions that receive NIH funding for any such research. Compliance with the *NIH Guidelines* is typically required as a term and condition of award of funding. Other Federal agencies may also require compliance with the *NIH Guidelines*.

The *NIH Guidelines* focus on the concepts of risk assessment, risk group classification of agents based on their ability to cause disease in humans and the availability of medical countermeasures, physical and biological containment levels, practices, personal protective equipment, and occupational health. To help ensure the safe conduct of this research, the *NIH Guidelines* specifies roles and responsibilities of investigators and institutions. Institutions subject to the *NIH Guidelines* must establish Institutional Biosafety Committees (IBCs) composed of members with appropriate expertise, to review and approve such research. IBCs provide local oversight and ensure compliance with the *NIH Guidelines*. Certain higher risk experiments require review by the Recombinant DNA Advisory Committee (RAC)[32] and specific approval by the NIH Director as Major Actions. These experiments involve the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if

[31] Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Middle East Respiratory Syndrome Coronavirus (MERS-CoV) – Version 2. http://www.cdc.gov/coronavirus/mers/guidelines-lab-biosafety.html [last updated June 18, 2015]

[32] The Recombinant DNA Advisory Committee (RAC) is a federal advisory committee that provides recommendations to the NIH Director related to basic and clinical research involving recombinant or synthetic nucleic acid molecules. See: http://osp.od.nih.gov/office-biotechnology-activities/biomedical-technology-assessment/hgt/rac

such acquisition could compromise the ability to control disease agents in humans, veterinary medicine or agriculture.

In order to continue to provide appropriate guidance for emerging pathogens or experimental approaches, the *NIH Guidelines* are updated periodically. The *NIH Guidelines* have been amended to include additional guidance for work with Risk Group 3 influenza viruses (1918 H1N1, H2N2, highly pathogenic avian influenza (HPAI) H5N1), to specify enhancements to biosafety level 3 containment, practices, and to incorporate occupational health requirements. In 2012, the *NIH Guidelines* were amended again to require further enhancements to facilities, biosafety equipment and practices, including occupational health practices, for research involving HPAI H5N1 strains transmissible among mammals by respiratory droplets.

Analysis: The *NIH Guidelines* provide guidance on risk assessment and appropriate containment and practices for conducting research involving recombinant or synthetic nucleic acids, which would apply to most government-funded GOF research. Some IBCs also review and approve non-recombinant pathogen research; however, not all institutions require their IBCs to do so. While the *NIH Guidelines* are often used as a model of biosafety guidance by the broader scientific community, compliance is required only by institutions receiving such funding from the NIH. Therefore, some GOF studies may not be subject to the *NIH Guidelines* depending on whether the institution where the research is being conducted is subject to the *NIH Guidelines*.

The Federal Select Agent Program

Subtitle A and B of the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 requires the U.S. Departments of Health and Human Services (HHS) and Agriculture (USDA) to establish and regulate a list of select agents, biological agents and toxins that have the potential to pose a severe threat to public health and safety or animal or plant health or animal or plant products. The Select Agent Program (SAP) is administered jointly by the HHS Centers for Disease Control and Prevention and USDA Animal and Plant Inspection Service. The SAP oversees the possession, use and transfer of biological select agents and toxins. The Select Agents and Toxins List is reviewed and updated biennially. Under the select agents regulations, individuals and institutions that possess, use, or transfer any select agent are required to be registered, follow appropriate biosafety procedures, and undergo periodic inspections. Individuals must be registered with the SAP to have access to select agents or toxins, which requires that they undergo a security risk assessment performed by the Federal Bureau of Investigation (FBI). There are legal penalties for failing to comply with the select agent regulations.

In addition to the agents and toxins on the list, the select agent regulations apply to some genetic elements, including nucleic acids that are immediate precursors to infectious forms of any select agent viruses (i.e., complete positive strand RNA viral genomes), as well as some nucleic acids that encode select toxins. Select agent regulations also apply to genetically modified select agents and toxins. Restricted experiments are described in the regulations and involve the deliberate transfer of or

selection for a drug resistance trait to select agents that are not known to acquire the trait naturally. If the acquisition of resistance is to a first-line drug that could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, the restricted experiment requires special review and approval by the SAP. Some attenuated strains of select agents may be excluded from the regulations based upon a determination that the attenuated strain or modified toxin does not pose a severe threat to public, plant, or animal health or safety. The Intragovernmental Select Agent and Toxin Technical Advisory Committee serves as an advisory group to the SAP. In the wake of the recent laboratory incidents at Federal facilities involving select agents, two advisory committees have issued recommendations for ways to strengthen the Select Agent Program.[33] [34] Plans to implement these recommendations are also in place.[35]

Analysis: Studies that could be considered GOF studies are subject to oversight by the SAP if they involve pathogens on the select agent list. Researchers and institutions performing such studies must receive favorable security risk assessments by the FBI, register with the SAP, receive training on the proper procedures and practices for handling such agents, and abide by other aspects of the regulations. SARS-CoV, HPAI H5N1 influenza, and 1918 influenza viruses are select agents and GOF studies involving these pathogens are subject to oversight by the SAP. Restricted experiments that would entail conferring antiviral resistance to these viruses would require additional review and approval prior to being conducted. However, MERS-CoV is not a select agent. GOF experiments involving MERS, and other agents not included on the select agent list, would not be subject to oversight by the SAP (though they could be subject to Federal and institutional biosafety oversight). The SAP is underpinned by a regulatory requirement that applies to non-USG funded (i.e., private sector funded) pathogen research.

Federal and Institutional Oversight of Life Science Dual Use Research of Concern

The U.S. government has issued two Federal policies for the oversight of life sciences DURC. These policies focus oversight on research involving 15 high-consequence pathogens and toxins[36] that involve seven categories of experimental activity, which are projects that can be reasonably anticipated to:

1. Enhance the harmful consequences of the agent or toxin;
2. Disrupt immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification;
3. Confer to the agent or toxin resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies;

[33] Report of the Federal Experts Security Advisory Panel, U.S. Government, December 2014.
[34] Fast Track Action Committee Report: Recommendations on the Select Agent Regulations Based on Broad Stakeholder Engagement, U.S. Government, October 2015.
[35] Lisa Monaco and John Holdren White House Memorandum, October 29, 2015, Next Steps to Enhance Biosafety and Biosecurity in the United States. https://www.whitehouse.gov/sites/default/files/docs/10-2015_biosafety_and_biosecurity_memo.pdf
[36] The agents within the scope of the USG DURC policies are the 13 Tier 1 select agents plus HPAI H5N1 and 1918 influenza virus.

4. Increase the stability, transmissibility, or the ability to disseminate the agent or toxin;
5. Alter the host range or tropism of the agent or toxin;
6. Enhance the susceptibility of a host population to the agent or toxin; or
7. Generate or reconstitute an eradicated or extinct agent or toxin listed above.

Projects involving any of the 15 agents and that could be anticipated to involve any of these seven experimental effects are then determined to be DURC if they then meet the definition of DURC listed in the policy.[37]

The DURC policies outline a coordinated approach to oversight involving the Federal funding agencies and institutions that conduct such research. The policy for Federal oversight, issued in March 2012, requires Federal agencies to review proposed and ongoing research projects to identify any that constitute DURC. The policy for institutional oversight, issued in September 2014, articulates responsibilities of research institutions in identifying and managing DURC. Research institutions are to establish an Institutional Review Entity (IRE) to review research subject to the policy to determine whether any such research involves any of the seven experimental effects, and if so, whether the research constitutes DURC. IREs may review projects not specifically covered under the DURC policies but such additional reviews are voluntary.

When DURC is identified—either by a funding agency or a research institution—the funder and institution are to work collaboratively to develop a risk mitigation plan to help ensure that the research is conducted and communicated in a responsible manner. DURC risk mitigation plans are approved by the Federal funding agency and are reviewed on an annual basis by the funder and the institution. Specific risk mitigation measures may be incorporated into a term of award. Risk mitigation may involve modifying the design or conduct of the research in order to address the same scientific question in a manner that poses fewer biosafety or biosecurity risks. Other measures may involve applying enhanced biosafety or biosecurity measures, evaluating the effectiveness of extant medical countermeasures prior to proceeding with particular studies, or establishing a more frequent schedule of DURC reviews to more closely monitor the research as it evolves. It is also expected that a communication plan is established to ensure that DURC is communicated in a responsible manner. Federal funding agencies can provide advice and guidance on responsible communication, but recommendations on how to communicate research typically are not binding; ultimately, investigators and journal editors decide on how to communicate the research.

Analysis: Some of the seven experimental effects within the scope of the DURC policies could be considered GOF studies. However, GOF projects that might involve these effects are only subject to DURC oversight if the study involves one of the 15 agents listed in the policy. Only two influenza

[37] The definition of dual use research of concern listed in the USG Policy for Oversight of Life Science DURC (USG, March 2012) and the USG Policy for Institutional Oversight of Life Sciences DURC (USG, September 2014) is "Life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security."

viruses are listed within the scope of these policies; SARS and MERS coronaviruses are not listed.[38] The DURC policies are also inherently subjective. While the list-based approach clearly delineates projects that are subject to oversight, the definition of DURC, and to a lesser extent, the seven experimental effects, all require significant judgment and interpretation.

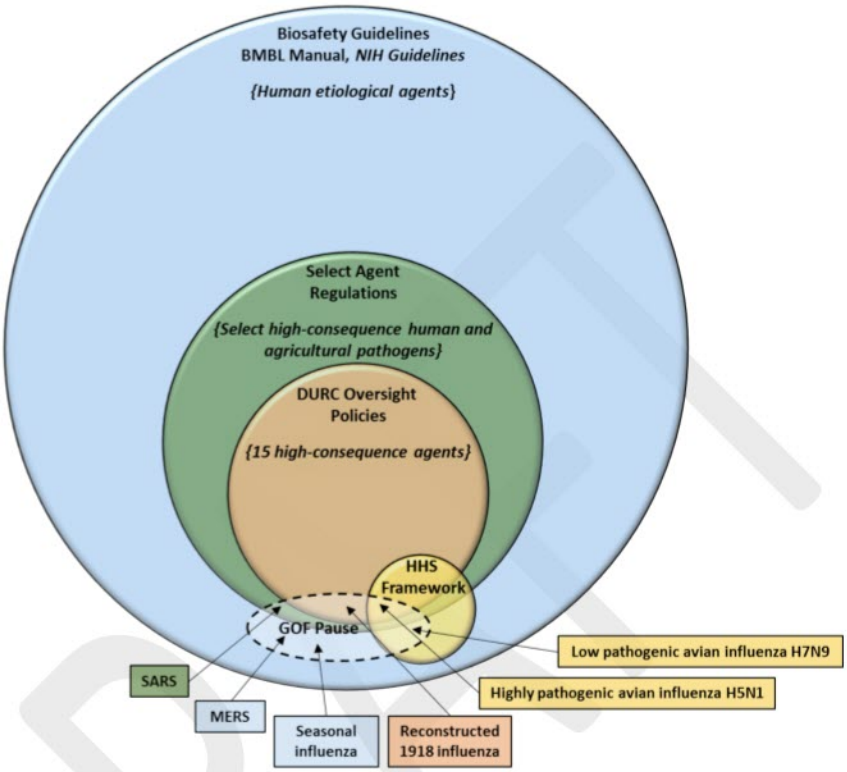

Figure 3. Comparison of the scope of different policies for the oversight of life sciences research involving pathogens. Oversight policies apply to research involving specified agents or procedures. GOF studies involving pathogens or manipulations covered under a given policy would be subject to oversight described by that policy.

Federal-Level Review of Certain Gain-of-Function Studies

The only U.S. Federal policy that specifically addresses GOF studies is the *Framework for Guiding U.S. Department of Health and Human Services Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets (HHS Framework)*, issued by the U.S. Department of Health and

[38] The policy for Federal DURC oversight requires Federal funding agencies to compile biannual inventories of projects identified as being subject to DURC oversight. As part of this process, Federal agencies have been identifying projects involving MERS and LPAI H7N9 influenza and proactively managing risks associated with those projects, as necessary.

Human Services in February, 2013. Under the *HHS Framework*[39,40] certain proposals with the potential for generating highly pathogenic avian influenza H5N1 viruses that are transmissible among mammals by respiratory droplets receive special review and approval before being funded by HHS. This policy was subsequently expanded to include review of similar proposals involving low pathogenic avian influenza H7N9 virus.[41]

Funding agencies within HHS (including NIH, CDC, and FDA) review relevant proposals for risks and benefits, and refer relevant studies to a Department-level review group, the HHS HPAI H5N1 Gain-of-Function Review Group, for advice prior to funding the proposal. The review group includes a wide range of interdisciplinary expertise from across HHS and the Federal government, if necessary. HHS reviews GOF research proposals that are subject to the *HHS Framework* and makes recommendations to HHS funding agencies about whether the study is acceptable for funding and whether additional measures may be needed to mitigate risks. HHS considers a number of factors including the following criteria, which must be met in order for a GOF study to be acceptable to receive HHS funding:

1. The virus anticipated to be generated could be produced through a natural evolutionary process;
2. The research addresses a scientific question with high significance to public health;
3. There are no feasible alternative methods to address the same scientific question in a manner that poses less risk than does the proposed approach;
4. Biosafety risks to laboratory workers and the public can be sufficiently mitigated and managed;
5. Biosecurity risks can be sufficiently mitigated and managed;
6. The research information is anticipated to be broadly shared in order to realize its potential benefits to global health; and
7. The research will be supported through funding mechanisms that facilitate appropriate oversight of the conduct and communication of the research

Analysis: The *HHS Framework* requires an explicit consideration of the risks and benefits associated with certain GOF studies prior to making a funding decision. This allows HHS to identify potential risks up front and make recommendations about risk mitigation—including consideration of alternative approaches or modifying the experimental design—at the outset. This review process also involves broader expertise including, ethical, legal, security, intelligence, and more. The criteria that must be met in order to receive funding are subject to judgment and interpretation. The scope of the *HHS Framework* is quite narrow and currently covers only projects involving two influenza viruses and that involve one specific experimental outcome (mammalian transmission by respiratory droplets); other GOF studies do not receive this pre-funding review.

[39] *A Framework for Guiding U.S. Department of Health and Human Services Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets*, U.S. Department of Health and Human Services, February, 2013. http://www.phe.gov/s3/dualuse/Documents/funding-hpai-h5n1.pdf

[40] Patterson, AP, et. al. A Framework for Decisions about Research with HPAI H5N1 Viruses. Science. 2013 Mar 1: 339(6123): 1036-1037.

[41] Jaffe H., et. al. Extra Oversight for H7N9 Experiments. Science. 2013 August 16: 341(6147):713-714.

Reviews under this framework are conducted by a group internal to the USG. Reviewing GOF studies in a confidential setting allows for the examination of potentially sensitive scientific, proprietary, and personal information, and allows discussions that may be sensitive from a national security or public health preparedness perspective. However, such reviews do not achieve the level of transparency desired by some stakeholders and also make it difficult to independently assess the effectiveness of the review process. Finally, the *HHS Framework* was in place for less than two years when the October 2014 funding pause was enacted and only a handful of GOF projects have been reviewed to date, making it difficult to fully evaluate this policy's strengths and limitations.

In response to the funding pause[42], the National Institute for Allergy and Infectious Diseases (NIAID), within the NIH, developed a process for considering on a case-by-case basis studies that might be subject to the GOF pause. Reviews by NIAID include a detailed consideration of the science, often including a specific examination of the viral strains in question and specific experiments being proposed. NIAID begins by consulting the investigators and an internal NIAID group determines whether the projects are subject to the pause. When identifying projects subject to the funding pause, NIAID has used a fairly broad interpretation of the language set forth in the pause statement and paused, at least initially, more projects than were ultimately determined to meet the scope of the pause policy. NIAID also sought exceptions (using a mechanism provided for in the USG's moratorium statement) for projects that were deemed critical to public health or national security. In determining whether an exception to the pause might be warranted, NIAID considers the intent of the research, the availability of countermeasures, potential alternative approaches, the risks of not conducting the research, and the available mechanisms for ongoing oversight. Exceptions may only granted by the NIH Director.

Analysis: NIAID's process for identifying GOF projects that are subject to the funding pause is rigorous and serves as an example of Federal-level identification and review of GOF studies of potential concern. It includes extensive scientific review and is performed by individuals with experience reviewing projects for DURC potential. It does not involve the same expertise that is provided under *HHS Framework* reviews such as national security, ethics, or legal. Given the limited number of projects that have been examined by NIAID it is difficult to fully evaluate how effective this approach is.

Sharing and Communicating Scientific Findings and Research Products

The majority of life sciences research is conducted in academic settings and the results are communicated openly in scientific journals and public forums. For a small subset of research with national security implications, there are policies in place to restrict access to scientific information or products. Under National Security Decision Directive (NSDD) 189, dissemination of fundamental research is to remain unrestricted to the maximum extent possible and in instances where restriction is

[42] U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses, U.S. Government, October 17, 2014. http://www.phe.gov/s3/dualuse/documents/gain-of-function.pdf

necessary for national security, classification is to be the appropriate mechanism for restricting access.[43] Life sciences research that requires classification is classified at its outset and conducted in designated facilities that are equipped with the infrastructure and personnel with appropriate level national security clearances to perform the research. Retroactively classifying research that was conducted in an unclassified setting is immensely challenging and may be unfeasible.

Export controls are Federal regulations that restrict exports that have national security or foreign policy implications. Certain materials and information related to biological agents and genetic elements, vaccines, equipment, and related technologies are covered by export control regulations. Furthermore, the transfer of controlled information to a foreign national within the United States is considered to be an export to that foreign national's country. The regulations are complex but, in general, they specify which items, when shipped to which destinations, will require export licenses. Life sciences research that is openly published is not subject to export controls, but information that is withheld from publication by the investigator or research institution based on security concerns may become subject to export control regulations, and an export license may be required before that information can be shared with foreign nationals. Most biological research activities that are subject to export controls fall under the Department of Commerce's Export Administration Regulations, which control items that have both military and civilian applications.[44] However, some might fall under the jurisdiction of the State Department's International Traffic in Arms Regulations.[45]

A number of scientific journals and families of journals have policies for identifying and reviewing manuscripts that raise biosecurity and biosafety concerns. These efforts are commendable but some have noted the challenges associated with trying to identify DURC or implement risk mitigation measures at the publication stage.[46,47] NSABB has previously developed strategies and a risk assessment tool to assist in the development of a responsible communication plan for DURC, which might include altering the content, distribution, or timing of a publication.[48] The U.S. government has no authority to mandate redaction, restriction, or classification of a scientific publication that it does not own or control, and the development of a mechanism for restricting communication of unclassified information to only those who require access, remain challenging and to date unsuccessful.[49]

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[43] NSDD 189 (September 21, 1985) defines fundamental research as "basic and applied research in science and engineering, the results of which ordinarily are published and shared broadly within the scientific community, as distinguished from proprietary research and from industrial development, design, production, and product utilization, the results of which ordinarily are restricted for proprietary or national security reasons." https://research.archives.gov/id/6879779

[44] Export Administration Regulations, 15 CFR Parts 730, 734, 736, 742, 744, and 745. https://www.bis.doc.gov/index.php/regulations/export-administration-regulations-ear

[45] International Traffic and Arms Regulations, 22 U.S.C. 2778 https://www.pmddtc.state.gov/regulations_laws/itar.html

[46] Casadevall A et al. Dual-Use Research of Concern Review at American Society for Microbiology Journals. mBio 6(4):e01236-15. 2015.

[47] Atlas et. al. Journal editors and authors group statement on scientific publication and security. Science, 299:1149. 2003.

[48] Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information. NSABB, June, 2007. http://osp.od.nih.gov/sites/default/files/resources/Framework%20for%20transmittal%20duplex%209-10-07.pdf

[49] Research information produced under a U.S. government grant is not considered to be owned or controlled by the Federal Government. However, under the Invention Secrecy Act, the U.S. government can nevertheless impose secrecy orders on patent applications if the publication or disclosure of the ensuing patent would be detrimental to national security.

Analysis: Once a study has been completed, it is difficult to limit the distribution of or access to the findings, particularly if the study was conducted in an open, academic environment. Oversight of DURC, and in particular GOF studies involving pathogens with pandemic potential, may be most feasible and effective if it occurs 1) upstream (i.e., during the review of proposed studies and before experiments are initiated) and 2) in an ongoing manner while the research is being conducted.

Classification may be an option for certain GOF studies, but this would entail that these studies be conducted in significantly different settings than they are conducted currently. Further, although certain GOF studies have raised concerns about whether they should be published, it is unlikely that such manuscripts would meet the criteria for classification under U.S. government classification authorities. It is conceivable that certain studies should not be undertaken at all or not published because of unanticipated findings. However, it may be very difficult to predict at the proposal stage whether findings of concern might arise during the experiment, and unanticipated findings that raise concern may be unavoidable. Individual investigators or journal editors have, on security grounds, decided to redact certain material from publication, possibly triggering export controls on the redacted material, but in general such a redaction could not be mandated by the U.S. government.

Broader U.S. Biosafety and Biosecurity Efforts

Parallel to the GOF deliberative process, the USG has also initiated additional, broader reviews of biosafety and biosecurity policies and procedures following a series of laboratory incidents occurring at federal institutions in 2014. The Holdren-Monoco memorandum[50] called for Federal and non-Federal reviews to provide recommendations to strengthen the biosafety and biosecurity practices and oversight system for USG funded research. The memo outlined three immediate actions for Federal Agencies:

1. Conduct a comprehensive review of current biosafety and biosecurity protocols to ensure adequacy and appropriateness for today's infectious disease research
2. Inventory and document culture collections
3. Increase attentiveness throughout research community to ensure the safety of laboratory workers and the American public.

In September 2015, The White House National Security Council tasked the Federal Experts Security Advisory Panel (FESAP) to 1) identify needs and gaps and make recommendations to optimize biosafety, biosecurity, oversight, and inventory management and control for biological select agents and toxins (BSAT); 2) identify actions and any regulatory changes to improve biosafety and biosecurity; and 3) identify an approach to determine the appropriate number of high-containment U.S. laboratories required to possess, use, or transfer BSAT. To obtain broad stakeholder recommendations, the National Science and Technology Council established the Fast Track Action Committee on Select Agent Regulations (FTAC-SAR). In October 2015, USG released the FESAP and FTAC-SAR recommendations[51] that address the culture of responsibility, oversight, outreach and education; applied biosafety research;

[50] https://www.whitehouse.gov/sites/default/files/microsites/ostp/enhancing_biosafety_and_biosecurity_19aug2014_final.pdf
[51] http://www.phe.gov/s3/Documents/fesap.pdf; http://www.phe.gov/s3/Documents/ftac-sar.pdf.

incident reporting; material accountability; inspection processes; and regulatory changes and guidance to improve biosafety and biosecurity. The USG has developed a plan to implement these recommendations.[52]

[52] Implementation of Recommendations of the Federal Experts Security Advisory Panel and the Fast Track Action Committee on Select Agent Regulations, October 2015. http://www.phe.gov/s3/Documents/fesap-ftac-ip.pdf

5. Findings

In developing the findings below (Box 2), the NSABB working group considered the results of the risk and benefit assessments, policy analysis and decision-making frameworks, discussions of ethics, and perspectives of domestic and international stakeholders.

Box 2. Summary of Findings

Finding 1: There are many types of GOF studies and not all of them have the same level of risks. Only a small subset of GOF research—GOF research of concern—entail risks that are potentially significant enough to warrant additional oversight.

Finding 2. The U.S. government has several policies in place for identifying and managing risks associated with life sciences research. There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOF research of concern could be implemented.

Finding 3. Oversight policies vary in scope and applicability, and do not cover all potential GOFROC, therefore, current oversight is not sufficient for all GOF research of concern.

Finding 4. An adaptive policy approach is a desirable way to ensure that oversight and risk mitigation measures remain commensurate with the risks associated with the research and the benefits of the research are being fully realized.

Finding 5. There are life sciences research studies, including possibly some GOF research of concern, that should not be conducted because the potential risks associated with the study are not justified by the potential benefits. Decisions about whether specific GOFROC should be permitted will entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of proposed studies but other considerations, including legal, ethical, public health, and societal values are also important and need to be taken into account.

Finding 6. Managing risks associated with GOF research of concern, like all life sciences research, requires both Federal-level and institutional oversight, awareness and compliance, and a commitment by all stakeholders to safety and security.

Finding 7. Funding and conducting GOF research of concern involves many issues that are international in nature.

Finding 1: There are many types of GOF studies and not all of them have the same level of risks. Only a small subset of GOF research—GOF research of concern—entail risks that are potentially significant enough to warrant additional oversight.

As with all life sciences research involving pathogens, GOF studies entail inherent biosafety and biosecurity risks. GOF research involving the generation of pathogens with pandemic potential involves the greatest risks. A laboratory accident involving such a pathogen could potentially release a pathogen that could spread rapidly and efficiently through the human population. A laboratory pathogen with enhanced characteristics could also, if malevolently used, pose a greater threat to national security or public health than similar misuse involving a wild type pathogen. The probability that such events would occur is low but non-zero and the potential consequences are uncertain but potentially significant.

Gryphon's biosafety risk assessment identified studies involving enhanced transmissibility, enhanced pathogenicity, and evasion of immunity as entailing the highest risks for coronaviruses, seasonal influenza, and avian influenza.[53] Manipulations that increase transmissibility, increase pathogenicity, and enable a pathogen to more readily spread through the population have the greatest potential to increase risk; in some strains even a moderate increase might be a concern.

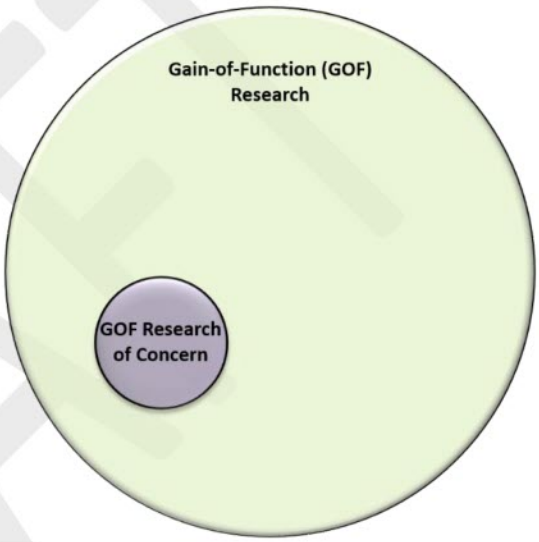

Figure 4. Conceptual categorization of GOF research involving human or animal pathogens. GOF research includes a broad range of experimental approaches, most of which do not raise significant concerns. GOF research of concern represents a small subset of all GOF research that can be reasonably anticipated to result in generation of a pathogen with pandemic potential, as described as a pathogen that is likely both highly transmissible and highly virulent in humans.

To help categorize studies based on the level of concern stemming from their associated risks, the working group has designated studies as: GOF research and GOF research of concern (GOFROC) (Figure 4). The term "GOF research" would encompass all studies whereby some characteristic of the pathogen is enhanced. The vast majority of GOF research does not raise any significant concerns; these studies do not entail novel or significant risks and are subject to oversight to manage risks. GOF research of concern, or GOFROC, represents the small subset of studies that result in the generation of a pathogen with pandemic potential—that is, a pathogen that is highly virulent and highly transmissible, as judged by its likely ability to spread among human populations (see Recommendation 1 for more thorough description of these attributes).

[53] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

Finding 2. The U.S. government has several policies in place for identifying and managing risks associated with life sciences research. There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOF research of concern could be implemented.

Federally-funded life sciences research in the U.S. is conducted in accordance with occupational health and safety laws and regulations, the *NIH Guidelines*, the BMBL, policies for the Federal and institutional oversight of DURC, the Select Agent Regulations, export control regulations, international treaties and agreements, and other relevant policies. HHS has also developed a framework for guiding funding decisions for certain GOF studies involving H5N1 and H7N9 influenza viruses. Together, these policies aim to mitigate biosafety risks, biosecurity risks, and other risks associated with life sciences research, including many of the GOF studies that have raised concerns.

U.S. policies involve oversight and help manage risks at several points throughout the research life cycle including the proposal review, the funding decision, the time during which the research is being conducted, and at the time at which the research is being communicated. There are also numerous entities that are responsible for providing oversight, managing risks or issuing guidance, including funding agencies, institutional review and compliance committees, individual investigators, federal advisory committees, and journal editors.

While effective implementation of these policy frameworks can manage much of the risk associated with life sciences research, including the risks of some GOFROC, some GOFROC is more thoroughly monitored than others. Additionally, coverage under current policies is incomplete (e.g., GOF research funded and conducted by/within the private sector may not be covered). Institutional oversight also varies. For example, IBCs differ in capabilities and expertise, and institutional resources and cultures vary. In addition, there is limited data describing the rate and extent of laboratory accidents, near-misses, and security breaches. Little comprehensive data about these critical issues exist, and no entity is currently authorized to collect all of the desirable information that would inform risk-benefit assessments.

Finding 3. Oversight policies vary in scope and applicability, and do not cover all potential GOFROC, therefore, current oversight is not sufficient for all GOF research of concern.

U.S. policies are applicable to some but not all GOFROC. Risks associated with GOFROC that do not involve select agents or pathogens subject to oversight under the USG DURC policies or the *HHS Framework*, would largely be managed at the institutional level, in accordance with guidance in the *NIH Guidelines* and BMBL. In general, GOFROC that is not conducted with U.S. government funds is not

subject to oversight by a Federal funding agency.[54] Other countries also fund and conduct life sciences research, including GOF studies, which are beyond the purview of the U.S. government as well.

In addition, the U.S. government's oversight policies vary. Different policies are aimed at managing different risks, and each is implemented by various Federal Departments and Agencies. This can result in redundancies as well as gaps in oversight, as the various policies have not been harmonized.

Finally, full compliance with policies is essential to their effectiveness. The effectiveness of policies can be enhanced by a commitment to proper implementation and enforcement at the Federal, institutional, and individual investigator levels. This can include training, education, codes of conduct, and other mechanisms for continuing to build a culture of responsibility.

Finding 4. An adaptive policy approach is a desirable way to ensure that oversight and risk mitigation measures remain commensurate with the risks associated with the research and the benefits of the research are being fully realized.

Many, but not all, of the policies that apply to GOF studies are adaptive in nature. The BMBL is updated periodically. The *NIH Guidelines* and the select agent programs are updated or revised periodically as well and both have processes for seeking external advice for informing policy development. The DURC policies and the *HHS Framework* do not have articulated mechanisms for seeking input on policy development, reviewing, or updating the policies, though both state an intention to be updated as necessary. Great uncertainty is inherent in conducting risk-benefit assessments with currently available data and several key parameters of the risk and benefit assessment made its interpretation challenging. Such uncertainty about risks and benefits may also make risk management difficult. An adaptive policy approach will facilitate refinement of GOF risk management as knowledge and experience are acquired.

Finding 5. There are life sciences research studies, including possibly some GOF research of concern, that should not be conducted because the potential risks associated with the study are not justified by the potential benefits. Decisions about whether specific GOFROC should be permitted will entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of proposed studies but other considerations, including legal, ethical, public health, and societal values are also important and need to be taken into account.

Examples of studies that should not be conducted for ethical reasons include those that: involve human subjects who have not been provided and signed an informed consent document approved by an IRB;

[54] Research involving a select agent, whose oversight is articulated in Federal statute and requires compliance from all researchers and institutions, would be subject to Federal oversight, regardless of the funding source. Some privately-funded research being conducted at institutions that receive Federal funding for that research may also be subject to oversight under the *NIH Guidelines*, USG DURC policies, or other policies.

are anticipated to cause undue harm to a human subject; or that entail benefits that are unjustifiable in the light of the risks. For example, the development of biological weapons is unethical and has been banned by international treaty.[55]

There may be GOFROC that should not be funded on ethical grounds but it is difficult to identify or describe such studies based on general or hypothetical descriptions. An ethical evaluation of a research study would entail an evaluation of the risks and benefits, which requires a thorough understanding of the scientific details of the proposal, including its aims and any foreseeable adverse consequences. In addition, the scientific, public health, and national security landscape is dynamic. Public health needs change as new diseases emerge. Risks may arise or diminish based on the availability (or lack) of effective countermeasures. Benefits may become more or less likely to be realized based on other enabling factors, such as new scientific findings or technologies. Decisions to fund GOF studies must take into account these nuances in the risk-benefit landscape.

The NSABB did not seek to develop a list of studies that should not be conducted but rather sought to develop general principles that describe what is acceptable and not acceptable for funding. A principle-based approach to guiding funding decisions is adaptable and likely more effective.

However, one example of a scientific study that should not be conducted might be the insertion of a virulence gene from an unrelated organism into the genome of a virus transmissible through the respiratory route, which would be highly unlikely to occur by natural recombination. This study, and others that involve the transfer of virulence genes between disparate microbes would appear to lack public health benefit, since the novel, laboratory-generated pathogen is unlikely to arise naturally and would therefore entail potentially significant and unnecessary risks.

Finding 6. Managing risks associated with GOF research of concern, like all life sciences research, requires both Federal-level and institutional oversight, awareness and compliance, and a commitment by all stakeholders to safety and security.

Biosafety and biosecurity risks associated with life sciences research are managed through engineering controls, laboratory practices, medical surveillance and support, appropriate training, and other interventions. However, GOFROC has the potential to generate strains with significant risks that may require additional oversight and containment mechanisms. Managing the risks associated with GOFROC in particular requires a commitment to safety and security at the Federal and institutional level that

[55] Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction. Signed at London, Moscow and Washington on 10 April 1972; entered into force on 26 March 1975. Depositaries: UK, US and Soviet governments. http://www.opbw.org/

includes a strong foundation of training and a demonstrated commitment to compliance by the research institution, and the individual investigators at the local level.

Finding 7. Funding and conducting GOF research of concern involves many issues that are international in nature. The potential risks and benefits associated with GOFROC are international in nature. Laboratory accidents and intentional misuse could have global consequences. The benefits of vaccine and other medical countermeasure development and disease surveillance likely also have important international implications. The research enterprise is international as well and GOFROC is being conducted in a number of countries already. While U.S. government funding policy regarding GOFROC only directly affects domestic and international research within the purview of the U.S. government, decisions made by the United States in this area can influence GOFROC oversight policies globally.

Notably, as highlighted during presentations at NSABB and NAS meetings, GOF research and GOFROC research is being conducted in a number of countries and a variety of oversight mechanisms at the national and regional level are in place. In addition, a number of countries and international scientific organizations have been considering issues related to biosafety, biosecurity, dual use research, and GOFROC.[56, 57, 58, 59, 60, 61] International perspectives are important to the development of U.S. policy in this area and global engagement is necessary to foster effective oversight mechanisms and an international culture of responsibility around research involving pathogens.

The U.S. government, often in concert with the NSABB, has been engaged with the international community for many years and continues to work with those governments and organizations now actively considering GOFROC-related issues. Presentations to the NSABB, its working groups, and at the NAS meetings have provided perspectives about the activities of foreign governments, international organizations, researchers and others have greatly aided the NSABB during the development of this report.

[56] *Gain-of-Function Research: Summary of the Second Symposium*, March 10-11, 2016. The National Academies of Sciences, Engineering, and Medicine. The National Academies Press, Washington DC.
[57] *Gain of function: experimental applications relating to potentially pandemic pathogens*. European Academies Science Advisory Council, EASAC policy report 27, October 2015. http://www.easac.eu/
[58] *Summary report: Dual Use Research On Microbes: Biosafety, Biosecurity, Responsibility.* December 10 – 12, 2014, Herrenhausen Palace, Hanover, Germany. https://www.volkswagenstiftung.de/dualuseresearch
[59] *France-US Bilateral Workshop on Dual Use Research Issues: Summary Report*, February 11, 2016. U.S. Department of State.
[60] Draghia-Akli, Ruxandra, Director of the Health Directorate at the Research DG, European Commission, presentation to NSABB working group, July 23, 2015.
[61] Donker, Marianne, Ministry of Health, Welfare and Sport, Netherlands, presentation to NSABB working group, July 23, 2015.

6. Recommendations of the NSABB

Based on its analyses and findings, the NSABB working group has developed the following recommendations to the U.S. government.

Box 3. Summary of Recommendations of the NSABB

Recommendation 1. Research proposals involving GOFROC entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the Federal and institutional levels.

Recommendation 2. An external advisory body that is designed for transparency and public engagement should be utilized as part of the U.S. government's ongoing evaluation of oversight policies for GOFROC.

Recommendation 3. In general, oversight mechanisms for GOFROC should be incorporated into existing policy frameworks when possible.

Recommendation 4. The U.S. government should pursue an adaptive policy approach to help ensure that oversight remains commensurate with the risks associated with the GOFROC.

