NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY

Written Public Comments (Dec. 13, 2015 – May 20, 2016)

The following are written comments submitted to the National Science Advisory Board for Biosecurity (NSABB) for the period December 13, 2015 – May 20, 2016.

Interested persons may file written comments with the Board at any time via an email sent to nsabb@od.nih.gov. Written statements should include the name, contact information, and when applicable, the professional affiliation of the interested person.

From: David Fedson [mailto:dfedson@wanadoo.fr]
Sent: Sunday, December 13, 2015 9:35 AM
To: Viggiani, Christopher (NIH/OD) [E]
Cc: Opal, Steven
Subject: NSABB Meeting on GOF research on January 7-8, 2016

Christopher Viggiani, Ph. D.
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Dear Dr. Viggiani,

I have reviewed the agenda of the NSABB meeting on January 7-8, 2016. At this meeting, the NSABB will discuss its Working Group's overview of progress, preliminary findings and draft working paper on Gain-of-Function (GOF) studies. The Gryphon Scientific report - "Risk and Benefit Analysis of Gain of Function Research, Final Report - December 2015" - will be presented at this meeting.

I would like to bring to your attention and that of the NSABB several important points.

1. If GOF research accidentally or deliberately creates a new highly virulent and highly transmissible influenza virus, it will spread throughout the world in a matter of months. The ensuing pandemic will be a global event, and it will require a global response.

2. Ron Fouchier has said that Mother Nature is the biggest bioterrorist. Pandemic influenza viruses can arise not only in nature but also in experimental circumstances. In a paper published 1974, Webster and Campbell described how they created in turkeys a new transmissible influenza reassortant virus that led to a 100% population die off (attachment 1). This GOF research was conducted more than 40 years ago.

3. In the event of a global pandemic caused by a highly virulent, highly transmissible influenza virus, regardless of its provenance, none of our current medical countermeasures (vaccines, antivirals) will be available to meet the needs of more than 90% of the world's people (attachment 2).

4. When a new pandemic virus appears, the most important question to ask is "what next?" In 2013, Professor Steven Opal at Brown University and I published a paper on GOF research in which we addressed this question. We described an approach to treating pandemic patients using widely available, inexpensive generic drugs that target the host response to infection, not the virus itself (attachment 3).

5. In late 2014, physicians in Sierra Leone treated approximately 100 patients with Ebola virus disease with a combination of a statin (atorvastatin) and an angiotensin receptor blocker (irbesartan). This treatment targets the host response to Ebola virus infection, not the Ebola

virus. Only three inadequately treated patients are known to have died (attachment 4). This treatment reverses the endothelial dysfunction that is central to the host response to Ebola virus disease. It could probably also be used to treat pandemic influenza, MERS, SARS, and other life-threatening diseases in which endothelial dysfunction leads to an increased risk of multi-organ failure and death.

5. Research on treating the host response to influenza and Ebola has been ignored by scientists and government agencies in the US and elsewhere. It is not on WHO's agenda for pandemic preparedness (see attachment 2) or the Ebola response. I have not read the complete Gryphon Scientific report, but the article in attachment 3 is not mentioned in any footnote in its first 486 pages, and it appears not to have been discussed in the text.

6. Given our inability to predict the specific pathogen that will cause the next epidemic, pandemic or biosecurity crisis, the only sensible way to prepare for this event is to identify effective medical countermeasures that address the pathophysiological disturbances common to them all.

Discussion of the risks and benefits of GOF research should focus on practical measures that could be used to counteract this and any other threat to biosecurity. Thus far, the NSABB has not done this. The need for research on treating the host response to emerging biosecurity threats should be discussed by the NSABB. It should be placed on the agenda of the Second Symposium on GOF Research that the National Academies will convene on March 10-11, 2016.

I would be grateful if you would forward copies of my letter and the attachments to Drs. Stanley, Berns and Kanabrocki.

If you have questions about any of these issues, please do not hesitate to write.

With best regards,

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Attachments

1. **A “bottom up” treatment for Ebola that could have been used in West Africa**
2. **How Will Physicians Respond to the Next Influenza Pandemic? -- CID, 2014**
3. **The controversy over H5N1 transmissibility research: An opportunity to define a practical response to a global threat -- Hum. Vaccin. Immunother., 2013**

A "bottom up" treatment for Ebola that could have been used in West Africa

More than 11,000 people have died as a result of the Ebola outbreak in West Africa. Aside from conventional supportive care, no specific treatment has been available. In most treatment units, more than 50% of the patients have died. This needn't have happened.

Patients who die of Ebola have elevated plasma levels of pro-inflammatory cytokines. The same thing is seen in patients with sepsis, and in sepsis patients these findings are associated with endothelial dysfunction and the loss of endothelial barrier integrity [1-3]. Careful studies of foreign healthcare workers who were infected with Ebola virus and evacuated from West Africa for medical care showed they had developed massive fluid losses. These losses were due to a dramatic increase in vascular permeability, a direct effect of the loss of endothelial barrier integrity.

Cardiovascular scientists have known for many years that several common drugs, among them statins and angiotensin receptor blockers, have the ability to stabilize or restore endothelial barrier integrity. These drugs are safe when given to patients with acute critical illness, and clinical studies suggest they might improve survival in patients with sepsis, pneumonia and influenza [1, 3]. For these reasons, in November local physicians in Sierra Leone treated consecutively approximately 100 Ebola patients with a combination of atorvastatin (40 mg orally /day) and irbesartan (150 mg orally/day) [4-7]. Only three inadequately treated patients are known to have died. Unfortunately, apart from a private donation of $25,000, there was no financial or logistical support to conduct a proper clinical trial. Surprisingly, physicians and health officials in Sierra Leone have refused to release information on this treatment experience. Nonetheless, letters and memoranda they have exchanged provide good evidence that treatment brought about "remarkable improvement" in these patients.

Unlike experimental treatments (antiviral drugs, convalescent plasma) currently being tested in Ebola patients, atorvastatin and irbesartan target the host response to the infection, not the virus itself [3-7]. By stabilizing endothelial function and restoring normal fluid balance, combination treatment allows patients to live long enough to develop immune responses of their own and get rid of the virus.

All physicians who treat patients with cardiovascular diseases are familiar with atorvastatin and irbesartan, and most of them have used these drugs to treat their patients. They are widely available as inexpensive generics in West Africa. A 10-day course of treatment for an individual Ebola patient would cost only a few dollars.

Details on the Ebola patients who were treated need to be released, and these findings need to be externally reviewed and validated. Surprisingly, no one seems interested in doing this [8]. If cases of Ebola continue to occur, combination treatment should be tested in a proper clinical trial. In the meantime, physicians should consider the possibility that this combination might be used to treat patients with any form of acute infectious disease, including pandemic influenza [9], in which failure to overcome endothelial dysfunction often leads to multi-organ failure and death.

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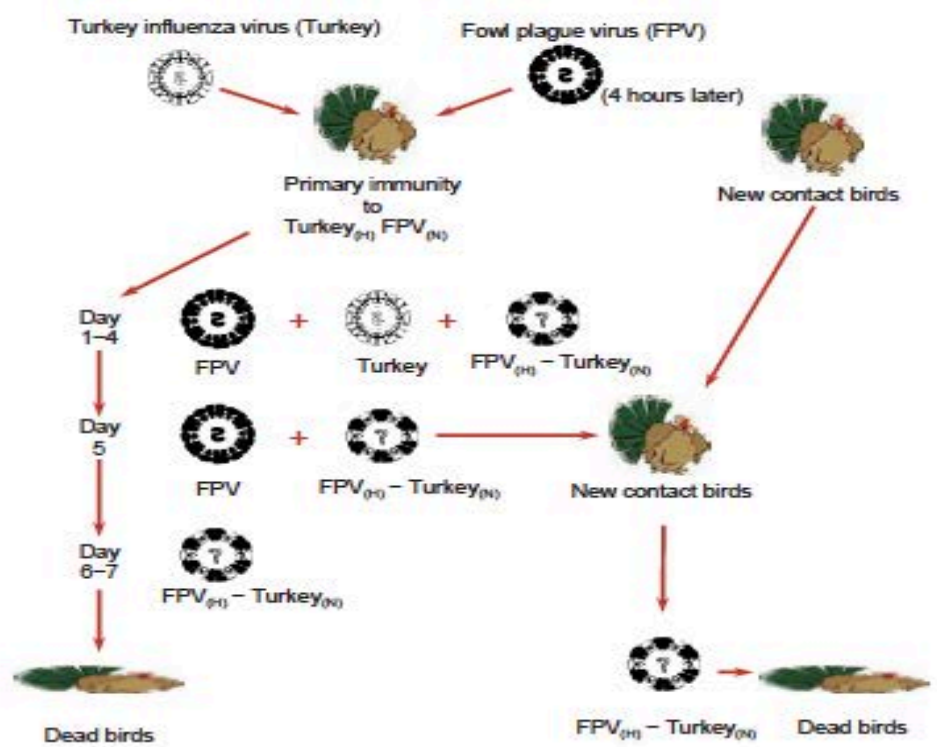