> **Recommendation 4.1.** The U.S. government should consider developing a system to collect and analyze data about laboratory safety incidents to inform GOFROC policy development over time.

Recommendation 5. The U.S. government should consider ways to ensure that all GOFROC conducted within the U.S. or by U.S. companies be subject to oversight, regardless of funding source.

Recommendation 6. The U.S. government should undertake broad efforts to strengthen laboratory biosafety and biosecurity and, as part of these efforts, seek to raise awareness about the specific issues associated with GOFROC.

Recommendation 7. The U.S. government should engage the international community in a dialogue about the oversight and responsible conduct of GOFROC.

Recommendation 1. Research proposals involving GOFROC entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the Federal and institutional levels.

GOFROC entails the generation of pathogens—perhaps novel pathogens—with anticipated pandemic potential. The associated risks associated with such studies are uncertain but potentially significant. It is possible that generating a laboratory pathogen with pandemic potential introduces a risk of a pandemic, albeit a low probability risk, that did not exist before that pathogen was generated. Therefore, a new, pre-funding review and approval mechanism is warranted before such studies should be undertaken. The NSABB working group proposes a conceptual approach for guiding funding decisions about GOFROC, which entails identifying GOFROC and subjecting such studies to an additional pre-funding review and approval process. The attributes that describe GOFROC, the principles that should guide funding decisions for GOFROC, and the steps in a proposed review/approval process for GOFROC are described below.

Identifying GOF research of concern

GOFROC is research that can be reasonably anticipated to generate a pathogen with pandemic potential. Determining whether a proposed research project is likely to do so will entail uncertainty and will require scientific and other expert judgment.

To be considered GOFROC, the research must, in a single step or over the course of manipulations, be reasonably anticipated to generate a pathogen with both of the following attributes:

i. **The pathogen generated is likely highly transmissible and likely capable of wide and uncontrollable spread in human populations.** To be considered "highly transmissible" the pathogen must be judged to have the capacity for sustained secondary transmission among humans, particularly but not exclusively by the respiratory route. Such a determination might be informed by data describing human infections by naturally-circulating isolates of the pathogen or studies in relevant experimental mammalian models that serve as a proxy for human infections. To be considered "capable of wide and uncontrollable spread in human populations" it must be judged that there would be limited options for controlling the spread of the pathogen other than patient isolation or quarantine. Such a determination might be made, for instance, if humans lack population immunity to the resulting pathogen, if the pathogen would evade or suppress the human immune response, if the pathogen would be resistant to medical countermeasures, or if existing countermeasures would be unavailable globally in sufficient quantities.

AND

ii. **The pathogen generated is likely highly virulent and likely to cause significant morbidity and/or mortality in humans.** To be considered "highly virulent" the pathogen must be judged to have the capacity for causing significant consequences in humans, such as severe disease and/or a high case fatality rate. Such a determination might be informed by data describing human infections by naturally-circulating strains of the pathogen or studies in relevant experimental mammalian models that serve as a proxy for human disease.

Any study involving the generation of a pathogen exhibiting the two attributes above would be considered GOFROC. However, it is generally anticipated that the following types of activities would not be considered GOFROC:

- Studies to characterize the virulence and transmission properties of circulating pathogens
- Surveillance activities, including sampling and sequencing
- Activities associated with developing and producing vaccines, such as generation of high-growth strains

Importantly, a proposed experiment need not involve the simultaneous enhancement of both phenotypes. For instance, research involving a naturally-occurring pathogen that exhibits one of the above attributes would be considered GOFROC if a study were anticipated to confer the second attribute to the agent (while retaining the first attribute). Other studies may generate a pathogen with the above attributes after a series of manipulations that enhance the phenotypes separately but ultimately result in a pathogen with both attributes. Any route of experimentation that is anticipated to ultimately generate a pathogen that exhibits both of the characteristics above would be considered GOFROC and should be reviewed carefully before it can be funded.

Appendix B describes examples of studies that would and would not be considered GOFROC. These examples are provided as guidance and are described in general terms. A more detailed consideration of the specific characteristics of a pathogen in question as well as the proposed experimental manipulations would be required to determine whether a research proposal is GOFROC.

Pre-funding review and approval of GOF research of concern

Proposals anticipated to involve GOFROC should be subject to additional review prior to making a funding decision and a high degree of Federal oversight throughout the course of the research, if funded. The working group has developed principles that should guide the review and funding of these proposals. There should be a high degree of confidence that a study will be conducted in accordance with these principles before determining whether the proposal is suitable for funding. Studies that cannot be or are not anticipated to be conducted in accordance with the principles below should not be funded.

Principles for guiding review and funding decisions

The NSABB working group has developed the following principles to guide funding decisions regarding GOFROC. Only projects that are in line with all of the following principles should be considered acceptable for funding. The principles below are intended to embody the substantive ethical values described in section 4.2 and the process of applying these principles would involve scientific, security, ethical, and other considerations.

i. **The research proposal has been evaluated by a peer-review process and determined to be scientifically meritorious, with high impact on the research field(s) involved.** If GOFROC is to be funded and conducted it must first and foremost address a valuable scientific question or public health need.

ii. **The pathogen that is anticipated to be generated must be judged, based on scientific evidence, to be able to arise by natural processes.** It is difficult to predict the types of pathogens that can or will emerge in nature. Nevertheless, before a pathogen with pandemic potential is generated through laboratory manipulations it is essential to consider whether such a pathogen could arise in nature. GOFROC may be permissible if the study were to generate a pathogen that is anticipated to arise in nature or if the study were to provide insight into natural evolutionary processes. GOFROC would not be permissible if it were to generate a laboratory pathogen that is highly unlikely to arise in nature.

iii. **An assessment of the overall potential risks and benefits associated with the project determines that the potential risks as compared to the potential benefits to society are justified**. Prior to funding GOFROC, the anticipated risks and potential benefits must be carefully evaluated. In general, the potential benefits associated with a research project should be commensurate with or exceed the presumed risks. Projects involving significant risks and little anticipated benefits are ethically unacceptable and should not be funded. If the potential risks appear high, the possible benefits should also appear high. Risks should be managed and should be mitigated whenever possible. The extent to which risks can be mitigated should factor into the assessment.

iv. **There are no feasible, equally efficacious alternative methods to address the same scientific question in a manner that poses less risk than does the proposed approach.** Alternative approaches must be explored and critically examined before funding GOFROC. It is possible that the proposed experimental approach that raises concern is the only feasible approach for addressing the scientific question at hand. In other cases, modifications of the experimental design, use of attenuated or other strains that pose fewer risks to humans, or different approaches with less risk that may provide the same or very similar information may be feasible. Lines of experimentation that entail less risk should be pursued whenever possible.

v. **The investigator and institution proposing the research have the demonstrated capacity and commitment to conduct it safely and securely, and have the ability to respond rapidly and adequately to laboratory accidents and security breaches.** Prior to funding, the risks associated with proposed GOFROC must be identified and assessed, and clear, realistic plans

for managing risks should be developed. In order to manage risks associated with GOFROC, an institution must have adequate facilities, resources, security, trained personnel, administrative structures, ongoing occupational health and safety monitoring procedures, relationships with local public health authorities and first responders, and the ability to adapt to unanticipated situations by increasing containment or adding additional safety or security features. In addition to adhering to standards of compliance, an institution (and the investigators proposing the study) should have a demonstrated commitment to laboratory safety and security, scientific integrity, and the responsible conduct of research. The researchers and institution should be committed to a culture of responsibility, perhaps demonstrated through adherence to a formal code of conduct or other measures.

vi. **The results of the research are anticipated to be broadly shared in compliance with applicable laws and regulations in order to realize its potential benefits to global health.** Prior to funding GOFROC, consideration should be given to the type of research-related information and products that are likely to be generated. The research-related information and products are expected to be shared appropriately and a responsible communication plan should be developed at the outset, as appropriate. NSABB[62] and the U.S. government[63] have issued guidance for developing communication plans for dual use research of concern that include consideration of the content, timing, and distribution of the research information.

vii. **The research will be supported through funding mechanisms that allow for appropriate management of risks and ongoing Federal and institutional oversight of all aspects of the research throughout the course of the project.** GOFROC should be funded through mechanisms to ensure that appropriate biocontainment conditions are utilized, adequate biosecurity precautions are in place, and that the data and materials generated will be shared appropriately. The funding mechanism should allow for modification of required mitigation and oversight features, as well as research objectives required during the course of the research, if needed.

viii. **The proposed research is ethically justifiable.** Determinations of whether proposed GOFROC should be undertaken involves value judgments to assess the potential risks and benefits and to determine whether any potential risks are justified. Non-maleficence, beneficence, justice, respect for persons, scientific freedom, and responsible stewardship are among the values that should be considered when ultimately making decisions about whether to fund GOFROC.

[62] Appendix 5, *Proposed Framework for the Oversight of Dual Use Research Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information*. National Science Advisory Board for Biosecurity, June, 2007.

[63] Section E, *Tools for the Identification, Assessment, Management, and Responsible Communication of Dual Use Research of Concern: A Companion Guide to the United States Government Policies for Oversight of Life Sciences Dual Use Research of Concern.* U.S. government, September, 2014.

Description of the Review Process for Proposals Involving GOF Research of Concern

The NSABB proposes the following conceptual approach for guiding funding decisions about GOFROC (Figure 5). Review of research projects that may involve GOFROC would involve five steps:

1. Investigators and research institutions identify proposed GOFROC, as described by the two attributes for identifying GOFROC.
2. Funding agencies identify or confirm proposed GOFROC.
3. A Department-level Federal panel with diverse expertise reviews proposals involving GOFROC to determine whether proposals meet the 8 principles for guiding funding decisions and make recommendations as to whether the proposed research is acceptable for funding.
4. Funding agencies make a funding decision, if funded, establish risk mitigation plans and issue the funding award with appropriate terms and conditions to help ensure ongoing oversight.
5. Investigators and institutions conduct the research in accordance with applicable Federal and local oversight policies and employ any necessary additional mitigation strategies. Federal agencies provide oversight to ensure adherence to established risk mitigation plans and funding terms.

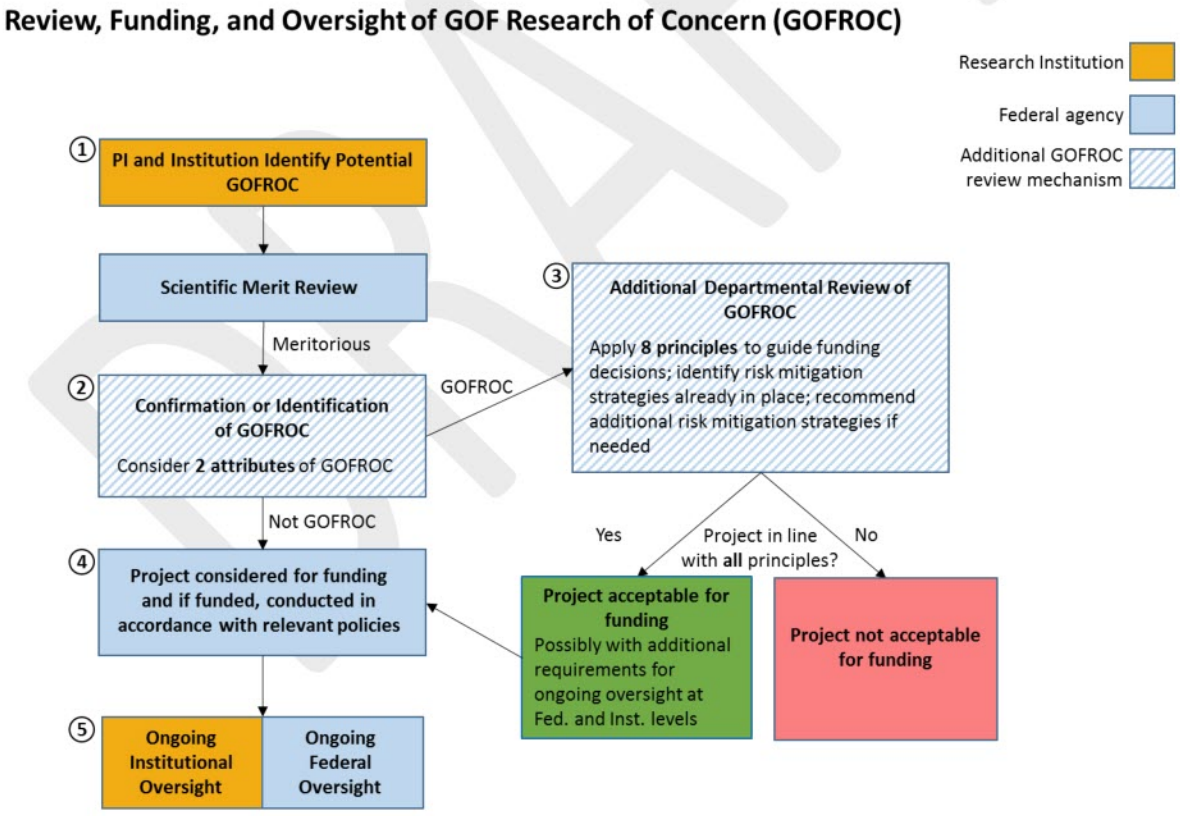

Figure 5. Proposed conceptual approach for guiding funding decisions for GOF research of concern.

Investigators and institutions identify GOFROC. Prior to submission of an application for funds, investigators and research institutions should identify possible GOFROC and submit with the research proposal any relevant information such as biosafety, biosecurity, or local public health response plans, descriptions of facilities available, a justification for the proposed approach that considers possible non-GOFROC alternatives that may be equally efficacious, and a discussion of the value and potential benefits of the proposed research. Identification of possible GOFROC should not affect a subsequent Federal scientific merit review either positively or negatively.

A need for guidance to investigators and institutions. The U.S. government should develop a "Points to Consider" document to provide guidance to investigators and institutions when preparing research proposals that may involve GOFROC. Such a document would describe to investigators any requirements for proposals involving GOFROC and provide guidance on the type of information that should be included in a proposal to facilitate its review. This document should be reviewed and updated as necessary. **NOTE: This para is formerly recommendation 5.1. As discussed, it was moved to the more logical location here, but it is no longer specified as its own recommendation. Is this acceptable?**

Department-level review of GOFROC. After the standard agency scientific merit review process, proposals that are determined to be scientifically meritorious and likely to be favorably considered for funding would also be reviewed by the funding agency to determine if they constitute GOFROC, as defined by whether the proposal can be anticipated to generate a pathogen that is highly transmissible and highly virulent, as described by the two attributes above. Prior to being determined acceptable for funding, proposals identified by a funding agency as involving GOFROC would require an additional, higher level, Departmental review. If a proposal does not involve GOFROC, it would proceed along the normal pathway for further evaluation and funding decisions.

The additional review of proposals involving GOFROC would determine whether the proposed research aligns with the 8 principles to guide funding decisions. Applying these principles will help to ensure that the GOFROC is scientifically and ethically acceptable, that the risk-benefit balance is favorable, that alternative approaches are explicitly considered, and that the research can be performed safely and securely. It is envisioned that the additional review of proposals involving GOFROC would involve diverse, multidisciplinary expertise including scientific, public health, biosafety, national security and intelligence, legal, bioethics, and other perspectives. To the extent possible, the review process should be efficient, transparent, well-documented, and adaptive. In addition, the process should be structured to avoid real or apparent conflicts of interest and to provide consistency across Federal agencies that might fund GOFROC. It is also envisioned that research institutions proposing the GOFROC might be asked for and would have an opportunity to provide any additional information that might be necessary for a thorough and substantive review of the research proposal.

Funding decision and risk mitigation. During the course of the Department-level review the relevant risk management plans should be critically evaluated and additional risk mitigation measures may be recommended in order for GOFROC to be considered acceptable. A satisfactory risk management plan would entail appropriate biocontainment facilities and biosafety practices, appropriate standard

operating procedures and administrative controls, occupational health and safety programs and security systems for protecting laboratory strains and reagents and promoting personal reliability. Some or all of the additional risk mitigation measures listed in Box 4 may also be recommended. These and a variety of additional measures could be required as a condition of funding.

NOTE: Box 4 was moved from its previous position in Recommendation 3 below because it seems to fit more naturally here. Is this acceptable?

Box 4. Additional risk mitigation measures to be employed, as appropriate, for GOF research of concern.

Risk mitigation features that should be considered prior to funding GOFROC may include requirements to:

- Provide additional training to researchers
- Enhance biosafety practices or features, as dictated by the specific strains and proposed manipulations
- Enhance security measures around strains, reagents, notebooks, and personnel
- Prohibit certain additional GOFROC experiments without prior approval
- Treat the research as if subject to the USG DURC policies, if it is not already
- Conduct more frequent institutional biosafety and biosecurity reviews of the research
- Conduct more frequent progress reports and discussions with Federal funding agency staff, particularly about unanticipated results that may raise concerns
- Conduct periodic site inspections/evaluations if not already required
- Identify certain experimental outcomes that would trigger a re-evaluation of the risks and benefits prior to proceeding with a study
- Develop a responsible communication plan, specifically, including a description of biosafety and biosecurity practices
- The institution to be in regular communication with local law enforcement and public health officials
- Conduct bioethics consultations at the local and Federal level throughout the lifecycle of the research
- The investigators to develop and/or adhere to an appropriate code of conduct

Ongoing oversight. Finally, throughout the course of the funding, both Federal and institutional oversight are critically important and the project should be carefully monitored to ensure that required conditions are met, that the principles guiding the decision to fund are still satisfied, and that any changes, significant developments, and publication/communication plans are discussed and addressed in a timely manner.

NOTE: NIH and WG co-chairs favored placing the FACA recommendation as a stand-alone Recommendation 2. It was suggested by WG that this rec be built into Rec 1 and into Figure 5 but in doing so, it diminished the strength of this important recommendation and also confused the role of the FACA committee. Is this acceptable?

Recommendation 2. An external advisory body that is designed for transparency and public engagement should be utilized as part of the U.S. government's ongoing evaluation of oversight policies for GOFROC. An external advisory body that is designed for transparency and public engagement should be utilized as part of the U.S. government's ongoing evaluation of oversight policies for GOFROC. An external advisory mechanism, such as a committee governed by the Federal Advisory Committee Act[64], would allow for an independent examination of the U.S. government's policies for reviewing, funding, and conducting GOFROC. Such a group could evaluate the additional review and funding processes for GOFROC to understand how decisions were made, identify challenges to implementing the policy, and provide recommendations, as needed. Importantly, this mechanism would also provide transparency, promote public engagement, and would facilitate continued dialogue about GOFROC. The NSABB is one such body that is well-suited to address this task.

Evaluation of Additional GOF Research of Concern (GOFROC) Review, Funding, and Oversight Process

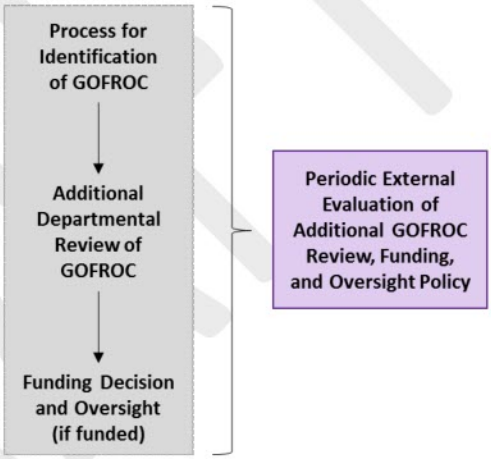

Figure 6. Independent evaluation of policies for the review, funding, and oversight of GOFROC. NOTE: Fig. 6 has not been discussed by WG yet. See also the alternate version of Figure 5, separate slide.

Recommendation 3. In general, oversight mechanisms for GOFROC should be incorporated into existing policy frameworks when possible.

Any additional oversight of GOFROC should be built into existing mechanisms rather than having the U.S. government develop a novel policy specific to GOFROC. Adapting or harmonizing current policies is preferable to developing entirely new oversight frameworks or wholly new approaches to manage the risks associated with these studies. There are precedents for additional Department-level pre-funding

[64] *Federal Advisory Committee Act*. http://www.gsa.gov/portal/content/100916

review of certain GOF studies (i.e. *HHS Framework*) as well as mechanisms for higher-level review and approval of certain studies (i.e., Major Actions, under the *NIH Guidelines*; restricted experiments, under the Select Agent Program). There are also mechanisms for continual Federal-level monitoring of biosafety and biosecurity risks for individual projects (i.e., USG Policy for Federal Oversight of DURC, select agent programs) and established mechanisms for ongoing institutional oversight (i.e., IREs under the USG Policy for Institutional Oversight of Life Sciences DURC; IBCs under the *NIH Guidelines*). Wherever possible, these mechanisms should be employed to ensure the initial and ongoing oversight of GOFROC.

Importantly, not all GOFROC would necessarily be subject to the entire suite of U.S. oversight policies. For instance, experimental manipulations with pathogens not included in the USG policies for DURC oversight or on the select agent list could conceivably generate a pathogen with pandemic potential. Additional oversight measures may need to be stipulated at the time of funding for proposals involving potential GOFROC that are not subject to a particular policy that is deemed necessary. For instance, specific, enhanced containment practices may be required or a project may require ongoing monitoring for DURC potential at the Federal and institutional level. Box 4 describes a number of potential risk mitigation measures that may be required for GOFROC that could potentially be implemented by leveraging existing policy frameworks.

Recommendation 4. The U.S. government should pursue an adaptive policy approach to help ensure that oversight remains commensurate with the risks associated with the GOFROC. The risk/benefit profile for GOFROC may change over time and should be re-evaluated periodically to ensure that the risks associated with such research are adequately managed and the benefits are being realized. An adaptive approach to the oversight of GOFROC would entail the continual evaluation of the risks and benefits associated with the research as well as the burdens and effectiveness of the additional proposal review process and ongoing oversight measures. An adaptive approach would allow policymakers to learn from experience and update policies accordingly as the risk/benefit landscape changes. For instance, the risks associated with a research proposal or project may change if newly developed countermeasures become available or if new information emerges to clarify certain risks or enable certain benefits.

> **Recommendation 4.1. The U.S. government should consider developing a system to collect and analyze data about laboratory safety incidents to inform GOFROC policy development over time.** Examining such data would provide a better understanding of the risks, inform future risk assessments, and allow for the refinement of oversight policies over time.

Recommendation 5. The U.S. government should consider ways to ensure that all GOFROC conducted within the U.S. or by U.S. companies be subject to oversight, regardless of funding source. GOFROC that is funded by the U.S. government or through private funding sources should be subject to

equivalent oversight to ensure that the associated risks are adequately managed. The U.S. government should consider providing oversight not only as a term and condition of a funding award but also via other mechanisms that would enable oversight of all relevant research activities, regardless of the funding source.

Recommendation 6. The U.S. government should undertake broad efforts to strengthen laboratory biosafety and biosecurity and, as part of these efforts, seek to raise awareness about the specific issues associated with GOFROC. Current discussions about GOFROC are related to broader domestic and international discussions about laboratory safety and security. A "top down" approach to managing the risks associated with GOFROC through Federal policies and oversight is appropriate. However, top-down approaches alone, in the form of Federal and/or institutional policies and leadership, will likely not be sufficient.. It is also critical to have adequately trained personnel that values safe and secure laboratory environments for conducting GOFROC. Therefore, it will also be important to facilitate a "bottom up" approach whereby scientific leaders and professional societies, as well as research staff involved in the design and conduct of GOFROC, are educated about biosafety, biosecurity, and the responsible conduct of their research. The U.S. government should engage the research community with the goal of promoting a culture of responsibility, or "citizenship," whereby all participants in the research enterprise have a sense of shared responsibility. Such a culture would incorporate and stress the values of safety, security, and compliance, and work to promote public trust in the scientific enterprise. For GOFROC, a combination of mandated and voluntary oversight and risk mitigation measures would be of great importance.

Recommendation 7. The U.S. government should engage the international community in a dialogue about the oversight and responsible conduct of GOFROC. Life sciences research is a global endeavor that continues to grow as more countries invest in their research capacities and as scientists move and collaborate across national boundaries. Life sciences research enables biomedical breakthroughs, pandemic preparedness, public health response efforts for emerging infectious diseases, and also provides an important economic driver. As more investigators undertake research involving pathogens, however, the associated risks become more likely to have international implications. The risks associated with GOFROC are especially international in nature since laboratory accidents or the deliberate misuse of pathogens with pandemic potential could have global consequences. Laboratories anywhere can undertake GOFROC and publications in the open scientific literature may enable others to generate pathogens with pandemic potential.

NSABB has benefitted greatly from the extensive input into its deliberations by experts representing foreign governments, international organizations, academia, and others during presentations and comments at its meetings and the NAS conferences.

The U.S. government should continue to engage the international community on issues related to dual use research, including policies, oversight mechanisms, science, research conduct, biosafety, biosecurity,

containment, publication, funding, and bioethics. These issues are important in general and, especially, as they are related to GOFROC. The U.S. government's international engagement efforts should seek to promote a global scientific culture of responsibility and enhance the quality, legitimacy and effectiveness of oversight processes.

The U.S. government should build these efforts on the substantial international engagement activities that it and the NSABB have carried out since the NSABB was established. Such efforts have included three international roundtable meetings on dual use research issues, a series of DURC-focused webinars focusing on different global regions, and an international consultative workshop on GOF issues[65]. In addition, the U.S. National Academy of Sciences and the European Academies Science Advisory Council have been engaged in the recent policy debates involving GOF studies and may be well positioned to continue the international dialogue on the issue in coordination with national governments and relevant international organizations. The USG is encouraged to participate in such activities.

[65] Information about these meetings and activities, including agendas, summaries, and archived videocasts, can be found on the NSABB website at: http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/nsabb/nsabb-meetings-and-conferences/international-engagement

7. Appendices

Appendix A. Detailed Description of NSABB Deliberations

NSABB Deliberations

The NSABB established two working groups to accomplish the two portions of its charge, which were to result in discrete work products.

- **Deliverable 1.** A report conveying NSABB's advice on the design, development, and conduct of the risk and benefit assessments.
- **Deliverable 2.** A report conveying NSABB's formal recommendations on the conceptual approach to the evaluation of proposed GOF studies.

DELIVERABLE 1: ADVISING ON THE RISK AND BENEFIT ASSESSMENTS

The first NSABB working group was tasked with advising on the design and conduct of the risk and benefit assessments. The group met between December 2014 and April 2015 and consisted of 13 NSABB voting members as well as non-voting *ex officio* members and other *ad hoc* members from Federal agencies. (Appendix A). The group convened by telephone conference calls and held a one-day in-person meeting.

The working group developed a draft *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*, which was presented to the full NSABB, which was developed further based on input from all Board members, and ultimately approved by the full Board on May 5, 2015. The recommendations in this framework were intended to inform the NIH as it guided the work of Gryphon Scientific in its risk and benefit assessments. The aim of the NSABB's framework was to help generate risk and benefit assessments that would provide information that would allow the NSABB to make sound, evidence-based recommendations.

The NSABB's framework describes: principles that should underpin the risk and benefit assessments; pathogens, pathogen characteristics, and types of GOF experiments and phenotypes that should be examined; the types of risks and benefits that should be analyzed; scenarios, conditions, and events to be examined; and approaches and methods that should be considered when analyzing risks and benefits. In order for the risk and benefit assessments to be grounded in scientific data and evidence, the assessments needed to focus on specific pathogens, experimental manipulations, and scenarios whose risks and benefits could be modeled and analyzed. The NSABB recommended that the risk and benefit assessments focus on studies involving influenza viruses (seasonal strains, as well as high and low pathogenic avian strains) and SARS and MERS coronaviruses. Given that most pandemics are associated with respiratory transmission, pathogens capable of airborne transmission were considered to be of most acute concern. NSABB recognized that the risk and benefit assessments would provide information specific to the pathogens and scenarios that were examined, but intended that the

assessment would generate information that could be more broadly interpreted and applied. Thus, NSABB's recommended approach to the risk and benefit assessments was intended to align with the USG's October 2014 statement, which states that while "gain-of-function studies that fall within the scope of research subject to the funding pause will be a starting point for deliberations, the suitability of other types of gain-of-function studies will be discussed."

DELIVERABLE 2: RECOMMENDATIONS ON A CONCEPTUAL APPROACH FOR EVALUATING PROPOSED GOF STUDIES

The second NSABB working group was tasked with developing draft recommendations on the conceptual approach for the evaluation of proposed GOF studies. The group met beginning in June 2015 and remains active the time of this writing. The working group consists of 18 NSABB voting members as well as non-voting *ex officio* members and other *ad hoc* members from Federal agencies. (Appendix A). The group convened by telephone conference calls and met twice in person.

In addition to the working group's primary task of developing draft recommendations, it continued to provide input on the conduct of the risk and benefit assessments. The working group also received periodic status updates on the risk and benefit assessments from NIH and Gryphon, as well as reports on the commissioned ethics analysis by Dr. Michael Selgelid, examined draft work products, and reported back to the full NSABB.

In developing draft recommendations on a conceptual framework for evaluating proposed GOF studies, the working group structured its deliberations into three phases.

Phase I. Policy examination, research, and information gathering
Phase II. Interpretation, analysis, and synthesis of information and results
Phase III. Development of recommendations

In Phase I the working group sought to 1) identify and examine the information necessary to inform development of recommendations and 2) begin to identify principles that should guide the development of NSABB recommendations. The working group began its deliberations by considering the topic areas discussed at the NSABB meeting in May 2015, which included examination of relevant U.S. and international policy and consideration of broader perspectives such as those from funding agencies, national security experts, journal editors and scientific publishers, ethicists, and others. The working group held an in-person meeting to consult with experts on many of these topics. The working group also examined a number of published GOF studies and discussed how current policies might apply to such studies to provide oversight and risk mitigation.

During Phase II the working group focused on translating information about risks and benefits as well as ethics into decisions and recommendations. It examined how current policies apply to GOF studies and began to develop preliminary observations and findings. The working group discussed the ethical issues

associated with funding and conducting GOF studies, particularly noting the values and ethical decision-frameworks that might be applied to policy decisions about GOF studies. The working group also developed analytic tools to assist it in systematically analyzing the results of the risk and benefit assessments. In November 2015, the working group began receiving briefings from Gryphon Scientific conveying the results of the risk and benefit assessments, as well as reports on ethics from Dr. Selgelid. The group sought to identify GOF studies that might raise particular concerns and may require additional oversight or consideration prior to being funded.

In Phase III, the working group developed its draft recommendations, based on its analysis of the risk and benefit assessments and the ethics report and consideration of all other information and perspectives that were examined.

Deliberations by the Full NSABB

The full NSABB convened times 5 times between October 2014 and January 2016. At these meetings the NSABB working groups provided progress updates and the full Board deliberated the issues further, consulted with various experts, and sought public feedback. Public comments made at NSABB meetings and delivered to the NSABB in writing were carefully considered by the Board during its deliberations. The articles, resources, and stakeholders consulted by the NSABB and its working groups throughout this process are listed in Appendix D.

On November 25, 2014, NSABB voted to approve a statement conveying to the USG concerns it heard regarding the implementation of the funding pause for certain GOF studies.[66] On May 5, 2015, NSABB voted to approve its *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*.[67] This working paper was shared for discussion by the full NSABB on January 7 & 8, 2016.

Role of the National Academies in the Deliberative Process

The National Academies play a critical role in the ongoing deliberative process. The National Research Council and the Institute of Medicine (now National Academy of Medicine) have been asked to convene two forums to engage the life sciences community and to solicit feedback from scientists, the public, and other stakeholders. These forums are to involve discussion of principles important for the design of risk and benefit assessments of GOF research and of NSABB draft recommendations.

[66] Statement of the National Science Advisory Board for Biosecurity Regarding the USG Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses. National Science Advisory Board for Biosecurity, November 25, 2014. http://osp.od.nih.gov/sites/default/files/resources/Final%20NSABB%20Funding%20Pause%20Statement_12-12-14_0.pdf

[67] http://osp.od.nih.gov/sites/default/files/resources/NSABB_Framework_for_Risk_and_Benefit_Assessments_of_GOF_Research-APPROVED.pdf

The first National Academies workshop was held on December 15 & 16, 2014 and focused on the potential risks and benefits associated with GOF studies, ways to assess risks and benefits, strengths and limitations of risk-benefit analyses, and the ethical and policy implications associated with funding and conducting GOF studies that have raised concerns.[68] The discussions at this meeting directly informed the development of NSABB recommendations for conducting the risk and benefit assessments and its subsequent deliberations. In particular, the discussions about the potential risks and benefits associated with GOF studies informed NSABB's recommendations for the types of risks and benefits that should be analyzed by Gryphon Scientific. A common theme at this National Academies meeting was also that the term "gain-of-function" is too broad and that in fact, only a subset of GOF studies truly raise concerns. NSABB applied this insight in its subsequent analysis of the risk and benefit assessments by seeking to identify the subset of GOF studies that raised significant or unique concerns. Finally, the legal and policy discussions that were initiated at this meeting prompted to the NSABB to explore these topics, as well as ethical issues, further.

The second National Academies meeting was held on March 10 & 11, 2016 and included a discussion of the completed risk and benefit assessments and NSABB's preliminary findings and draft recommendations. NSABB's proposed attributes for identifying GOFROC were a major discussion point at this meeting, which resulted in NSABB refining and clarifying these attributes. In addition, there was significant discussion about the desirability of an adaptive policy approach, the need for data to inform policy decisions, and the role that a Federal advisory committee might play in evaluating GOFROC or GOFROC policy. This meeting also had a significant focus on international issues and perspectives, with specific discussion of ongoing and potential future international activities in this area.

The Risk and Benefit Assessments of GOF Studies

NIH commissioned Gryphon Scientific to perform a formal risk and benefit assessments to provide the NSABB with qualitative and quantitative information about the risks and benefits associated with conducting certain GOF studies. Dr. Rocco Casagrande, the principal investigator for the study, presented to the NSABB on May 5, 2015 an overview of Gryphon's approach to conducting the risk and benefit assessments, which included a quantitative biosafety risk assessment, a semi-quantitative biosecurity risk assessment, and a qualitative benefit assessment. Prior to voting to finalize its *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*, NSABB discussed with Dr. Casagrande its draft recommendations and how Gryphon's proposed approach aligned with NSABB's proposed recommendations. In June 2015, Dr. Casagrande presented and discussed a more detailed work plan with the NSABB working group. Over the course of the study, the NSABB working group received occasional progress reports from Gryphon and NIH staff, and were provided draft sections of the risk and benefit assessments. In November 2015 the NSABB working group began

[68] Potential Risks and Benefits of Gain-of-Function Research: Summary of a Workshop. National Research Council and the Institute of Medicine of the National Academies. The National Academies Press, Washington D.C., 2015. www.nap.edu.

receiving the results of the completed risk and benefit assessments. Gryphon's final draft report was posted in advance of the NSABB meeting in January, 2016.[69]

The NIH Office of Science Policy managed the contract with Gryphon Scientific. NIH staff met weekly with Gryphon to accomplish the goals of the Statement of Work and to ensure the recommendations provided in the NSABB's *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research* continued to inform the conduct of the risk and benefit assessments, as appropriate. NIH staff also consulted with NSABB *Ex officio* members to get broader expertise and advice, and to help ensure that the risk and benefit assessments would yield information that would inform subsequent policy deliberations by the U.S. government.