Figure. Genetic reassortment and genesis of a new pandemic influenza virus. This study was designed to determine whether the selection and transmission of a new reassortant influenza A virus could occur under experimental conditions in vivo that mimic what might occur in nature. Reassortment between 2 antigenically distinct influenza A viruses was studied in turkeys that had been previously immunized to induce low levels of antibodies to the hemagglutinin (H) of a nonlethal turkey influenza virus (Turkey), and to the neuraminidase (N) of a fowl plague virus (FPV), an avian virus that is highly pathogenic for chickens. Twenty-eight days after immunization, the immunized turkeys were sequentially infected, first with the Turkey virus and 4 h later with FPV. During the first few days, both parent viruses were isolated from the infected turkeys, but by day 4 a reassortant virus containing the FPV hemagglutinin and the Turkey neuraminidase ($FPV_{(H)}$–$Turkey_{(N)}$) was also isolated; within 2 days it became the dominant virus. All infected turkeys died, and only the $FPV_{(H)}$–$Turkey_{(N)}$ reassortant virus could be recovered. In a separate experiment, similarly immunized turkeys were again sequentially infected, but on day 5 a group of nonimmunized or selectively immunized turkeys ($Turkey_{(H)}$ $FPV_{(N)}$) were placed in the same room. All contact birds soon died of fulminant infection caused by the $FPV_{(H)}$–$Turkey_{(N)}$ reassortant virus. These experiments demonstrated that under conditions of selective primary immunity, a new virus could be generated through genetic reassortment in vivo and that this reassortant virus could be readily transmitted to contacts. The reassortant virus caused uniformly fatal disease in primary infected and contact birds. Thus, under the conditions of these experiments, genetic reassortment gave rise to a new influenza virus that led to a total population collapse. Adapted from Webster and Campbell (9).

Fedson DS. Meeting the challenge of pandemic preparedness in developing countries Emerg Infect Dis 2009; 15: 365-71. Adapted from Webster RG, Campbell CH. Studies on the origin of pandemic influenza. IV. Selection and transmission of 'new' influenza viruses in vivo. Virology 174: 62: 404-13.9

How Will Physicians Respond to the Next Influenza Pandemic?

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The emergence of the H7N9 virus in China is another reminder of the threat of a global influenza pandemic. Many believe we could confront a pandemic by expanding our capacity to provide timely supplies of affordable pandemic vaccines and antiviral agents. Experience in 2009 demonstrated that this cannot and will not be done. Consequently, physicians may have little more to offer their patients than they had in the 1918 pandemic. Fortunately, several modern drugs (eg, statins, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors) can modify the host response to inflammatory illness, and laboratory and clinical studies suggest they might be used to treat pandemic patients. Unfortunately, little attention has been given to the research needed to support their use in patient care. There is no guarantee these drugs will work, but physicians will never know unless those responsible for pandemic preparedness recognize and act on the extraordinary possibility that they might save lives.

Keywords. pandemic influenza; statins; immunomodulatory agents; public health.

The recent emergence of the influenza A(H7N9) virus in China has led to a limited outbreak of disease that has been associated with an overall mortality of approximately 30% [1–3]. The impact has been especially severe among the elderly. It is widely known that influenza viruses can modify or exchange their genes, and these changes often yield new viruses with altered virulence and/or transmissibility. An experiment published in 1974 showed that infecting turkeys with 2 different influenza viruses generated a new reassortant virus that killed all of the infected birds and all of their contacts—a 100% population collapse [4]. The influenza pandemic of 1918 killed between 50–100 million people worldwide, and epidemiologists estimate that a similar pandemic today could kill 62 million people [5], almost twice the number that have ever died of AIDS. Since 1997 there has been deep concern about the high mortality (≥50%) seen in human infection with the avian influenza A(H5N1) virus, and recent controversy over H5N1 gain-of-function research has heightened this concern [6]. Billions of dollars have been spent preparing for an H5N1 pandemic. It is no wonder that scientists and health officials are worried about the H7N9 virus [7].

Several commentators writing in journals that target practicing physicians in the United States have expressed concern that the H7N9 virus could evolve to become easily transmissible and lead to a devastating global pandemic [8–10]. Many believe that the most effective way to respond to the next pandemic would be to greatly expand our capacity to rapidly produce influenza vaccines. They have been encouraged by new developments in influenza vaccinology, especially those based on antibodies and cytotoxic T lymphocytes that mediate heterotypic protection against influenza virus infection [11]. Targets for these new vaccines include the stem cell region of the hemagglutinin molecule and several internal proteins (eg, M2e, NP, M1, and NA). Many believe that research on these targets could lead to a universal influenza vaccine that would obviate the need for annual immunization and provide a foundation of protection against the next pandemic. Other developments in influenza vaccinology include (1) rapid

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preparation of seed strains for vaccine production using reverse genetics; (2) expanded cell culture vaccine production facilities; (3) recombinant glycoprotein HA antigens produced in pharmaceutical bioreactors; (4) antigen-sparing adjuvants that increase the number of vaccine doses that could be produced; and (5) monovalent live attenuated pandemic vaccines [12]. However, enthusiasm for these new developments in influenza vaccinology must be tempered by recognizing that they alone will not guarantee the success of pandemic vaccination.

If vaccination against a global pandemic is to succeed, other measures will be required [12]. New facilities for vaccine formulation and filling will be needed, experienced production technicians must be trained, supplies of syringes and needles for administering inactivated vaccines must be secured, clinical trials of candidate vaccines must be supported, procedures for rapid regulatory certification must be put in place, commercial arrangements between vaccine companies and patent holders must be worked out, advanced purchasing agreements and prices must be negotiated between companies and governments, the logistics of vaccine distribution must be set up, and a human infrastructure for vaccination programs must be established. In each country, the cumulative impact of these factors will directly affect the ability of vaccination programs to successfully confront the next pandemic [12].

The most important factor that will determine the global success of pandemic vaccination will be the level of expansion of seasonal influenza vaccination programs, especially in countries that currently use little vaccine [12]. This will require better understanding of the burden of influenza disease and the effectiveness of influenza vaccination. Remarkably, in recent years the global production capacity for seasonal influenza vaccines has increased to the point where it exceeds world demand, yet there is little evidence that demand will soon match production capacity [13]. In all likelihood, expansion of seasonal vaccination will depend on whether governments in low-use countries recommend and purchase influenza vaccines. In the absence of such decisions, implementing new advances in influenza vaccinology "will depend on company assessments of their individual scientific, technical and commercial advantages. These assessments will be viewed within the context of seasonal not pandemic vaccination" [12].

The global vaccination response to the influenza A(H1N1) pandemic in 2009 offers little encouragement that things will be much better for the next pandemic [14]. In the United States, because pandemic vaccines were not available in time, vaccination affected only 2%–4% of all pandemic cases, hospitalizations, and deaths (see Tables 3–5 of [15]). Consequently, health officials had to advise people to wash their hands and limit social contacts, a throwback to 19th-century public health "technologies." Although the vaccine and antiviral response in the United States was minimally effective, for most of the world it was a comprehensive failure: >90% of the world's people had no access to timely supplies of affordable pandemic vaccines [16].