Considering Ethical Issues Associated with GOF Studies

To guide the NSABB's evaluation of the risks and benefits associated with GOF studies and its development of recommendations, the Board sought additional ethical input and analysis. NIH commissioned Dr. Michael Selgelid, Monash University, to examine the literature regarding the ethical issues associated with funding and conducting GOF research and to explore different ethical frameworks that might be utilized when considering how to evaluate the potential risk and benefits associated with GOF studies. Dr. Selgelid was also asked to provide an ethical decision-making framework that NSABB could consider using when analyzing the information provided in the risk and benefit assessments of GOF studies. The decision framework was to identify and consider ethical values that may not be fully captured by a risk-benefit analysis. Dr. Selgelid's analysis was to be accomplished in a neutral, objective manner, without making any definitive recommendations on whether and how to fund or conduct certain GOF studies or what policy course might be the most appropriate. Dr. Selegelid presented his initial work to the NSABB in September 2015 and delivered to the NIH a draft paper in December 2015, which was conveyed to the NSABB working group and posted in advance of the NSABB meeting in January, 2016. [70]

[69] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

[70] Selgelid, Michael. Gain-of-Function Research: Ethical Analysis. December 7, 2015. http://osp.od.nih.gov/sites/default/files/GOF%20%20White%20Paper%20by%20Michael%20Selgelid_0.pdf

Appendix B. Examples of Studies that would and would not be expected to entail GOFROC

Examples of studies that would and would not be expected to entail GOFROC	
Experiment that is anticipated to entail GOFROC and therefore require additional pre-funding review and approval	**Rationale**
An experiment that is anticipated to generate avian influenza viruses that are transmissible by the respiratory route in mammals if the starting virus is highly virulent in humans.	**Attribute 1.** The experiment is anticipated to increase transmissibility by the respiratory route in a relevant experimental mammalian model. Further, altering the host range from birds to mammals could generate a virus to which there is no existing population immunity resulting in a virus capable of wide and potentially uncontrollable spread among humans. **Attribute 2.** Since the starting virus is highly virulent in humans it can be reasonably anticipated that the resulting virus will remain highly virulent in humans.
Reassortant studies involving avian and human influenza virus strains to identify reassortants with pandemic potential that could arise naturally.	**Attribute 1.** Given the starting viruses and the goal of the experiment to identify/select for reassortants that are potentially highly transmissible in mammals, it can be reasonably expected that one or more of the resulting pathogens could be highly transmissible in humans. Since the resulting viruses are reassortants between bird and human influenza viruses, it can be anticipated that the antigenicity of at least some resulting viruses will remain avian-specific such that human populations would not be expected to have been exposed to such a strain or have pre-existing immunity. Therefore resulting in a virus that is capable of wide and uncontrollable spread. **Attribute 2.** Whether or not any of the starting viruses are highly virulent in humans, it can be reasonably anticipated that the expression of novel combinations of gene segments, derived from different influenza strains, in reassortant viruses could result in a range of characteristics that includes high virulence.
Studies that would result in strain of *Yersinia pestis* would be more likely to cause pneumonic forms of infection and would be resistant to antibiotics.	**Attribute 1.** Given that ease of transmission of Yersinia pestis in previous pandemics, manipulations that would enhance its ability to spread by respiratory droplets and cause pneumonic infections would generate a highly transmissible pathogen. In addition, if this manipulation were performed in a strain that was resistant to antibiotics, there would be limited options for controlling the spread of the pathogen among humans. **Attribute 2.** Since the starting agent is highly virulent in humans, particularly when spread through the respiratory route, it can be reasonably anticipated that the resulting agent will remain highly virulent in humans.

NOT anticipated to entail GOFROC and therefore not require additional pre-funding review and approval	**Rationale**
Studies aimed at generating a mouse-adapted MERS-CoV or other emerging human respiratory pathogen	**Not Attribute 1.** The starting virus is transmissible by the respiratory route among humans but is not highly transmissible. MERS-CoV transmission usually occurs as a result of close contact (e.g. providing unprotected care to an infected patient). Sustained community transmission has not been observed. Furthermore, the proposed adaptation to recapitulate human disease symptoms in mice would not be reasonably anticipated to enhance transmissibility thus the resulting virus would not be anticipated to be capable of wide and uncontrollable spread. **Possibly Attribute 2.** The starting virus is already highly virulent in humans and is associated with significant morbidity and mortality. However, it should also be noted that a mouse-adapted strain is likely to be less virulent in humans.
Studies enhancing the growth of seasonal influenza viruses, which may be performed during vaccine production	**Not Attribute 1.** The starting seasonal influenza virus is highly transmissible by the respiratory route in humans however, population immunity is likely to exist against circulating (and recently circulated) strains. Enhancement of growth is unlikely to result in a virus that can evade immunity, thus a virus capable of wide and uncontrollable spread would not be likely. **Possibly attribute 2.** Increasing seasonal virus' ability to replicate could potentially result in its increased ability to cause disease, which could result in highly virulent strains. **Note:** If this experiment were to involve an attenuated strain, as is often the case with vaccine production, it would be unlikely to result in a virus that is highly virulent in humans.
Antigenic drift studies whereby seasonal influenza viruses that are no longer neutralized by vaccine-induced immunity are generated and selected for in the laboratory.	**Not Attribute 1.** The starting seasonal influenza virus is highly transmissible by the respiratory route in humans. However, antigenic drift studies generate influenza viruses with some resistance to a specific immunization but do not change the antigenic character of the virus to a degree such that it would no longer be recognized by the human immune system. Given that the starting virus is a human virus—not one that naturally infects birds or other non-human hosts—there would likely be some pre-existing population immunity to the resulting strains. **Possibly attribute 2.** The experimental manipulation would not be anticipated to increase the virulence of the virus. The resulting strains are likely to exhibit a similar level of virulence as the starting strain. Whether its virulence is considered high or low would depend on the specific initial strain used.

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Appendix C. Summaries of Stakeholder Perspectives

The NSABB consulted a wide range of experts and stakeholder groups including not only scientists and institutions that fund and conduct life sciences research, but a much larger and diverse array of groups including public health officials, medical practitioners, emergency responders, vaccine developers, scientific journals, as well as the general public, non-governmental organizations, individuals with international perspectives and others. To accomplish this, NSABB organized meetings with expert presentations and panels that offered opportunities for interested groups there and for individuals and organizations to express their views and contribute throughout the deliberative process in ways that have informed the NSABB deliberations. These include: several public full NSABB advisory committee meetings that included sessions dedicated to obtaining public comment, two public symposia hosted by the National Academies that obtained comments from the public at the meetings and online, as well as comments submitted to the NIH/OSP and NSABB by email, and discussions with subject matter experts during NSABB WG conference calls and in-person meetings. Also included below are views expressed in some of the articles that have been published on this topic. A complete list of the individuals consulted and articles examined by NSABB are listed in Appendix D. Note that Gryphon Scientific also conducted extensive consultations with experts as part of their risk and benefit assessments. Those experts are not listed here but a listing is available in Gryphon's report. [71]

The following is a synthesis of stakeholder ideas and opinions expressed during the deliberative process. Many of these points were conveyed in more than one venue and by more than one person or group.

Scientists and Others Favoring GOF Research

A variety of influenza and coronavirus researchers who conduct GOF research, and other life sciences researchers have stated that GOF studies are widely used and fundamental for understanding viruses, and therefore are crucial to undertake. This group generally favors conducting such research because it aims to benefit society. In their view, such research can be safely conducted under current oversight frameworks and further restrictions will impede valuable work that will lead to important scientific information about these viruses, leading to better drugs and vaccines, as well as to improving the specificity of surveillance, particularly for influenza. In addition, some GOF studies are viewed as essential, specifically those that alter host range or enhance pathogenicity in order to develop animal models of disease (for example, with SARS-CoV) or GOF studies that generate drug or countermeasure resistance, which are important in satisfying various FDA requirements for marketing approval. Those who support GOF studies also point out that such studies are needed for predicting what amino acid changes are important for human transmission and therefore are important for the selection of candidate vaccine viruses. They also argue that GOF studies are important for prioritizing viruses for risk management (surveillance) and that further work will make these applications more robust. The risks

[71] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

associated with not doing GOF research (generally due to a lack of preparedness for natural public health threats) must also be considered.

While acknowledging there are risks associated with GOF research, proponents believe those risks are manageable and have been overstated by some, as evidenced by the fact that laboratory acquired infections are rare and infections in the community as a result of releases from a laboratory are almost unknown. While risk cannot be zero, the work can be conducted safely and securely with appropriate risk mitigation including containment along with good training and with the implementation of robust occupational medicine programs. Alternatives to GOF do not always provide the full answer to key questions and may yield misinformation. Supporters of GOF studies have also expressed concerns about the effects of the current funding pause and possible additional oversight on the field of virology and young researchers, and feel that there are costs of not undertaking the work in question. A major need is for better definition of what is meant by GOF with a clear distinction between GOF studies and GOF studies of concern. Some have suggested that only viruses with increased transmissibility and pathogenicity represent risks that exceed those of other infectious diseases research. They have also noted that SARS and MERS viruses are different from influenza, and require a different risk assessment approach since they are already virulent human pathogens; GOF research is needed to develop animal models that will benefit development of countermeasures for coronaviruses. Some supporters have acknowledged that there may be some experiments that should not be done. Finally, proponents of GOF research have stated that the risks from naturally occurring influenza viruses, which they argue could be reduced through GOF work, are greater than risks from performing GOF studies.

Scientists and Others Critical of GOF Studies

Opponents and critics of GOF research have generally focused their concern on a subset of GOF studies—those that involve enhancing the pathogenicity and/or transmissibility in mammals (particularly by the respiratory route), which may result in the generation of novel pathogens with pandemic potential. Critics have argued that the generation of novel laboratory pathogens with pandemic potential poses major public health risks and some have argued such studies should not be conducted. They have presented and published calculations that suggest a high probability of global outbreaks of influenza that might kill hundreds of millions of people, as a result of the release from a laboratory of a novel GOF virus. There is some disagreement about these estimates and how likely a pandemic might be, but opponents generally argue that even a relatively low probability of a potentially massive outbreak with major consequences is unacceptable. Some critics of GOF studies have acknowledged that there are a number of GOF studies that can and should be conducted.

Opponents of certain GOF studies have also argued that the benefits of GOF studies have been overstated, or are questionable, and that the benefits generally do not outweigh the biosafety risks. They also question claims about the effectiveness of risk mitigation strategies, since human factors and human error are unavoidable and hard to control, and institutional compliance and competence may vary. Critics have disputed the value of GOF studies to surveillance stating that it is not possible to predict phenotype from genotype; therefore predicting the pandemic risk of newly emergent strains is

not achievable given the current state of knowledge. Also, in their view, controlling outbreaks doesn't require GOF research.

Opponents of GOF research tend to favor alternative types of research that, in their view, can provide the same public health benefits without the large risks. It was suggested that the approach should be on reducing the risk by reducing the hazard, as opposed to focusing on mitigation of the risk. For example, if a universal influenza vaccine was developed, the need for many GOF experiments would be eliminated. Critics want to see funds currently used for GOF work provided to other types of research, which would be a better use of scarce resources in their view. Overall, they view preventing major public health problems as paramount, and see a need to define a critical set of experiments that should not be done, or only be done with additional strong oversight. Opponents are also concerned about proliferation and other factors that may lead to misuse and biosecurity threats. Finally, opponents have pointed out a moral issue if risks and benefits of certain GOF studies are not fairly distributed globally.

Funding Agencies

Public and private funding agencies support GOF research that has raised concerns with the goal of improving public health and well-being. These organizations in the US and abroad are aware of the issues surrounding DURC/GOF studies and are working diligently to implement and comply with existing policies in their countries. Most funders have requirements and procedures in place as they apply policies and guidance to evaluate proposed work and to oversee funded work. Current approaches involve education and awareness campaigns, project risk evaluation, ethics reviews, development of risk mitigation plans, and post-award monitoring. Funders believe they can contribute to the GOF deliberative process as a result of their practical, on-the-ground experience with DURC and GOF. They are concerned that interpreting policy can be very challenging, since it requires considerable expertise and judgment. They would welcome workable policies with clear guidance and have noted some unintended consequences of the funding pause, which affected some GOF projects that had not raised particular concerns. Some foreign government funders view government funding as a poor control mechanisms because this does not cover privately funded research and research funded by other entities. National legislation, regulations, compliance, training, awareness-raising, and self-monitoring have been noted as important.

Biosecurity Experts and Others Concerned about National Security

The ultimate goal of national security professionals, as it pertains to life sciences research, is to protect public health from natural or man-made health threats. Those concerned with national security aim to prevent terrorists and others with malicious intent or misguided motives from using products or information from GOF research to cause harm. This may include deliberate release of pathogens into the community, targeting of researchers or research facilities, or interference with on-going research activities. GOF research represents biosecurity risks in addition to biosafety risks; these overlap but are different with regard to important legal, policy and regulatory issues. Managing biosafety risks may or may not also manage biosecurity risks; GOF policy must take both types of risk into account.

When trying to assess biosecurity threats, security professionals have noted the importance of avoiding assumptions and predictions about the motives and capabilities of those who might be planning biosecurity actions. Those in the security field gather a large variety of data, but often their information is imprecise and may require consideration of what is feasible and plausible. Because of the paucity of biosecurity events, it is very difficult to evaluate and predict the likelihood and consequences of a deliberate release or determine how to prevent and/or mitigate one, and different experts view this issue very differently. It was stated that research policy in itself is not be the appropriate solution to prevent specific biological threats but specific research policies could help raise awareness of security issues among researchers, which would be important.

Security and intelligence professionals have described the challenges associated with using classification as a potential risk mitigation strategy. Classification would effectively restrict access to sensitive research information and research products and would limit the number of laboratories able to perform the studies. This could be described as both a strength and a limitation, depending on one's perspective. Life sciences research that requires classification is typically classified at the outset; the retroactive classification of research that had been conducted in an open, academic setting is exceedingly difficult.

Scientific and Medical Journals

Scientific and medical journals have been at the forefront of the GOF issue. While a number of jounrnals and families of journals have procedures in place for identifying DURC, including GOF and other biosecurity concerns in submitted manuscripts, many journal editors are not entirely comfortable with their role. Their mission is to transmit scientific information, not control it, and they may not have the security expertise or the access to such expertise to make the necessary judgments and decisions about risks associated with communicating certain research findings. Rejection and redaction are the major tools journals have to control dissemination of dual use information, and neither may actually address the concerns; they are also impractical to implement effectively. One suggestion voiced was to require that a description of the steps that were taken during conduct of the research to ensure safety be included in all manuscripts. Some journal editors and staff expressed a desire to get help in evaluating risks and mitigation strategies from an independent national group such as the NSABB and to involve them earlier in the overall process. Most think the publication stage is not the best point to exercise control or prevent misuse of data from GOF studies but realize they are the final gatekeepers. Earlier identification of DURC/GOF along with risk mitigation earlier in the research life cycle would reduce the burden on them. Also, new technology and novel publication venues make controlling information increasingly difficult, and, as noted above, not all journals are able to or choose to impose a rigorous review of manuscripts.

Countermeasure Developers

Companies and others that are attempting to develop vaccines and drugs against pathogens were represented in several discussions. Medical countermeasure (MCM) developers expressed quite divergent views and opinions. Those favoring GOF research argued that such work is absolutely

necessary for antiviral drug development because GOF experiments to select for drug resistant mutants as well as to develop animal models are part of the critical path to marketing approval. In their view, GOF studies also have had a major influence on developing influenza vaccines, both seasonal and pandemic, and are likely to result in improved ways to make even better vaccines in the future. GOF experiments are required for selection of strains with better growth properties, with key mutations that alter important phenotypes needed in the vaccine strain, and with incorporating characteristics of strains that are likely to emerge into proven backbones. It was noted that GOF studies that enhance virulence can help inform vaccine designers about which mutations to avoid incorporating into vaccine strains. This group is concerned that their efforts to improve public health may be limited or impeded by new policies and urge careful consideration of their needs as decisions are made.

Conversely, other MCM developers expressed the view that vaccine production now is little dependent on GOF research and that any possible benefits will be far into the future, although some feel long-term potential is there. Those who criticize GOF studies on these grounds have argued that vaccines are developed in response to strains that emerge as threats, rather than preemptively based on strains that might be predicted as threats. Rather than supporting GOF studies to enhance vaccine production and drug development, it has been suggested that the other constraints that impede MCM development be addressed, such as streamlining FDA approval procedures and improving manufacturing processes, which would have a much greater impact. These critics suggest limiting current GOF-related efforts and focusing attention and resources in other directions. Overall, they believe that impact of GOF research on vaccine and drug development has been overstated, and that the benefits articulated are more theoretical than practical.

The General Public and Organizations Representing their Views.

A number of stakeholders stressed the importance of having meaningful public engagement with input and participation as part of the deliberative process. It is important that communities that might be affected by accidents or the misuse of research have a say in the research that is being conducted, however, but this may not generally be the case in their view. Real transparency, with the public good as the foremost consideration, must be part of a truly independent decision-making process. They note that it is important to maintain public trust in the scientific enterprise by involving non-scientists at stages when their views can still have an impact on policy-making. Public opinion of science is harmed when decisions that influence public health and safety are made without such input or the input has no real impact. Conversely, effective community engagement can convert sceptics to supporters. More than one participant raised the concern that if risks and benefits are not equitably distributed, it is a serious ethical issue[72].

Other issues that were mentioned include: how harms will be compensated if a laboratory incident were to affect the surrounding community; the need for enough resources to conduct research safely; and the opportunity to learn from other industries such as nuclear industry.

[72] The ethical issues are discussed in more depth elsewhere, notably, Dr. Michael Selgelid's ethical analysis and the section of this report on Ethical Values and Decision-Making Frameworks.

Research Institutions

Representatives of universities and other research institutions generally noted that there is already significant oversight of DURC and GOF at both the Federal and institutional levels. Biosafety professionals noted that potentially high risk projects would receive thorough scientific review and risk assessment, resulting in the development of risk mitigation plans, and on-going monitoring as a result of policies and requirements that are already in place. They cited concerns over any increase in compliance that would impose burdens on their already-limited resources or impede researchers from doing valuable work. They have difficulty, at times, deciding what is DURC when reviewing specific projects and would welcome more specificity and guidance. Many emphasized the need for policies that are unambiguous and straightforward to implement.

Public Health Officials

Public health officials have expressed diverse opinions. Some believe that GOF research has and can continue to improve surveillance efforts, as well as vaccine and therapeutic development. Others expressed concerns that an accident involving a laboratory pathogen for which there are no countermeasures would be very concerning and difficult to respond to. At the local level it is important to have public health involvement in the decision-making process because they will be incident responders. Strong connections with state and local laboratories should be established for sharing information and might include involving them in the review process. It was also noted that GOF and related policies may impact sample sharing and impede international relations relating to public health efforts.

International Perspectives

A number of participants noted that there is much interest in the GOF/DURC issue internationally, and the international community is looking to see what the USG will do as a result of the deliberative process. It was noted that U.S. policy often influences policies globally and the international ramifications should be considered. Recent biosafety incidents in U.S. Federal labs have raised concerns among many in other countries about the ability of the U.S. to adequately manage risks. A number of countries have well-developed systems of policy and regulation that would address many or some GOF and DURC issues, though international policy approaches are generally somewhat different from those in the U.S. International experiences, activities, and perspectives were cited as important to consider in the deliberative process. A collaborative approach and active attempts to engage the international community was viewed as the most effective way to benefit all. Many favored launching an international dialogue soon, with development of broad concepts and points of agreement that could be shared by all, while still respecting national differences. In addition, it was suggested that academies of science and multi-national organizations such as WHO can play an important role in such interactions at the right time. Those with a particular interest in the international aspects of GOF research also cited ethical issues associated with the unequal distribution of risks and benefits across rich and poor

countries. It was noted that the European Commission uses a comprehensive ethics process for screening and monitoring DURC/GOF in research projects.[73]

Those with an Interest in the Deliberative Process Itself

A broad group of individuals offered comments on the deliberative process itself. This included: federal government personnel, ethicists, decision-making experts, policy experts, other scientists, and includes people who are also members of the previously-mentioned groups. Those concerned with the deliberative process generally called for a well-planned and executed, thorough, scientifically rigorous, and impartial RBA that is technically sound and socially acceptable. They favored a democratic deliberative process and a policy that incorporates decisions made by neutral parties. Policy should be created using risk-based and value-based approaches to achieve desired outcomes. They want the final policy resulting from the deliberative process to be capable of reasonably identifying and mitigating risks related to GOF while protecting scientific autonomy, research progress, discovery and innovation, public health, national security, and other critical interests.

Many see an adaptive process as desirable, and recommend collecting appropriate data about laboratory accidents and mitigation effectiveness. It was noted that risks and benefits will change as science advances. The funding decision-making process should be accountable and limit inherent conflicts of interest; the individuals or entities that make decisions is critical. Most favor using existing policies as the basis of policy for GOF, while acknowledging that current frameworks are not entirely adequate. The question of how to incorporate non-USG funded research into an acceptable framework was raised several times. Deciding how to decide is a key point.

Both proponents and critics of GOF studies criticized the term "gain-of-function" as being too broad and not descriptive enough. There was much discussion about the appropriate definition of GOF research of concern; many strong, often conflicting, views were expressed. Unfortunately while it is important to have a working definition and criteria for what is GOF of concern as opposed to GOF, a binary distinction needed for deciding what requires extra scrutiny, GOF experiments are actually a continuum of increasing risk.

The funding pause was criticized for being too broad, and some described it as disruptive to scientific process. Finally, some feel that a definitive quantitative risk assessment is not possible because of the very large uncertainties and lack of critical information associated with doing such studies, and they question the value of any studies that are done.

[73] The EU Framework Programme for Research and Innovation, Horizon 2020. How to complete your ethics self-assessment, version 1.0, 11 July 2014. http://ec.europa.eu/research/participants/data/ref/h2020/call_ptef/pt/h2020-call-pt-ria-ia_en.pdf#page=27

Appendix D. Consultations, Comments, and Sources Consulted During NSABB Deliberations

NOTE: We are breaking this into two tables. One table will list all of the invited speakers who were consulted at WG, NSABB, and NAS meeting. The second will list all of the individuals and organizations that submitted public comments or made comments during a public comment session.

Table 1. Experts consulted by NSABB or the NSABB working groups. Individuals listed here addressed the NSABB or NSABB working group in their individual or professional capacities. Members of the NSABB or an NSABB working group are listed if they presented as a subject matter expert on a specific topic.

Speaker/Commenter	Affiliation/Location	Venue
Regine Aalders, M.Sc.	Embassy of the Netherlands, Washington, D.C.	NSABB Full Board Meeting (January 7-8, 2016), Public Comment
Richard Adams		Public Comment
Nisreen AL-Hmoud, Ph.D, M.Phil.	Royal Scientific Society of Jordan	National Academies Workshop (March 10-11, 2016)
Ronald Atlas, Ph.D.	University of Louisville	National Academies Workshop (December 15, 2014)
Ralph Baric, Ph.D.	University of North Carolina	National Academies Workshop (December 15, 2014), Public Comment
Kavita Berger, Ph.D.	Gryphon Scientific	NSABB Full Board Meeting (September 28, 2015), In-person WG Meeting (November 9, 2015)
Kenneth W. Bernard, M.D.	US Public Health Service (ret.)	Public Comment
Thomas Briese, Ph.D.	Columbia University	National Academies Workshop (December 15, 2014)
Michael Callahan, M.D., D.T.M.&H., M.S.P.H.	Massachusetts General Hospital and Harvard Medical School	National Academies Workshop (March 10-11, 2016)
Arturo Casadevall, M.D., Ph.D.	Albert Einstein College of Medicine; mBio	NSABB Full Board Meeting (October 22, 2014), In-person WG Meeting (July 23, 2015), Public Comment
Rocco Casagrande, Ph.D.	Gryphon Scientific	NSABB Full Board Meetings (September 28, 2015 and January 7-8, 2016), In-person WG Meeting (November 9, 2015), National Academies Workshop (March 10-11, 2016)
R. Alta Charo, J.D.	University of Wisconsin–Madison	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016)
Susan Coller-Monarez, Ph.D.	Office of Science and Technology Policy	In-person WG Meeting (July 23, 2015)
Louis (Tony) Cox, Ph.D., S.M.	Cox Associates	National Academies Workshop (March 10-11, 2016)
Derrin Culp	White Plains, New York	Public Comment
Mark Denison, M.D.	Vanderbilt University	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016), Public Comment
Dennis Dixon, Ph.D.	HHS/National Institutes of Health	NSABB Full Board Meeting (November 25, 2014)

Marianne Donker, Ph.D.	Ministry of Health, Welfare and Sport, Netherlands	In-person WG Meeting (July 23, 2015)
Philip Dormitzer, M.D., Ph.D.	Novartis Vaccines	National Academies Workshop (December 15, 2014)
Ruxandra Draghia-Akli, M.D., Ph.D.	European Commission	In-person WG Meeting (July 23, 2015), National Academies Workshop (March 10-11, 2016)
Rebecca Dresser, J.D.	Washington University in St. Louis	NSABB Full Board Meeting (September 28, 2015)
Paul Duprex, Ph.D.	Boston University, NEIDL Institute	NSABB Full Board Meeting (October 22, 2015)
Gerald Epstein, Ph.D.	Department of Homeland Security	In-person WG Meeting (July 23, 2015)
Stephen Eubank, Ph.D.	Virginia Polytechnic Institute and State University	NSABB Full Board Meetings (October 22, 2014 and January 7-8, 2016), Public Comment
Nicholas Evans, Ph.D.	University of Pennsylvania	Public Comment
David S. Fedson, M.D.	Sergy Haut, France	Public Comment
Scott Ferson, Ph.D.	Applied Biomathematics	NSABB Full Board Meeting (October 22, 2014), Public Comment
David Fidler, J.D., M.Phil.	Indiana University, Bloomington	NSABB Full Board Meeting (January 7-8, 2016)
Harvey Fineberg M.D, Ph.D.	University of California, San Francisco	National Academies Workshops (December 15, 2014 and March 10-11, 2016)
Adam Finkel, Sc.D., M.P.P.	University of Pennsylvania Law School	National Academies Workshops (March 10-11, 2016)
Baruch Fischhoff, Ph.D.	Carnegie Mellon University	NSABB Full Board Meeting (October 22, 2014); National Academies Workshop (December 15, 2014)
Robert Fisher, Ph.D.	U.S. Food and Drug Administration	National Academies Workshop (March 10-11, 2016)
Ron Fouchier, Ph.D.	Erasmus Medical Center	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016), Public Comment
Gregory Frank, Ph.D.	Infectious Diseases Society of America	Public Comment
David Franz, D.V.M., Ph.D.	Former Commander, United States Army Medical Research Institute for Infectious Diseases	In-person WG Meeting (July 23, 2015)
Christophe Fraser, Ph.D.	Imperial College	National Academies Workshop (December 15, 2014)
Matt Frieman, Ph.D.	University of Maryland	Public Comment
Richard Frothingham	Duke University	National Academies Workshop (March 10-11, 2016)
Keiji Fukuda, M.D., M.P.H.	World Health Organization	National Academies Workshop (March 10-11, 2016)
George F. Gao, D.V.M., D.Phil.	Chinese Academy of Sciences; Chinese Center for Disease Control and Prevention	National Academies Workshop (March 10-11, 2016)
Gigi Kwik Gronvall, Ph.D.	University of Pittsburgh Medical Center (UPMC) Center for Health Security	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016)
Charles Haas, Ph.D.	Drexel University	National Academies Workshop (December 15, 2014)
Peter Hale	Foundation for Vaccine Research	Public Comment
Elizabeth Hart	Adelaide, South Australia	Public Comment

Andrew M. Hebbeler, Ph.D.	White House Office of Science and Technology Policy	NSABB Full Board Meeting (October 22, 2014), National Academies Workshop (December 15, 2014)
Denise Hein		Public Comment
Ruthanne Huising, Ph.D., M.Sc.	McGill University	National Academies Workshop (March 10-11, 2016)
Gavin Huntley-Fenner, Ph.D.	Huntley-Fenner Advisors	National Academies Workshops (December 15, 2014 and March 10-11, 2016)
Jo Husbands, Ph.D.	Board on Life Sciences of the US National Academy of Sciences	In-person WG Meeting (July 23, 2015), NSABB Full Board Meeting (January 7-8, 2016)
Michael Imperiale, Ph.D.	University of Michigan	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016), Public Comment
Thomas Inglesby, M.D.	University of Pittsburgh	NSABB Full Board Meeting (October 22, 2014 and January 7-8, 2016), Public Comment
Barbara Jasny, Ph.D.	Science	In-person WG Meeting (July 23, 2015), NSABB Full Board Meeting (January 7-8, 2016), Public Comment
Daniel Jernigan, M.D., M.P.H.	Centers for Disease Control and Prevention	NSABB Full Board Meeting (January 7-8, 2016)
Barbara Johnson, Ph.D., R.B.P.	Biosafety Biosecurity International	National Academies Workshop (December 15, 2014)
John Kadvany, Ph.D.	Independent consultant on decision science	Full Board Meeting (January 7-8, 2016)
Joseph Kanabrocki, Ph.D., C.B.S.P.	University of Chicago	In-person WG Meeting (January 22, 2015), In-person WG Meeting (July 23, 2015)
Isidoros Karatzas, Ph.D.	European Commission	WG Meeting (February 16, 2016)
Yoshihiro Kawaoka, D.V.M., Ph.D.	University of Wisconsin, Madison	NSABB Full Board Meetings (October 22, 2014 and January 7-8, 2016), National Academies Workshop (December 15, 2014), Public Comment
George Kemble, Ph.D.	3-V Biosciences	National Academies Workshop (December 15, 2014)
Lawrence Kerr, Ph.D.	National Security Council Staff	WG Meeting (November 5, 2015), National Academies Workshop (March 10-11, 2016)
Andy Kilianski, Ph.D.	National Research Council Fellow at US Army	Public Comment
Lynn Klotz, Ph.D.	Center for Arms Control and Non-proliferation	Public Comment
Gregory Koblentz, Ph.D., M.P.P.	George Mason University	National Academies Workshop (December 15, 2014)
Todd Kuiken, Ph.D.	The Wilson Center	In-person Meeting (July 23, 2015)
Robert Lamb, Ph.D., Sc.D.	Northwestern University; Howard Hughes Medical Institute	National Academies Workshop (December 15, 2014)
Linda Lambert, Ph.D.	HHS/National Institutes of Health	In-person WG Meeting (July 23, 2015)
Gabriel Leung, M.D., M.P.H.	University of Hong Kong	National Academies Workshop (March 10-11, 2016)
Carol Linden, Ph.D.	HHS/Biomedical Advanced Research and Development Authority	National Academies Workshop (December 15, 2014)
W. Ian Lipkin, M.D.	Columbia University	NSABB Full Board Meeting (October 22, 2014)
Marc Lipsitch, Ph.D.	Harvard School of Public Health	NSABB Full Board Meetings (October 22, 2014 and January 7-8, 2016), National Academies Workshop (December 15, 2014), Public Comment

Patricia Long, J.D., LL.M.	HHS/Office of Security and Strategic Information	In-person WG Meeting (July 24, 2015)
Nicole Lurie, M.D., M.S.P.H.	HHS/Assistant Secretary for Preparedness and Response	NSABB Full Board Meeting (October 22, 2014); In-person WG Meeting (July 23, 2015)
Eric Meslin, Ph.D.	Indiana University School of Medicine	NSABB Full Board Meeting (September 28, 2015)
Corey Meyer, Ph.D.	Gryphon Scientific	NSABB Full Board Meeting (September 28, 2015), In-person WG Meeting (November 9, 2015)
Jonathan Moreno, Ph.D.	University of Pennsylvania	NSABB Full Board Meeting (January 7-8, 2016), National Academies Workshop (March 10-11, 2016)
Kara Morgan, Ph.D., M.S.E.S.	Battelle	National Academies Workshop (March 10-11, 2016)
Rebecca Moritz, M.S., C.B.S.P., S.M.(NRCM)	University of Wisconsin–Madison	National Academies Workshop (December 15, 2014)
Peter Murakami	Baltimore, Maryland	Public Comment
Kalyani Narasimhan, Ph.D.	Nature Publishing Group	In-person WG Meeting (July 23, 2015)
Daniel O'Connell	Albany, Oregon	Public Comment
Kimberly Orr, Ph.D.	US Department of Commerce	In-person WG Meeting (July 23, 2015)
Michael Osterholm, Ph.D., M.P.H.	University of Minnesota	NSABB Full Board Meeting (October 22, 2015)
Kenneth Oye, Ph.D.	Massachusetts Institute of Technology	In-person WG Meeting (July 23, 2015)
Megan Palmer, Ph.D.	Center for International Security and Cooperation, Stanford University	Public Comment
Christopher Park	U.S. Department of State	In-person WG Meeting (July 23, 2015)
Jean Patterson, Ph.D.	Texas Biomedical Research institute	In-person WG Meeting (January 22, 2015)
Daniel Perez, Ph.D.	University of Maryland	NSABB Full Board Meeting (October 22, 2014)
Janet Peterson, C.B.S.P.	University of Maryland	NSABB Full Board Meeting (October 22, 2014)
Dustin Phillips	Louisville, Kentucky	Public Comment
Stanley Plotkin, M.D.	University of Pennsylvania	Public Comment
Philip Potter, Ph.D.	St. Jude Children's Research Hospital	NSABB Full Board Meeting (January 7-8, 2016), National Academies Workshop (March 10-11, 2016)
David Relman, M.D.	Stanford University	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016)
David B. Resnik, J.D., Ph.D.	HHS/National Institutes of Health	NSABB Full Board Meeting (October 22, 2014)
George Rudy	Frederick County & City Containment Laboratory Community Advisory Committee	Public Comment
Colin Russell, Ph.D.	University of Cambridge	National Academies Workshop (December 15, 2014)
Steven L. Salzberg, Ph.D.	Johns Hopkins University School of Medicine	Public Comment

Monica Schoch-Spana, Ph.D.	University of Pittsburgh Medical Center (UPMC) Center for Health Security	National Academies Workshops (December 15, 2014 and March 10-11, 2016)
Stacey Schultz-Cherry, Ph.D.	St. Jude Children's Research Hospital	NSABB Full Board Meeting (October 22, 2014), National Academies Workshop (December 15, 2014)
Shannon Scott		Public Comment
Michael Selgelid, Ph.D.	Monash University	NSABB Full Board Meetings (September 28, 2015 and January 7-8, 2016), National Academies Workshop (March 10-11, 2016), Public Comment
Billie Sellers		Public Comment
Ethan Settembre, Ph.D.	Seqirus	National Academies Workshop (March 10-11, 2016)
Richard Sever, Ph.D.	Cold Spring Harbor Laboratories Press bioRxiv	In-person WG Meeting (July 23, 2015)
Michael Shaw, Ph.D.	Centers for Disease Control and Prevention	In-person WG Meeting (July 23, 2015)
Bill Sheridan, M.B., B.S.	BioCryst Pharmaceuticals Inc.	NSABB Full Board Meeting (October 22, 2014)
Lone Simonsen, Ph.D.	George Washington University	Public Comment
Andrew Snyder-Beattie	Future of Humanity Institute, University of Oxford	Public Comment
Charles Stack, M.P.H.	University of Illinois at Chicago	Public Comment
John Steel, Ph.D.	Emory University	Public Comment
Kanta Subbarao, M.B.B.S., M.P.H.	HHS/National Institutes of Health	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016), Public Comment
Jill Taylor, Ph.D.	Wadsworth Center, NYS Department of Public Health	NSABB Full Board Meeting (January 7-8, 2016), Public Comment
Robert Temple, M.D.	Food and Drug Administration	In-person WG Meeting (July 23, 2015)
Volker ter Meulen, M.D., Ph.D.	European Academies Science Advisory Council	National Academies Workshop (March 10-11, 2016)
Eileen Thacker, D.V.M., Ph.D., D.A.C.V.M.	Department of Agriculture	In-person WG Meeting (July 23, 2015)
Silja Vöneky, (credentials)	University of Freiburg and German Ethics Council	National Academies Workshop (March 10-11, 2016)
Kimball Ward		Public Comment
Robert Webster, Ph.D.	St. Jude Children's Research Hospital	National Academies Workshop (December 15, 2014)
Jerry Weir, Ph.D.	Food and Drug Administration	National Academies Workshop (December 15, 2014)
Robbin Weyant, Ph.D., R.B.P. (ABSA)	Center for Disease Control and Prevention	National Academies Workshop (December 15, 2014), In-person WG Meeting (July 23,2015)
Gary Whittaker, Ph.D.	Cornell University	Public Comment
Beth Willis	Co-founder, Frederick Citizens for Bio-lab Safety	NSABB Full Board Meeting (January 7-8, 2016)
Carrie Wolinetz, Ph.D.	HHS/National Institutes of Health	NSABB Full Board Meetings (May 5, 2015 and January 7-8, 2016)
American Association of Immunologists (AAI)	American Association of Immunologists	Public Comment
Infectious Diseases Society of America (IDSA)	Infectious Diseases Society of America	Public Comment

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Table 2. Sources consulted by NSABB and NSABB working groups include but are not limited to the following

NOTE: This table is being reformatted to list full citations and links where possible

Authors	Title
Baek, Y.H., et al., 2015	Profiling and Characterization of Influenza Virus N1 Strains Potentially Resistant to Multiple Neuraminidase Inhibitors
Boddie, C., et al., 2015	Assessing the bioweapons threat
Cambridge Working Group, 2014	Cambridge Working Group statement (July 2014)
Casadevall, A., and Imperiale, M.J., 2014	Risks and benefits of gain-of-function experiments with pathogens of pandemic potential, such as influenza virus: A call for a science-based discussion
Casadevall, A., et al., 2014	An epistemological perspective on the value of gain-of-function experiments involving pathogens with pandemic potential
Doshi, P., 2008	Trends in Recorded Influenza Mortality - United States 1900–2004
Duprex, P., and Casadevall, A., 2014	Falling down the Rabbit Hole: aTRIP Toward Lexiconic Precision in the "Gain-of-Function" Debate
Environmental Protection Agency Science Policy Council, 2000	Risk Characterization - EPA Science Policy Council Handbook
European Academies Science Advisory Council, 2015	Gain of function: experimental applications relating to potentially pandemic pathogens
European Center for Disease Prevention and Control, 2012	Risk Assessment: Laboratory-created A(H5N1) viruses transmissible between ferrets
European Commission, 2015	Guidance — How to complete your ethics self-assessment (ver. 4.01)
European Commission	Exploratory Guidance note — Research involving dual-use items
European Commission	Exploratory Guidance note — Research with an exclusive focus on civil applications
European Commission	Exploratory Guidance note — Potential misuse of research
Evans, N.G., 2013.	Great expectations - Ethics, avian flu and the value of progress
Evans, N.G., et al., 2015	The ethics of biosafety considerations in gain-of-function research resulting in the creation of potential pandemic pathogens
Fedson, D.S., and Opal, S.M., 2013	The controversy over H5N1 transmissibility research
Fedson, D.S., 2013	How Will Physicians Respond to the Next Influenza Pandemic?
Fouchier, R., et al., 2012	Preventing Pandemics - The fight over flu
German Ethics Council, 2014	Biosecurity — Freedom and Responsibility of Research