The threat of another influenza pandemic, H7N9 or otherwise, is real [4–10]. If it is severe, hospitals and intensive care units will be swamped with patients. Extracorporeal membrane oxygenation treatment will help only a few. Even if excellent medical care (including antiviral agents) is available, experience with H7N9 and H5N1 influenza has shown that mortality rates could still be high. Wherever such care is not available, especially in low- and middle-income countries, the mortality impact of a global pandemic could be devastating. Although physicians in most countries will find themselves in healthcare settings much different from those in 1918, their experiences and those of their patients could be much the same [17]. Given this possibility, physicians everywhere need to ask whether agents they already know and use in the routine care of their patients might also be used to treat those who become seriously ill with pandemic influenza.

Until now, health officials have relied on influenza scientists—primarily virologists and epidemiologists—to guide pandemic preparedness efforts. Virologists who have adopted a systems approach to discovery have made important contributions to explaining influenza virus–host interactions and the consequences of these interactions for the pathogenesis of disease [18]. Nonetheless, they have yet to suggest agents that would be available to physicians who will be called upon to manage severely ill pandemic patients. Fortunately, investigators in other fields, especially cardiovascular and metabolic diseases, have developed several groups of drugs whose "pleiotropic" activities modify the innate and adaptive immune response to acute inflammatory illness. These drugs might be used for pandemic treatment and prophylaxis. Statins were the first group suggested [19], and since then angiotensin II receptor blockers (ARBs), angiotensin-converting enzyme (ACE) inhibitors, peroxisome proliferator-activated receptor (PPAR) γ and PPARα agonists (glitazones and fibrates, respectively), and adenosine monophosphate–activated kinase agonists (eg, metformin) have emerged as additional candidate agents. These developments have been comprehensively reviewed in a recent publication [16]. Laboratory studies of acute lung injury, sepsis, and other forms of acute systemic inflammation have shown that these drugs control damaging inflammation, promote its resolution, and improve survival [16, 20, 21]. The benefits of treatment may have little to do with the effects of these drugs on influenza virus–infected cells [16]. Instead, they might improve survival by maintaining or restoring pulmonary microvascular barrier integrity [22], accelerating the early return of mitochondrial biogenesis [23], and/or promoting beneficial changes in immunometabolism [24–26]. Laboratory and clinical research on these agents might help us understand why influenza mortality rates are lower in children

than in adults [16], and perhaps show that "disease tolerance" in children with influenza is a defense strategy that reflects the heritage of human evolution [16, 27–29].

Clinical studies support laboratory findings on the effectiveness of inpatient treatment with 3 groups of these agents (reviewed in [16]). For example, an observational study of 3043 patients hospitalized with laboratory-confirmed seasonal influenza showed that statin treatment was associated with a 41% reduction in 30-day mortality [30]. This reduction was in addition to any that might have been attributable to previous vaccination and antiviral treatment. Another observational study showed that inpatient treatment with ARBs, ACE inhibitors, and statins reduced 30-day pneumonia mortality by 53%, 42%, and 32%, respectively [31]. Importantly, a randomized controlled trial in 100 statin-naive patients (untreated for at least 2 weeks) who were hospitalized with sepsis showed that inpatient atorvastatin (40 mg per day) reduced progression to severe sepsis by 83% (24% in control patients vs 4% in treated patients; $P = .007$) [32].

Statins and other immunomodulatory agents that might benefit influenza patients are used by physicians every day to treat millions of patients with cardiovascular diseases and diabetes. For statins, long-term treatment is safe and effective in improving cardiovascular outcomes, and the benefits greatly outweigh the modestly increased risks of statin-associated diabetes, elevated liver enzymes, and myopathy [33], adverse events that are easily managed. Cases of severe liver injury or rhabdomyolysis are rare. For short-term inpatient treatment, cardiologists routinely initiate statin treatment in patients hospitalized with acute coronary syndrome (ACS), and such treatment has shown to be safe and effective in reducing hospital and 30-day ACS mortality (reviewed in [16]). This experience suggests that studies of treating influenza patients with statins and other immunomodulatory agents should focus on those with illness serious enough to require hospitalization, and an agenda for such research has recently been presented [16]. This research will allow physicians to carefully assess the clinical and immunological effects of treatment while monitoring patients for any signs of adverse events or drug–drug interactions. Special attention will have to be given to the safety of treating pregnant women and children.

Several small-scale studies of statin treatment in humans with experimental acute lung injury, sepsis, and pneumonia have been published (reviewed in [16]). Although these studies were too small to show evidence of clinical benefit, no adverse reactions were noted and several parameters associated with immune dysregulation showed improvement. If statins or other immunomodulatory agents could be shown to be safe and effective, treatment for most patients (especially those who are not older adults) would probably be limited to the duration of the hospital stay and would not need to be continued after hospital discharge. For hospitalized patients who have previously received outpatient treatment with any of these agents, continued treatment after hospital admission would probably be indicated, just as it is for ACS patients who have received outpatient statins [16].

All of the immunomodulatory agents discussed above are now produced as inexpensive generics in developing countries, and global supplies are huge [16]. If 1 or more of them were shown to be safe and clinically effective in treating severe influenza (or in the syndromic treatment of acute critical illness due to other causes such as pneumococcal pneumonia [34]), they would be immediately available to physicians in any country with a basic healthcare system. The cost of treating an individual patient would probably be less than $1.00 [16]. Nonetheless, the laboratory and clinical research needed to justify using these agents to treat influenza patients must be initiated and supported by governments and/or nongovernmental institutions; it cannot be left to pharmaceutical companies because the drugs are no longer of commercial interest.

In the United States, the Assistant Secretary for Preparedness and Response (ASPR), Department of Health and Human Services, joined by the directors of the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health, recently published a set of key components for a research response to public health emergencies [35]. After listing the research failures during the influenza A(H1N1) pandemic in 2009, the authors called for several actions to be taken before the next emergency event. These actions include (1) identifying potential knowledge gaps and research questions; (2) developing and preapproving generic study protocols; (3) obtaining approval for these protocols from institutional review boards; (4) using prefunded research networks and preawarded just-in-time research contracts; and (5) developing an on-call "ready reserve" of clinicians, scientists, and other experts to undertake this research. The essential elements of ASPR's research response plan as they might apply to influenza pandemic preparedness were outlined in an article published in 2009 [36]. Unfortunately, none of ASPR's proposed actions has been implemented, and no plans have been made to study immunomodulatory agents (D.S. Fedson, unpublished observation).

The statins/influenza study mentioned earlier [30] was conducted by the CDC's Emerging Infections Program, but CDC's Influenza Division has not initiated studies to confirm or extend its findings (D.S. Fedson, unpublished observation). In September 2012, the Infectious Diseases Society of America (IDSA) published its US action plan for pandemic and seasonal influenza [10, 37]. The plan focuses on vaccines, antiviral agents, better diagnostics, improved surveillance, and more effective risk communication. The IDSA report briefly mentions

immunomodulatory treatment, but a careful reading indicates that research on these agents is not central to the IDSA's action plan. At the global level, the pandemic preparedness efforts of the World Health Organization (WHO) remain focused on vaccines and antiviral agents [38]. WHO has paid no attention to immunomodulatory treatment, and it was not discussed at the World Health Assembly meeting this past May [39].

George Orwell once wrote that "to see what is front of one's nose needs a constant struggle" [40]. Physicians inevitably will be called upon to care for patients in the next pandemic. They need to ask why influenza scientists and health officials who support their work have not undertaken pragmatically focused laboratory and clinical research to see if statins and other promising immunomodulatory agents could be used to reduce influenza-related mortality. There is no guarantee that any of these drugs will work, but physicians will never know unless those responsible for pandemic preparedness recognize and act on the extraordinary possibility that these agents might save lives.

Note

Potential conflicts of interest. The author has previously received honoraria and travel expenses from Sanofi Pasteur, Sanofi Pasteur MSD, and Merck, Inc, for speaking engagements on influenza and pneumococcal vaccination.