Gronvall, G., 2013	H5N1: A case study for dual-use research
Gronvall, G., and Rozo, M., 2015	A Synopsis of Biological Safety and Security Arrangements
Guthrie, S., et al., 2013	Measuring Research - A guide to research evaluation frameworks and tools
Herfst, S., et al., 2012	Airborne transmission of influenza A/H5N1 virus between ferrets
Imai, M., et al., 2012	Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to reassortant H5 HA/H1N1 virus in ferrets
Imperiale, M.J., and Casadevall, A., 2015	A New Synthesis for Dual Use Research of Concern
Inglesby, T.V., and Relman, D.A., 2015	How likely is it that biological agents will be used deliberately to cause widespread harm?
Jaffe, H., et al., 2013	Extra oversight for H7N9 experiments
Kilianski, A., et al., 2015	Gain-of-Function Research and the Relevance to Clinical Practice
Kilianski, A., and Murch, R.S., 2015	When gain-of-function research is not "gain-of-function" research
Linster, M., et al., 2014	Identification, characterization, and natural selection of mutations driving airborne transmission of A/H5N1 virus
Lipsitch, M., and Bloom, B.R., 2012	Rethinking Biosafety in research on potential pandemic pathogens
Lipsitch, M., and Galvani, A., 2014	Ethical alternatives to experiments with novel potential pandemic pathogens
Lipsitch, M., and Relman, D.A., 2015	New Game, New Rules - Limiting the Risks of Biological Engineering
Lipsitch, M., et al., 2016	Six policy options for conducting gain-of-function research
Maines, T.R., et al., 2011	Effect of receptor binding domain mutations on receptor binding and transmissibility of avian influenza H5N1 viruses
Miller, M., and Palese, P., 2014	Peering into the crystal ball: Influenza pandemics and vaccine efficacy
National Research Council/Institute of Medicine, 2015	Potential Risks and Benefits of GOF Research – NRC/IOM Workshop Summary (Full Report)
Nature Editorial, 2014	A ripe time for gaining ground
NIH Blue Ribbon Panel Slide Presentation, 2008	Blue Ribbon Panel Scientific Subcommittee Teleconference slide presentation (May 2008)
Osterholm, M., and Relman, D., 2012	Creating mammalian-transmissible A/H5N1 influenza virus: Social contracts, prudence, and alternative perspectives
Palmer, M.J., et al., 2015	A more systematic approach to biological risk
Pascua, P.N., et al., 2012	Virulence and transmissibility of H1N2 influenza virus in ferrets imply the continuing threat of triple-reassortant swine viruses
Patterson, A., et al., 2013	A framework for decisions about research with HPAI H5N1 viruses
Patterson, A., et al., 2014	Biocontainment laboratory risk assessment: perspectives and considerations

Peng, X., et al., 2016	Amino acid substitutions occurring during adaptation of an emergent H5N6 avian influenza virus to mammals
Presidential Commission for the Study of Bioethical Issues, 2010	New Directions: The Ethics of Synthetic Biology and Emerging Technologies
Richard, M. et al., 2013	Limited airborne transmission of H7N9 influenza A virus between ferrets
Roberts, A., et al., 2007	A Mouse-Adapted SARS-Coronavirus Causes Disease and Mortality in BALB/c Mice
Rozell, D.J., 2015	Assessing and Managing the Risks of Potential Pandemic Pathogen Research
Rozo, M., and Gronvall, G., 2015	The Reemergent 1977 H1N1 Strain and the Gain-of-Function Debate
Russell, C., et al., 2012	The potential for respiratory droplet-transmissible A/H5N1 influenza virus to evolve in a mammalian host
Russell, C., et al., 2014	Improving pandemic influenza risk assessment
Schultz-Cherry, S., et al., 2014	Influenza Gain-of-Function Experiments: Their Role in Vaccine Virus Recommendation and Pandemic Preparedness
Scientific Management Review Board Report, 2014	Approaches to Assess the Value of NIH-Supported Research
Scientists for Science, 2014	Scientists for Science statement (July 2014)
Stern, P.C., and Fineberg, H.V., 1996	Understanding Risk - Informing Decisions in a Democratic Society
Sullivan, M., et al., 2013 (RMS White Paper)	Influenza Pandemic Risk - The Contribution of Laboratory Pathogens to Excess Mortality Risk
Sutton, T., et al., 2014	Airborne transmission of highly pathogenic H7N1 influenza virus in ferrets
Taubenberger, J., et al., 2012	Reconstruction on the 1918 influenza virus: Unexpected rewards from the past
Tharakaraman, K., et al., 2014	Structural determinants for naturally evolving H5N1 hemagglutinin to switch its receptor specificity
Trevan, T., 2015	Rethink Biosafety
Trock, S., et al., 2015	Development of Framework for Assessing Influenza Virus Pandemic Risk
USG (June 2013)	Biological Safety Guidance for Research with Risk Group 3 Influenza Viruses - Human H2N2, 1918 H1N1, and HPAI H5N1
USG (December 2009)	Biosafety in Microbiological and Biomedical Laboratories BMBL (5th Edition)
USG (September 2014)	Companion Guide to the USG Policies for Oversight of Life Sciences Dual Use Research of Concern
USG (February 2005)	Environmental Impact Statement For the Galveston National Laboratory for Biodefense and Emerging Infectious Diseases
USG (as of July 2015)	Federal Select Agents and Toxins List
USG (July 2012)	Final Supplementary Risk Assessment for the Boston University National Emerging Infectious Diseases Laboratories (NEIDL)
USG (February 2016)	France-US Bilateral Workshop on Dual Use Research Issues: Summary Report

USG (August 2013)	HHS Funding Framework for HPAI H5N1 Studies
USG (February 2013)	NIH Guidelines for Research Involving Recombinant DNA Molecules - Amendment Notice. February 21, 2013
USG (November 2013)	NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
USG (October 2014)	USG Gain-of-function GOF Deliberative Process and Funding Pause Statement
USG (September 2014)	USG Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern
USG (March 2012)	USG Policy for Oversight of Life Sciences Dual Use Research of Concern
United Nations (April 1972)	Biological Weapons Convention
Volkswagen Foundation and Max Plank Society, 2014	Dual Use Research on Microbes - Biosafety, Biosecurity, Responsibility - Hanover Symposium Summary Report
Watanabe, T., et al., 2014	Circulating Avian Influenza Viruses closely related to the 1918 virus have pandemic potential
Zhang, Y., et al., 2013	H5N1 hybrid viruses bearing 2009/H1N1 virus genes transmit in guinea pigs by respiratory droplet

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Appendix E. National Science Advisory Board for Biosecurity Roster

National Science Advisory Board for Biosecurity Roster

[Ŧ] NSABB Working Group Co-chair
[†] NSABB Working Group on the Design and Conduct of Risk and Benefit Assessments of Gain-of-Function Studies
[‡] NSABB Working Group on Evaluating the Risks and Benefits of Gain-of-Function Studies
[*] NSABB Member, Retired

NOTE: Please send any updates to your titles/affiliations.

NSABB Voting Members

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Office of the President
Stony Brook University

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Distinguished Professor
Dept. of Molecular Genetics & Microbiology
Genetics Institute
College of Medicine
University of Florida

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Eberly Chair in Biochemistry and Molecular Biology
The Pennsylvania State University

Andrew (Drew) Endy, Ph.D.[†‡]
Assistant Professor
Stanford Bioengineering
Stanford University

J. Patrick Fitch, Ph.D.[†]
Laboratory Director
National Biodefense Analysis & Countermeasures Center
President, Battelle National Biodefense Institute, LLC

Christine M. Grant, J.D.[†‡]
CEO/Founder
InfecDetect Rapid Diagnostic Tests, LLC

Marie-Louise Hammarskjöld, M.D., Ph.D.[†‡]
Charles H. Ross Jr. Professor
and Professor of Microbiology, Immunology and Cancer Biology,
Associate Director of the Myles H. Thaler Center
University of Virginia School of Medicine

Clifford W. Houston, Ph.D.[‡]
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Herman Barnett Distinguished Professorship in Microbiology and Immunology
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Joseph Kanabrocki, Ph.D., NRCM(SM) [Ŧ†‡]
Associate Vice President for Research Safety
Professor of Microbiology
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Theresa M. Koehler, Ph.D.[‡]
Chair, Department of Microbiology and Molecular Genetics
Herbert L. and Margaret W. DuPont Distinguished Professor in Biomedical Science
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Marcelle C. Layton, M.D.[‡]
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Jan Leach, Ph.D.
University Distinguished Professor
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James W. LeDuc, Ph.D.[‡]
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Margie D. Lee, D.V.M., Ph.D.[†‡]
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College of Veterinary Medicine
The University of Georgia

Francis L. Macrina, Ph.D.[‡]
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Joseph E. McDade, Ph.D.[†‡]
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Texas Biomedical Research Institute

I. Gary Resnick, Ph.D.[†‡]
President, IGR Consulting
Guest Scientist
Global Security Directorate
Los Alamos National Laboratory

Susan M. Wolf, J.D.[†‡]
McKnight Presidential Professor of Law,
Medicine & Public Policy
Faegre Baker Daniels Professor of Law
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DELIBERATIVE DRAFT

~~Policy~~ Recommendations for the Evaluation and Oversight of Proposed Gain-of-Function Research

A Draft Report of the NSABB Working Group ~~on Evaluating the Potential Risks and Benefits of Gain-of-Function Studies~~

Version: April 2~~5~~9, 2016

Preface for NSABB Meeting on May 24, 2016

This draft report was developed by the NSABB working group tasked with evaluating the risks and benefits associated with gain-of-function studies and developing draft recommendations on a conceptual approach for the evaluation of proposed gain-of-function studies. The first version of this document was discussed at the NSABB meeting on January 7 & 8, 2016 and again at the symposium hosted by the National Academies on March 10 & 11, 2016. This version represents an updated draft of that initial working paper. This document is still pre-decisional and intended as a deliberative document to be discussed at the meeting of the full NSABB on May 24, 2016. This is document is not a formal NSABB work product and should not be considered to be official NSABB findings or recommendations to the U.S. government. This document does not represent official policy of the U.S. government.

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Executive Summary

~~NOTE: Executive Summary will be updated to reflect any changes in the rest of the document.~~

Research involving pathogens is essential to global health and security. Such research provides insight into the fundamental nature of human-pathogen interactions, enables the assessment of the pandemic potential of emerging infectious agents, and informs public health and preparedness efforts, including the development of medical countermeasures. Several policies are in place to help ensure that pathogen research is conducted safely and in ways to minimize the risks of laboratory accidents and security risks. Recently, and in the wake of a number of biosafety incidents at Federal facilities, concerns have been raised about certain "gain-of-function" (GOF) studies with the potential to generate pathogens with ~~enhanced pathogenicity or transmissibility in mammals~~pandemic potential. The concerns center on whether a pathogen with enhanced ~~characteristics~~ transmissibility and/or virulence could be accidentally or intentionally released from a laboratory, potentially exposing surrounding populations to a pathogen with pandemic potential.

The U.S. Government (USG), as part of its continued focus on biosafety and biosecurity, has undertaken a deliberative process to carefully examine the risks and benefits associated with certain GOF studies. The deliberative process involves the National Science Advisory Board for Biosecurity (NSABB), which has been tasked with making recommendations to the USG on this topic, and the National Academy of Sciences (NAS), which was tasked to convene two public symposia to generate broad discussion on the relevant issues. To further inform NSABB deliberations, the National Institutes of Health (NIH) commissioned Gryphon Scientific to perform an independent assessment of the risks and benefits associated with GOF studies and an ethical analysis of the issues related to funding and conducting such studies.

The NSABB was charged with ~~1)~~ advising on the design~~, development, and conduct~~ of the risk and benefit assessments (RBA) for GOF studies~~,~~ and ~~2)~~with providing recommendations to the USG on a conceptual approach ~~to the~~for evaluating~~on of~~ proposed GOF studies. ~~The NSABB established two working groups to address its tasks and the full Board convened publically five times between October 2014 and January 2016.~~ In May 2015 the NSABB issued its *Framework for Guiding the Conduct of Risk and Benefit Assessments of Gain-of-Function Research*, which guided NIH in overseeing the contractor conducting the risk and benefit assessments. In May 2016, informed by the results of the RBA as well as its analysis of the current policy landscape, consideration of relevant ethical issues, and consulatations with domestic and international stakeholders, the NSABB working group will present this draft report for consideration and finalization by the full NSABB.

~~The working group tasked with issuing recommendations on an approach to evaluating proposed GOF studies considered four major areas: the current policy landscape as it pertains to pathogen research, the results of the risk and benefit assessments, the analysis of relevant ethical issues, and broad stakeholder perspectives on the issues at hand. This working paper describes the working group's process, analysis, preliminary findings, and draft recommendations to date. This paper is not a final NSABB work product and does not represent NSABB recommendations to the U.S. government. This interim report is offered by the working group to the full NSABB, and the broader stakeholder community, to serve as a springboard for discussion at the NSABB meeting in May, 2016.~~

The NSABB working group has developed ~~X~~ 7 major~~key~~ findings:

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Key Finding 1: There are many types of GOF research and not all of them have the same level of risks. Only a small subset of GOF research—GOF research of concern (GOFROC)—entail risks that are potentially significant enough to warrant additional oversight.

Key Finding 2. The U.S. government has several policy frameworks in place for identifying and managing risks associated with life sciences research. There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOFROC could be applied.

Key Finding 3. Oversight policies vary in scope and applicability, and are not sufficiently harmonized; therefore, current oversight is not sufficient for all GOFROC.

Key Finding 4. An adaptive policy approach is a desirable way to ensure that oversight and risk mitigation measures remain commensurate with the risks associated with the research and the benefits of the research are being fully realized.

Key Finding 5. There are life sciences research studies, including possibly some GOFROC, that should not be conducted on ethical or public health grounds if the potential risks associated with the study are not justified by the potential benefits. Decisions about whether GOFROC should be permitted will entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of proposed studies but other considerations, including legal, ethical, and societal values are also important.

Key Finding 6. Managing risks associated with GOFROC, like all life sciences research, requires Federal-level and institutional oversight, awareness and compliance, and a commitment by all stakeholders to safety and security.

Key Finding 7. Consideration of the international dimensions associated with funding and conducting GOF research of concern is important. It is important to engage with and to continue an active dialogue with the international community on GOFORC as well as on DUR/DURC.

~~Based on its analyses thus far, the NSABB working group has formulated the following draft recommendations for discussion~~The NSABB working group has developed 7 recommendations to the U.S. government:

Recommendation 1. Research proposals involving GOFROC entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the Federal and institutional levels.

As part of this recommendation, the NSABB working group has proposed a conceptual approach for guiding funding decisions about GOFROC. First, the working group identified the attributes of GOFROC,

which is research that could generate a pathogen that is: 1) highly transmissible and likely capable of wide and uncontrollable spread in human populations; and 2) highly virulent and likely to cause significant morbidity and/or mortality in humans. Next, the working group identified a set of principles that should guide funding decisions for GOFROC. Only research that is determined to be in line with these principles should be funded. Additional risk mitigation measures may be required for certain research studies to be deemed acceptable for funding.

Recommendation 2. An external advisory body that is designed for transparency and public engagement should be utilized as part of the U.S. government's ongoing evaluation of oversight policies for GOFROC.

Recommendation 3. In general, oversight mechanisms for GOFROC should be incorporated into existing policy frameworks when possible.

Recommendation 4. The U.S. government should pursue an adaptive policy approach to help ensure that oversight remains commensurate with the risks associated with the GOFROC.

Recommendation 4.1. The U.S. government should consider developing a system to collect and analyze data about laboratory safety incidents to inform GOFROC policy development over time.

Recommendation 5. The U.S. government should consider ways to ensure that all GOFROC conducted within the U.S. or by U.S. companies be subject to oversight, regardless of funding source.

Recommendation 6. The U.S. government should undertake broad efforts to strengthen laboratory biosafety and biosecurity and, as part of these efforts, seek to raise awareness about the specific issues associated with GOFROC.

Recommendation 7. The U.S. government should engage the international community in a dialogue about the oversight and responsible conduct of GOFROC.

1. Introduction

A robust life sciences research enterprise is necessary to counter the continually evolving threats to public health and national security posed by endemic and emerging pathogens, as well as malicious biological threats. By helping to define the nature of human-pathogen interactions, life sciences research promotes public health and national security not only by enhancing our understanding of pathogen biology and disease pathogenesis, but also by informing biosurveillance and medical countermeasure development. Such research can also aid in the assessment of the pandemic potential of emerging infectious agents, thereby underpinning health policy decisions and preparedness and response efforts.

While the ultimate goal of life sciences research involving pathogens is the protection and promotion of public health, there are inherent associated biosafety and biosecurity risks. Potential risks might arise from laboratory accidents or security breaches that result in laboratory acquired infections or the accidental or deliberate release of a pathogen from containment. Life sciences research has "dual use" potential. That is, legitimate research may generate information, products or technologies that could be misused to threaten public health or national security. To mitigate such dual use concerns, as well as potential biosafety and biosecurity risks, research involving pathogens is subject to multiple layers of Federal and institutional oversight.

The Gain-of-Function Debate and the USG Response

Experimental techniques and approaches that modify the genome of microorganisms are routinely employed in pathogen research to ascertain the roles of genes and their functional products. Such studies are fundamental to the field of microbial genetics and facilitate correlation of genetic and phenotypic characteristics – a critical step in deciphering the complex nature of host-pathogen interactions that underlie transmission, infection, and pathogenesis. Such genetic manipulations can result in either diminished (loss-of-function) or enhanced (gain-of-function) biological phenotypes.

Studies that result in the generation of pathogens with enhanced, or gain-of-function (GOF), phenotypes are conducted for a number of valid scientific purposes. Such studies provide information that adds to the scientific knowledge base and can inform biosurveillance, medical countermeasure development, and public policy decision-making related to public health and preparedness. The vast majority of such GOF studies do not raise significant safety or security concerns. However, certain GOF studies involving pathogens have raised significant concerns about whether a laboratory-generated pathogen with pandemic potential could be accidentally or intentionally released, resulting in significant consequences to public, or perhaps, global health. Concerns have also been raised about whether certain GOF studies could generate information that could enable individuals with malevolent intent to generate a pathogen with pandemic potential (see Box 1).

The controversy over certain GOF studies arose after two groups demonstrated that highly pathogenic avian influenza H5N1 viruses with a small number of engineered mutations became transmissible between mammals by respiratory droplets.[1,2] In 2012, in response to the controversy associated with publishing the manuscripts describing these findings, the influenza community initiated a voluntary suspension of certain GOF studies involving highly pathogenic avian influenza H5N1 viruses. During that time, policymakers considered whether certain GOF studies should be conducted using Federal funds, and if so, how those studies could be safely conducted. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) issued new biosafety guidelines for working with highly pathogenic avian influenza strains.[3,4] The U.S. Department of Health and Human Services (HHS) developed a framework for guiding its funding decisions about GOF projects that may generate H5N1 or H7N9 avian influenza viruses that are transmissible between mammals by respiratory droplets.[5]

Concerns regarding laboratory safety and biosecurity associated with GOF studies were renewed following a number of biosafety incidents at U.S. Federal laboratories during the summer of 2014. The incidents did not involve GOF studies *per se* but raised broader concerns about laboratory safety and security as it applies to pathogen research.

Box 1. Gain-of-Function Research

Recently, the phrase "gain-of-function research" has become synonymous with certain studies that enhance the ability of pathogens to cause disease. However, gain-of-function studies, as well as loss-of-function studies, are common in molecular and microbiology and form the foundation of microbial genetics. Changes to the genome of an organism, whether naturally occurring or directed through experimental manipulations in the laboratory, can result in altered phenotypes as biological functions are lost or gained. Investigators routinely conduct loss- and gain-of-function experiments to understand the complex nature of host-pathogen interactions that underlie transmission, infection, and pathogenesis.

The term "gain-of-function" is generally used to refer to changes resulting in the acquisition of new, or an enhancement of existing, biological phenotypes. This report further defines "gain-of-function research of concern" to describe the subset of studies that have been the subject of recent debate regarding potential biosafety and biosecurity implications -- that is, gain-of-function studies with the potential to generate pathogens with pandemic potential in humans by exhibiting high transmissibility and high virulence. See Section 6 for a more rigorous description of GOF research of concern (GOFROC).

As one component of comprehensive efforts to review and enhance laboratory biosafety and biosecurity, the U.S. government (USG) embarked on a deliberative process to re-evaluate the risks and benefits of certain GOF research with a goal of developing policy governing the funding and conduct of

[1] Imai et al. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 486, 21 June 2012

[2] Herfst et al. Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets. *Science* 336, 22 June 2012

[3] Gangadharan D, Smith J, and Weyant R. Biosafety Recommendations for Work with Influenza Viruses Containing a Hemagglutinin from the A/goose/Guangdong/1/96 Lineage, Morbidity and Mortality Weekly Report 62(RR06); 1-7. http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6206a1.htm

[4] *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

[5] Framework for Guiding Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets, February 21, 2013. http://www.phe.gov/s3/dualuse/Documents/funding-hpai-h5n1.pdf

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such research.[6] The deliberative process involves the National Science Advisory Board for Biosecurity (NSABB), which serves as the official Federal advisory body for providing advice in this area, and the National Academy of Sciences (NAS), which is to foster broader scientific and public discussions on the topics. To inform NSABB deliberations, NIH commissioned formal risk and benefit assessments (RBA) of GOF research involving pathogens with pandemic potential and an analysis of ethical issues surrounding the conduct of such studies. Stakeholder input is also essential to the process and has been received throughout NSABB's deliberative process.

The deliberative process is accompanied by a pause in the provision of new federal funds for certain GOF research involving influenza, Middle East Respiratory Syndrome (MERS) or Severe Acute Respiratory Syndrome (SARS) viruses—pathogens determined to have pandemic potential. Specifically:

> *New USG funding will not be released for gain-of-function research projects that may be reasonably anticipated to confer attributes to influenza, MERS, or SARS viruses such that the virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route. This restriction would not apply to characterization or testing of naturally occurring influenza, MERS, and SARS viruses, unless the tests are reasonably anticipated to increase transmissibility and/or pathogenicity.*[7]

In parallel, the USG has encouraged the research community (both those who receive USG funding and those who do not) to join in adopting a voluntary pause on any ongoing research that involves the types of studies that are subject to the funding restriction above.

NSABB recommendations will inform the USG as it develops policies about whether certain types of GOF studies on pathogens with pandemic potential should be supported and, if so, how such research proposals should be evaluated to inform funding and oversight decisions. It is expected that the temporary funding pause will be lifted and/or replaced by a decision or policy that addresses GOF research involving the generation of pathogens with pandemic potential.

2. NSABB Charge

On October 22, 2014, as part of the USG's deliberative process for GOF studies, the NSABB was issued its charge to:

1. Advise on the design, development, and conduct of risk and benefit assessments for GOF studies, and
2. Provide recommendations to the U.S. government on a conceptual approach to the evaluation of proposed GOF studies

In developing its recommendations the NSABB was asked to consider: the results of the risk and benefit assessments; the discussions hosted by the National Academies; the spectrum of potential risks and

[6] U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses, U.S. Government, October 17, 2014. http://www.phe.gov/s3/dualuse/documents/gain-of-function.pdf

[7] Ibid.

benefits associated with GOF studies; and any alternative methods that may be employed to yield similar scientific insights or benefits, while reducing potential risks.

Since gain-of-function studies encompass a broad spectrum of pathogens and experimental manipulations, the NSABB discussed its charge and sought to identify the appropriate scope of its deliberations. Since the experiments that initiated the controversy involved the generation of pathogens that were concerning from a human health perspective, NSABB deliberations and recommendations focus for pathogens that pose risks to human populations. NSABB deliberations also focused on guiding U.S. funding decisions but Board also considered issues associated with non-Federally funded research and international research.

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3. NSABB Deliberative Approach

The deliberative process (Figure 1) initiated by the USG to evaluate the risks and benefits of GOF studies involves the NSABB and the National Academies. To address its charge, NSABB formed two working groups to develop draft recommendations, which were discussed by the full Board[8]. The National Academies convened public forums to generate broad discussions and receive additional stakeholder input ~~on the topic~~. The first forum was held early in the deliberative process and a second was held in March 2016; both were designed to inform NSABB deliberations.

To inform the deliberative process further, NIH commissioned two additional analyses: 1) qualitative and quantitative risk and benefit assessments, conducted by Gryphon Scientific, and 2) a review of the ethical considerations associated with the GOF issue and an analysis of relevant ethical decision-making frameworks, conducted by Dr. Michael Selgelid.

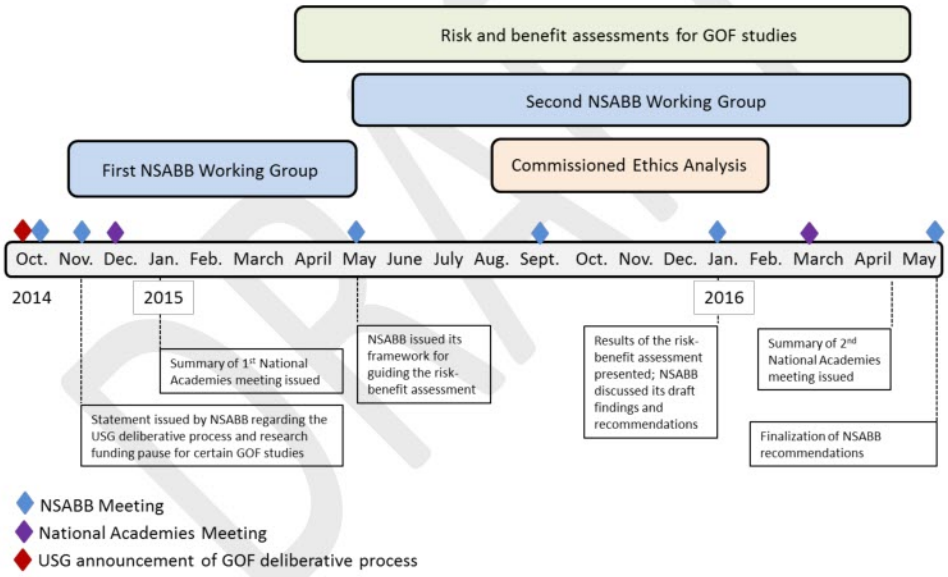

Figure 1. Timeline and major events of the GOF deliberative process.

[8] Information about these meetings and activities, including agendas, summaries, and archived videocasts, can be found on the NSABB website at: http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/nsabb/nsabb-meetings-and-conferences/past-meetings

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The NIH Office of Science Policy, which administers the NSABB, managed the overall deliberative process. NIH oversaw the work of its contractors, Gryphon Scientific and Dr. Michael Selgelid, and interfaced between the NSABB and contracted entities.

See Appendices ~~A, B, C, and E~~ for ~~the NSABB~~ more information. Appendix A provides a detailed description of the NSABB's deliberative approach. Appendix B describes examples of studies that would or would not be considered GOF research of concern. Appendix C provides an overview of the stakeholder views that were presented and considered by NSABB. Appendix D lists the experts and sources consulted by NSABB, including those who submitted public comments. Appendix E and F list the~~and~~ NSABB roster and charter.~~working group roster, a detailed description of the NSABB's deliberative approach, an overview of stakeholder views presented and considered by NSABB, and a list of the experts and sources consulted, including those who submitted public comments. NOTE: Need to more clearly state what each appendix is instead of lumping all together here.~~

Guiding Principles for NSABB Deliberations

Early in its deliberations the NSABB developed the principles below to guide its deliberations and underpin its analysis of the risk and benefit assessments.

1. The NSABB deliberations should focus on defining the GOF problem then include broad consideration of possible solutions. A range of approaches and decision-making frameworks will be considered, and the NSABB will take into account these various approaches when developing its recommendations.

2. NSABB will consider the potential risks and benefits of a broad range of GOF studies involving influenza, SARS, and MERS viruses in order to identify those that may raise significant concerns that should be addressed. However, the NSABB will aim to develop recommendations that are grounded in broadly-applicable concepts and principles that could, if necessary, apply to GOF studies involving other pathogens that may require evaluation in the future.

3. Similarly, NSABB will consider the risks and benefits associated with alternative research approaches to GOF research to understand whether or not these may substitute for or complement GOF studies.

4. NSABB recommendations will be informed by data and information about potential risks and benefits as well as values that will guide the evaluation and comparison of these risks and benefits. Ethical, societal, and legal considerations will also contribute to the development of recommendations and these inputs should be explicitly identified, discussed, and prioritized.

5. NSABB recognizes that not all analyses relevant to its task are quantitative and that uncertainties inherent in any quantitative analysis may remain. NSABB will seek to document important areas of uncertainty in any data or analysis when necessary.

6. NSABB should publicly debate its draft recommendations and describe in its report any dissenting views that may vary substantially from the Board's recommendations.

7. NSABB should consider current USG policies and guidelines, determine whether they adequately address risks associated with GOF research (in light of potential benefits), and make recommendations that are consistent with that determination. Current policies may be adequate or require only minor changes; alternatively, significant enhancements may be needed. The adequacy of current policy to cover GOF studies may vary by pathogen. Recognizing the paramount importance of ensuring safety, security, and public health, policies should also minimize the burdens placed upon the conduct of science.

8. NSABB recommendations will inform the development of U.S. government policy, which will apply to research funded, conducted, or overseen by the U.S. government either domestically or internationally. NSABB will be mindful in its deliberations of the likelihood that the Board's recommendations and U.S. policy decisions will also influence other governments and non-USG funders of life sciences research.

9. The NSABB will also consider whether there are certain studies that should not be conducted under any circumstances, and if so, articulate the critical characteristics of such studies.

10. Maintaining public trust and confidence in life sciences research is critical and must be taken into account as recommendations are formulated.

4. Analysis

~~The NSABB working group tasked with~~In developing recommendations on a conceptual approach for evaluating GOF proposals, NSABB examined three major areas: the current policy landscape for overseeing research involving pathogens, ethical issues associated with funding and conducting GOF studies, and the results of Gryphon's risk and benefit assessments. In addition, the NSABB considered broad stakeholder perspectives through presentations from domestic and international experts at Working Group and full NSABB meetings, expert consultations, individual NSABB member participation in and ~~review~~ ideas and views from~~of~~ the National Academies workshops and proceedings, analysis of published articles, and comments from attendees at NSABB meetings ~~and~~ or public comments submitted to the Board.

4.1. Analysis and Interpretation of the Risk and Benefit Assessment

The NSABB working group has reviewed the risk and benefit assessments (RBA) conducted by Gryphon Scientific, which were designed to evaluate the risks and benefits of GOF research in a manner that encompassed both benign and worrisome aspects of a broader range of GOF studies than those that have raised concern. The RBA analyzed biosafety and biosecurity risks as well as possible benefits. Overall, the RBA includes a commendable amount of sophisticated work and analysis, is generally well-done, and largely achieves the goals it was intended to address. Gryphon's draft RBA report was made publically available in December 2015 and key results were presented and discussed at NSABB and NAS meetings. The final report is available on Gryphon's website.[9]

Strengths of the Risk and Benefit Assessments

The RBA has ~~numerous~~ significant strengths. It is a thorough and extensive analysis of the risks and benefits of GOF work in the context of the guidance provided in the NSABB *Framework for Conducting Risk and Benefits Assessments of Gain-of-Function Research* (May 2015)[10]. It takes into account the principles articulated in the framework and includes the agents, categories of possible risks, types of possible benefits, and possibly concerning scenarios and phenotypes that were laid out in the *Framework*. A few items from the *Framework* were eliminated from consideration at the meeting of the NSABB where the framework was voted on[11], so that the most probable issues of concern could be thoroughly addressed within the available time and resources.

The biosafety risk assessment does a credible job of defining the relative risks associated with potential laboratory accidents involving GOF manipulations of pathogens with enhanced characteristics as compared to wild-type pathogens. This analysis is performed in a semi-quantitative way; it uses

[9] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

[10] Framework for Conducting Risk and Benefits Assessments of Gain-of-Function Research, May 2015. http://osp.od.nih.gov/sites/default/files/resources/NSABB_Framework_for_Risk_and_Benefit_Assessments_of_GOF_Research-APPROVED.pdf

[11] National Science Advisory Board for Biosecurity Meeting, May 5, 2015. http://osp.od.nih.gov/office-biotechnology-activities/event/2015-05-05-120000-2015-05-05-200000/national-science-advisory-board-biosecurity-nsabb-meeting

appropriate, established, peer-reviewed methods to the extent available. The parametric approach employed is powerful and allows consideration of many situations of interest.

The report effectively illustrates that the harmful events being modeled are low probability (see Figures 6.2 and 6.4 in Gryphon's report). Only a small fraction of laboratory accidents would result in a loss of containment; of those, only a small fraction would result in a laboratory acquired infection, and of those, only a fraction would spread throughout the surrounding community (or to the global population). The working group recognizes the challenge of analyzing low-probability, high-consequence events for which little data exists and appreciates attempts to make this point clear in the RBA.

The biosecurity risk assessment is primarily qualitative, and highlights analysis of previous malevolent events and evasions of security systems, likely capabilities and motivations of various possible actors, and an evaluation of the systems in place to prevent biosecurity breaches. Information was obtained from a survey of literature and discussions with biosecurity, intelligence, and law enforcement professionals. It is an extensive gathering of a wide range of information that has not been presented before in one place.

The information risk assessment (an element of the biosecurity risk assessment) is a qualitative analysis of risks that may result from the misuse of information derived from certain GOF studies that might be published in the future. It identifies information that might be attractive to malicious actors and compares it to other sources of information they might find attractive.

The benefits assessment uses a novel approach to assess benefits of GOF studies, a difficult task without much prior methodology to draw upon. The results are not quantitative, and attempts to quantify would have been appreciated. However, as is, the assessment may be the best that can be done with the available information and analytic tools. The benefits assessment ~~effectively~~ thoroughly analyzed the possible benefits of alternatives to GOF studies and identified areas where GOF research appears to provide unique benefits (i.e., benefits that are not attainable without the use of GOF), either currently or in the near future.

The RBA contains a number of other useful analyses as well, including background and contextual information on the biology of influenza and coronavirus, historical analysis of naturally-occurring seasonal and pandemic influenza and coronavirus outbreaks, an examination of the potential proliferation of GOF research, and analysis of the potential loss of public trust in science that could result if a laboratory incident involving GOF research were to occur. Significantly, the historical analysis notes that each year, influenza infects 5 – 10% of the world's population, resulting in significant morbidity and mortality (up to 500,000 deaths per year). This description of naturally-occurring influenza (and coronavirus) infections helps to establish the extant risks associated with these infectious diseases to which the risks associated with GOF studies might be compared.

Overall, the RBA is comprehensive, objective, reasonable, and generally extensively documented.

Limitations of the Risk and Benefit Assessments

The RBA also has some weaknesses and limitations that should be noted. First, the RBA was limited to the types of labs traditionally funded by the Federal government, which may not be representative of other settings where GOF research may be conducted. Every attempt was made to base the analyses in the RBA on scientific information and data. Nevertheless, data on the properties of the various pathogens being examined, events such as laboratory accidents or security breaches, or possible future acts of terrorism are limited in some cases and unavailable in principle in others. Therefore, assumptions and estimations were necessary. For this reason, the biosafety risk assessment is not fully quantitative, primarily because absolute, quantitative baselines for the risk of work with wild-type pathogens could not be estimated with any certainty. Thus, the data presented are primarily comparative, and provide relative, not absolute values, for the risks associated with laboratory accidents involving GOF studies. Gryphon compared the risks associated with potential lab accidents involving a GOF strain with the risks associated with the same accident involving a wild-type strain. This comparative approach is adequate for some instances but inadequate for others. For instance, an increased risk associated with a GOF study that is relatively large (5-10-fold or greater) may appear significant, but if this increase is in comparison to a very small risk baseline, the overall risk associated with the GOF study may not be significant or concerning. Similarly, small increases in risk over a higher risk baseline, in fact, may be concerning. Additionally, differences in risk that are relatively small (~2-fold) are difficult to interpret because such changes may fall within the limits of uncertainty for the analysis. Attempts to include some absolute baseline estimates of risk (an admittedly difficult task) were included in Section 6.8 of Gryphon's report. However, the lack of comprehensive estimates of baseline risk make interpreting the biosafety risks a challenge.