The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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REVIEW

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The controversy over H5N1 transmissibility research

An opportunity to define a practical response to a global threat

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Since December 2011, influenza virologists and biosecurity experts have been engaged in a controversial debate over research on the transmissibility of H5N1 influenza viruses. Influenza virologists disagreed with the NSABB's recommendation not to publish experimental details of their findings, whereas biosecurity experts wanted the details to be withheld and future research restricted. The virologists initially declared a voluntary moratorium on their work, but later the NSABB allowed their articles to be published, and soon transmissibility research will resume. Throughout the debate, both sides have had understandable views, but both have overlooked the more important question of whether anything could be done if one of these experimentally derived viruses or a naturally occurring and highly virulent influenza virus should emerge and cause a global pandemic. This is a crucial question, because during the 2009 H1N1 influenza pandemic, more than 90% of the world's people had no access to timely supplies of affordable vaccines and antiviral agents. Observational studies suggest that inpatient statin treatment reduces mortality in patients with laboratory-confirmed seasonal influenza. Other immunomodulatory agents (glitazones, fibrates and AMPK agonists) improve survival in mice infected with influenza viruses. These agents are produced as inexpensive generics in developing countries. If they were shown to be effective, they could be used immediately to treat patients in any country with a basic health care system. For this reason alone, influenza virologists and biosecurity experts need to join with public health officials to develop an agenda for laboratory and clinical research on these agents. This is the only approach that could yield practical measures for a global response to the next influenza pandemic.

Introduction

In December 2011, the National Science Advisory Board for Biosecurity (NSABB) in the US recommended restricting publication of the experimental details of A/H5N1 influenza virus transmissibility research conducted by Ron Fouchier, Yoshi Kawaoka and their colleagues.[1,2] Fouchier had presented the results of his studies at a scientific meeting in September 2011 and his findings had received considerable attention among influenza virologists. However, following the announcement of the NSABB recommendation, there was widespread comment in major scientific journals and in the media, and the NSABB's decision quickly became controversial.[3]

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H5N1 Transmissibility Research and the NSABB

In response to the NSABB decision, Fouchier and Kawaoka reluctantly agreed to a voluntary moratorium on publishing their findings and continuing their research.[4] They and many other virologists were concerned that science was being censored.[1,2,5-9] In contrast, the NSABB[10,11] and others regarded as biosecurity experts[12-15] worried that a highly transmissible H5N1 virus could be released accidentally or deliberately among human populations. In February 2012, the World Health Organization (WHO) convened an international technical consultation that included the principal scientists involved in this controversy.[16] One month later, the NSABB received reassuring new data from Fouchier and Kawaoka. Moreover, intelligence officials had concluded that H5N1 transmissibility research did not present a biosecurity threat. Accordingly, the NSABB revised its earlier decision and unanimously recommended full publication of Kawaoka's findings,[17] which were subsequently published.[18] There was less than complete agreement on whether to publish Fouchier's findings, but after extensive revision his manuscript too was published.[19] The US Government also issued revised recommendations on its oversight of "dual use research of concern"; i.e., research that is considered scientifically useful but could also be used deliberately or accidentally to cause harm.[20]

Influenza virologists believe that publication of their findings will have several benefits. For example, Kawaoka has said, "The amino acid changes identified here will help individuals conducting surveillance in regions with circulating H5N1 viruses … to recognize key residues that predict the pandemic potential of

isolates. Rapid responses in a potential pandemic situation are essential in order to generate appropriate vaccines and initiate other public health measures to control infection. Furthermore, our findings are of critical importance to those making public health and policy decisions."[18] However, many influenza scientists doubt this research will yield any practical benefits for influenza virus surveillance or for developing vaccines and antiviral agents, at least in the foreseeable future.[21,22]

The ability of influenza viruses to mutate and yield new viruses that might be more virulent or more easily transmitted was earlier demonstrated in vivo for the 2009 pandemic A (H1N1) (pH1N1) virus in mice[23] and ferrets.[24-26] These reports appeared before the H5N1 studies of Fouchier and Kawaoka came to NSABB and public attention. A more recent study has reported the in vitro evolution of two mutant H5N1 viruses, one that was transmissible by direct contact and another that was partially transmissible by droplets in ferrets.[27] Fouchier and Kawaoka found that only 3 to 5 mutations were required to generate respiratory transmissible H5N1 viruses. Other investigators using mathematical models have concluded, "the remaining mutations could evolve within a single mammalian host, making the possibility of a respiratory droplet–transmissible A/H5N1 virus evolving in nature a potentially serious threat."[28]

The H5N1 transmissibility research controversy is slowly moving toward resolution. Eventually, new rules for this and other types of "dual use research of concern" will be formulated. In the meantime, it is worth asking whether this controversy has something else to teach us.[29]

Adequate Global Supplies of Vaccines and Antiviral Agents won't be Available for a Global Response to the Next Pandemic

The concerns expressed by influenza virologists and biosecurity experts about H5N1 transmissibility research are understandable. However, both groups have overlooked a far more important question: could an effective global response be mounted to confront a pandemic caused by a new highly transmissible and virulent influenza virus, regardless of whether it is a laboratory-generated H5N1 virus or (more likely) a naturally derived variant of the currently circulating H5N1 or seasonal influenza viruses? This question is critically important, for if a virus as virulent as the one that caused the pandemic in 1918 were to emerge today, it might kill 62 million people worldwide.[30]

The global response to the relatively mild H1N1 influenza pandemic in 2009 amply demonstrated that scientists, companies and public health officials working together lacked the capacity to rapidly develop,[31] produce[32] and distribute[33-35] affordable supplies of pandemic vaccines and antiviral agents in time to mitigate the pandemic's impact on more than 90% of the world's people. This is incontrovertible evidence that in the event of a new and more severe influenza pandemic, regardless of its provenance, it will be impossible to successfully implement an effective global public health response that targets only the virus.

Clinical and Epidemiologic Findings Suggest an Alternative Approach to a Pandemic

If vaccines and antiviral agents will be unavailable to most of the world's people when the next pandemic virus emerges, would it be possible to confront the pandemic using an alternative approach that targets the host response to the virus? A clue to the promise of this approach promise can be seen in the disparity in the case fatality rates of children and young adults in the 1918 influenza pandemic.[36] This pandemic caused exceptional mortality in young adults but not in children. Some scientists have ascribed the high mortality in young adults to secondary bacterial pneumonia,[37-39] but this explanation fails to account for the more frequent infection of children with the virus that killed young adults and the (almost certain) more frequent colonization of their nasopharyngeal passages with the same bacteria found in the lungs of young adults who died (**Fig. 1**).[36,40]

Influenza virologists recognize that children were not protected from infection, but "… for reasons that are as mysterious today as they were in 1918, they were able to cope with the disease much better than their adult counterparts."[41] Although these virologists have made extraordinary contributions to our understanding of the 1918, H5N1 and other influenza viruses, they have been unable to answer the question, "Why did young adults die." The more important question is "Why did children live?" The different case fatality rates in children and young adults in 1918 might have been due to characteristics specific to host responses of children and young adults that differentially affected their risks of dying.[36,40] Clinicians and epidemiologists have documented similar differences in the case fatality rates of children and adults in several other infectious and non-infectious conditions.[40] These differences might have arisen during the course of human evolution. Yet, influenza virologists, immunologists and evolutionary biologists appear to have given little attention to studying the mechanisms underlying these differences.

In older adults, mortality due to seasonal and pandemic influenza largely affects those with underlying high-risk conditions: cardiopulmonary diseases, diabetes and renal disease. In younger adults those with obesity, asthma and pregnancy are affected. In both young and old, these conditions share one feature in common: each is characterized by alterations in innate immunity that in many instances constitute a form of low-grade inflammation known to cardiovascular scientists as "metabolic syndrome."[42-46] Among children who die of influenza, most have known immune disorders. In those with fatal influenza and no recognized disturbance in immune function, it is possible that unrecognized antecedent events have induced cytokine dysregulation and increased their vulnerability to influenza-related complications and death. In all likelihood, all of these individuals are at increased risk because their "innate immune rheostats" have been set at different and more precarious levels, making them more vulnerable to a loss of innate immune homeostasis.[47]