Given the comparative approach undertaken for the biosafety risk assessment, the implications of the results of this analysis depend a great deal on the wild-type comparator strains that were selected for the analysis. For instance, for pandemic influenza Gryphon initially selected the 1918 influenza strain as the comparator. Gryphon regarded this strain as embodying the maximum risk for influenza, yet a level of risk that is also deemed as acceptable given that research with this strain is permitted. However, using 1918 influenza as the comparator for the analysis compares GOF risks to a relatively high level of baseline risk, making the changes in risk associated with GOF manipulations comparatively small. Utilizing different comparator strains alters the relative risks associated with GOF manipulations; using a high-risk baseline strain may obscure significant risks associated with GOF studies whereas using a low-risk baseline strain may inflate the potential risks associated with GOF studies.

Little data exists about the probabilities of the accidents that initiate the chain of events that may lead to a pandemic and therefore, the quantitative probability of these accidents could not be incorporated into the biosafety risk assessment. The modeling of secondary spread of a pathogen through populations once it is released from a laboratory allows for some estimation of the consequences of an event but without a better understanding of the likelihood that an accident would result in loss of containment or a laboratory acquired infection, it is difficult to make judgments about the overall risk. Gryphon's analysis accounts for this by presenting relative, actuarial risk. However, this approach results in the challenges associated with comparing relative risks described above. There are large uncertainties in most of the input parameters that are the basis for the biosafety risk calculations.

Uncertainties about inferring absolute risk from these relative risks exist and should be kept in mind as any conclusions are reached.

The biosecurity risk assessment attempts to examine how GOF studies add to the risk of malevolent acts. Portions of the biosecurity risk assessment focus on GOF studies but others describe the type of threats that could occur against any high-containment laboratory. The semi-quantitative portion of the biosecurity risk assessment estimates the number of infections that could occur if a pathogen with various enhanced characteristics were intentionally released. However, this analysis (see section 7.4.2 and Table 7.7 in Gryphon's report) assumes that 1 or 10 individuals are initially infected as a result of a malicious act with no indication of how likely such an event would be, since there is no way to make such an estimate.

While exhaustively documented, the RBA is not always transparent about data reliability. In particular, interviews were used to gather much critical information, and this was not always well documented in a way that reflects how robust the resulting information may be. For peer-reviewed publications, this is less of a concern.

While evaluation of the benefits of alternatives to GOF studies was extensive, evaluation of risks of alternative approaches was not as thorough. In addition, risks and benefits have not been presented in comparable terms, making it a challenge to determine whether certain risks are justified by potential benefits. Significantly, the benefit assessment is not quantitative and there is no probability analysis or attempt to estimate the likelihood that a certain benefit would be realized or what its impact might be.

Key Results of the Risk and Benefit Assessments

While NSABB has examined all of the analyses in the RBA, some results are important to highlight. In general, the RBA examined risks and benefits associated with the major GOF phenotypes with the intention of identifying types of studies that would be most and least concerning, based particularly on their risk profile.

With regard to biosafety risks, only some potential GOF phenotypes represent substantially increased (5- to 10-fold or more) risks over the starting strain. Two-fold changes most likely fall within the uncertainty of the data, and while small differences might be important if it could be shown that they are significant, this demonstration is probably difficult. For coronaviruses, GOF studies that would create strains with increased transmissibility among mammals may entail significant risks if they also increase human transmission. The risks, were this combination to occur, would include increased probability of an outbreak escaping local control and increased likelihood of global consequences. In addition, experiments that enhance coronavirus growth in culture would likely increase the possibility of laboratory acquired infections.

For seasonal influenza, the GOF-generated phenotypes entailing the greatest risks include enhanced transmission in mammals (assuming this increases transmission in humans), enhanced virulence, and evasion of immunity. Enhanced pathogenicity might significantly increase the global consequences of an outbreak. For pandemic influenza, no GOF-generated phenotypes led to greatly increased risk, but

that is based on using 1918 influenza as the comparator; because the risk associated with the wild-type 1918 strain is already so great it is difficult to increase risk substantially. If less transmissible and/or less virulent wild-type strains were used as the basis of comparison, the risks of GOF studies with pandemic strains might appear higher. For avian influenza, the GOF experiments that lead to enhanced transmissibility in mammals (and presumably humans) would likely lead to an increased probability of local and widespread outbreaks, as well as increased global consequences. More subtle aspects of these very general conclusions may be found in the biosafety risk section of the Executive Summary of Gryphon's RBA report.

In general, GOF studies that were not considered by the working group to entail significant risks were those that would: adapt human pathogens to mammals to generate animal models; enhance the growth of attenuated vaccine strains; and antigenic drift or immune evasion studies that are commonly used to guide vaccine selection.

The biosecurity risk assessment shows that the most probable threats involve insiders who have direct access to dangerous pathogens or outsiders who collaborate with or subvert insiders. If currently mandated biosecurity systems are effective, outsiders have little chance of causing harm on their own. The RBA report also concludes that the risks associated with information from future GOF studies with influenza, SARS and MERS appear small; this is because most of the information of interest is already published, or non-GOF information relating to pathogens that are more attractive agents of harm is readily available. However, future scientific advancements could alter this assessment.

Most GOF studies provide benefits in the form of new scientific knowledge, and some of these benefits are unique (i.e., unable to be achieved by alternative, non-GOF approaches). While some GOF studies are likely to provide unique near-term benefits, these are associated with specific agents and phenotypes. With regard to more applied benefits, such as countermeasure development and biosurveillance, the most clear-cut situation is experiments that increase growth of seasonal influenza vaccine candidates in culture; these studies provide unique benefits to current production of seasonal influenza vaccines, and likely will in the future. Another reasonably clear unique benefit is derived from experiments that enhance mammalian pathogenicity for coronavirus as a means of developing animal models for studying disease and developing countermeasures. GOF studies that yield phenotypes that provide unique benefits to countermeasure development include enhanced pathogenicity, evasion of vaccines, and evasion of therapeutics. For several other potential benefits with seasonal influenza, either the potential benefit is long term, or alternative approaches may yield the same or similar benefits. Interestingly, few unique benefits pertaining to GOF studies involving pandemic influenza were identified. There are several types of GOF studies that entail generating avian influenza strains with phenotypes that may be valuable for surveillance and preparedness efforts, although other advances are needed to fully realize such benefits. This point is controversial, with strong proponents and critics. Additionally, a variety of benefits were identified that may also be provided to some extent by alternative approaches. It should be noted that no attempt was made to provide a probability assessment based on historical data for potential benefits; hence no direct comparison of risk to benefit for a proposed research project is possible.

4.2. Consideration of Ethical Values

The risk and benefit assessments provide information about the potential risks and benefits associated with conducting GOF research. However, determinations about whether such studies should be undertaken will involve value judgments when weighing the risks and benefits. The NSABB identified a number of values (that are applicable to the decisions about whether to fund certain GOF studies and how to oversee them. Sources of these values include the Belmont Report,[12] the literature on public health ethics,[13] and the literature on oversight of emerging technologies,[14] as well as the literature specifically debating appropriate approaches to overseeing DURC and GOF research that has raised concern.[15,16,17,18,19] The commissioned ethics analysis conducted by Dr. Michael Selgelid also describes additional decision-making frameworks and values to be considered.[20]

Substantive values

The following values are important to consider when considering funding of a research proposal involving GOF studies that might entail significant risks.

Non-maleficence: not causing harm. Harm might include: losing lives; causing disease; damage to the economy, national or international security, or agriculture; or loss of public trust in science or governance structures. There are inherent risks associated with research involving pathogens that could result in harm. Approaches aimed at preventing harm and mitigating potential risks should be considered and applied to the design, conduct, and communication of research involving pathogens in GOF studies.

Beneficence: promoting beneficial outcomes while preventing harmful outcomes; appropriately balancing benefits and risks; formulating policy that maximizes public benefit while minimizing public harm. Benefits might include: saving lives, preventing disease, improving public health; enhancing the economy, national and international security, or public trust in science and

[12] The Belmont Report. Office of the Secretary, U.S. Department of Health and Human Services. Ethical Principles and Guidelines for the Protection of Human Subjects Research. The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, April 18, 1979. http://www.hhs.gov/ohrp/humansubjects/guidance/belmont.html
[13] Kass NE. An Ethics Framework for Public Health. *American Journal of Public Health*. 2001;91(11):1776-1782. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1446875/
[14] New Directions. The Ethics of Synthetic Biology and Emerging Technologies. Presidential Commission for the Study of Bioethical Issues, December 2010. http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf
[15] Resnik DB. H5N1 Avian flu research and the ethics of knowledge. Hastings Center Report 2013; 43, 2: 22-33.
[16] Kelle A. Beyond patchwork precaution in the dual-use governance of synthetic biology. Sci Eng Ethics. 2013 Sep;19(3):1121-39.
[17] Kuhlau F, Höglund AT, Evers K, Eriksson S. A precautionary principle for dual use research in the life sciences. Bioethics. 2011 Jan;25(1):1-8.
[18] Biotechnology Research in the Age of Terrorism. The National Academies, 2004. http://www.nap.edu/catalog/10827/biotechnology-research-in-an-age-of-terrorism
[19] Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information. National Science Advisory Board for Biosecurity, June, 2007. http://osp.od.nih.gov/sites/default/files/resources/Framework%20for%20transmittal%20duplex%209-10-07.pdf
[20] Selgelid, Michael. Gain-of-Function Research: Ethical Analysis. December 7, 2015. http://osp.od.nih.gov/sites/default/files/GOF%20%20White%20Paper%20by%20Michael%20Selgelid_0.pdf

548 governance structures. When the ultimate goals of the research are to improve public health, public
549 health ethics would ask how effective the research is likely to be in achieving those goals, what are
550 the known or potential burdens of the research, can those burdens be minimized, whether there are
551 alternative approaches that are less risky or burdensome, and how can the potential benefits and
552 burdens of the research be fairly balanced. The work of the Presidential Commission for the Study
553 of Bioethical Issues suggests that those formulating and effectuating government policy on scientific
554 research and emerging technologies have a duty of public beneficence – a duty "to promote
555 individual activities and institutional practices…that have great potential to improve the public's
556 well-being," while being "vigilant about risks and harms, [and] standing ready to revise policies that
557 pursue potential benefits with insufficient caution."[21] Both risks and benefits have associated
558 probabilities, magnitudes, and uncertainties. In some instances, it may be justifiable to pursue
559 benefits despite the potential risks; in others, the potential benefits may be foregone due to
560 possible risks.

561 **Social justice**: distributing potential benefits and harms fairly (distributive justice) and selecting
562 participants in research fairly, as well as those who may potentially be exposed to risk. There are
563 many different approaches to social justice, such as egalitarianism, utilitarianism, and
564 libertarianism,[22] to name but a few. Decisions about whether to fund research that entails some risk
565 should consider how the risks and benefits associated with conducting that research will be
566 distributed, with an effort to distribute risks and benefits as fairly as possible. When considering
567 pandemic potential, fair distribution of risks and benefits must be considered on a global scale.
568 Those who will potentially be exposed to risk, through participation in research or other avenues of
569 exposure, should be selected equitably.

570 **Respect for persons**: allowing competent individuals to make informed choices, and ensuring that
571 the representatives of individuals lacking capacity to choose can make choices in keeping with the
572 wishes, values, or interests of those represented. Autonomy generally requires informing human
573 research participants, laboratory workers, and the public about the risks of research and eliciting
574 their free and uncoerced decision about whether to subject themselves to those risks. In the case of
575 the public, mechanisms for representative decision-making and publicly accountable governance
576 may be needed, as getting consent directly from the members of the public may be impracticable.

577 **Scientific Freedom**: avoiding unnecessary interference with scientific research, debate, or
578 publication. Scientific freedom includes an entitlement to avoid interference unless necessary
579 (negative freedom), but not the affirmative right to receive funding or other forms of support for a
580 particular project (positive freedom). Scientific freedom is compatible with norms and regulation to
581 promote the responsible conduct of research and protect participants in research and the public. As
582 a corollary to the principle of scientific or intellectual freedom, the Presidential Commission

[21] New Directions. The Ethics of Synthetic Biology and Emerging Technologies. Presidential Commission for the Study of Bioethical Issues, December 2010. http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf
[22] Nozick R. Anarchy, State, and Utopia. New York: Basic Books, 1974.

endorses a principle of regulatory parsimony, requiring "only as much oversight as is truly necessary to ensure justice, fairness, security, and safety while pursuing the public good."[23]

Responsible Stewardship: acting in a way that shows concern for children, future generations, and the environment. The Presidential Commission emphasizes that this is both a domestic and global responsibility that requires "prudent vigilance, establishing processes for assessing likely benefits along with assessing safety and security risks both before and after projects are undertaken."[24]

Procedural Values

The following values apply to the process of decision-making about GOF research and are important to consider when establishing mechanisms to review and/or approve the funding of research proposals involving gain-of-function studies that may entail significant risks.

Public participation & democratic deliberation: making decisions with participation from the public, utilizing respectful debate and inclusive deliberation. Life sciences research is largely a publicly-supported endeavor; therefore, those who allocate funds and conduct life sciences have a responsibility to be good stewards of public funds and to respond to the interests and concerns of the public. Many, if not all, members of society have a stake in the life sciences enterprise and will be affected directly or indirectly by the benefits and risks stemming from such research. This stakeholder community has diverse values and tolerances for risk, which are important to consider when making decisions about funding and overseeing life sciences research. Some forms of public participation include: oversight by the legislative or executive branches of government, public membership and input on government science advisory committees, other mechanisms of public governance, surveys of public opinion on science policy issues, research models such as community-based participatory research, and efforts by scientists and government officials to share information with the public and better understand the public's interests and concerns. The Presidential Commission urges the importance of democratic deliberation, as "[a]n inclusive process of deliberation, informed by relevant facts and sensitive to ethical concerns, promotes an atmosphere for debate and decision making that looks for common ground wherever possible and seeks to cultivate mutual respect where irreconcilable differences remain."[25]

Accountability: taking responsibility for one's actions and being prepared to justify or explain them to others. It is important that decisions to fund research are justifiable to the public and others. Decisions should be justified in terms of substantive and procedural values.

Transparency: sharing with the public the information and assumptions used to make a decision, including uncertainties, controversies, and limitations of analyses. Transparency is an important

[23] New Directions. The Ethics of Synthetic Biology and Emerging Technologies. Presidential Commission for the Study of Bioethical Issues, December 2010. http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf, p5.
[24] Ibid., p5.
[25] Ibid., p5.

part of accountability and public participation. It allows review and reconsideration of policy over time as new facts emerge and analysis evolves.

4.3. Decision-Making Strategies and Frameworks for Evaluating and Managing Risks and Developing Policy

The NSABB working group identified a number of approaches or frameworks that may be used to guide the making of complex decisions with ethical implications, particularly in the face of uncertainty. These may also be used in developing policies such as that for managing GOF research. Different strategies reflect different attitudes toward risk-taking. Some may be more appropriate in some situations than others. The NSABB working group examined a number of such strategies as it attempted to determine the best option as relates to GOF research that has raised concerns. These options are not mutually exclusive, and elements from more than one may be used together to develop a path forward. The following are decision-making frameworks that were considered.

Maximax: This involves choosing the option with the best possible outcome. Maximax is a relatively simple strategy that focuses on choosing the option with the best possible outcomes While maximax may be appropriate for making some types of personal choices (e.g. playing games with nothing of value to lose), it may not be appropriate for making science and technology policy decisions because most people would want to take appropriate steps to prevent or mitigate risks regardless of benefits. For GOF studies, use of maximax would involve identifying research with the best possible benefits, generally regardless of risks.

Maximin: This involves choosing the option with best outcome among the worst possible outcomes. Maximin is a risk-averse approach because it aims to avoid the worst possible outcomes. Maximin is another relatively simple approach, but may present difficulties in making science and technology policy decisions, because it would recommend not developing a new technology if this decision could lead to the worst possible outcome. Since all technologies (and scientific ideas) can conceivably lead to good and bad outcomes, strict adherence to maximin would imply a very cautious approach to science and technology development. For GOF studies, use of maximin would involve identifying studies with risks, and choosing the least risky regardless of benefits.

Expected Utility Theory: This involves choosing the option that maximizes expected utility, where expected utility for a possible outcome = probability x utility. Expected utility theory involves a quantitative balancing of risks and benefits and is inherently a more complex process. Cost-benefit analysis in economics is a form of expected utility theory. A problem with expected utility theory is that sufficient evidence may not always be available to confidently estimate the probabilities involved in the utility calculus. When this is the case, other approaches may be appropriate. For GOF studies, use of expected utility theory would require determining quantitatively the likelihood of risks and benefits and calculating the resulting utility.

Precautionary approach: This approach involves taking reasonable measures to prevent, minimize, or mitigate risks that are significant and plausible. A measure is "reasonable" if it: 1) appropriately balances the values at stake in the risk management; 2) is proportional to nature of the risk (i.e. greater risks require stronger measures); and 3) is likely to be effective. A risk is "plausible" if there is some scientific evidence that it could occur even if the probability of the risk cannot be confidently estimated. There are many versions of the precautionary principle, including ones that are more or less risk-averse.[26,27] A precautionary approach, in general, would limit an activity unless the environment, health, or security, are clearly protected. This approach can recognize a potential problem early and prevent harm from occurring but may lead to regulatory burdens or unnecessarily limit activities. This approach might restrict potential GOF research unless the studies are demonstrated to be safe.

Permissive approach: This approach, in general, would allow an activity unless the environment, health, or security, are clearly compromised. This approach may reduce unnecessary regulatory burdens but can result in after-the-fact reaction to harms. This approach might allow certain GOF studies to proceed until they are demonstrated to entail significant risk.

Planned adaptation or risk-based approach: This approach provides a systematic way to deal with managing risks in the face of uncertainty. It involves: 1) preparation to identify the risks and regulatory gaps, including getting input from a broad range of perspectives; 2) putting measures in place to control risk based on the best information available at the time; 3) systematically gathering data and observing effects of policies; and 4) updating and revising policy as needed. An example of an adaptive approach is the life cycle approach taken by the Food and Drug Administration when making decisions about whether to approve drugs, when that includes post-market surveillance.[28] For GOF studies, this approach might entail allowing GOF studies of potential concern—or certain GOF studies—to proceed under defined conditions, then evaluating the risk-benefit landscape periodically to determine whether the GOF studies that are permitted should continue, be expanded, or be restricted.

Threshold approach: This approach would entail identifying a risk threshold beyond which, certain studies are given special attention or subject to additional scrutiny or oversight and might preclude certain studies. Implementation would involve defining or describing the studies that would require additional oversight as well as a description of what that oversight would entail. This approach would allow for the identification of studies of concern but might need to be reevaluated if the risk landscape changes and the threshold that was identified is no longer appropriate. For GOFROC, this would entail identifying the characteristics of studies involving significant risks that may not be

[26] Resnik DB. Environmental Health Ethics, New York: Oxford University Press, 2013.
[27] Munthe C. The Price of Precaution and the Ethics of Risks. Dordrecht: Springer, 2011.
[28] FDA determinations about whether a new drug is safe and effective are complex, address uncertainty, and involve ongoing monitoring to assess risks and benefits and take appropriate post-marketing actions as necessary. See: *Structured Approach to Benefit-Risk Assessment in Drug Regulatory Decision-Making*, 2013 http://www.fda.gov/downloads/ForIndustry/UserFees/PrescriptionDrugUserFee/UCM329758.pdf

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adequately managed and then stipulating further oversight or determining that they should not be conducted.

Point-source approach: This approach would involve controlling where certain studies are conducted and under what conditions. This approach would centralize certain research activities, restricting them to designated locations or facilities. For GOFROC this might involve requiring that certain studies only be conducted in facilities with certain biocontainment conditions, biosafety practices, and security measures.

The NSABB working group used ideas from a number of frameworks to inform its findings and deliberations (Sections 5 and 6). The criteria for identifying GOF research of concern (see Recommendation 1) reflect a threshold approach. The principles for guiding funding decisions for GOF research of concern entails elements from several of the decision frameworks above. For instance, an explicit call for a risk-benefit analysis (Recommendation 1, Guiding Principle 3) reflects expected utility theory, however, a strict quantitative calculation is probably not possible. The principles to guide funding decisions that call for risk mitigation and a restriction to laboratories with a demonstrated capacity to safely carry out the studies (Recommendation 1, Guiding Principles 4 and 5) incorporate elements of point-source and precautionary approaches. An adaptive approach was considered particularly attractive and appropriate for policies aimed at providing oversight of GOF research (see Recommendation 3).

4.4. Examination of the Current Policy Landscape

Many Federal agencies fund life sciences research in furtherance of their specific missions. In general, research supported by the USG is founded on the principle of scientific merit and goals of the funding agency. Multiple complementary layers of oversight are in place to manage laboratory and other risks associated with Federally-funded life sciences research. These policies are intended to provide oversight at various points throughout the research life cycle, from research conception to its publication and translation into practice. These policies include a foundation of occupational health and medicine (for laboratory and clinical workers), laboratory biosafety practices, and policies that address biosecurity risks. Below is a description of the oversight policies in place for Federally-funded life sciences research involving pathogens, with discussion of whether and how such policies apply to GOF studies. This analysis is illustrated in Figures 2 and 3 and summarized in Appendix D.

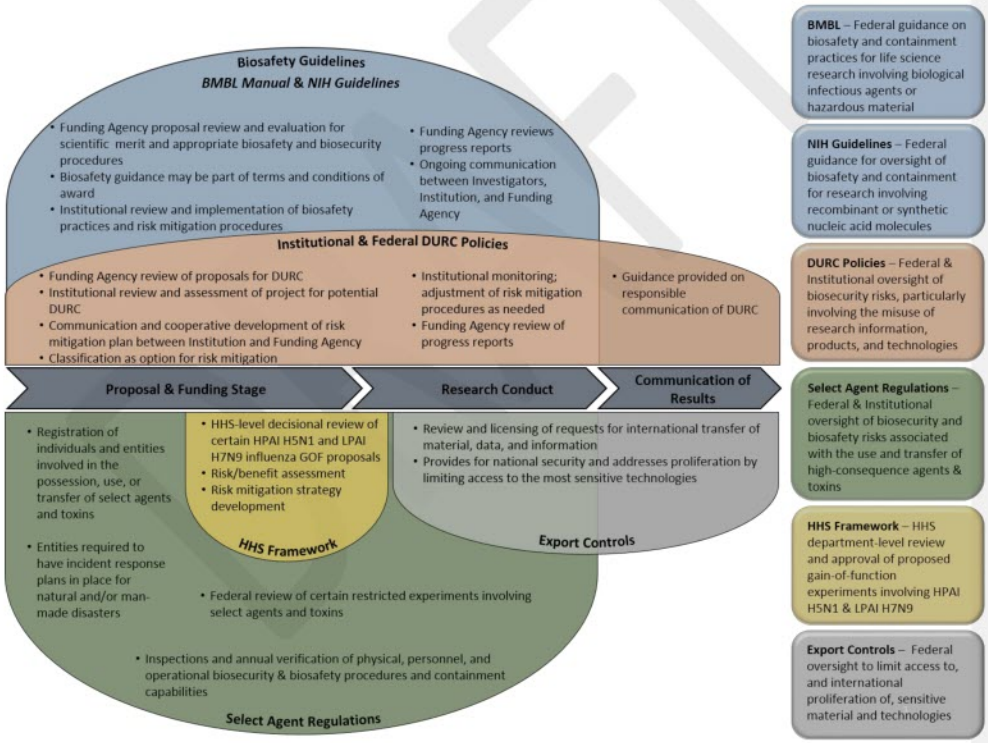

Figure 2. U.S. government oversight of life sciences research involving pathogens. Oversight policies apply at different stages and occur at different levels throughout the research life cycle. See text and Appendix D for descriptions of each policy. The policies depicted in this figure are defined by different applicability and scope requirements and therefore do not apply to all life sciences (or GOF) research projects.

Scientific Merit Review

Departments and agencies within the U.S. government fund diverse portfolios of life sciences research. Funding decisions are based on the scientific merit of a given proposal and the ability of a project to advance the agency's strategic mission. The U.S. government funds life sciences research through a variety of mechanisms including grants, contracts, and cooperative agreements. Each funding agency has its own processes for evaluating research proposals and awarding funds but, in general, proposals are subject to rigorous scientific review by Federal agency staff and often, scientific peers. NIH grant proposals, for example, undergo two levels of review. The first evaluation is by a panel of scientific peer reviewers who score proposals based on scientific merit and other criteria. The second round of review includes discussion of meritorious proposals at public meetings of advisory councils, specific to individual funding institutes and centers within NIH, to determine how proposals fit within their broader strategic objectives.

Biosafety Oversight

Oversight of pathogen research focuses first on ensuring the safe handling of biological agents through appropriate biosafety practices and containment measures, which are addressed by the *Biosafety in Microbiological and Biomedical Laboratories* (*BMBL*)[29], the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*)[30], and other documents. The BMBL and the *NIH Guidelines* provide for Federal and institutional biosafety oversight and guidance involving biosafety practices and containment features that are based on risk assessments for specific projects. Such determinations are typically made at the institutional level and are guided by Federal guidelines and policies, which are updated as necessary to provide additional guidance for research involving emerging pathogens or technologies. Biosafety is achieved by conducting research under appropriate physical and biological containment levels and employing practices that help to ensure a safe working laboratory environment.

The BMBL is a CDC-NIH guidance document that is generally considered the authoritative reference for laboratory biosafety. The BMBL provides summary statements for many bacterial, fungal, parasitic, rickettsial, viral, and other agents. These statements describe the characteristics of the pathogen, its natural mode of infection, potential occupational hazards with the agent, and recommendations for laboratory safety and containment. It also describes the fundamentals of biological containment, which includes descriptions of proper microbiological practices, safety equipment, and facility safeguards that protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory. It describes the process of biological risk

[29] Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition. http://www.cdc.gov/biosafety/publications/bmbl5/

[30] The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), November 2013. http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html

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assessment, which enables the appropriate selection of microbiological practices, safety equipment, and facility safeguards that can prevent laboratory-associated infections. It also describes occupational health, immunoprophylaxis, and principles for laboratory biosecurity. The BMBL is updated periodically to refine guidance based on new knowledge and experiences and to address contemporary issues that present new risks that confront laboratory workers and the public health.

Analysis: The BMBL does not address GOF studies *per se* but does include summary statements and biocontainment guidance for research involving various influenza strains (including contemporary and non-contemporary human, high and low pathogenic avian, swine, the 1918 influenza strain, and reassortant viruses) and SARS-CoV. MERS-CoV had not emerged at the time of the last BMBL update, but interim laboratory biosafety guidance was issued by CDC.[31]

The BMBL is not a regulatory document. U.S. funding agencies may require it be followed as a term and condition of awards but in general, compliance with the BMBL is voluntary. In addition, the BMBL provides general biosafety guidance but does not describe detailed procedures or experiment-specific containment protocols.

The *NIH Guidelines* specify the practices for safely constructing and handling recombinant nucleic acid molecules; synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules; and cells, organisms, and viruses containing such molecules. The *NIH Guidelines* apply to basic and clinical recombinant or synthetic nucleic acid research conducted at or sponsored by institutions that receive NIH funding for any such research. Compliance with the *NIH Guidelines* is typically required as a term and condition of award of funding. Other Federal agencies may also require compliance with the *NIH Guidelines*.

The *NIH Guidelines* focus on the concepts of risk assessment, risk group classification of agents based on their ability to cause disease in humans and the availability of medical countermeasures, physical and biological containment levels, practices, personal protective equipment, and occupational health. To help ensure the safe conduct of this research, the *NIH Guidelines* specifies roles and responsibilities of investigators and institutions. Institutions subject to the *NIH Guidelines* must establish Institutional Biosafety Committees (IBCs) composed of members with appropriate expertise, to review and approve such research. IBCs provide local oversight and ensure compliance with the *NIH Guidelines*. Certain higher risk experiments require review by the Recombinant DNA Advisory Committee (RAC)[32] and specific approval by the NIH Director as Major Actions. These experiments involve the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if

[31] Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Middle East Respiratory Syndrome Coronavirus (MERS-CoV) – Version 2. http://www.cdc.gov/coronavirus/mers/guidelines-lab-biosafety.html [last updated June 18, 2015]

[32] The Recombinant DNA Advisory Committee (RAC) is a federal advisory committee that provides recommendations to the NIH Director related to basic and clinical research involving recombinant or synthetic nucleic acid molecules. See: http://osp.od.nih.gov/office-biotechnology-activities/biomedical-technology-assessment/hgt/rac

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such acquisition could compromise the ability to control disease agents in humans, veterinary medicine or agriculture.

In order to continue to provide appropriate guidance for emerging pathogens or experimental approaches, the *NIH Guidelines* are updated periodically. The *NIH Guidelines* have been amended to include additional guidance for work with Risk Group 3 influenza viruses (1918 H1N1, H2N2, highly pathogenic avian influenza (HPAI) H5N1), to specify enhancements to biosafety level 3 containment, practices, and to incorporate occupational health requirements. In 2012, the *NIH Guidelines* were amended again to require further enhancements to facilities, biosafety equipment and practices, including occupational health practices, for research involving HPAI H5N1 strains transmissible among mammals by respiratory droplets.

Analysis:

The *NIH Guidelines* provide guidance on risk assessment and appropriate containment and practices for conducting research involving recombinant or synthetic nucleic acids, which would apply to most government-funded GOF research. Some IBCs also review and approve non-recombinant pathogen research; however, not all institutions require their IBCs to do so. While the *NIH Guidelines* are often used as a model of biosafety guidance by the broader scientific community, compliance is required only by institutions receiving such funding from the NIH. Therefore, some GOF studies may not be subject to the *NIH Guidelines* depending on whether the institution where the research is being conducted is subject to the *NIH Guidelines*.

The Federal Select Agent Program

Subtitle A and B of the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 requires the U.S. Departments of Health and Human Services (HHS) and Agriculture (USDA) to establish and regulate a list of select agents, biological agents and toxins that have the potential to pose a severe threat to public health and safety or animal or plant health or animal or plant products. The Select Agent Program (SAP) is administered jointly by the HHS Centers for Disease Control and Prevention and USDA Animal and Plant Inspection Service. The SAP oversees the possession, use and transfer of biological select agents and toxins. The Select Agents and Toxins List is reviewed and updated biennially. Under the select agents regulations, individuals and institutions that possess, use, or transfer any select agent are required to be registered, follow appropriate biosafety procedures, and undergo periodic inspections. Individuals must be registered with the SAP to have access to select agents or toxins, which requires that they undergo a security risk assessment performed by the Federal Bureau of Investigation (FBI). There are legal penalties for failing to comply with the select agent regulations.

In addition to the agents and toxins on the list, the select agent regulations apply to some genetic elements, including nucleic acids that are immediate precursors to infectious forms of any select agent viruses (i.e., complete positive strand RNA viral genomes), as well as some nucleic acids that encode select toxins. Select agent regulations also apply to genetically modified select agents and toxins.

Restricted experiments are described in the regulations and involve the deliberate transfer of or selection for a drug resistance trait to select agents that are not known to acquire the trait naturally. If the acquisition of resistance is to a first-line drug that could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, the restricted experiment requires special review and approval by the SAP. Some attenuated strains of select agents may be excluded from the regulations based upon a determination that the attenuated strain or modified toxin does not pose a severe threat to public, plant, or animal health or safety. The Intragovernmental Select Agent and Toxin Technical Advisory Committee serves as an advisory group to the SAP. In the wake of the recent laboratory incidents at Federal facilities involving select agents, two advisory committees have issued recommendations for ways to strengthen the Select Agent Program.[33] [34] Plans to implement these recommendations are also in place.[35]

Analysis: Studies that could be considered GOF studies are subject to oversight by the SAP if they involve pathogens on the select agent list. Researchers and institutions performing such studies must receive favorable security risk assessments by the FBI, register with the SAP, receive training on the proper procedures and practices for handling such agents, and abide by other aspects of the regulations. SARS-CoV, HPAI H5N1 influenza, and 1918 influenza viruses are select agents and GOF studies involving these pathogens are subject to oversight by the SAP. Restricted experiments that would entail conferring antiviral resistance to these viruses would require additional review and approval prior to being conducted. However, MERS-CoV is not a select agent. GOF experiments involving MERS, and other agents not included on the select agent list, would not be subject to oversight by the SAP (though they could be subject to Federal and institutional biosafety oversight). The SAP is underpinned by a regulatory requirement that applies to non-USG funded (i.e., private sector funded) pathogen research.

Federal and Institutional Oversight of Life Science Dual Use Research of Concern

The U.S. government has issued two Federal policies for the oversight of life sciences DURC. These policies focus oversight on research involving 15 high-consequence pathogens and toxins[36] that involve seven categories of experimental activity, which are projects that can be reasonably anticipated to:

1. Enhance the harmful consequences of the agent or toxin;
2. Disrupt immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification;

[33] Report of the Federal Experts Security Advisory Panel, U.S. Government, December 2014.
[34] Fast Track Action Committee Report: Recommendations on the Select Agent Regulations Based on Broad Stakeholder Engagement, U.S. Government, October 2015.
[35] Lisa Monaco and John Holdren White House Memorandum, October 29, 2015, Next Steps to Enhance Biosafety and Biosecurity in the United States. https://www.whitehouse.gov/sites/default/files/docs/10-2015_biosafety_and_biosecurity_memo.pdf
[36] The agents within the scope of the USG DURC policies are the 13 Tier 1 select agents plus HPAI H5N1 and 1918 influenza virus.

3. Confer to the agent or toxin resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies;
4. Increase the stability, transmissibility, or the ability to disseminate the agent or toxin;
5. Alter the host range or tropism of the agent or toxin;
6. Enhance the susceptibility of a host population to the agent or toxin; or
7. Generate or reconstitute an eradicated or extinct agent or toxin listed above.

Projects involving any of the 15 agents and that could be anticipated to involve any of these seven experimental effects are then determined to be DURC if they then meet the definition of DURC listed in the policy.[37]

The DURC policies outline a coordinated approach to oversight involving the Federal funding agencies and institutions that conduct such research. The policy for Federal oversight, issued in March 2012, requires Federal agencies to review proposed and ongoing research projects to identify any that constitute DURC. The policy for institutional oversight, issued in September 2014, articulates responsibilities of research institutions in identifying and managing DURC. Research institutions are to establish an Institutional Review Entity (IRE) to review research subject to the policy to determine whether any such research involves any of the seven experimental effects, and if so, whether the research constitutes DURC. IREs may review projects not specifically covered under the DURC policies but such additional reviews are voluntary.

When DURC is identified—either by a funding agency or a research institution—the funder and institution are to work collaboratively to develop a risk mitigation plan to help ensure that the research is conducted and communicated in a responsible manner. DURC risk mitigation plans are approved by the Federal funding agency and are reviewed on an annual basis by the funder and the institution. Specific risk mitigation measures may be incorporated into a term of award. Risk mitigation may involve modifying the design or conduct of the research in order to address the same scientific question in a manner that poses fewer biosafety or biosecurity risks. Other measures may involve applying enhanced biosafety or biosecurity measures, evaluating the effectiveness of extant medical countermeasures prior to proceeding with particular studies, or establishing a more frequent schedule of DURC reviews to more closely monitor the research as it evolves. It is also expected that a communication plan is established to ensure that DURC is communicated in a responsible manner. Federal funding agencies can provide advice and guidance on responsible communication, but recommendations on how to communicate research typically are not binding; ultimately, investigators and journal editors decide on how to communicate the research.

[37] The definition of dual use research of concern listed in the USG Policy for Oversight of Life Science DURC (USG, March 2012) and the USG Policy for Institutional Oversight of Life Sciences DURC (USG, September 2014) is "Life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security."

Analysis: Some of the seven experimental effects within the scope of the DURC policies could be considered GOF studies. However, GOF projects that might involve these effects are only subject to DURC oversight if the study involves one of the 15 agents listed in the policy. Only two influenza viruses are listed within the scope of these policies; SARS and MERS coronaviruses are not listed.[38] The DURC policies are also inherently subjective. While the list-based approach clearly delineates projects that are subject to oversight, the definition of DURC, and to a lesser extent, the seven experimental effects, all require significant judgment and interpretation.

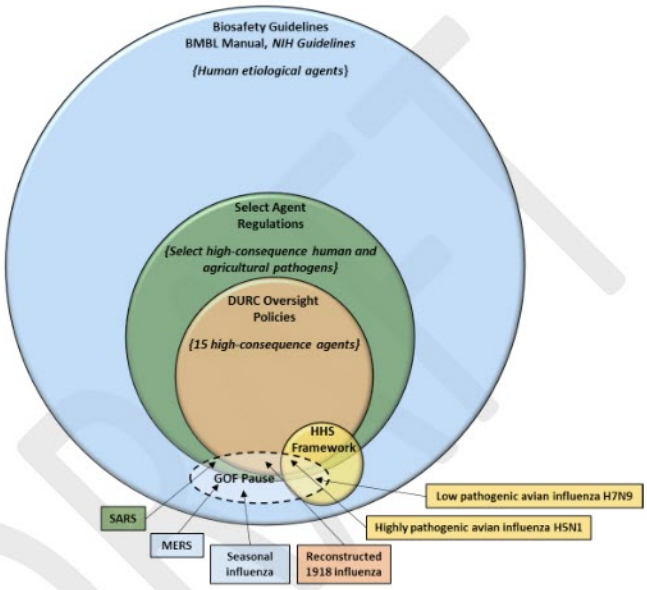

Figure 3. Comparison of the scope of different policies for the oversight of life sciences research involving pathogens. Oversight policies apply to research involving specified agents or procedures. GOF studies involving pathogens or manipulations covered under a given policy would be subject to oversight described by that policy.

Federal-Level Review of Certain Gain-of-Function Studies

The only U.S. Federal policy that specifically addresses GOF studies is the *Framework for Guiding U.S. Department of Health and Human Services Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets (HHS Framework)*, issued by the U.S. Department of Health and

[38] The policy for Federal DURC oversight requires Federal funding agencies to compile biannual inventories of projects identified as being subject to DURC oversight. As part of this process, Federal agencies have been identifying projects involving MERS and LPAI H7N9 influenza and proactively managing risks associated with those projects, as necessary.

Human Services in February, 2013. Under the *HHS Framework*[39,40] certain proposals with the potential for generating highly pathogenic avian influenza H5N1 viruses that are transmissible among mammals by respiratory droplets receive special review and approval before being funded by HHS. This policy was subsequently expanded to include review of similar proposals involving low pathogenic avian influenza H7N9 virus.[41]

Funding agencies within HHS (including NIH, CDC, and FDA) review relevant proposals for risks and benefits, and refer relevant studies to a Department-level review group, the HHS HPAI H5N1 Gain-of-Function Review Group, for advice prior to funding the proposal. The review group includes a wide range of interdisciplinary expertise from across HHS and the Federal government, if necessary. HHS reviews GOF research proposals that are subject to the *HHS Framework* and makes recommendations to HHS funding agencies about whether the study is acceptable for funding and whether additional measures may be needed to mitigate risks. HHS considers a number of factors including the following criteria, which must be met in order for a GOF study to be acceptable to receive HHS funding:

1. The virus anticipated to be generated could be produced through a natural evolutionary process;
2. The research addresses a scientific question with high significance to public health;
3. There are no feasible alternative methods to address the same scientific question in a manner that poses less risk than does the proposed approach;
4. Biosafety risks to laboratory workers and the public can be sufficiently mitigated and managed;
5. Biosecurity risks can be sufficiently mitigated and managed;
6. The research information is anticipated to be broadly shared in order to realize its potential benefits to global health; and
7. The research will be supported through funding mechanisms that facilitate appropriate oversight of the conduct and communication of the research

Analysis: The *HHS Framework* requires an explicit consideration of the risks and benefits associated with certain GOF studies prior to making a funding decision. This allows HHS to identify potential risks up front and make recommendations about risk mitigation—including consideration of alternative approaches or modifying the experimental design—at the outset. This review process also involves broader expertise including, ethical, legal, security, intelligence, and more. The criteria that must be met in order to receive funding are subject to judgment and interpretation. The scope of the *HHS Framework* is quite narrow and currently covers only projects involving two influenza viruses and that involve one specific experimental outcome (mammalian transmission by respiratory droplets); other GOF studies do not receive this pre-funding review.

[39] *A Framework for Guiding U.S. Department of Health and Human Services Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets*, U.S. Department of Health and Human Services, February, 2013. http://www.phe.gov/s3/dualuse/Documents/funding-hpai-h5n1.pdf

[40] Patterson, AP, et. al. A Framework for Decisions about Research with HPAI H5N1 Viruses. Science. 2013 Mar 1: 339(6123): 1036-1037.

[41] Jaffe H., et. al. Extra Oversight for H7N9 Experiments. Science. 2013 August 16: 341(6147):713-714.

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Reviews under this framework are conducted by a group internal to the USG. Reviewing GOF studies in a confidential setting allows for the examination of potentially sensitive scientific, proprietary, and personal information, and allows discussions that may be sensitive from a national security or public health preparedness perspective. However, such reviews do not achieve the level of transparency desired by some stakeholders and also make it difficult to independently assess the effectiveness of the review process. Finally, the *HHS Framework* was in place for less than two years when the October 2014 funding pause was enacted and only a handful of GOF projects have been reviewed to date, making it difficult to fully evaluate this policy's strengths and limitations.

In response to the funding pause[42], the National Institute for Allergy and Infectious Diseases (NIAID), within the NIH, developed a process for considering on a case-by-case basis studies that might be subject to the GOF pause. Reviews by NIAID include a detailed consideration of the science, often including a specific examination of the viral strains in question and specific experiments being proposed. NIAID begins by consulting the investigators and an internal NIAID group determines whether the projects are subject to the pause. When identifying projects subject to the funding pause, NIAID has used a fairly broad interpretation of the language set forth in the pause statement and paused, at least initially, more projects than were ultimately determined to meet the scope of the pause policy. NIAID also sought exceptions (using a mechanism provided for in the USG's moratorium statement) for projects that were deemed critical to public health or national security. In determining whether an exception to the pause might be warranted, NIAID considers the intent of the research, the availability of countermeasures, potential alternative approaches, the risks of not conducting the research, and the available mechanisms for ongoing oversight. Exceptions may only granted by the NIH Director.

Analysis: NIAID's process for identifying GOF projects that are subject to the funding pause is rigorous and serves as an example of Federal-level identification and review of GOF studies of potential concern. It includes extensive scientific review and is performed by individuals with experience reviewing projects for DURC potential. It does not involve the same expertise that is provided under *HHS Framework* reviews such as national security, ethics, or legal. Given the limited number of projects that have been examined by NIAID it is difficult to fully evaluate how effective this approach is.

Sharing and Communicating Scientific Findings and Research Products

The majority of life sciences research is conducted in academic settings and the results are communicated openly in scientific journals and public forums. For a small subset of research with national security implications, there are policies in place to restrict access to scientific information or products. Under National Security Decision Directive (NSDD) 189, dissemination of fundamental research is to remain unrestricted to the maximum extent possible and in instances where restriction is

[42] U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses, U.S. Government, October 17, 2014. http://www.phe.gov/s3/dualuse/documents/gain-of-function.pdf

necessary for national security, classification is to be the appropriate mechanism for restricting access.[43] Life sciences research that requires classification is classified at its outset and conducted in designated facilities that are equipped with the infrastructure and personnel with appropriate level national security clearances to perform the research. Retroactively classifying research that was conducted in an unclassified setting is immensely challenging and may be unfeasible.

Export controls are Federal regulations that restrict exports that have national security or foreign policy implications. Certain materials and information related to biological agents and genetic elements, vaccines, equipment, and related technologies are covered by export control regulations. Furthermore, the transfer of controlled information to a foreign national within the United States is considered to be an export to that foreign national's country. The regulations are complex but, in general, they specify which items, when shipped to which destinations, will require export licenses. Life sciences research that is openly published is not subject to export controls, but information that is withheld from publication by the investigator or research institution based on security concerns may become subject to export control regulations, and an export license may be required before that information can be shared with foreign nationals.

Most biological research activities that are subject to export controls fall under the Department of Commerce's Export Administration Regulations, which control items that have both military and civilian applications.[44] However, some might fall under the jurisdiction of the State Department's International Traffic in Arms Regulations.[45]

A number of scientific journals and families of journals have policies for identifying and reviewing manuscripts that raise biosecurity and biosafety concerns. These efforts are commendable but some have noted the challenges associated with trying to identify DURC or implement risk mitigation measures at the publication stage.[46,47] NSABB has previously developed strategies and a risk assessment tool to assist in the development of a responsible communication plan for DURC, which might include altering the content, distribution, or timing of a publication.[48] The U.S. government, in most cases, has no authority to mandate redaction, restriction, or classification of a scientific publication that it does not

[43] NSDD 189 (September 21, 1985) defines fundamental research as "basic and applied research in science and engineering, the results of which ordinarily are published and shared broadly within the scientific community, as distinguished from proprietary research and from industrial development, design, production, and product utilization, the results of which ordinarily are restricted for proprietary or national security reasons." https://research.archives.gov/id/6879779

[44] Export Administration Regulations, 15 CFR Parts 730, 734, 736, 742, 744, and 745. https://www.bis.doc.gov/index.php/regulations/export-administration-regulations-ear

[45] International Traffic and Arms Regulations, 22 U.S.C. 2778 https://www.pmddtc.state.gov/regulations_laws/itar.html

[46] Casadevall A et al. Dual-Use Research of Concern Review at American Society for Microbiology Journals. mBio 6(4):e01236-15. 2015.

[47] Atlas et. al. Journal editors and authors group statement on scientific publication and security. Science, 299:1149. 2003.

[48] Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information. NSABB, June, 2007. http://osp.od.nih.gov/sites/default/files/resources/Framework%20for%20transmittal%20duplex%209-10-07.pdf

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own or control, and the development of a mechanism for restricting communication of unclassified information to only those who require access, remain challenging and to date unsuccessful.[49]

Analysis: Once a study has been completed, it is difficult to limit the distribution of or access to the findings, particularly if the study was conducted in an open, academic environment. Oversight of DURC, and in particular GOF studies involving pathogens with pandemic potential, may be most feasible and effective if it occurs 1) upstream (i.e., during the review of proposed studies and before experiments are initiated) and 2) in an ongoing manner while the research is being conducted.

Classification may be an option for certain GOF studies, but this would entail that these studies be conducted in significantly different settings than they are conducted currently. Further, although certain GOF studies have raised concerns about whether they should be published, it is unlikely that such manuscripts would meet the criteria for classification under U.S. government classification authorities. It is conceivable that certain studies should not be undertaken at all or not published because of unanticipated findings. However, it may be very difficult to predict at the proposal stage whether findings of concern might arise during the experiment, and unanticipated findings that raise concern may be unavoidable. Individual investigators or journal editors have, on security grounds, decided to redact certain material from publication, possibly triggering export controls on the redacted material, but in general such a redaction could not be mandated by the U.S. government.

Broader U.S. Biosafety and Biosecurity Efforts

~~In p~~Parallel to the GOF deliberative process~~es~~, the USG has also initiated additional, broader reviews of biosafety and biosecurity policies and procedures following a series of laboratory incidents occurring at federal institutions in 2014 ~~**[REF needed]**~~. The Holdren-Monoco memorandum[50] called for Federal and non-Federal reviews to provide recommendations to strengthen the biosafety and biosecurity practices and oversight system for USG funded research. The memo outlined three immediate actions for Federal Agencies:

1. Conduct a comprehensive review of current biosafety and biosecurity protocols to ensure adequacy and appropriateness for today's infectious disease research
2. Inventory and document culture collections
3. Increase attentiveness throughout research community to ensure the safety of laboratory workers and the American public.

In September 2015, The White House National Security Council tasked the Federal Experts Security Advisory Panel (FESAP) to 1) identify needs and gaps and make recommendations to optimize biosafety, biosecurity, oversight, and inventory management and control for biological select agents and toxins (BSAT); 2) identify actions and any regulatory changes to improve biosafety and biosecurity; and 3) identify an approach to determine the appropriate number of high-containment U.S. laboratories

[49] Research information produced under a U.S. government grant is not considered to be owned or controlled by the Federal Government. However, under the Invention Secrecy Act, the U.S. government can nevertheless impose secrecy orders on patent applications if the publication or disclosure of the ensuing patent would be detrimental to national security.
[50] https://www.whitehouse.gov/sites/default/files/microsites/ostp/enhancing_biosafety_and_biosecurity_19aug2014_final.pdf

required to possess, use, or transfer BSAT. To obtain broad stakeholder recommendations, the National Science and Technology Council established the Fast Track Action Committee on Select Agent Regulations (FTAC-SAR). In October 2015, USG released the FESAP and FTAC-SAR recommendations[51] that address the culture of responsibility, oversight, outreach and education; applied biosafety research; incident reporting; material accountability; inspection processes; and regulatory changes and guidance to improve biosafety and biosecurity. The USG has developed a plan to implement these recommendations ~~in order to improve biosafety and biosecurity practices along with oversight~~.[52]

[51] http://www.phe.gov/s3/Documents/fesap.pdf; http://www.phe.gov/s3/Documents/ftac-sar.pdf.
[52] Implementation of Recommendations of the Federal Experts Security Advisory Panel and the Fast Track Action Committee on Select Agent Regulations, October 2015. http://www.phe.gov/s3/Documents/fesap-ftac-ip.pdf

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5. Findings

In developing the findings below (Box 2), the NSABB working group considered the results of the risk and benefit assessments, policy analysis and decision-making frameworks, discussions of ethics, and perspectives of domestic and international stakeholders.

Box 2. Summary of Findings

Finding 1: There are many types of GOF studies and not all of them have the same level of risks. Only a small subset of GOF research—GOF research of concern—entail risks that are potentially significant enough to warrant additional oversight.

Finding 2. The U.S. government has several policies in place for identifying and managing risks associated with life sciences research. There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOF research of concern could be implemented.

Finding 3. Oversight policies vary in scope and applicability, and do not cover all potential GOFROC, therefore, current oversight is not sufficient for all GOF research of concern.

Finding 4. An adaptive policy approach is a desirable way to ensure that oversight and risk mitigation measures remain commensurate with the risks associated with the research and the benefits of the research are being fully realized.

Finding 5. There are life sciences research studies, including possibly some GOF research of concern, that should not be conducted because the potential risks associated with the study are not justified by the potential benefits. Decisions about whether specific GOFROC should be permitted will entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of proposed studies but other considerations, including legal, ethical, public health, and societal values are also important and need to be taken into account.

Finding 6. Managing risks associated with GOF research of concern, like all life sciences research, requires both Federal-level and institutional oversight, awareness and compliance, and a commitment by all stakeholders to safety and security.

Finding 7. Funding and conducting GOF research of concern involves many issues that are international in nature.

~~Key~~ Finding 1: There are many types of GOF studies and not all of them have the same level of risks. Only a small subset of GOF research—GOF research of concern—entail risks that are potentially significant enough to warrant additional oversight.

As with all life sciences research involving pathogens, GOF studies entail inherent biosafety and biosecurity risks. GOF research involving the generation of pathogens with pandemic potential involves the greatest risks. A laboratory accident involving such a pathogen could potentially release a pathogen that could spread rapidly and efficiently through the human population. A laboratory pathogen with enhanced characteristics could also, if malevolently used, pose a greater threat to national security or public health than similar misuse involving a wild type pathogen. The probability that such events would occur is low but non-zero and the potential consequences are uncertain but potentially significant.

Gryphon's biosafety risk assessment identified studies involving enhanced transmissibility, enhanced pathogenicity, and evasion of immunity as entailing the highest risks for coronaviruses, seasonal influenza, and avian influenza.[53] Manipulations that increase transmissibility, increase pathogenicity, and enable a pathogen to more readily spread through the population have the greatest potential to increase risk; in some strains even a moderate increase might be a concern.

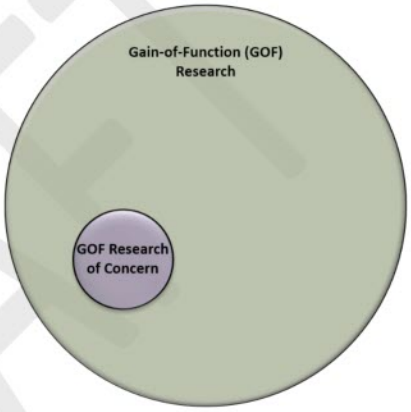

Figure 4. Conceptual categorization of GOF research involving human or animal pathogens. GOF research includes a broad range of experimental approaches, most of which do not raise significant concerns. GOF research of concern represents a small subset of all GOF research that can be reasonably anticipated to result in generation of a pathogen with pandemic potential, as described as a pathogen that is likely both highly transmissible and highly virulent in humans.

To help categorize studies based on the level of concern stemming from their associated risks, the working group has designated studies as: GOF research and GOF research of concern (GOFROC) (Figure 4). The term "GOF research" would encompass all studies ~~involving human or animal pathogens~~ whereby some characteristic of the pathogen is enhanced. The vast majority of GOF research does not raise any significant concerns; these studies do not entail novel or significant risks and are subject to ~~layers of~~ oversight to manage risks. GOF research of concern, or GOFROC, represents the small subset of studies that result in the generation of a pathogen with pandemic potential—that is, a pathogen that is highly virulent and highly transmissible, as judged by its likely ability to spread among human populations (see Recommendation 1 for more thorough description of these attributes).

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[53] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

~~Key~~ Finding 2. The U.S. government has several policies ~~cy frameworks~~ in place for identifying and managing risks associated with life sciences research. There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOF research of concern could be implemented.

Federally-funded life sciences research in the U.S. is conducted in accordance with occupational health and safety laws and regulations, the *NIH Guidelines*, the BMBL, policies for the Federal and institutional oversight of DURC, the Select Agent Regulations, export control regulations, international treaties and agreements, and other relevant policies. HHS has also developed a framework for guiding funding decisions for certain GOF studies involving H5N1 and H7N9 influenza viruses. Together, these policies aim to mitigate biosafety risks, biosecurity risks, and other risks associated with life sciences research, including many of the GOF studies that have raised concerns.

U.S. policies ~~apply~~ involve oversight and help manage risks at several points throughout the research life cycle including the proposal review, the funding decision, the time during which the research is being conducted, and at the time at which the research is being communicated. There are also numerous entities that are responsible for providing oversight, managing risks or issuing guidance, including funding agencies, institutional review and compliance committees, individual investigators, federal advisory committees, and journal editors.

While effective implementation of these policy frameworks can manage much of the risk associated with life sciences research, including the risks of some GOFROC, ~~there remains variability in how policies are applied~~some GOFROC is more thoroughly monitored than others. Additionally, ~~and~~ coverage under current policies is incomplete (e.g., GOF research funded and conducted by/within the private sector may not be covered). Institutional oversight also varies. For example, IBCs differ in capabilities and expertise, and institutional resources and cultures vary. In addition, there is limited data describing the rate and extent of laboratory accidents, near-misses, and security breaches. Little comprehensive data about these critical issues exist, and no entity is currently authorized to collect all of the desirable information that would inform risk-benefit assessments~~what would be desirable~~.

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~~Key~~ Finding 3. Oversight policies vary in scope and applicability, and do not cover all potential GOFROC, ~~therefore, current oversight is not sufficient for all GOF research of concern.~~

U.S. policies are applicable to some but not all GOFROC. Risks associated with GOFROC that do not involve select agents or pathogens subject to oversight under the USG DURC policies or the *HHS Framework*, would largely be managed at the institutional level, in accordance with guidance in the *NIH Guidelines* and BMBL. In general, GOFROC that is not conducted with U.S. government funds is not

subject to oversight by a Federal funding agency.[54] Other countries also fund and conduct life sciences research, including GOF studies, which are beyond the purview of the U.S. government as well.

~~Further~~In addition, the U.S. government's oversight policies vary. Different policies are aimed at managing different risks, and each is implemented by various Federal Departments and Agencies. This can result in redundancies as well as gaps in oversight, as the various policies have not been harmonized.

~~In addition~~Finally, full compliance with policies is essential to their effectiveness. The effectiveness of policies can be enhanced by a commitment to proper implementation and enforcement at the Federal, institutional, and individual investigator levels. This can include training, education, codes of conduct, and other mechanisms ~~that are valuable tools~~ for continuing to build a culture of responsibility ~~among researchers~~.

~~Key~~ **Finding 4. An adaptive policy approach is a desirable way to ensure that oversight and risk mitigation measures remain commensurate with the risks associated with the research and the benefits of the research are being fully realized.**

Many, but not all, of the policies that apply to GOF studies are adaptive in nature. The BMBL is updated periodically. The *NIH Guidelines* and the select agent programs are updated or revised periodically as well and both have processes for seeking external advice for informing policy development. The DURC policies and the *HHS Framework* do not have articulated mechanisms for seeking input on policy development, reviewing, or updating the policies, though both state an intention to be updated as necessary. Great uncertainty is inherent in conducting risk-benefit assessments with currently available data and ~~was identified with~~ several key parameters of the ~~effecting GOF~~ risk and benefit assessment made its interpretation challenging. Such uncertainty about risks and benefits may also make~~, and thereby~~ risk management difficult. An adaptive policy approach will facilitate refinement of GOF risk management as knowledge and experience are acquired.

~~Key~~ **Finding 5. There are life sciences research studies, including possibly some GOF research of concern, that should not be conducted ~~if~~ because the potential risks associated with the study are not justified by the potential benefits. Decisions about whether specific GOFROC should be permitted will entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of**

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[54] Research involving a select agent, whose oversight is articulated in Federal statute and requires compliance from all researchers and institutions, would be subject to Federal oversight, regardless of the funding source. Some privately-funded research being conducted at institutions that receive Federal funding for that research may also be subject to oversight under the *NIH Guidelines*, USG DURC policies, or other policies.

proposed studies but other considerations, including legal, ethical, public health, and societal values are also important and need to be taken into account.

Examples of studies that should not be conducted for ethical reasons include those that: involve human subjects who have not been provided and signed an informed consent document approved by an IRB; are anticipated to cause undue harm to a human subject; or that entail benefits that are unjustifiable in the light of the risks. For example, the development of biological weapons is unethical and has been banned by international treaty.[55]

There may be GOFROC that should not be funded on ethical grounds but it is difficult to identify or describe such studies based on general or hypothetical descriptions. An ethical evaluation of a research study would entail an evaluation of the risks and benefits, which requires a thorough understanding of the scientific details of the proposal, including its aims and any foreseeable adverse consequences ~~that could be foreseen~~. In addition, the scientific, public health, and national security landscape is dynamic. Public health needs change as new diseases emerge. Risks may arise or diminish based on the availability (or lack) of effective countermeasures. Benefits may become more or less likely to be realized based on other enabling factors, such as new scientific findings or technologies. Decisions to fund GOF studies must take into account ~~this anticipated variability~~these nuances in the risk-benefit landscape.

The NSABB did not seek to develop a list of studies that should not be conducted but rather sought to develop general principles that describe what is acceptable and not acceptable for funding. A principle-based approach to guiding funding decisions is adaptable and likely more effective ~~than a list of specific studies that should not be funded~~.

However, one example of a scientific study that should not be conducted might be the insertion of a virulence gene from an unrelated organism into the genome of a virus transmissible through the respiratory route, which would be highly unlikely to occur by natural recombination. This study, and others that involve the transfer of virulence genes between disparate microbes would appear to lack public health benefit, since the novel, laboratory-generated pathogen is unlikely to arise naturally and would therefore entail potentially significant and unnecessary risks.

~~Key~~ **Finding 6. Managing risks associated with GOF research of concern, like all life sciences research, requires both Federal-level and institutional oversight, awareness and compliance, and a commitment by all stakeholders to safety and security.**

[55] Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction. Signed at London, Moscow and Washington on 10 April 1972; entered into force on 26 March 1975. Depositaries: UK, US and Soviet governments. http://www.opbw.org/

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Biosafety and biosecurity risks associated with life sciences research are managed through engineering controls, laboratory practices, medical surveillance and support, appropriate training, and other interventions~~controls~~. However, GOFROC has the potential to generate strains with significant risks that may require additional oversight and containment mechanisms. Managing the risks associated with GOFROC in particular requires a commitment to safety and security at the Federal and institutional level that includes a strong foundation of training and a demonstrated commitment to compliance by the research institution, and the individual investigators at the local level.

~~Key~~ **Finding 7. ~~F~~~~The~~ unding and conducting GOF research of concern involves ~~scientific, biosafety, biosecurity, ethical, funding, and policy~~ many issues that are international in nature.** The potential risks and benefits associated with GOFROC are international in nature. Laboratory accidents and intentional misuse could have global consequences. The benefits of vaccine and other medical countermeasure development and disease surveillance ~~could~~ likely also have important international implications. The ~~GOFROC~~ research enterprise is international as well and ~~;~~ GOFROC is being conducted in a number of countries already. While U.S. government funding policy regarding GOFROC only directly affects domestic and international research within the purview of the U.S. government, decisions made by the United States in this area can influence GOFROC oversight policies globally.

~~Importantly~~Notably, as highlighted during~~per~~ presentations at NSABB and NAS meetings, GOF research and ~~/~~GOFROC research is ~~-~~being conducted in a number of countries and a variety of oversight mechanisms at the national and regional level are in place. In addition, a number of countries and international scientific organizations have been considering issues related to biosafety, biosecurity, dual use research, and GOFROC ~~**[REFS, or reference section in this paper]**~~.[56, 57, 58, 59, 60, 61] International perspectives are important to the development of U.S. policy in this area and global engagement is necessary to foster effective oversight mechanisms and an international culture of responsibility around research involving pathogens.

The U.S. government, often in concert with the NSABB, has been engaged with the international community for many years and continues to work with those governments and organizations now actively considering GOFROC-related issues. Presentations to the NSABB GOF Working Group ~~to~~ and at the NAS meetings have provided ~~a broad range of views and~~perspectives about the activities of foreign governments, international organizations, researchers and others have ~~been critical in informing~~greatly aided -the NSABB ~~views and its~~during the development of this report. ~~The value of international~~

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[56] *Gain-of-Function Research: Summary of the Second Symposium*, March 10-11, 2016. The National Academies of Sciences, Engineering, and Medicine. The National Academies Press, Washington DC.

[57] *Gain of function: experimental applications relating to potentially pandemic pathogens*, European Academies Science Advisory Council, EASAC policy report 27, October 2015. http://www.easac.eu/

[58] *Summary report: Dual Use Research On Microbes: Biosafety, Biosecurity, Responsibility*, December 10 – 12, 2014, Herrenhausen Palace, Hanover, Germany. https://www.volkswagenstiftung.de/dualuseresearch

[59] *France-US Bilateral Workshop on Dual Use Research Issues: Summary Report*, February 11, 2016. U.S. Department of State.

[60] Draghia-Akli, Ruxandra, Director of the Health Directorate at the Research DG, European Commission, presentation to NSABB working group, July 23, 2015.

[61] Donker, Marianne, Ministry of Health, Welfare and Sport, Netherlands, presentation to NSABB working group, July 23, 2015.

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~~engagement and continued dialogue related to DUR/DURC and GOFROC is substantial and should be continued by the USG.~~

6. Recommendations of the NSABB

Based on its analyses and findings, the NSABB ~~has formulated the following recommendations.~~working group has developed the following recommendations to the U.S. government.

Box 3. Summary of Recommendations of the NSABB

Recommendation 1. Research proposals involving GOFROC entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the Federal and institutional levels.

Recommendation 2. An external advisory body that is designed for transparency and public engagement should be utilized as part of the U.S. government's ongoing evaluation of oversight policies for GOFROC.

Recommendation 3. In general, oversight mechanisms for GOFROC should be incorporated into existing policy frameworks when possible.

Recommendation 4. The U.S. government should pursue an adaptive policy approach to help ensure that oversight remains commensurate with the risks associated with the GOFROC.

> **Recommendation 4.1.** The U.S. government should consider developing a system to collect and analyze data about laboratory safety incidents to inform GOFROC policy development over time.

Recommendation 5. The U.S. government should consider ways to ensure that all GOFROC conducted within the U.S. or by U.S. companies be subject to oversight, regardless of funding source.

Recommendation 6. The U.S. government should undertake broad efforts to strengthen laboratory biosafety and biosecurity and, as part of these efforts, seek to raise awareness about the specific issues associated with GOFROC.

Recommendation 7. The U.S. government should engage the international community in a dialogue about the oversight and responsible conduct of GOFROC.

~~NOTE: Box to be updated as Recs finalized~~

Recommendation 1. Research proposals involving GOFROC entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the Federal and institutional levels.

GOFROC entails the generation of pathogens—perhaps novel pathogens—with anticipated pandemic potential. The associated risks associated with such studies ~~generating pathogens with pandemic potential~~ are uncertain but potentially significant. It is possible that generating a laboratory pathogen with pandemic potential introduces a risk of a pandemic, albeit a low probability risk, that did not exist before that pathogen was generated. Therefore, a new, pre-funding review and approval mechanism is warranted before such studies should be undertaken. The NSABB working group proposes a conceptual approach for guiding funding decisions about GOFROC~~. This conceptual approach~~, which entails identifying GOFROC and subjecting such studies to an additional pre-funding review and approval process. The attributes that describe~~ing~~ GOFROC, the principles that should guide funding decisions for GOFROC, and the steps in a proposed ~~features of the proposed~~ review/approval process for GOFROC are described below.

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Identifying GOF research of concern

GOFROC is research that can be reasonably anticipated to generate a pathogen with pandemic potential. Determining whether a proposed research project is likely to ~~generate a pathogen with pandemic potential, as described by the attributes below,~~do so will entail uncertainty and will require scientific and other expert judgment.

To be considered GOFROC, the research must, in a single step or over the course of manipulations, be reasonably anticipated to generate a pathogen with both of the following attributes:

i. **The pathogen generated is likely highly transmissible and likely capable of wide and uncontrollable spread in human populations.** To be considered "highly transmissible" the pathogen must be judged to have the capacity for sustained secondary transmission among

humans, particularly but not exclusively by the respiratory route. Such a determination might be informed by data describing human infections by naturally-circulating isolates of the pathogen or studies in relevant experimental mammalian models that serve as a proxy for human infections. To be considered "capable of wide and uncontrollable spread in human populations" it must be judged that there would be limited options for controlling the spread of the pathogen other than patient isolation or quarantine. Such a determination might be made, for instance, if humans lack population immunity to the resulting pathogen, if the pathogen would evade or suppress the human immune response, if the pathogen would be resistant to medical countermeasures, or if existing countermeasures would be unavailable globally in sufficient quantities.

AND

ii. **The pathogen generated is likely highly virulent and likely to cause significant morbidity and/or mortality in humans.** To be considered "highly virulent" the pathogen must be judged to have the capacity for causing significant consequences in humans, such as severe disease and/or a high case fatality rate. Such a determination might be informed by data describing human infections by naturally-circulating ~~isolates~~ strains of the pathogen or studies in relevant experimental mammalian models that serve as a proxy for human disease.

Any study involving the generation of a pathogen exhibiting the two attributes above would be considered GOFROC. However, it is generally anticipated that the following types of activities would not be considered GOFROC:

- Studies to characterize the virulence and transmission properties of circulating pathogens
- Surveillance activities, including sampling and sequencing
- Activities associated with developing and producing vaccines, such as generation of high-growth strains

Importantly, a proposed experiment need not involve the simultaneous enhancement of both phenotypes. For instance, research involving a naturally-occurring pathogen that exhibits one of the above attributes would be considered GOFROC if a study were anticipated to confer the second attribute to the agent (while retaining the first attribute). Other studies may generate a pathogen with the above attributes after a series of manipulations that enhance the phenotypes separately but ultimately result in a pathogen with both attributes. Any route of experimentation that is anticipated to ultimately generate a pathogen that exhibits both of the characteristics above would be considered GOFROC and should be reviewed carefully before it can be funded.

Appendix B describes examples of studies that would and would not be considered GOFROC. These examples are provided as guidance and are described in general terms. A more detailed consideration of the specific characteristics of a pathogen in question as well as the proposed experimental manipulations would be required to determine whether a research proposal is ~~likely to entail~~ GOFROC.

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~~The specific nature of a given pathogen or manipulation could alter the determination about whether or not a study constitutes GOFROC.~~

Pre-funding review and approval of GOF research of concern

Proposals anticipated to involve GOFROC should be subject to additional review prior to making a funding decision and a high degree of Federal oversight throughout the course of the research, if funded. The working group has developed principles that should guide the review and funding of these proposals. There should be a high degree of confidence that a study will be conducted in accordance with these principles before determining whether the proposal is suitable for funding. Studies that cannot be or are not anticipated to be conducted in accordance with the principles below should not be funded.

Principles for guiding review and funding decisions

The NSABB working group has developed the following principles ~~below~~ to guide funding decisions regarding GOFROC. Only projects that are in line with all of the following principles should be considered acceptable for funding. The principles below are intended to embody the substantive ethical values described in section 4.2 and the process of applying these principles would involve scientific, security, ethical, and other considerations.

i. **The research proposal has been evaluated by a peer-review process and determined to be scientifically meritorious, with high impact on the research field(s) involved.** If GOFROC is to be funded and conducted it must first and foremost address a valuable scientific question or public health need.

ii. **The pathogen ~~with pandemic potential~~ that is anticipated to be generated must be judged, based on scientific evidence, to be able to arise by natural processes.** It is difficult to predict the types of pathogens that can or will emerge in nature. Nevertheless, before a pathogen with pandemic potential is generated through laboratory manipulations it is essential to consider whether such a pathogen could arise in nature. GOFROC may be permissible if the study were to generate a pathogen that is anticipated to arise in nature or if the study were to provide insight into natural evolutionary processes. GOFROC would not be permissible if it were to generate a laboratory pathogen that is highly unlikely to arise in nature ~~(e.g., combining virulence factors of two viruses that are highly unlikely to recombine in nature).~~

iii. **An assessment of the overall potential risks and benefits associated with the project determines that the potential risks as compared to the potential benefits to society are justified**. Prior to funding GOFROC, the anticipated risks and potential benefits must be carefully evaluated~~considered~~. In general, the potential benefits associated with a research project should be commensurate with or exceed the presumed risks. Projects involving

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significant risks and little anticipated benefits are ethically unacceptable and should not be funded. If the potential risks appear high, the possible benefits should also appear high. Risks should be managed and should be mitigated whenever possible. The extent to which risks can be mitigated should factor into the assessment.

iv. **There are no feasible, equally efficacious alternative methods to address the same scientific question in a manner that poses less risk than does the proposed approach.** Alternative approaches must be explored and critically examined before funding GOFROC. It is possible that the proposed experimental approach that raises concern is the only feasible approach for addressing the scientific question at hand. In other cases, modifications of the experimental design, ~~selection~~ use of attenuated or other strains that pose fewer risks to humans, or different approaches with less risk that may provide the same or very similar information may be feasible. Lines of experimentation that entail less risk should be pursued whenever possible.

v. **The investigator and institution proposing the research have the demonstrated capacity and commitment to conduct~~arry~~ it ~~out~~ safely and securely, and have the ability to respond rapidly and adequately to laboratory accidents and security breaches.** Prior to funding, the risks associated with proposed GOFROC must be identified and assessed, and clear, realistic plans for managing risks should be developed. In order to manage risks associated with GOFROC, an institution must have adequate facilities, resources, security, trained personnel, administrative structures, ongoing occupational health and safety monitoring procedures, relationships with local public health authorities and first responders, and the ability to adapt to unanticipated situations~~results~~ by increasing containment or adding additional safety or security features. In addition to adhering to standards of compliance, an institution (and the investigators proposing the study) should have a demonstrated commitment to laboratory safety and security, scientific integrity, and the responsible conduct of research. The researchers and institution should be committed to a culture of responsibility, perhaps demonstrated through adherence to a formal code of conduct or other measures.

vi. **The ~~benefits~~ results of the research are anticipated to be broadly shared in compliance with applicable laws and regulations in order to realize its potential benefits to global health.** Prior to funding GOFROC, consideration should be given to the type of research-related information and products that are likely to be generated. The research-related information and products are expected to be shared appropriately and a responsible communication plan should be developed at the outset, as appropriate. NSABB[62] and the U.S. government[63] have ~~developed~~ issued guidance for developing communication plans for dual use research of concern that include consideration of the content, timing, and distribution of the research information.

[62] Appendix 5, *Proposed Framework for the Oversight of Dual Use Research Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information*. National Science Advisory Board for Biosecurity, June, 2007.
[63] Section E, *Tools for the Identification, Assessment, Management, and Responsible Communication of Dual Use Research of Concern: A Companion Guide to the United States Government Policies for Oversight of Life Sciences Dual Use Research of Concern*. U.S. government, September, 2014.