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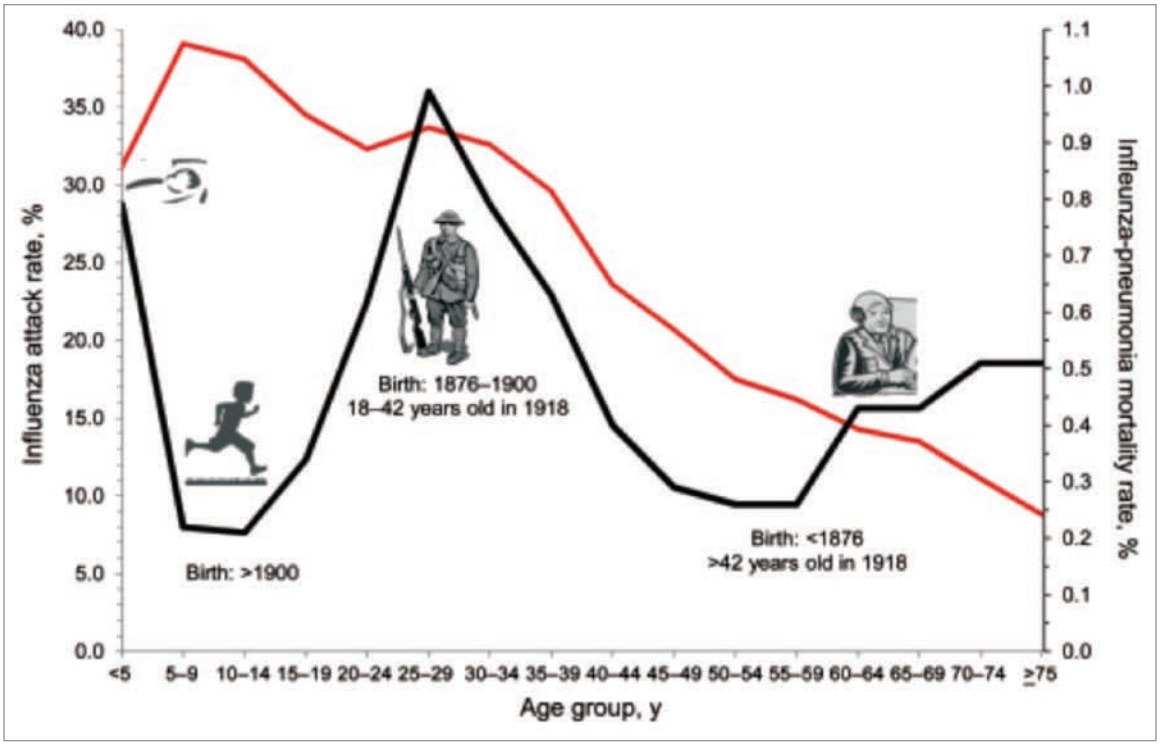

Figure 1. Discrepancy between clinical influenza attack rates and influenza pneumonia mortality rates in the 1918 influenza pandemic (adapted from ref. 38).

The Host Response to Influenza

Human influenza is associated with elevated levels of pro- and anti-inflammatory cytokines and chemokines, and the greater the degree of dysregulation, the greater the likelihood of severe or fatal illness.[48] Even in patients with mild illness, elevated cytokine levels distinguish between those who develop symptoms and those who have asymptomatic infection.[49] Few people with fatal influenza die during the first few days of illness when a pro-inflammatory response dominates. Instead, like patients with sepsis,[50] most die during the second week or later when an anti-inflammatory response and immunosuppression become dominant and virus replication has decreased.[36,40] These changes in the host response have been demonstrated in studies of H5N1 and non-H5N1 influenza viruses in mice,[51] ferrets[52] and non-human primates,[53] and interactions between virus and host factors that determine the course of illness have been discussed extensively by influenza virologists.[54-57]

Many influenza virologists are convinced that virus factors - infecting dose, extent of replication and degree of virulence - principally determine the outcome in influenza, hence their emphasis on controlling the disease with vaccines and antiviral agents.[57-59] No one would argue seriously that these factors are unimportant. Nonetheless, they cannot explain why an inactivated H5N1 virus can cause fatal acute lung injury in mice,[60] nor why survival in the acute lung injury seen in sepsis, pneumonia and influenza is determined by active resolution of inflammation,[61,62] the restoration of pulmonary endothelial barrier integrity,[63] mitochondrial biogenesis[64-66] and changes in energy metabolism.[67,68] Most of all, it is difficult to imagine how factors intrinsic to the virus could have been solely responsible for the different mortality rates seen in children and adults in the 1918 pandemic.[36,40]

A dysregulated host response appears to be the principal factor responsible for fatal influenza. Since timely and affordable supplies of vaccines and antiviral agents won't be available when the next pandemic virus emerges, the challenge to laboratory and clinical investigators is to identify existing agents that can reestablish the host's capacity for self-regulated homeostasis. An abundance of clinical and laboratory research indicates this can be done.

Targeting the Host Response to Pneumonia and Influenza with Immunomodulatory Agents

A growing body of evidence suggests it should be possible to modify the dysregulated host response of patients with community-acquired pneumonia and influenza and improve their survival.[36] For many years, physicians have used 3-hydroxymethyl-3-glutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), peroxisome proliferator activator receptor (PPAR)α and PPARγ agonists (fibrates and glitazones, respectively) and AMP kinase agonists (metformin) to treat the dysregulated host responses of patients with chronic heart diseases and diabetes mellitus. The clinical benefits and safety of these immunomodulatory agents are widely known. In addition to their effectiveness when given as long-term treatment, they have beneficial effects when given acutely; for example, when statins are given to patients within 24 h following hospitalization for acute myocardial infarction,

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they significantly reduce hospital mortality.[69] These agents have also been shown to have overlapping anti-inflammatory and immunomodulatory (pleiotropic) activities in mouse models of systemic inflammation, both sterile [e.g., after endotoxin (LPS) treatment] and infection-induced [e.g., cecal ligation and puncture (CLP)] sepsis.[36]

Observational studies in humans have evaluated the effects of statins in patients with pneumonia (there are no studies of fibrates, glitazones or metformin). Most but not all of these studies have shown that outpatients taking statins (almost certainly for cardiovascular reasons) have reduced rates of pneumonia hospitalization and death.[70-75] Three observational studies have documented the effects of inpatient statin treatment on pneumonia mortality. In one study of 1985 patients, continued statin use in the hospital reduced hospital mortality by 27% [adjusted odds ratio (OR) 0.73; 95% confidence interval (CI) 0.47–1.13; p = 0.15].[76] In a second study of 121,254 inpatients, statin treatment reduced hospital mortality in those not admitted to intensive care by 21% (adjusted OR 0.79; 95% CI 0.71–0.87), but it had no effect on mortality in those who required intensive care (adjusted OR 0.93; 95% CI 0.81–1.06).[77] The third study reported the results of a propensity matched case-control study that used a Department of Veterans Affairs administrative database of patients ≥ 65 y of age hospitalized with pneumonia (11,498 cases and 11,498 controls).[78] Inpatient statin treatment was associated with a 32% reduction in 30-d mortality (adjusted OR 0.68; 95% CI 0.59–0.78). In addition, outpatient statins were associated with a 26% reduction in 30-d mortality (adjusted OR 0.74; 95% CI 0.68–0.82). Outpatient and inpatient use of angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) were also associated with significant reductions in 30-d mortality, but there was no analysis of combination treatment with a statin and either an ACE inhibitor or an ARB.[78]

No reports have been published of randomized controlled trials of statin treatment of patients with pneumonia. However, a single center clinical trial conducted in 100 patients hospitalized with sepsis has shown that atorvastatin (40 mg/day) significantly reduced progression to severe sepsis (4% in treated patients vs. 24% in controls; p = 0.007).[79]

Immunomodulatory Treatment of Pandemic Influenza

In 2004, it was suggested that statins might be useful in reducing mortality from pandemic influenza.[80] This idea was based on the well-established phenotypic benefits of acute statin treatment in patients with acute myocardial infarction, and the possibility that similar benefits might be seen in patients with severe influenza. Over the next few years, several influenza virologists failed to show that statins could reduce influenza mortality in mice, although none of their studies has been published (DS Fedson, unpublished observations).