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vii. **The research will be supported through funding mechanisms that allow for appropriate management of risks and ongoing Federal and institutional oversight of all aspects of the research throughout the course of the project.** GOFROC should be funded through mechanisms to ensure that appropriate biocontainment conditions are utilized, adequate biosecurity precautions are in place, and that the data and materials generated will be shared appropriately. The funding mechanism should allow for modification of required mitigation and oversight features, as well as research objectives ~~the implementation of additional risk mitigation measures to be~~ required during the course of the research, if needed.

viii. **The proposed research is ethically justifiable.** Determinations ~~about~~ of whether proposed GOFROC should be undertaken ~~will~~ involves value judgments to assess the potential risks and benefits and to determine whether any potential risks are justified. Non-maleficence, beneficence, justice, respect for persons, scientific freedom, and responsible stewardship are among the values that should be considered when ultimately making decisions about whether to fund GOFROC.

Description of the Review Process for Proposals Involving GOF Research of Concern

NOTE: Previous Recommendation 5.1 ("Points to consider guidance") was integrated into this section.

The NSABB proposes the following conceptual approach for guiding funding decisions about GOFROC (Figure 5). Review of research projects that may involve GOFROC would involve five steps:

1. Investigators and research institutions identify proposed GOFROC, as described by the two attributes for identifying GOFROC.
2. Funding agencies identify or confirm proposed GOFROC.
3. A Department-level Federal panel with diverse expertise reviews proposals involving GOFROC to determine whether proposals~~it~~ meet~~s~~ the 8 principles for guiding funding decisions and make recommendations as to whether the proposed research is acceptable for funding.
4. Funding agencies make a funding decision, ~~and~~ if funded, establish risk mitigation plans and issue the funding award with appropriate terms and conditions ~~and other conditions if the GOFROC is determined suitable for funding~~to help ensure ongoing oversight.
5. Investigators and institutions conduct the research in accordance with applicable Federal and local oversight policies and employ any necessary additional mitigation strategies. Federal agencies provide oversight to ensure adherence to established risk mitigation plans and funding terms.

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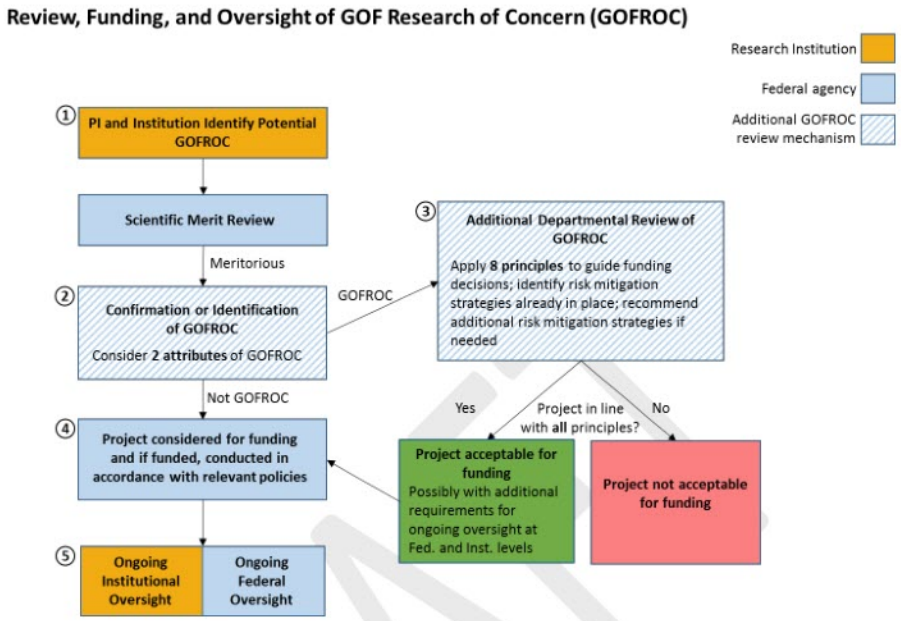

Figure 5. Proposed conceptual approach for guiding funding decisions for GOF research of concern.

Investigators and institutions identify GOFROC. Prior to submission of an application for funds, investigators and research institutions should identify possible GOFROC and submit with the research proposal any relevant information such as biosafety, biosecurity, or local public health response plans, descriptions of facilities available, a justification for the proposed approach that considers possible non-GOFROC alternatives that may be equally efficacious, and a discussion of the value and potential benefits of the proposed research. Identification of possible GOFROC should not affect a subsequent Federal scientific merit review either positively or negatively.

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A need for guidance to investigators and institutions. The U.S. government should develop a "Points to Consider" document to provide guidance to investigators and institutions when preparing research proposals that may involve GOFROC. Such a document would describe to investigators any requirements for proposals involving GOFROC and provide guidance on the type of information that should be included in a proposal to facilitate its review. This document should be reviewed and updated as necessary. **NOTE: This para is formerly recommendation 5.1. As discussed, it was moved to the more logical location here, but it is no longer specified as its own a recommendation. Is this acceptable?**

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Department-level review of GOFROC. After the standard agency scientific merit review process, proposals that are determined to be scientifically meritorious and likely to be favorably considered for funding would also be reviewed by the funding agency to determine if they constitute GOFROC, as defined by whether the proposal can be anticipated to generate a pathogen that is highly transmissible and highly virulent, as described by the ~~exhibiting the~~ two attributes above. Prior to being determined acceptable for funding, proposals identified by a funding agency as involving GOFROC would require an additional, higher level, Departmental review. If a proposal does not involve GOFROC, it would proceed along the normal pathway for further evaluation and funding decisions.

The additional review of proposals involving GOFROC would ~~be to~~ determine whether the proposed research aligns with the 8 principles to guide funding decisions. Applying these principles will help to ensure that the GOFROC is scientifically and ethically acceptable, that the risk-benefit balance is favorable, that alternative approaches are explicitly considered, and that the research can be performed safely and securely. It is envisioned that the additional review of proposals involving GOFROC would involve diverse, multidisciplinary expertise including scientific, public health, biosafety, national security and intelligence, legal, bioethics, and other perspectives. To the extent possible, the review process should be efficient, transparent, well-documented, and adaptive. In addition, the process should be structured to avoid real or apparent conflicts of interest and to provide consistency across Federal agencies that might fund GOFROC. It is also envisioned that research institutions proposing the GOFROC might be asked for and would have an opportunity to provide any additional information that might be necessary for a thorough and substantive review of the research proposal.

Funding decision and risk mitigation. During the course of the Department-level review the relevant risk management plans should be critically evaluated and additional risk mitigation measures may be ~~deemed necessary~~recommended in order for GOFROC to be considered acceptable~~funded~~. A satisfactory risk management plan would entail appropriate biocontainment facilities and biosafety practices, appropriate standard operating procedures and administrative controls, occupational health and safety programs and security systems~~features aimed at~~ for protecting laboratory strains and reagents and promoting personal reliability. Some or all of the additional risk mitigation measures listed in Box 4 may also be recommended~~equired~~. These and ~~A~~a variety of additional measures could be required as a condition of funding ~~such as more frequent institutional and Federal reviews of progress, site inspections, prohibition of adding new GOFROC experiments without approval, requirements to report unanticipated results, and/or Federal review of communication plans.~~ .

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NOTE: Box 4 was moved from its previous position in Recommendation 3 below because it seems to fit more naturally here. Is this acceptable?

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Box 4. Additional risk mitigation measures to be employed, as appropriate, for GOF research of concern.

Risk mitigation features that should be considered prior to funding GOFROC may include requirements to:

- Provide additional training to researchers
- Enhance biosafety practices or features, as dictated by the specific strains and proposed manipulations
- Enhance security measures around strains, reagents, notebooks, and personnel
- Prohibit certain additional GOFROC experiments without prior approval
- Treat the research as if subject to the USG DURC policies, if it is not already
- Conduct more frequent institutional biosafety and biosecurity reviews of the research
- Conduct more frequent progress reports and discussions with Federal funding agency staff, particularly about unanticipated results that may raise concerns
- Conduct periodic site inspections/evaluations if not already required
- Identify certain experimental outcomes that would trigger a re-evaluation of the risks and benefits prior to proceeding with a study
- Develop a responsible communication plan, specifically, including a description of biosafety and biosecurity practices
- The institution to be in regular communication with local law enforcement and public health officials
- Conduct bioethics consultations at the local and Federal level throughout the lifecycle of the research
- The investigators to develop and/or adhere to an appropriate code of conduct

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Ongoing oversight. Finally, throughout the course of the funding, both Federal and institutional oversight are critically important and the project should be carefully monitored to ensure that required conditions are met, that the principles guiding the decision to fund are still satisfied, and that any changes, significant developments, and publication/communication plans are discussed and addressed in a timely manner.

NOTE: NIH and WG co-chairs favored placing the FACA recommendation as a stand alone Recommendation 2. It was suggested that this rec be built into Rec 1 and Figure 5 but in doing so, it diminished the strength of this important recommendation and also confused the role of the FACA committee. Is this acceptable?

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Recommendation 2. An external advisory body that is designed for transparency and public engagement should be utilized as part of the U.S. government's ongoing evaluation of oversight policies for GOFROC. An external advisory body that is designed for transparency and public engagement should be utilized as part of the U.S. government's ongoing evaluation of oversight policies for GOFROC. An external advisory mechanism, such as a committee governed by the Federal Advisory Committee Act[64], would allow for an independent examination of the U.S. government's policies for reviewing, funding, and conducting GOFROC. Such a group could evaluate the additional review and funding processes for GOFROC to understand how decisions were made, identify challenges to implementing the policy, and provide recommendations, as needed. Importantly, this mechanism would also provide transparency, promote public engagement, and would facilitate continued dialogue about GOFROC. The NSABB is one such body that is well-suited to address this task.

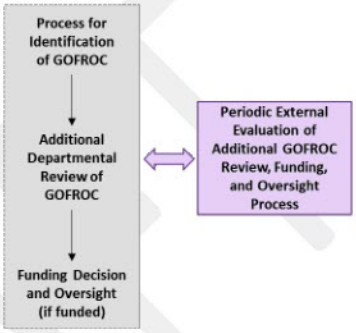

Figure 6. Independent evaluation of policies for the review, funding, and oversight of GOFROC. NOTE: Fig. 6 has not been discussed by WG yet ~~and likely requires revision~~. See also the alternate version of Figure 5, separate slide.

Recommendation 3. In general, oversight mechanisms for GOFROC should be incorporated into existing policy frameworks when possible ~~but additional risk mitigation may need to be utilized when there are gaps in coverage~~.

Any additional oversight of GOFROC should be built into existing mechanisms rather than having the U.S. government develop a novel ~~regime~~ policy specific to GOFROC. Adapting or harmonizing current policies is preferable to developing entirely new oversight frameworks or wholly new approaches to manage the risks associated with these studies. There are precedents for additional Department-level pre-funding review of certain GOF studies (i.e. *HHS Framework*) as well as mechanisms for higher-level

[64] *Federal Advisory Committee Act*. http://www.gsa.gov/portal/content/100916

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review and approval of certain studies (i.e., Major Actions, under the *NIH Guidelines*; restricted experiments, under the Select Agent Program). There are also mechanisms for continual Federal-level monitoring of biosafety and biosecurity risks for individual projects (i.e., USG Policy for Federal Oversight of DURC, select agent programs) and established mechanisms for ongoing institutional oversight (i.e., IREs under the USG Policy for Institutional Oversight of Life Sciences DURC; IBCs under the *NIH Guidelines*). Wherever possible, these mechanisms should be employed to ensure the initial and ongoing oversight of GOFROC.

Importantly, not all GOFROC would necessarily be subject to the entire suite of U.S. oversight policies. For instance, experimental manipulations with pathogens not included in the USG policies for DURC oversight or on the select agent list could conceivably generate a pathogen with pandemic potential. Additional oversight measures may need to be stipulated at the time of funding for proposals involving potential GOFROC that are not subject to a particular policy that is deemed necessary. For instance, specific, enhanced containment practices may be required or a project may require ongoing monitoring for DURC potential at the Federal and institutional level. Box 4 describes a number of potential risk mitigation measures that may be required for GOFROC that could potentially be implemented by leveraging existing policy frameworks.

Recommendation 4. The U.S. government should pursue an adaptive policy approach to help ensure that oversight remains commensurate with the risks associated with the GOFROC. The risk/benefit profile for GOFROC may change over time and should be re-evaluated periodically to ensure that the risks associated with such research are adequately managed and the benefits are being realized. An adaptive approach to the oversight of GOFROC would entail the continual evaluation of the risks and benefits associated with the research as well as the burdens and effectiveness of the additional proposal review process and ongoing oversight measures. An adaptive approach would allow policymakers to learn from experience and update policies accordingly as the risk/benefit landscape changes. For instance, the risks associated with a research proposal or project may change if newly developed countermeasures become available or if new information emerges to clarify certain risks or enable certain benefits.

Recommendation 4.1. The U.S. government should consider developing a system to collect and analyze data ~~associated with~~about laboratory -safety incidents to inform GOFROC policy development over time ~~for GOFROC~~. Examining such data would provide a better understanding of the risks, inform future risk assessments, and allow for the refinement of oversight policies over time.

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Recommendation 5. The U.S. government should consider ways to ensure that all GOFROC conducted within the U.S. or by U.S. companies be subject to oversight, regardless of funding source. GOFROC that is funded by the U.S. government or through private funding sources should be subject to

equivalent oversight to ensure that the associated risks are adequately managed. The U.S. government should consider providing oversight not only as a term and condition of a funding award but also via other mechanisms that would enable oversight of all relevant research activities, regardless of the funding source. ~~Section 4.4 of this report examines the scope and applicability of established policy frameworks relevant to the funding and oversight of GOFROC. Of these, only the Select Agent Program is underpinned by legislation and regulation that apply to pathogen research regardless of funding.~~ ~~NOTE: The last two sentences were requested by the WG. They are true statements, but calling out the SAP raises several questions. WHY is it called out? Is NSABB suggesting GOFROC oversight should be part of the SAP? Are they suggesting a need for legislation? Are they suggesting other oversight mechanisms are inadequate or should be expanded? Are these last sentences needed or can they be modified?~~

Recommendation 6. The U.S. government should undertake broad efforts to strengthen laboratory biosafety and biosecurity and, as part of these efforts, seek to raise awareness about the specific issues associated with GOFROC. Current discussions about GOFROC are related to broader domestic and international discussions about laboratory safety and security. A "~~T~~top ~~D~~down" approach to managing the risks associated with GOFROC through Federal policies and oversight is appropriate. However, top-down approaches alone, in the form of Federal and/or institutional policies and leadership, will likely not be sufficient~~, to fully address the associated risks~~. It is also critical to have adequately trained personnel that values safe and secure laboratory environments for conducting GOFROC. Therefore, it will also be important to facilitate a "~~B~~bottom ~~U~~up" approach whereby scientific ~~and institutional~~ leaders and professional societies, as well as research staff involved in the design and conduct of GOFROC, are educated about biosafety, biosecurity, and the responsible conduct of their research. The U.S. government should engage the research community with the goal of promoting a culture of responsibility, or "citizenship," whereby all participants in the research enterprise have a sense of shared responsibility ~~for its continued beneficial contribution~~. Such a culture would incorporate and stress the values of safety, security, and compliance, and work to promote public trust in the scientific enterprise. For GOFROC, a combination of mandated and voluntary oversight and risk mitigation measures would be of great importance.

Recommendation 7. The U.S. government should engage the international community in a dialogue about the oversight and responsible conduct of GOFROC. Life sciences research is a global endeavor that continues to grow as more countries invest in their research capacities and as scientists move and collaborate across national boundaries. Life sciences research enables biomedical breakthroughs, pandemic preparedness, public health response efforts for emerging infectious diseases, and also provides an important economic driver. As more investigators undertake research involving pathogens, however, the associated risks become more likely to have international implications. The risks

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associated with GOFROC are especially international in nature since laboratory accidents or the deliberate misuse of pathogens with pandemic potential could have global consequences. Laboratories anywhere can undertake GOFROC and publications in the open scientific literature ~~could~~ may enable others to generate pathogens with pandemic potential.

NSABB has benefitted greatly from the extensive input into its deliberations by experts representing foreign governments, international organizations, academia, and others during ~~from~~ presentations and comments at its meetings and the NAS conferences ~~by experts representing foreign governments, international organizations, academia, and others. This input has informed NSABB recommendations.~~ .

The U.S. government should continue to engage the international community on issues related to dual use research, including policies, oversight mechanisms, science, research conduct, biosafety, biosecurity, containment, publication, funding, and bioethics. These issues are important in general and, especially, as they are related to GOFROC. The U.S. government's international engagement efforts should seek to promote a global scientific culture of responsibility and enhance the quality, legitimacy and effectiveness of oversight processes.

The U.S. government should build these efforts on the substantial international engagement activities that it and the NSABB have carried out since the NSABB was established. Such efforts have included three international roundtable meetings on dual use research issues, a series of DURC-focused webinars focusing on different global regions, and an international consultative workshop on GOF issues[65]. In addition, the U.S. National Academy of Sciences and the European Academies Science Advisory Council have been engaged in the recent policy debates involving GOF studies and may be well positioned to continue the international dialogue on the issue in coordination with national governments and relevant international organizations. The USG is encouraged to participate in such activities.

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[65] Information about these meetings and activities, including agendas, summaries, and archived videocasts, can be found on the NSABB website at: http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/nsabb/nsabb-meetings-and-conferences/international-engagement

7. Appendices

~~Note: Appendices have been updated but are not complete.~~

Appendix A. Detailed Description of NSABB Deliberations

NSABB Deliberations

The NSABB established two working groups to accomplish the two portions of its charge, which were to result in discrete work products.

- **Deliverable 1.** A report conveying NSABB's advice on the design, development, and conduct of the risk and benefit assessments.
- **Deliverable 2.** A report conveying NSABB's formal recommendations on the conceptual approach to the evaluation of proposed GOF studies.

DELIVERABLE 1: ADVISING ON THE RISK AND BENEFIT ASSESSMENTS

The first NSABB working group was tasked with advising on the design and conduct of the risk and benefit assessments. The group met between December 2014 and April 2015 and consisted of 13 NSABB voting members as well as non-voting *ex officio* members and other *ad hoc* members from Federal agencies. (Appendix A). The group convened by telephone conference calls and held a one-day in-person meeting.

The working group developed a draft *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*, which was presented to the full NSABB, which was developed further based on input from all Board members, and ultimately approved by the full Board on May 5, 2015. The recommendations in this framework were intended to inform the NIH as it guided the work of Gryphon Scientific in its risk and benefit assessments. The aim of the NSABB's framework was to help generate risk and benefit assessments that would provide information that would allow the NSABB to make sound, evidence-based recommendations.

The NSABB's framework describes: principles that should underpin the risk and benefit assessments; pathogens, pathogen characteristics, and types of GOF experiments and phenotypes that should be examined; the types of risks and benefits that should be analyzed; scenarios, conditions, and events to be examined; and approaches and methods that should be considered when analyzing risks and benefits. In order for the risk and benefit assessments to be grounded in scientific data and evidence, the assessments needed to focus on specific pathogens, experimental manipulations, and scenarios whose risks and benefits could be modeled and analyzed. The NSABB recommended that the risk and benefit assessments focus on studies involving influenza viruses (seasonal strains, as well as high and low pathogenic avian strains) and SARS and MERS coronaviruses. Given that most pandemics are associated with respiratory transmission, pathogens capable of airborne transmission were considered to be of most acute concern. NSABB recognized that the risk and benefit assessments would provide

information specific to the pathogens and scenarios that were examined, but intended that the assessment would generate information that could be more broadly interpreted and applied. Thus, NSABB's recommended approach to the risk and benefit assessments was intended to align with the USG's October 2014 statement, which states that while "gain-of-function studies that fall within the scope of research subject to the funding pause will be a starting point for deliberations, the suitability of other types of gain-of-function studies will be discussed."

DELIVERABLE 2: RECOMMENDATIONS ON A CONCEPTUAL APPROACH FOR EVALUATING PROPOSED GOF STUDIES

The second NSABB working group was tasked with developing draft recommendations on the conceptual approach for the evaluation of proposed GOF studies. The group met beginning in June 2015 and remains active the time of this writing. The working group consists of 18 NSABB voting members as well as non-voting *ex officio* members and other *ad hoc* members from Federal agencies. (Appendix A). The group convened by telephone conference calls and met twice in person.

In addition to the working group's primary task of developing draft recommendations, it continued to provide input on the conduct of the risk and benefit assessments. The working group also received periodic status updates on the risk and benefit assessments from NIH and Gryphon, as well as reports on the commissioned ethics analysis by Dr. Michael Selgelid, examined draft work products, and reported back to the full NSABB.

In developing draft recommendations on a conceptual framework for evaluating proposed GOF studies, the working group structured its deliberations into three phases.

Phase I. Policy examination, research, and information gathering
Phase II. Interpretation, analysis, and synthesis of information and results
Phase III. Development of recommendations

In Phase I the working group sought to 1) identify and examine the information necessary to inform development of recommendations and 2) begin to identify principles that should guide the development of NSABB recommendations. The working group began its deliberations by considering the topic areas discussed at the NSABB meeting in May 2015, which included examination of relevant U.S. and international policy and consideration of broader perspectives such as those from funding agencies, national security experts, journal editors and scientific publishers, ethicists, and others. The working group held an in-person meeting to consult with experts on many of these topics. The working group also examined a number of published GOF studies and discussed how current policies might apply to such studies to provide oversight and risk mitigation.

During Phase II the working group focused on translating information about risks and benefits as well as ethics into decisions and recommendations. It examined how current policies apply to GOF studies and

began to develop preliminary observations and findings. The working group discussed the ethical issues associated with funding and conducting GOF studies, particularly noting the values and ethical decision-frameworks that might be applied to policy decisions about GOF studies. The working group also developed analytic tools to assist it in systematically analyzing the results of the risk and benefit assessments. In November 2015, the working group began receiving briefings from Gryphon Scientific conveying the results of the risk and benefit assessments, as well as reports on ethics from Dr. Selgelid. The group sought to identify GOF studies that might raise particular concerns and may require additional oversight or consideration prior to being funded.

In Phase III, the working group developed its draft recommendations, based on its analysis of the risk and benefit assessments and the ethics report and consideration of all other information and perspectives that were examined.

Deliberations by the Full NSABB

The full NSABB convened times 5 times between October 2014 and January 2016. At these meetings the NSABB working groups provided progress updates and the full Board deliberated the issues further, consulted with various experts, and sought public feedback. Public comments made at NSABB meetings and delivered to the NSABB in writing were carefully considered by the Board during its deliberations. The articles, resources, and stakeholders consulted by the NSABB and its working groups throughout this process are listed in Appendix D.

On November 25, 2014, NSABB voted to approve a statement conveying to the USG concerns it heard regarding the implementation of the funding pause for certain GOF studies.[66] On May 5, 2015, NSABB voted to approve its *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*.[67] This working paper was shared for discussion by the full NSABB on January 7 & 8, 2016.

Role of the National Academies in the Deliberative Process

The National Academies play a critical role in the ongoing deliberative process. The National Research Council and the Institute of Medicine (now National Academy of Medicine) have been asked to convene two forums to engage the life sciences community and to solicit feedback from scientists, the public, and other stakeholders. These forums are to involve discussion of principles important for the design of risk and benefit assessments of GOF research and of NSABB draft recommendations.

[66] Statement of the National Science Advisory Board for Biosecurity Regarding the USG Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses. National Science Advisory Board for Biosecurity, November 25, 2014. http://osp.od.nih.gov/sites/default/files/resources/Final%20NSABB%20Funding%20Pause%20Statement_12-12-14_0.pdf

[67] http://osp.od.nih.gov/sites/default/files/resources/NSABB_Framework_for_Risk_and_Benefit_Assessments_of_GOF_Research-APPROVED.pdf

The first National Academies workshop was held on December 15 & 16, 2014 and focused on the potential risks and benefits associated with GOF studies, ways to assess risks and benefits, strengths and limitations of risk-benefit analyses, and the ethical and policy implications associated with funding and conducting GOF studies that have raised concerns.[68] The discussions at this meeting directly informed the development of NSABB recommendations for conducting the risk and benefit assessments and its subsequent deliberations. In particular, the discussions about the potential risks and benefits associated with GOF studies informed NSABB's recommendations for the types of risks and benefits that should be analyzed by Gryphon Scientific. A common theme at this National Academies meeting was also that the term "gain-of-function" is too broad and that in fact, only a subset of GOF studies truly raise concerns. NSABB applied this insight in its subsequent analysis of the risk and benefit assessments by seeking to identify the subset of GOF studies that raised significant or unique concerns. Finally, the legal and policy discussions that were initiated at this meeting prompted to the NSABB to explore these topics, as well as ethical issues, further.

The second National Academies meeting was held on March 10 & 11, 2016 and included a discussion of the completed risk and benefit assessments and NSABB's preliminary findings and draft recommendations. NSABB's proposed attributes for identifying GOFROC were a major discussion point at this meeting, which resulted in NSABB refining and clarifying these attributes. In addition, there was significant discussion about the desirability of an adaptive policy approach, the need for data to inform policy decisions, and the role that a Federal advisory committee might play in evaluating GOFROC or GOFROC policy. This meeting also had a significant focus on international issues and perspectives, with specific discussion of ongoing and potential future international activities in this area. ~~**NOTE: This is being expanded slightly to reflect discussion from NAS.**~~

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The Risk and Benefit Assessments of GOF Studies

NIH commissioned Gryphon Scientific to perform a formal risk and benefit assessments to provide the NSABB with qualitative and quantitative information about the risks and benefits associated with conducting certain GOF studies. Dr. Rocco Casagrande, the principal investigator for the study, presented to the NSABB on May 5, 2015 an overview of Gryphon's approach to conducting the risk and benefit assessments, which included a quantitative biosafety risk assessment, a semi-quantitative biosecurity risk assessment, and a qualitative benefit assessment. Prior to voting to finalize its *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*, NSABB discussed with Dr. Casagrande its draft recommendations and how Gryphon's proposed approach aligned with NSABB's proposed recommendations. In June 2015, Dr. Casagrande presented and discussed a more detailed work plan with the NSABB working group. Over the course of the study, the NSABB working group received occasional progress reports from Gryphon and NIH staff, and were provided draft sections of the risk and benefit assessments. In November 2015 the NSABB working group began

[68] Potential Risks and Benefits of Gain-of-Function Research: Summary of a Workshop. National Research Council and the Institute of Medicine of the National Academies. The National Academies Press, Washington D.C., 2015. www.nap.edu.

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receiving the results of the completed risk and benefit assessments. Gryphon's final draft report was posted in advance of the NSABB meeting in January, 2016.[69]

The NIH Office of Science Policy managed the contract with Gryphon Scientific. NIH staff met weekly with Gryphon to accomplish the goals of the Statement of Work and to ensure the recommendations provided in the NSABB's *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research* continued to inform the conduct of the risk and benefit assessments, as appropriate. NIH staff also consulted with NSABB *Ex officio* members to get broader expertise and advice, and to help ensure that the risk and benefit assessments would yield information that would inform subsequent policy deliberations by the U.S. government.

Considering Ethical Issues Associated with GOF Studies

To guide the NSABB's evaluation of the risks and benefits associated with GOF studies and its development of recommendations, the Board sought additional ethical input and analysis. NIH commissioned Dr. Michael Selgelid, Monash University, to examine the literature regarding the ethical issues associated with funding and conducting GOF research and to explore different ethical frameworks that might be utilized when considering how to evaluate the potential risk and benefits associated with GOF studies. Dr. Selgelid was also asked to provide an ethical decision-making framework that NSABB could consider using when analyzing the information provided in the risk and benefit assessments of GOF studies. The decision framework was to identify and consider ethical values that may not be fully captured by a risk-benefit analysis. Dr. Selgelid's analysis was to be accomplished in a neutral, objective manner, without making any definitive recommendations on whether and how to fund or conduct certain GOF studies or what policy course might be the most appropriate. Dr. Selegelid presented his initial work to the NSABB in September 2015 and delivered to the NIH a draft paper in December 2015, which was conveyed to the NSABB working group and posted in advance of the NSABB meeting in January, 2016. [70]

[69] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

[70] Selgelid, Michael. Gain-of-Function Research: Ethical Analysis. December 7, 2015. http://osp.od.nih.gov/sites/default/files/GOF%20%20White%20Paper%20by%20Michael%20Selgelid_0.pdf

Appendix B. Examples of Studies that would and would not be expected to entail GOFROC

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Examples of studies that would and would not be expected to entail GOFROC	
Experiment that is anticipated to entail GOFROC and therefore require additional pre-funding review and approval	**Rationale**
An experiment that is anticipated to generate avian influenza viruses that are transmissible by the respiratory route in mammals if the starting virus is highly virulent in humans.	**Attribute 1.** The experiment is anticipated to increase transmissibility by the respiratory route in a relevant experimental mammalian model. Further, altering the host range from birds to mammals could generate a virus to which there is no existing population immunity resulting in a virus capable of wide and potentially uncontrollable spread among humans. **Attribute 2.** Since the starting virus is highly virulent in humans it can be reasonably anticipated that the resulting virus will remain highly virulent in humans.
Reassortant studies involving avian and human influenza virus strains to identify reassortants with pandemic potential that could arise naturally.	**Attribute 1.** Given the starting viruses and the goal of the experiment to identify/select for reassortants that are potentially highly transmissible in mammals, it can be reasonably expected that one or more of the resulting pathogens could be highly transmissible in humans. Since the resulting viruses are reassortants between bird and human influenza viruses, it can be anticipated that the antigenicity of at least some resulting viruses will remain avian-specific such that human populations would not be expected to have been exposed to such a strain or have pre-existing immunity. Therefore resulting in a virus that is capable of wide and uncontrollable spread . **Attribute 2.** Whether or not any of the starting viruses are highly virulent in humans, it can be reasonably anticipated that the expression of novel combinations of gene segments, derived from different influenza strains, in reassortant viruses could result in a range of characteristics that includes high virulence.
Studies that would result in strain of *Yersinia pestis* would be more likely to cause pneumonic forms of infection and would be resistant to antibiotics. ~~Studies utilizing a strain of *SARS-CoV, or some other emerging human respiratory pathogen*, which will be modified in ways that can be anticipated to render humans more susceptible to infection by for instance, introducing resistance to a~~	**Attribute 1.** -Given that ease of transmission of Yersinia pestis in previous pandemics, manipulations that would enhance its ability to spread by respiratory droplets and cause pneumonic infections would generate a highly transmissible pathogen. In addition, if this manipulation were performed in a strain that was resistant to antibiotics, there would be limited options for controlling the spread of the pathogen among humans. **Attribute 2.** Since the starting agent is highly virulent in humans, particularly when spread through the respiratory route, it can be reasonably anticipated that the resulting agent will remain highly virulent in humans.

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~~countermeasure (were countermeasures available). [NOTE: this example will be replace with bacterial resp. pathogen]~~	
NOT anticipated to entail GOFROC and therefore not require additional pre-funding review and approval	**Rationale**
Studies aimed at generating a mouse-adapted MERS-CoV or other emerging human respiratory pathogen	**Not Attribute 1.** The starting virus is transmissible by the respiratory route among humans but is not highly transmissible. MERS-CoV transmission usually occurs as a result of close contact (e.g. providing unprotected care to an infected patient). Sustained community transmission has not been observed. Furthermore, the proposed adaptation to recapitulate human disease symptoms in mice would not be reasonably anticipated to enhance transmissibility thus the resulting virus would not be anticipated to be capable of wide and uncontrollable spread. **Possibly Attribute 2.** The starting virus is already highly virulent in humans and is associated with significant morbidity and mortality. However, it should also be noted that a mouse-adapted strain is likely to be less virulent in humans.
Studies enhancing the growth of seasonal influenza viruses, which may be performed during vaccine production	**Not Attribute 1.** The starting seasonal influenza virus is highly transmissible by the respiratory route in humans however, population immunity is likely to exist against circulating (and recently circulated) strains. Enhancement of growth is unlikely to result in a virus that can evade immunity, thus a virus capable of wide and uncontrollable spread would not be likely. **Possibly attribute 2.** Increasing seasonal virus' ability to replicate could potentially result in its increased ability to cause disease, which could result in highly virulent strains. **Note:** If this experiment were to involve an attenuated strain, as is often the case with vaccine production, it would be unlikely to result in a virus that is highly virulent in humans.
Antigenic drift studies whereby seasonal ~~or pandemic~~ influenza viruses that are no longer neutralized by vaccine-induced	**Not Attribute 1.** The starting seasonal ~~or pandemic~~ influenza virus is highly transmissible by the respiratory route in humans. However, antigenic drift studies generate influenza viruses with some resistance to a specific immunization but do not change the antigenic character of the virus to a degree such that it would no longer be recognized by the human immune system. Given that the starting virus is a human virus—not

immunity are generated and selected for in the laboratory.	one that naturally infects birds or other non-human hosts—there would likely be some pre-existing population immunity to the resulting strains. **Possibly attribute 2.** The experimental manipulation would not be anticipated to increase the virulence of the virus. The resulting strains are likely to exhibit a similar level of virulence as the starting strain. Whether its virulence is considered~~this is~~ high or low would depend on the specific initial strain used.

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Appendix C. Summaries of Stakeholder Perspectives

The NSABB consulted a wide range of experts and stakeholder groups including not only scientists and institutions that fund and conduct life sciences research, but a much larger and diverse array of groups including public health officials, medical practitioners, emergency responders, vaccine developers, scientific journals, as well as the general public, non-governmental organizations, individuals with international perspectives and others. To accomplish this, NSABB organized meetings with expert presentations and panels that offered ~~provided a variety of~~ opportunities for interested groups there and for individuals and organizations to express their views and contribute throughout the deliberative process in ways that have informed the NSABB deliberations. These include: several public full NSABB advisory committee meetings that included ~~with~~ sessions dedicated to obtaining public comment, two public symposia hosted by the National Academies that obtained comments from the public at the meetings and online, as well as comments submitted to the NIH/OSP and NSABB by email, and discussions with subject matter experts during NSABB WG conference calls and in-person meetings. Also included below are views expressed in some of the articles that have been published on this topic. A complete list of the individuals consulted and articles examined by NSABB are listed in Appendix D. Note that Gryphon Scientific also conducted extensive consultations with experts as part of their risk and benefit assessments. Those experts are not listed here but a listing is available in Gryphon's report. [71]

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The following is a synthesis of stakeholder ideas and opinions expressed during the deliberative process. Many of these points were conveyed in more than one venue and by more than one person or group.

Scientists and Others Favoring GOF Research

A variety of influenza and coronavirus researchers who conduct GOF research, and other life sciences researchers have stated that GOF studies are widely used and fundamental for understanding viruses, and therefore are crucial to undertake. This group generally favors conducting such research because it aims to benefit society. In their view, such research can be safely conducted under current oversight frameworks and further restrictions will impede valuable work that will lead to important scientific information about these viruses, leading to better drugs and vaccines, as well as to improving the specificity of surveillance, particularly for influenza. In addition, some GOF studies are viewed as essential, specifically those that alter host range or enhance pathogenicity in order to develop animal models of disease (for example, with SARS-CoV) or GOF studies that generate drug or countermeasure resistance, which are important in satisfying various FDA requirements for marketing approval. Those who support GOF studies also point out that such studies are needed for predicting what amino acid changes are important for human transmission and therefore are important for the selection of candidate vaccine viruses. They also argue that GOF studies are important for prioritizing viruses for risk

[71] Risk and Benefit Analysis of Gain-of-Function Research, Final Draft Report. Gryphon Scientific, December, 2015. http://osp.od.nih.gov/sites/default/files/Risk%20and%20Benefit%20Analysis%20of%20Gain%20of%20Function%20Research%20-%20Draft%20Final%20Report.pdf

management (surveillance) and that further work will make these applications more robust. The risks associated with not doing GOF research (generally due to a lack of preparedness for natural public health threats) must also be considered.

While acknowledging there are risks associated with GOF research, proponents believe those risks are manageable and have been overstated by some, as evidenced by the fact that laboratory acquired infections are rare and infections in the community as a result of releases from a laboratory are almost unknown. While risk cannot be zero, the work can be conducted safely and securely with appropriate risk mitigation including containment along with good training and with the implementation of robust occupational medicine programs. Alternatives to GOF do not always provide the full answer to key questions and may yield misinformation. Supporters of GOF studies have also expressed concerns about the effects of the current funding pause and possible additional oversight on the field of virology and young researchers, and feel that there are costs of not undertaking the work in question. A major need is for better definition of what is meant by GOF with a clear distinction between GOF studies and GOF studies of concern. Some have suggested that only viruses with increased transmissibility and pathogenicity represent risks that exceed those of other infectious diseases research. They have also noted that SARS and MERS viruses are different from influenza, and require a different risk assessment approach since they are already virulent human pathogens; GOF research is needed to develop animal models that will benefit development of countermeasures for coronaviruses. Some supporters have acknowledged that there may be some experiments that should not be done. Finally, proponents of GOF research have stated that the risks from naturally occurring influenza viruses, which they argue could be reduced through GOF work, are greater than risks from performing GOF studies.