Two recent studies failed to show that statins reduce mortality in mouse models of influenza. In one report, rosuvastatin (administered in the diet) failed to protect C57Bl/6 mice infected with H3N2 and WSN influenza viruses, but the infecting doses of virus were very high (LD_{100}) and there was clear evidence that after one or two days the mice stopped eating, and therefore were no longer being treated.[81] In a much larger study, several different statins were tested against several different influenza viruses in BALB/c mice.[82] No meaningful evidence of protection was shown, but again the infecting dose of virus was highly lethal. Moreover, treatment was given for only a few days, and it is well known that early cessation of statin treatment during an inflammatory illness in both mice and humans leads to a rebound hypercytokinemia and increased mortality.[83]

A limited number of laboratory studies have shown the effectiveness of other immunomodulatory agents in mouse models of influenza. Post-infection treatment with resveratrol (a plant polyphenol with immunomodulatory activities)[84] and gemfibrozil[85] significantly improved survival in influenza virus-infected mice, and similar improvements have been demonstrated for pre-infection treatment with pioglitazone[86] and pioglitazone combined with AICAR, a metformin-like drug.[87] In two studies that evaluated the effects of treatment on virus replication, pulmonary virus levels were either unchanged[86] or reduced.[84] A more recent study has shown that treatment of mice with the PPARγ agonist 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), starting one day after infection, improved survival from 14% to 79% and markedly reduced.[88] Surprisingly, 15d-PGJ_2 treatment started on day 0 was not protective. Moreover, although protection by 15d-PGJ_2 could be reversed by a specific PPARγ antagonist, treatment with rosiglitazone (a clinical PPARγ agonist that also has non PPARγ activities) on day 0 or day 1 was not protective. In another study, a highly active glutathione derivative (glutathione is an important intracellular antioxidant) strongly inhibited PR8 influenza virus replication in vitro by blocking cytoplasmic maturation of the virus hemagglutinin, and treatment of influenza virus-infected mice reduced mortality 4-fold.[89] Statins, glitazones, fibrates and metformin all upregulate glutathione activity.[90] It is important to note that none of these experimental studies included co-treatment with a recognized antiviral agent.

Reports on the effects of immunomodulatory agents in human influenza are limited to statins. Two reports have appeared on the effects of statins on laboratory-confirmed human influenza. In an observational study of 1520 patients hospitalized in 2009 with pH1N1, preadmission statins were associated with a statistically nonsignificant 28% reduction in hospital mortality (adjusted OR 0.72; 95% CI 0.38–1.33).[91] Unfortunately, the investigators gathered no data on inpatient statin use. More important, an observational study has reported on statin treatment of 3043 older adults hospitalized in 2007–2008 with laboratory-confirmed seasonal influenza.[92] Statins were begun as outpatient treatment in 96% of patients and were either continued or started after hospital admission in 87%. Statin use was associated with a statistically significant 41% reduction in mortality within 30 d of a positive test for influenza virus (adjusted OR 0.59; 95% CI 0.29–0.92; deaths occurred either in the hospital or shortly after discharge). The results of this pivotal study provide compelling evidence to support the concept that immunomodulatory treatment of influenza should work.

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Table 1. Cell signaling pathways that might be targeted by immunomodulatory treatment*

• Upregulate HO-1† and decrease TLR signaling by PAMPs and DAMPs
• Downregulate NF-kappaB and pro-inflammatory cytokines (e.g., TNFα, IL-1, IL-6)
• Upregulate anti-inflammatory cytokines (IL-10, TGFβ)
• Upregulate pro-resolution factors (lipoxin A4, resolvin E1)
• Downregulate HMGB1/RAGE and late mediators of inflammation
• Upregulate adipokines (adiponectin) that decrease inflammation
• Upregulate eNOS, downregulate iNOS, restore iNOS/eNOS balance and stabilize cardiovascular function
• Decrease formation of reactive oxygen species and reduce oxidative stress
• Decrease tissue factor and its associated pro-thrombotic state
• Attenuate the C5a-C5aR-related increase in vascular endothelial permeability
• Stabilize the actin cytoskeleton and adherens and tight junctions in endothelial cells, increase pulmonary barrier integrity and decrease vascular leak
• Attenuate acute disease-associated pulmonary hypertension
• Restore the balance between Th17 and Treg cells
• Differentially modify caspase activation and apoptosis in epithelial and endothelial cells, macrophages, neutrophils and lymphocytes in the lung and other organs
• Upregulate AMPK and PGC-1α, improve mitochondrial function and restore mitochondrial biogenesis and metabolic homeostasis

*Adapted from references 36 and 96 and DS Fedson, unpublished observations. †HO-1, heme oxygenase -1; TLR, Toll-like receptor; PAMP, pathogen-associated molecular pattern; DAMP, damage associated molecular pattern; NF-kappaB, nuclear factor kappaB; TNFα, tumor necrosis factor α; IL-1, Interleukin-1; TGFβ, transforming growth factor β; HMGB1, high molecular group box-1; RAGE, receptor for advanced glycation end products; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; C5aR, C5a receptor; Treg, T regulatory; AMPK, adenosine monophosphate-activated protein kinase; PGC-1α, peroxisome-proliferator-activated receptor (PPAR)γ coactivator-1α.

Questions about the Effectiveness of Statins in Treating Influenza

The results of this pivotal study have been questioned because it is thought that patients who received statins were "healthy users."[93] The same reason has been used to claim that observational studies showing the effectiveness of influenza vaccination in reducing hospitalizations and deaths are similarly biased; in other words, vaccination appears to be effective (but is not) because relatively healthy older adults take better care of their health (and get more vaccines) than those who are less healthy, and thus they are more likely not to be hospitalized or die because they are healthier, not because they have been vaccinated.[94] The statins investigators responded to this criticism by listing the steps they took in their analysis to control for healthy user bias.[95] The critics failed to mention that the healthy user bias had already been accounted for by the investigators in their adjusted analysis: the 41% reduction in mortality with statin treatment was in addition to any reduction that might have been attributable to previous influenza vaccination and antiviral treatment.[92]

The results of most observational studies demonstrate the phenotypic effects of statin treatment in reducing pneumonia and influenza mortality. To date, no such studies have been reported on the effects of glitazones, fibrates or metformin, although observational studies of large groups of diabetic patients would be informative. Nonetheless, the known immunomodulatory effects of these agents in other conditions characterized by cytokine dysregulation (e.g., cardiovascular disease, metabolic syndrome, diabetes) as well as their effects in several experimental models of infection and inflammation have provided insights into some of their potential mechanisms of action (**Table 1**; refs. 36, 96, 97 and DS Fedson, unpublished data). Other immunomodulatory agents have been suggested as candidates for influenza treatment.[98] ACE inhibitors and ARBs are among the most promising agents,[78] but there are no studies of their use in experimental models of influenza. Among other agents that are licensed, (e.g., macrolides, cyclooxygenase-2 inhibitors), few data support their use. For other candidate agents (e.g., anti-TNF therapy, mesenchymal stem cells, angiopoeitin-1, high mobility group box-1 antagonists), limited supplies, high costs and/or their investigational status mean that many years will pass before any of them can be considered seriously for clinical trials in influenza patients.

We already have an indication that immunomodulatory treatment might reduce the higher influenza mortality rates of younger adults. In an experiment published in 2008, "children" and "young adult" mice were subjected to ischemia reperfusion injury of the liver.[99] (In "young adult" mice more so than in "children," this condition is highly inflammatory and often fatal). In this study, pre-treatment with rosiglitazone was able to "roll back" the harmful inflammatory response of young adults to the more benign response of children. This important experiment could have implications for patient care in an influenza pandemic. In a study comparing the effects of pH1N1 virus infection in newly weaned and adult ferrets, the immunological and pathological findings in newly weaned ferrets were less severe and the clinical illness was much milder.[100]

The four groups of the immunomodulatory agents mentioned above are now produced as inexpensive generics in developing countries. If these agents could be shown convincingly to reduce mortality in patients with severe influenza, they would be

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available to treat patients in any country with a basic health care system on the first pandemic day. For each patient, the cost of this "bottom up" approach would be less than one dollar.[36]

Corticosteroid Treatment of Influenza: A Cautionary Note

Physicians often use corticosteroids to treat patients with sepsis, severe acute lung injury and acute respiratory distress syndrome in the hope that the anti-inflammatory effects of these agents will improve survival. Unfortunately, the evidence supporting their use is weak.[101,102] This includes observational studies in 6650 patients and ten randomized controlled trials involving 1090 patients hospitalized with pneumonia due to pandemic H1N1 virus infection.[102] Some of these studies have even shown that corticosteroids were harmful,[103,104] leading to a spirited discussion of the pros and cons of steroid treatment for viral pneumonia.[105,106]

A full discussion of corticosteroid treatment lies outside the bounds of this review. Nonetheless, it is worth noting the considerable overlap in their cell-signaling pathways and those for the immunomodulatory agents under discussion here (**Table 1** and ref. 106). There is also considerable molecular crosstalk between PPAR agonists and the glucocorticoid receptor.[107,108] Thus, despite encouraging results from the observational studies reviewed above, these similarities argue for caution regarding benefits that might be anticipated from treating influenza patients with statins and these other agents. That being said, fibrates and statins enhance the signaling effects of corticosteroids,[108,109] so combination treatment that includes a corticosteroid might be more beneficial than single agent treatment. In addition, a direct comparison of dexamethasone and pioglitazone treatment of smoke-exposed mice infected with H1N1 influenza A virus showed greater efficacy for pioglitazone.[110]

A Research Agenda for Immunomodulatory Treatment of Influenza Patients

Several years ago, a five-point research agenda was proposed for identifying one or more immunomodulatory agents that might be used to manage patients with pandemic influenza (**Table 2** and ref. 36). If immunomodulatory agents could be shown to be effective, they would be used primarily to treat pandemic patients with severe, life-threatening illness, although for special groups (e.g., health care workers or very high-risk patients) they might also be used for prophylaxis, especially when vaccines and antiviral agents are unavailable.

Since this agenda was first presented, there has been progress on several fronts. We now have good international information on the companies that produce statins, glitazones, fibrates and metformin. We also have information on quantities produced each year, distribution channels and wholesale prices for branded and generic products. For example, a few years ago it was estimated that in 2012, 48 billion doses of statins would be distributed throughout the world (DS Fedson, unpublished observation). Of these doses, 77% would be produced as generics, and the average price per generic dose would be $0.17. Almost 20 billion doses would be distributed in countries outside the United States, Canada and Western and Central Europe. If it were assumed that in a pandemic, 5% (350 million) of the world's 7.0 billion people would need to be treated for ten days (a deliberately exaggerated assumption), 3.5 billion doses would be required. This would account for approximately 7% of the annual consumption of statins worldwide. Information on statins and the other immunomodulatory agents mentioned above needs to be updated. Nonetheless, it is already evident that these drugs are currently available as generics wherever there are physicians who treat patients with cardiovascular diseases and diabetes. In most countries, expensive programs for stockpiling them would not be needed.

Soon after the H1N1 pandemic virus emerged in 2009, several groups of intensive care specialists tried unsuccessfully to initiate randomized controlled trials of statins in pH1N1-infected, ICU-admitted patients.[111,112] The focus on statins was based largely on encouraging findings from observational studies of statins use in patients with sepsis and pneumonia (no such information was available for the other agents). Nonetheless, there is broad agreement that randomized controlled trials will be needed to determine whether immunomodulatory treatments are efficacious. In anticipation of the next pandemic, clinical trials should be organized beforehand so they can be started immediately after the emergence of a new pandemic virus. In the meantime, similar trials conducted in patients with seasonal influenza should be undertaken. Investigators will have to decide whether the trials should be restricted to ICU-admitted patients, who might not benefit,[76,77,113] or include all hospitalized patients at risk of rapidly developing more serious illness.[79] Regardless of their design, the trials will be expensive, so animal studies comparing different immunomodulatory agents will be needed to guide the choice of which agent(s) to evaluate in clinical trials.

Animal Studies of Immunomodulatory Treatment of Influenza

Investigators will need to proceed with caution because the results of laboratory studies might be difficult to interpret.[81,82] For example, studies by several virologists have yet to show that statins are effective in mouse models of influenza, yet many human studies suggest that they are (see above). There is no ready explanation for these discordant results, but it is worth noting that although the molecular mechanisms for the inflammatory responses of humans and mice are in many ways similar, they are quantitatively very different. For example, a comparison of the response of human and mouse macrophages to LPS-induced inflammation showed that the human response was 10,000 times more sensitive to LPS than that of mice.[114]

In mouse models of immunomodulatory treatment, choosing a test virus that more clearly mimics human influenza virus infection could be important (**Table 2**). For example, the mouse-adapted PR8 virus is highly lethal for mice, but markedly less so for man, so a pH1N1 virus might be a better choice. Likewise, choosing an appropriate infecting dose is also

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Table 2. Research to identify immunomodulatory agents that might be used to treat pandemic influenza patients*

• Test candidate agents in mice, ferrets and non-human primates to identify agents that might be used to manage patients
• Later study these agents in cell culture and animals to identify molecular mechanisms that explain their beneficial effects
• Document where these agents are produced as generics and determine quantities produced, surge capacities, patterns of distribution and costs to public programs
• Establish a process for managing their global stockpiling before a pandemic or distribution once a pandemic begins
• Plan randomized controlled trials of promising agents to begin immediately upon the emergence of a new pandemic virus

*Adapted from reference 36.

probably important; an illness caused by a dose that is 100% lethal in mice will probably not reflect the spectrum of human influenza because not all patients with severe illness die. The choice of mouse strain might also be critical. Influenza virologists usually use either inbred BALB/c or C57Bl/6 mice,[115] and these two strains have been used in all experimental studies of immunomodulatory agents.[84-89] These strains might not be optimal for determining which agent might best counteract the more intense inflammatory response in man. For example, in a study of host factors involved in the pathogenesis of pH1N1 virus influenza, BALB/c mice, which have a Th-2 bias, were shown to be less suitable than C57Bl/6 mice, which have a Th-1 bias.[116] Neither strain might be as suitable as DBA/2J mice, which have a more intense inflammatory response to influenza virus infection.[117-119] Investigators should also consider testing immunomodulatory agents in mice that have the same high-risk conditions as humans; e.g., pregnancy,[62] obesity[120] and cardiovascular disease.[121] Once the most promising immunomodulatory agent (or combination of agents) has been identified, it should then be studied in ferrets and, if necessary, in non-human primates. In all of these studies it will be important to compare responses in "children" and "adults."

The Broader Implications of Immunomodulatory Treatment for Global Health

Despite compelling arguments for undertaking the laboratory and clinical research needed to show definitively whether immunomodulatory agents would improve survival in severe influenza, virologists and public health officials, including those at the World Health Organization, remain focused on targeting the virus. Yet success with treating the host response to influenza might be extended to the management of several other diseases in which cytokine dysregulation and the loss of homeostatic defense mechanisms leads to poor outcomes; for example, pneumococcal pneumonia,[122] severe malaria,[123] dengue hemorrhagic fever[124] and critical illness associated with trauma[125,126] and burn injury.[127,128]

Almost a half-century ago, physicians and public health officials learned that syndromic treatment of the host response to severe acute diarrheal illness could be accomplished with an inexpensive and universally available oral rehydration solution (ORS).[129] Although vaccines that target a few of the pathogens responsible for diarrheal disease have been developed since then (e.g., cholera and rotavirus vaccines), it is syndromic treatment with ORS that has saved millions of lives. Had decisions been made long ago to ignore the possibility of simple and inexpensive treatment and instead focus only on developing vaccines, these millions would have died. Scientists and health officials responsible for developing a practical response to a global influenza pandemic should learn from this history.

Conclusion

The dysregulated host response seen in severe influenza (and many other conditions) might be treatable with safe, inexpensive generic immunomodulatory agents. Whether these agents will actually be effective in routine clinical care needs to be demonstrated in further laboratory and clinical research. Nonetheless, it should be clear to everyone that such treatment would be of immense practical importance to global public health. Until now, influenza virologists have been reluctant to undertake experiments to identify potentially useful and widely available agents that investigators could test in clinical trials and physicians could use to manage their patients. Until they do, public health officials will have no alternative but to recommend that most of the world's people confront the next global influenza pandemic with little more than hand washing and social distancing. These "technologies" represent the best of 19th Century public health practice. In the 21st Century, we can and should do much better.[36,130]

The debate about H5N1 transmissibility research should be about more than how to define its boundaries, important though this may be. The controversy presents influenza virologists, bio-security experts and public health officials with a new opportunity to jointly define a research agenda to identify existing immunomodulatory agents that could be used in a practical response to a global influenza pandemic. This opportunity must not be wasted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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From: Stanley Plotkin [mailto:stanley.plotkin@vaxconsult.com]
Sent: Monday, December 21, 2015 12:26 PM
To: National Science Advisory Board for Biosecurity (NIH/OD) <NSABB@od.nih.gov>
Subject: gain of function

Dear Committee:

I have perused the document produced by Gryphon Scientific and chose to comment on the section titled "Benefits." My comments are contained in the attachment.