Scientists and Others Critical of GOF Studies

Opponents and critics of GOF research have generally focused their concern on a subset of GOF studies—those that involve enhancing the pathogenicity and/or transmissibility in mammals (particularly by the respiratory route), which may result in the generation of novel pathogens with pandemic potential. Critics have argued that the generation of novel laboratory pathogens with pandemic potential poses major public health risks and some have argued such studies should not be conducted. They have presented and published calculations that suggest a high probability of global outbreaks of influenza that might kill hundreds of millions of people, as a result of the release from a laboratory of a novel GOF virus. There is some disagreement about these estimates and how likely a pandemic might be, but opponents generally argue that even a relatively low probability of a potentially massive outbreak with major consequences is unacceptable. Some critics of GOF studies have acknowledged that there are a number of GOF studies that can and should be conducted.

Opponents of certain GOF studies have also argued that the benefits of GOF studies have been overstated, or are questionable, and that the benefits generally do not outweigh the biosafety risks. They also question claims about the effectiveness of risk mitigation strategies, since human factors and human error are unavoidable and hard to control, and institutional compliance and competence may vary. Critics have disputed the value of GOF studies to surveillance stating that it is not possible to predict phenotype from genotype; therefore predicting the pandemic risk of newly emergent strains is

not achievable given the current state of knowledge. Also, in their view, controlling outbreaks doesn't require GOF research.

Opponents of GOF research tend to favor alternative types of research that, in their view, can provide the same public health benefits without the large risks. It was suggested that the approach should be on reducing the risk by reducing the hazard, as opposed to focusing on mitigation of the risk. For example, if a universal influenza vaccine was developed, the need for many GOF experiments would be eliminated. Critics want to see funds currently used for GOF work provided to other types of research, which would be a better use of scarce resources in their view. Overall, they view preventing major public health problems as paramount, and see a need to define a critical set of experiments that should not be done, or only be done with additional strong oversight. Opponents are also concerned about proliferation and other factors that may lead to misuse and biosecurity threats. Finally, opponents have pointed out a moral issue if risks and benefits of certain GOF studies are not fairly distributed globally.

Funding Agencies

Public and private funding agencies support GOF research that has raised concerns with the goal of improving public health and well-being. These organizations in the US and abroad are aware of the issues surrounding DURC/GOF studies and are working diligently to implement and comply with existing policies in their countries. Most funders have requirements and procedures in place as they apply policies and guidance to evaluate proposed work and to oversee funded work. Current approaches involve education and awareness campaigns, project risk evaluation, ethics reviews, development of risk mitigation plans, and post-award monitoring. Funders believe they can contribute to the GOF deliberative process as a result of their practical, on-the-ground experience with DURC and GOF. They are concerned that interpreting policy can be very challenging, since it requires considerable expertise and judgment. They would welcome workable policies with clear guidance and have noted some unintended consequences of the funding pause, which affected some GOF projects that had not raised particular concerns. Some foreign government funders view government funding as a poor control ~~point~~ mechanisms because this does not cover privately funded research and research funded by other entities. National legislation, regulations, compliance, training, awareness-raising, and self-monitoring have been noted as important.

Biosecurity Experts and Others Concerned about National Security

The ultimate goal of national security professionals, as it pertains to life sciences research, is to protect public health from natural or man-made health threats. Those concerned with national security aim to prevent terrorists and others with malicious intent or misguided motives from using products or information from GOF research to cause harm. This may include deliberate release of pathogens into the community, targeting of researchers or research facilities, or interference with on-going research activities. GOF research represents biosecurity risks in addition to biosafety risks; these overlap but are different with regard to important legal, policy and regulatory issues. Managing biosafety risks may or may not also manage biosecurity risks; GOF policy must take both types of risk into account.

When trying to assess biosecurity threats, security professionals have noted the importance of avoiding assumptions and predictions about the motives and capabilities of those who might be planning biosecurity actions. Those in the security field gather a large variety of data, but often their information is imprecise and may require consideration of what is feasible and plausible. Because of the paucity of biosecurity events, it is very difficult to evaluate and predict the likelihood and consequences of a deliberate release or determine how to prevent and/or mitigate one, and different experts view this issue very differently. It was stated that research policy in itself is not be the appropriate solution to prevent specific biological threats but specific research policies could help raise awareness of security issues among researchers, which would be important.

Security and intelligence professionals have described the challenges associated with using classification as a potential risk mitigation strategy. Classification would effectively restrict access to sensitive research information and research products and would limit the number of laboratories able to perform the studies. This could be described as both a strength and a limitation, depending on one's perspective. Life sciences research that requires classification is typically classified at the outset; the retroactive classification of research that had been conducted in an open, academic setting is exceedingly difficult.

Scientific and Medical Journals

Scientific and medical journals have been at the forefront of the GOF issue. While ~~several~~ a number of jounrnals and families of journals have ~~in place~~ procedures in place for identifying DURC, including GOF and other biosecurity concerns in submitted manuscripts, many journal editors are not entirely comfortable with their role. Their mission is to transmit scientific information, not control it, and they may not have the security expertise or the access to such expertise to make the necessary judgments and decisions about risks associated with communicating certain research findings. Rejection and redaction are the major tools journals have to control dissemination of dual use information, and neither may actually address the concerns; they are also impractical to implement effectively. One suggestion voiced was to require that a description of the steps that were taken during conduct of the research to ensure safety be included in all manuscripts. Some journal editors and staff expressed a desire to get help in evaluating risks and mitigation strategies from an independent national group such as the NSABB and to involve them earlier in the overall process. Most think the publication stage is not the best point to exercise control or prevent misuse of data from GOF studies but realize they are the final gatekeepers. Earlier identification of DURC/GOF along with risk mitigation earlier in the research life cycle would reduce the burden on them. Also, new technology and novel publication venues make controlling information increasingly difficult, and, as noted above, not all journals are able to or choose to impose a rigorous review of manuscripts.

Countermeasure Developers

Companies and others that are attempting to develop vaccines and drugs against pathogens were represented in several discussions. Medical countermeasure (MCM) developers expressed quite divergent views and opinions. Those favoring GOF research argued that such work is absolutely

necessary for antiviral drug development because GOF experiments to select for drug resistant mutants as well as to develop animal models are part of the critical path to marketing approval. In their view, GOF studies also have had a major influence on developing influenza vaccines, both seasonal and pandemic, and are likely to result in improved ways to make even better vaccines in the future. GOF experiments are required for selection of strains with better growth properties, with key mutations that alter important phenotypes needed in the vaccine strain, and with incorporating characteristics of strains that are likely to emerge into proven backbones. It was noted that GOF studies that enhance virulence can help inform vaccine designers about which mutations to avoid incorporating into vaccine strains. This group is concerned that their efforts to improve public health may be limited or impeded by new policies and urge careful consideration of their needs as decisions are made.

Conversely, other MCM developers expressed the view that vaccine production now is little dependent on GOF research and that any possible benefits will be far into the future, although some feel long-term potential is there. Those who criticize GOF studies on these grounds have argued that vaccines are developed in response to strains that emerge as threats, rather than preemptively based on strains that might be predicted as threats. Rather than supporting GOF studies to enhance vaccine production and drug development, it has been suggested that the other constraints that impede MCM development be addressed, such as streamlining FDA approval procedures and improving manufacturing processes, which would have a much greater impact. These critics suggest limiting current GOF-related efforts and focusing attention and resources in other directions. Overall, they believe that impact of GOF research on vaccine and drug development has been overstated, and that the benefits articulated are more theoretical than practical.

The General Public and Organizations Representing their Views.

A number of stakeholders stressed the importance of having meaningful public engagement with input and participation as part of the deliberative process. It is important that communities that might be affected by accidents or the misuse of research have a say in the research that is being conducted, however, but this may not generally be the case in their view. Real transparency, with the public good as the foremost consideration, must be part of a truly independent decision-making process. They note that it is important to maintain public trust in the scientific enterprise by involving non-scientists at stages when their views can still have an impact on policy-making. Public opinion of science is harmed when decisions that influence public health and safety are made without such input or the input has no real impact. Conversely, effective community engagement can convert sceptics to supporters. More than one participant raised the concern that if risks and benefits are not equitably distributed, it is a serious ethical issue[72].

Other issues that were mentioned include: how harms will be compensated if a laboratory incident were to affect the surrounding community; the need for enough resources to conduct research safely; and the opportunity to learn from other industries such as nuclear industry.

[72] The ethical issues are discussed in more depth elsewhere, notably, Dr. Michael Selgelid's ethical analysis and the section of this report on Ethical Values and Decision-Making Frameworks.

Research Institutions

Representatives of universities and other research institutions generally noted that there is already significant oversight of DURC and GOF at both the Federal and institutional levels. Biosafety professionals noted that potentially high risk projects would receive thorough scientific review and risk assessment, resulting in the development of risk mitigation plans, and on-going monitoring as a result of policies and requirements that are already in place. They cited concerns over any increase in compliance that would impose burdens on their already-limited resources or impede researchers from doing valuable work. They have difficulty, at times, deciding what is DURC when reviewing specific projects and would welcome more specificity and guidance. Many emphasized the need for policies that are unambiguous and straightforward to implement.

Public Health Officials

Public health officials have expressed diverse opinions. Some believe that GOF research has and can continue to improve surveillance efforts, as well as vaccine and therapeutic development. Others expressed concerns that an accident involving a laboratory pathogen for which there are no countermeasures would be very concerning and difficult to respond to. At the local level it is important to have public health involvement in the decision-making process because they will be incident responders. Strong connections with state and local laboratories should be established for sharing information and might include involving them in the review process. It was also noted that GOF and related policies may impact sample sharing and impede international relations relating to public health efforts.

International Perspectives

A number of participants noted that there is much interest in the GOF/DURC issue internationally, and the international community is looking to see what the USG will do as a result of the deliberative process. It was noted that U.S. policy often influences policies globally and the international ramifications should be considered. Recent biosafety incidents in U.S. Federal labs have raised concerns among many in other countries about the ability of the U.S. to adequately manage risks. A number of countries have well-developed systems of policy and regulation that would address many or some GOF and DURC issues, though international policy approaches are generally somewhat different from those in the U.S. International experiences, activities, and perspectives were cited as important to consider in the deliberative process. A collaborative approach and active attempts to engage the international community was viewed as the most effective way to benefit all. Many favored launching an international dialogue soon, with development of broad concepts and points of agreement that could be shared by all, while still respecting national differences. In addition, it was suggested that academies of science and multi-national organizations such as WHO can play an important role in such interactions at the right time. Those with a particular interest in the international aspects of GOF research also cited ethical issues associated with the unequal distribution of risks and benefits across rich and poor

countries. It was noted that the European Commission uses a comprehensive ethics process for screening and monitoring DURC/GOF in research projects.[73]

Those with an Interest in the Deliberative Process Itself

A broad group of individuals offered comments on the deliberative process itself. This included: federal government personnel, ethicists, decision-making experts, policy experts, other scientists, and includes people who are also members of the previously-mentioned groups. Those concerned with the deliberative process generally called for a well-planned and executed, thorough, scientifically rigorous, and impartial RBA that is technically sound and socially acceptable. They favored a democratic deliberative process and a policy that incorporates decisions made by neutral parties. Policy should be created using risk-based and value-based approaches to achieve desired outcomes. They want the final policy resulting from the deliberative process to be capable of reasonably identifying and mitigating risks related to GOF while protecting scientific autonomy, research progress, discovery and innovation, public health, national security, and other critical interests.

Many see an adaptive process as desirable, and recommend collecting appropriate data about laboratory accidents and mitigation effectiveness. It was noted that risks and benefits will change as science advances. The funding decision-making process should be accountable and limit inherent conflicts of interest; the individuals or entities that make decisions is critical. Most favor using existing policies as the basis of policy for GOF, while acknowledging that current frameworks are not entirely adequate. The question of how to incorporate non-USG funded research into an acceptable framework was raised several times. Deciding how to decide is a key point.

Both proponents and critics of GOF studies criticized the term "gain-of-function" as being too broad and not descriptive enough. There was much discussion about the appropriate definition of GOF research of concern; many strong, often conflicting, views were expressed. Unfortunately while it is important to have a working definition and criteria for what is GOF of concern as opposed to GOF, a binary distinction needed for deciding what requires extra scrutiny, GOF experiments are actually a continuum of increasing risk.

The funding pause was criticized for being too broad, and some described it as disruptive to scientific process. Finally, some feel that a definitive quantitative risk assessment is not possible because of the very large uncertainties and lack of critical information associated with doing such studies, and they question the value of any studies that are done.

[73] The EU Framework Programme for Research and Innovation, Horizon 2020. How to complete your ethics self-assessment, version 1.0, 11 July 2014. http://ec.europa.eu/research/participants/data/ref/h2020/call_ptef/pt/h2020-call-pt-ria-ia_en.pdf#page=27

Appendix D. Consultations, Comments, and Sources Consulted During NSABB Deliberations

NOTE: We are breaking this into two tables. One table will list all of the invited speakers who were consulted at WG, NSABB, and NAS meeting. The second will list all of the individuals and organizations that submitted public comments or made comments during a public comment session.

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Table 1. Experts consulted by NSABB or the NSABB working groups. Individuals listed here addressed the NSABB or NSABB working group in their individual or professional capacities. Members of the NSABB or an NSABB working group are listed if they presented as a subject matter expert on a specific topic.

Speaker/Commenter	Affiliation/Location	Venue
Regine Aalders, M.Sc.	Embassy of the Netherlands, Washington, D.C.	NSABB Full Board Meeting (January 7-8, 2016), Public Comment
Richard Adams		Public Comment
Nisreen AL-Hmoud, Ph.D, M.Phil.	Royal Scientific Society of Jordan	National Academies Workshop (March 10-11, 2016)
Ronald Atlas, Ph.D.	University of Louisville	National Academies Workshop (December 15, 2014)
Ralph Baric, Ph.D.	University of North Carolina	National Academies Workshop (December 15, 2014), Public Comment
Kavita Berger, Ph.D.	Gryphon Scientific	NSABB Full Board Meeting (September 28, 2015), In-person WG Meeting (November 9, 2015)
Kenneth W. Bernard, M.D.	US Public Health Service (ret.)	Public Comment
Thomas Briese, Ph.D.	Columbia University	National Academies Workshop (December 15, 2014)
Michael Callahan, M.D., D.T.M.&H., M.S.P.H.	Massachusetts General Hospital and Harvard Medical School	National Academies Workshop (March 10-11, 2016)
Arturo Casadevall, M.D., Ph.D.	Albert Einstein College of Medicine; mBio	NSABB Full Board Meeting (October 22, 2014), In-person WG Meeting (July 23, 2015), Public Comment
Rocco Casagrande, Ph.D.	Gryphon Scientific	NSABB Full Board Meetings (September 28, 2015 and January 7-8, 2016), In-person WG Meeting (November 9, 2015), National Academies Workshop (March 10-11, 2016)
R. Alta Charo, J.D.	University of Wisconsin–Madison	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016)
Susan Coller-Monarez, Ph.D.	Office of Science and Technology Policy	In-person WG Meeting (July 23, 2015)
Louis (Tony) Cox, Ph.D., S.M.	Cox Associates	National Academies Workshop (March 10-11, 2016)
Derrin Culp	White Plains, New York	Public Comment
Mark Denison, M.D.	Vanderbilt University	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016), Public Comment
Dennis Dixon, Ph.D.	HHS/National Institutes of Health	NSABB Full Board Meeting (November 25, 2014)

Marianne Donker, Ph.D.	Ministry of Health, Welfare and Sport, Netherlands	In-person WG Meeting (July 23, 2015)
Philip Dormitzer, M.D., Ph.D.	Novartis Vaccines	National Academies Workshop (December 15, 2014)
Ruxandra Draghia-Akli, M.D., Ph.D.	European Commission	In-person WG Meeting (July 23, 2015), National Academies Workshop (March 10-11, 2016)
Rebecca Dresser, J.D.	Washington University in St. Louis	NSABB Full Board Meeting (September 28, 2015)
Paul Duprex, Ph.D.	Boston University, NEIDL Institute	NSABB Full Board Meeting (October 22, 2015)
Gerald Epstein, Ph.D.	Department of Homeland Security	In-person WG Meeting (July 23, 2015)
Stephen Eubank, Ph.D.	Virginia Polytechnic Institute and State University	NSABB Full Board Meetings (October 22, 2014 and January 7-8, 2016), Public Comment
Nicholas Evans, Ph.D.	University of Pennsylvania	Public Comment
David S. Fedson, M.D.	Sergy Haut, France	Public Comment
Scott Ferson, Ph.D.	Applied Biomathematics	NSABB Full Board Meeting (October 22, 2014), Public Comment
David Fidler, J.D., M.Phil.	Indiana University, Bloomington	NSABB Full Board Meeting (January 7-8, 2016)
Harvey Fineberg M.D, Ph.D.	University of California, San Francisco	National Academies Workshops (December 15, 2014 and March 10-11, 2016)
Adam Finkel, Sc.D., M.P.P.	University of Pennsylvania Law School	National Academies Workshops (March 10-11, 2016)
Baruch Fischhoff, Ph.D.	Carnegie Mellon University	NSABB Full Board Meeting (October 22, 2014); National Academies Workshop (December 15, 2014)
Robert Fisher, Ph.D.	U.S. Food and Drug Administration	National Academies Workshop (March 10-11, 2016)
Ron Fouchier, Ph.D.	Erasmus Medical Center	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016), Public Comment
Gregory Frank, Ph.D.	Infectious Diseases Society of America	Public Comment
David Franz, D.V.M., Ph.D.	Former Commander, United States Army Medical Research Institute for Infectious Diseases	In-person WG Meeting (July 23, 2015)
Christophe Fraser, Ph.D.	Imperial College	National Academies Workshop (December 15, 2014)
Matt Frieman, Ph.D.	University of Maryland	Public Comment
Richard Frothingham	Duke University	National Academies Workshop (March 10-11, 2016)
Keiji Fukuda, M.D., M.P.H.	World Health Organization	National Academies Workshop (March 10-11, 2016)
George F. Gao, D.V.M., D.Phil.	Chinese Academy of Sciences; Chinese Center for Disease Control and Prevention	National Academies Workshop (March 10-11, 2016)
Gigi Kwik Gronvall, Ph.D.	University of Pittsburgh Medical Center (UPMC) Center for Health Security	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016)
Charles Haas, Ph.D.	Drexel University	National Academies Workshop (December 15, 2014)
Peter Hale	Foundation for Vaccine Research	Public Comment
Elizabeth Hart	Adelaide, South Australia	Public Comment

Andrew M. Hebbeler, Ph.D.	White House Office of Science and Technology Policy	NSABB Full Board Meeting (October 22, 2014), National Academies Workshop (December 15, 2014)
Denise Hein		Public Comment
Ruthanne Huising, Ph.D., M.Sc.	McGill University	National Academies Workshop (March 10-11, 2016)
Gavin Huntley-Fenner, Ph.D.	Huntley-Fenner Advisors	National Academies Workshops (December 15, 2014 and March 10-11, 2016)
Jo Husbands, Ph.D.	Board on Life Sciences of the US National Academy of Sciences	In-person WG Meeting (July 23, 2015), NSABB Full Board Meeting (January 7-8, 2016)
Michael Imperiale, Ph.D.	University of Michigan	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016), Public Comment
Thomas Inglesby, M.D.	University of Pittsburgh	NSABB Full Board Meeting (October 22, 2014 and January 7-8, 2016), Public Comment
Barbara Jasny, Ph.D.	Science	In-person WG Meeting (July 23, 2015), NSABB Full Board Meeting (January 7-8, 2016), Public Comment
Daniel Jernigan, M.D., M.P.H.	Centers for Disease Control and Prevention	NSABB Full Board Meeting (January 7-8, 2016)
Barbara Johnson, Ph.D., R.B.P.	Biosafety Biosecurity International	National Academies Workshop (December 15, 2014)
John Kadvany, Ph.D.	Independent consultant on decision science	Full Board Meeting (January 7-8, 2016)
Joseph Kanabrocki, Ph.D., C.B.S.P.	University of Chicago	In-person WG Meeting (January 22, 2015), In-person WG Meeting (July 23, 2015)
Isidoros Karatzas, Ph.D.	European Commission	WG Meeting (February 16, 2016)
Yoshihiro Kawaoka, D.V.M., Ph.D.	University of Wisconsin, Madison	NSABB Full Board Meetings (October 22, 2014 and January 7-8, 2016), National Academies Workshop (December 15, 2014), Public Comment
George Kemble, Ph.D.	3-V Biosciences	National Academies Workshop (December 15, 2014)
Lawrence Kerr, Ph.D.	National Security Council Staff	WG Meeting (November 5, 2015), National Academies Workshop (March 10-11, 2016)
Andy Kilianski, Ph.D.	National Research Council Fellow at US Army	Public Comment
Lynn Klotz, Ph.D.	Center for Arms Control and Non-proliferation	Public Comment
Gregory Koblentz, Ph.D., M.P.P.	George Mason University	National Academies Workshop (December 15, 2014)
Todd Kuiken, Ph.D.	The Wilson Center	In-person Meeting (July 23, 2015)
Robert Lamb, Ph.D., Sc.D.	Northwestern University; Howard Hughes Medical Institute	National Academies Workshop (December 15, 2014)
Linda Lambert, Ph.D.	HHS/National Institutes of Health	In-person WG Meeting (July 23, 2015)
Gabriel Leung, M.D., M.P.H.	University of Hong Kong	National Academies Workshop (March 10-11, 2016)
Carol Linden, Ph.D.	HHS/Biomedical Advanced Research and Development Authority	National Academies Workshop (December 15, 2014)
W. Ian Lipkin, M.D.	Columbia University	NSABB Full Board Meeting (October 22, 2014)
Marc Lipsitch, Ph.D.	Harvard School of Public Health	NSABB Full Board Meetings (October 22, 2014 and January 7-8, 2016), National Academies Workshop (December 15, 2014), Public Comment

Patricia Long, J.D., LL.M.	HHS/Office of Security and Strategic Information	In-person WG Meeting (July 24, 2015)
Nicole Lurie, M.D., M.S.P.H.	HHS/Assistant Secretary for Preparedness and Response	NSABB Full Board Meeting (October 22, 2014); In-person WG Meeting (July 23, 2015)
Eric Meslin, Ph.D.	Indiana University School of Medicine	NSABB Full Board Meeting (September 28, 2015)
Corey Meyer, Ph.D.	Gryphon Scientific	NSABB Full Board Meeting (September 28, 2015), In-person WG Meeting (November 9, 2015)
Jonathan Moreno, Ph.D.	University of Pennsylvania	NSABB Full Board Meeting (January 7-8, 2016), National Academies Workshop (March 10-11, 2016)
Kara Morgan, Ph.D., M.S.E.S.	Battelle	National Academies Workshop (March 10-11, 2016)
Rebecca Moritz, M.S., C.B.S.P., S.M.(NRCM)	University of Wisconsin–Madison	National Academies Workshop (December 15, 2014)
Peter Murakami	Baltimore, Maryland	Public Comment
Kalyani Narasimhan, Ph.D.	Nature Publishing Group	In-person WG Meeting (July 23, 2015)
Daniel O'Connell	Albany, Oregon	Public Comment
Kimberly Orr, Ph.D.	US Department of Commerce	In-person WG Meeting (July 23, 2015)
Michael Osterholm, Ph.D., M.P.H.	University of Minnesota	NSABB Full Board Meeting (October 22, 2015)
Kenneth Oye, Ph.D.	Massachusetts Institute of Technology	In-person WG Meeting (July 23, 2015)
Megan Palmer, Ph.D.	Center for International Security and Cooperation, Stanford University	Public Comment
Christopher Park	U.S. Department of State	In-person WG Meeting (July 23, 2015)
Jean Patterson, Ph.D.	Texas Biomedical Research institute	In-person WG Meeting (January 22, 2015)
Daniel Perez, Ph.D.	University of Maryland	NSABB Full Board Meeting (October 22, 2014)
Janet Peterson, C.B.S.P.	University of Maryland	NSABB Full Board Meeting (October 22, 2014)
Dustin Phillips	Louisville, Kentucky	Public Comment
Stanley Plotkin, M.D.	University of Pennsylvania	Public Comment
Philip Potter, Ph.D.	St. Jude Children's Research Hospital	NSABB Full Board Meeting (January 7-8, 2016), National Academies Workshop (March 10-11, 2016)
David Relman, M.D.	Stanford University	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016)
David B. Resnik, J.D., Ph.D.	HHS/National Institutes of Health	NSABB Full Board Meeting (October 22, 2014)
George Rudy	Frederick County & City Containment Laboratory Community Advisory Committee	Public Comment
Colin Russell, Ph.D.	University of Cambridge	National Academies Workshop (December 15, 2014)
Steven L. Salzberg, Ph.D.	Johns Hopkins University School of Medicine	Public Comment

Monica Schoch-Spana, Ph.D.	University of Pittsburgh Medical Center (UPMC) Center for Health Security	National Academies Workshops (December 15, 2014 and March 10-11, 2016)
Stacey Schultz-Cherry, Ph.D.	St. Jude Children's Research Hospital	NSABB Full Board Meeting (October 22, 2014), National Academies Workshop (December 15, 2014)
Shannon Scott		Public Comment
Michael Selgelid, Ph.D.	Monash University	NSABB Full Board Meetings (September 28, 2015 and January 7-8, 2016), National Academies Workshop (March 10-11, 2016), Public Comment
Billie Sellers		Public Comment
Ethan Settembre, Ph.D.	Seqirus	National Academies Workshop (March 10-11, 2016)
Richard Sever, Ph.D.	Cold Spring Harbor Laboratories Press bioRxiv	In-person WG Meeting (July 23, 2015)
Michael Shaw, Ph.D.	Centers for Disease Control and Prevention	In-person WG Meeting (July 23, 2015)
Bill Sheridan, M.B., B.S.	BioCryst Pharmaceuticals Inc.	NSABB Full Board Meeting (October 22, 2014)
Lone Simonsen, Ph.D.	George Washington University	Public Comment
Andrew Snyder-Beattie	Future of Humanity Institute, University of Oxford	Public Comment
Charles Stack, M.P.H.	University of Illinois at Chicago	Public Comment
John Steel, Ph.D.	Emory University	Public Comment
Kanta Subbarao, M.B.B.S., M.P.H.	HHS/National Institutes of Health	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016), Public Comment
Jill Taylor, Ph.D.	Wadsworth Center, NYS Department of Public Health	NSABB Full Board Meeting (January 7-8, 2016), Public Comment
Robert Temple, M.D.	Food and Drug Administration	In-person WG Meeting (July 23, 2015)
Volker ter Meulen, M.D., Ph.D.	European Academies Science Advisory Council	National Academies Workshop (March 10-11, 2016)
Eileen Thacker, D.V.M., Ph.D., D.A.C.V.M.	Department of Agriculture	In-person WG Meeting (July 23, 2015)
Silja Vöneky, (credentials)	University of Freiburg and German Ethics Council	National Academies Workshop (March 10-11, 2016)
Kimball Ward		Public Comment
Robert Webster, Ph.D.	St. Jude Children's Research Hospital	National Academies Workshop (December 15, 2014)
Jerry Weir, Ph.D.	Food and Drug Administration	National Academies Workshop (December 15, 2014)
Robbin Weyant, Ph.D., R.B.P. (ABSA)	Center for Disease Control and Prevention	National Academies Workshop (December 15, 2014), In-person WG Meeting (July 23,2015)
Gary Whittaker, Ph.D.	Cornell University	Public Comment
Beth Willis	Co-founder, Frederick Citizens for Bio-lab Safety	NSABB Full Board Meeting (January 7-8, 2016)
Carrie Wolinetz, Ph.D.	HHS/National Institutes of Health	NSABB Full Board Meetings (May 5, 2015 and January 7-8, 2016)
American Association of Immunologists (AAI)	American Association of Immunologists	Public Comment
Infectious Diseases Society of America (IDSA)	Infectious Diseases Society of America	Public Comment

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Table 2. Sources consulted by NSABB and NSABB working groups include but are not limited to the following

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Authors	Title
Baek, Y.H., et al., 2015	Profiling and Characterization of Influenza Virus N1 Strains Potentially Resistant to Multiple Neuraminidase Inhibitors
Boddie, C., et al., 2015	Assessing the bioweapons threat
Cambridge Working Group, 2014	Cambridge Working Group statement (July 2014)
Casadevall, A., and Imperiale, M.J., 2014	Risks and benefits of gain-of-function experiments with pathogens of pandemic potential, such as influenza virus: A call for a science-based discussion
Casadevall, A., et al., 2014	An epistemological perspective on the value of gain-of-function experiments involving pathogens with pandemic potential
Doshi, P., 2008	Trends in Recorded Influenza Mortality - United States 1900–2004
Duprex, P., and Casadevall, A., 2014	Falling down the Rabbit Hole: aTRIP Toward Lexiconic Precision in the "Gain-of-Function" Debate
Environmental Protection Agency Science Policy Council, 2000	Risk Characterization - EPA Science Policy Council Handbook
European Academies Science Advisory Council, 2015	Gain of function: experimental applications relating to potentially pandemic pathogens
European Center for Disease Prevention and Control, 2012	Risk Assessment: Laboratory-created A(H5N1) viruses transmissible between ferrets
European Commission, 2015	Guidance — How to complete your ethics self-assessment (ver. 4.01)
European Commission	Exploratory Guidance note — Research involving dual-use items
European Commission	Exploratory Guidance note — Research with an exclusive focus on civil applications
European Commission	Exploratory Guidance note — Potential misuse of research
Evans, N.G., 2013.	Great expectations - Ethics, avian flu and the value of progress
Evans, N.G., et al., 2015	The ethics of biosafety considerations in gain-of-function research resulting in the creation of potential pandemic pathogens
Fedson, D.S., and Opal, S.M., 2013	The controversy over H5N1 transmissibility research
Fedson, D.S., 2013	How Will Physicians Respond to the Next Influenza Pandemic?
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Appendix E. Policy Analysis Summary Table

Oversight Measures	Risks Addressed	Description of Oversight	Analysis/Applicability to GOF Studies
Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition (December 2009) http://www.cdc.gov/biosafety/publications/bmbl5/index.htm	Biosafety risks	**Applies to:** Life sciences research involving infectious microorganisms or hazardous biological materials **Description:** General biosafety practices and biological containment for various classifications (risk groups) of microorganisms and etiological agents	BMBL does not describe GOF studies per se but does include summary statements and biocontainment guidance for research involving various influenza strains (including contemporary and non-contemporary human, high and low pathogenic avian, swine, the 1918 influenza strain, and reassortant viruses) and SARS-CoV. MERS-CoV had not emerged at the time of the last BMBL update but interim laboratory biosafety guidance was issued by CDC and is referenced by BMBL. BMBL is a guidance document and generally considered the authoritative reference for laboratory biosafety but it is not a regulatory document; compliance is voluntary.
NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (November 2013) http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines	Biosafety risks	**Applies to:** Basic or clinical life sciences research that involves recombinant or synthetic nucleic acid molecules and is conducted at an institution receiving NIH funding for any such research **Description:** Describes roles and responsibilities of institutions and investigators in safely conducting research. Requires institutional review with a focus on the concepts of risk assessment, risk group classification of agents, physical and biological containment levels, practices, personal protective equipment, and occupational health. **Advised by:** NIH Recombinant DNA Advisory Committee (RAC)	The NIH Guidelines have been amended to include additional guidance for work with Risk Group 3 influenza viruses (1918 H1N1, H2N2, highly pathogenic avian influenza (HPAI) H5N1) to specify enhancements to biosafety level 3 containment, practices, and occupational health requirements. NIH Guidelines were amended again to require further enhancements to facilities, biosafety equipment and practices, including occupational health practices, for research involving HPAI H5N1 strains transmissible among mammals by respiratory droplets. NIH Guidelines are often used as a model of biosafety guidance by the broader scientific community but compliance is required only by institutions receiving such funding from the NIH. The scope is also limited to research involving recombinant or synthetic nucleic acids. Some IBCs also review and approve non-recombinant pathogen research; however, not all institutions require their IBCs to do so.
HHS and USDA Select Agent Program (as of July 2014) http://www.selectagents.gov/regulations.html	Biosecurity (physical and personnel) and biosafety risks	**Applies to:** Biological agents and toxins that have the potential to pose a severe threat to public health and safety, based on a set of criteria. **Description:** Regulates the possession, use, and transfer of select agents and toxins. Overseen by the Federal Select Agent Program. Requires registration of individuals and entities; federal background investigations; federal review of restricted experiments; training; institutional compliance; etc. **Advised by:** Intragovernmental Select Agents and Toxins Technical Advisory Committee (ISATTAC)	Studies that could be considered GOF studies, which involve pathogens on the select agent list, are subject to oversight by the SAP. Researchers and institutions performing such studies must receive favorable security risk assessments by the FBI, register with the SAP, receive training on the proper procedures and practices for handling such agents, and abide by other aspects of the regulations. SARS-CoV, HPAI H5N1 influenza, and 1918 influenza viruses are select agents and GOF studies involving these pathogens are subject to oversight by the SAP. Restricted experiments that would entail conferring antiviral resistance to these viruses would require additional review and approval prior to being conducted.

			GOF experiments involving MERS, and other agents not included on the select agent list, would not be subject to oversight by the SAP.
USG Policy for Federal Oversight of DURC (March 2012) http://www.phe.gov/s3/dualuse/Pages/USGOversightPolicy.aspx	Biosecurity risks, particularly involving misuse of research information, products, and technologies (DURC)	**Applies to:** Life sciences research conducted at an institution receiving USG funding that involves any of 15 agents that pose the greatest risk of deliberate misuse with most significant potential for mass casualties or devastating effects.	The federal DURC policy requires identification and oversight of certain pathogen research involving 7 experimental types, some of which can be described as GOF experiments (i.e., enhancing the harmful consequences of an agent; increase transmissibility; alter host range; etc.) by Federal funding agencies. DURC policies only apply to research involving 15 pathogens. Institutions may review other studies for DURC potential but are not required to do so. Certain GOF studies that involve other agents would not be subject to DURC oversight under the policies.
USG Policy for Institutional Oversight of DURC (September 2014) http://www.phe.gov/s3/dualuse/Pages/InstitutionalOversight.aspx	Biosecurity risks, particularly involving misuse of research information, products, and technologies (DURC)	**Applies to:** Life sciences research conducted at an institution receiving USG funding that involves any of 15 agents that pose the greatest risk of deliberate misuse with most significant potential for mass casualties or devastating effects.	The institutional DURC policy requires federally funded institutions to establish a system for the identification and oversight of certain pathogen research involving 7 experimental types, some of which can be described as GOF experiments (i.e., enhancing the harmful consequences of an agent; increase transmissibility; alter host range; etc.) DURC policies only apply to research involving 15 pathogens. Institutions may review other studies for DURC potential but are not required to do so. Certain GOF studies that involve other agents would not be subject to DURC oversight under the policies.
HHS Funding Framework for GOF studies (August 2013) http://www.phe.gov/s3/dualuse/Pages/HHSh5n1Framework.aspx	Biosafety and biosecurity risks associated with certain GOF experiments involving agents with pandemic potential	**Applies to:** Gain-of-function studies that are reasonably anticipated to generate HPAI H5N1 viruses that are transmissible, and LPAI H7N9 viruses that have increased transmissibility, between mammals by respiratory droplets **Description:** Describes an HHS Department-level review pre-funding review and approval process for certain GOF studies, which can result in funding, not funding, or funding with certain conditions and ongoing oversight.	The only policy focused specifically on funding decisions related to the types of GOF studies that have raised concern. Narrowly focused only on specific GOF studies (enhancing mammalian transmissibility) on two avian influenza viruses; other GOF studies may raise concern and would not be reviewed under this framework.
USG Export Controls (as of July 2014) http://www.bis.doc.gov/index.php/regulations/export-administration-regulations-ear		**Applies to:** Export or release of equipment, software and technology, chemicals, microorganisms, toxins, and other materials and information deemed dual use or strategically important to U.S. national security, economic, and/or foreign policy interests	Comprehensive set of federal regulations that control and restrict the export and release of sensitive equipment, software and technology; chemical, biological, and other materials and information as a means to promote national security interests and foreign policy objectives.

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DELIBERATIVE DRAFT

Appendix ~~F~~E. National Science Advisory Board for Biosecurity Roster

National Science Advisory Board for Biosecurity Roster

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† NSABB Working Group on the Design and Conduct of Risk and Benefit Assessments of Gain-of-Function Studies
‡ NSABB Working Group on Evaluating the Risks and Benefits of Gain-of-Function Studies
* NSABB Member, Retired

NOTE: Please send any updates to your titles/affiliations.

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