Yours truly,
Stanley A Plotkin, MD

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ALLEGED GoF BENEFITS	
Excerpts from RBA Report	**Plotkin comments**
GoF approaches that alter host range and enhance virulence uniquely enable the development of animal model systems that recapitulate human disease pathogenesis	True for enhancement of animal virulence, but issue is increasing human virulence, which is not the same, and infectiousness is just as important, as shown by high virulence but low spread of avian strains.
GoF approaches that enhance virulence are also uniquely capable of showing that live attenuated vaccines (LAVs) do not recover virulence upon growth *in vivo*	LAVs are not made that way, they are made with RNA segments of attenuated virus and RNA segments of current virus that give immunogenicity. There is no example of LAV becoming more virulent in vivo
This particular type of experiment simply increases the human health risk of the attenuated strain to approach that of wild type strains	Not true if HA made hypervirulent.
GoF that lead to evasion of therapeutics are critical for the development and regulatory approval of new therapeutics	Nonsense. Resistance to neuraminidase inhibiitors has not heeded approval.
Of note, adaptation to a new host typically attenuates virulence in the original host (in the case of SARS and MERS-CoV, humans)	Don't understand this. Adaptation to humans of SARS resulted in more virulence for humans. MERS is more virulent for humans than camels.
GoF can enhance virus production	No relationship to enhancement of virulence
GoF approaches that enhance the infectivity, transmissibility and virulence of influenza viruses inform pandemic risk assessments of circulating influenza viruses	So far this is unproven.
These risk assessments facilitate more rapid initiation of response activities such as pre-pandemic vaccine	Only true if there is natural increase of virulence. In any case, avian flu has high mortality but has yet to become epidemic
GoF approaches also guide selection of viruses used as the basis of pre-pandemic vaccines	No truth to this. Antigenic match is more important than virulence match
GoF approaches that lead to evasion of vaccines are uniquely capable of determining whether viruses can acquire mutations to escape neutralization of candidate broad-spectrum or universal influenza vaccines, a critical aspect of testing the potential field efficacy of vaccines in development	This is tautology. This is the unproven argument for GoF. We do not know if causing evasion in the lab predicts what will happen in nature.
No increase in human health risk is posed by strains that can overcome the protection afforded by universal vaccines because the latter are not available.	Don't understand logic. If a strain evades future vaccines it is perforce a threat to health if it escapes.
GoF approaches that lead to evasion of existing natural or induced immunity have potential to improve the efficacy of seasonal influenza vaccines	I suppose there is that potential, but no proof as yet and danger of escape.

From: D Gold
Sent: Wednesday, December 30, 2015 6:08 PM
To: National Science Advisory Board for Biosecurity (NIH/OD) <NSABB@od.nih.gov>
Subject: Comments on GOF Risk Benefit Report

Dear Dr. Viggiani,

Attached are my comments on the Gryphon Scientific risk-benefit analysis. I am very concerned about the short time-frame provided for public comment. I believe this important issue deserves a thorough review, not only by the scientific community immediately involved in the issue, but by a lot of other interested people, such as myself, who do not have the resources to review a 1000 page document, plus additional material, in less than 30 days.

Thank you for your consideration.

Deborah Gold, MPH, CIH

Deborah Gold

December 30, 2015

Christopher Viggiani, Ph.D.
Executive Director, NSABB
NIH Office of Science Policy
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Via email: viggianic@od.nih.gov, nsabb@od.nih.gov

Dear Dr. Viggiani and Members of the Board:

I am writing in regards to the recently published draft report by Gryphon Scientific, *Risk and Benefit Analysis of Gain of Function Research* (Report). My comments today are based on my 21 years of experience with Cal/OSHA, which began as an industrial hygienist in the Enforcement unit, and ended as Deputy Chief for Health, from which I retired in December 2014.

I think the less than 30 day period provided between the publication of the Report and the January 7-8 meeting is completely inadequate for a thorough review. For that reason I strongly suggest that you allow a public comment period of no less than 90 days, which would be more typical for such a significant project that has occupational as well as local, regional and world-wide public health implications. The Report has a number of significant gaps and unsubstantiated assertions, which will require time and research to address. The document doesn't address a number of risks, such as occupational risks to many categories of workers (which I will briefly explain below). It also does a poor job of explaining any true benefits to be achieved from this research. In this letter, I am addressing only the issues of biosafety as they apply to occupational exposures both immediate and distant from the laboratory. I will not try to restate the excellent discussion by the Cambridge Working Group (CWG), and encourage you to address the issues that they raise.

As a person who has been involved in public health as an advocate and as an occupational safety and health professional for decades, I am particularly appalled that in 2015, a government agency would consider basing a decision on a report that discounts the global risk from intentional development of drug resistant viruses with the following statement:

> *"The creation of an antiviral resistant strain could increase the consequences of a global outbreak, but only in more economically developed countries where caches of these antivirals could be handed out to a significant fraction of the infected population. A strain of seasonal influenza that can overcome protective vaccination could also increase the consequences of an outbreak in high income countries, which has the resources to vaccinate their population quickly."* (Executive Summary, page 2)

This is an extremely cynical statement, particularly in the light of the recent experience with Ebola Virus Disease (EVD), in which it became abundantly clear that countries with more resources MUST find ways to make care and treatment available for infectious diseases throughout the world, if for no other reason than their own self-interest. One would hope that should a pandemic influenza strain emerge in lower income countries, the US, in particular, would make sure that all relevant treatments were made available to reduce loss of life and improve outcomes in impacted countries.

Biosafety Risks

I believe that the Report fails to take seriously the biosafety hazards that currently exist in research laboratories. It states that the *"state of knowledge of the rates and consequences of human errors in life science laboratories is too poor to develop robust predictions of the absolute frequency with which laboratory accidents will lead to laboratory acquired infections."* This is an understatement regarding the lack of information, which is due both to lack of recognition of laboratory acquired infections (LAIs) as well as under-reporting. There is no public means of tracking other losses of containment, although there is apparently some tracking under the select agents program, which does not include all pathogens under consideration. A Report by the National Research Council (Review of Risk Assessment Work Plan for the Medical Countermeasures Test and Evaluation Facility at Fort Detrick: A Letter Report) cited unpublished 2010 CDC data, which found 395 reports of potential release events of select agents from 2003 to 2009.

The scientific and popular literature describe a plethora of laboratory incidents. For example, in 2012, an employee at the San Francisco Veterans Administration laboratory conducting research to develop a meningitis vaccine contracted meningitis and died. The joint investigations conducted by Cal/OSHA, OSHA, and the California Department of Public Health, found numerous problems in biosafety protocols, including unverified biosafety cabinets, during the investigation. In 2004, workers at the Children's Hospital Oakland Research Institute had to undergo chemoprophylaxis to prevent development of anthrax after it was determined that a shipment of purportedly deactivated *B. anthracis* had caused the death of some laboratory animals injected with the material. In 2014, CDC workers were exposed to live anthrax, and in 2015, the US Department of Defense was initially reported to have sent live (instead of deactivated) anthrax spores to labs in 9 states; this estimate was later revised to include labs in all 50 states and 9 countries. Mistaken shipments of pandemic or other virulent influenza strains have also been documented.

High containment laboratories, particularly BSL 3 laboratories, have proliferated in the past two decades, and on several occasions the US General Accounting Office has warned of the hazards associated with the lack of centralized regulation. Nancy Kingsburg, speaking on behalf of the GAO at a 2014 Congressional hearing following the anthrax exposures at the CDC explained some of their findings:

> *"The number of biosafety level (BSL)-3 and BSL-4 laboratories (high-containment laboratories) began to rise in the late 1990s, accelerating after the anthrax attacks throughout the United States. The laboratories expanded across federal, state,*

academic, and private sectors. Information about their number, location, activities, and ownership is available for high-containment laboratories registered with CDC's Division of Select Agent and Toxins (DSAT) or the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) as part of the Federal Select Agent Program. These entities register laboratories that work with select agents that have specific potential human, animal, or plant health risks…

"According to most experts that we have spoken to in the course of our work, a baseline risk is associated with any high-containment laboratory. Although technology and improved scientific practice guidance have reduced the risk in high-containment laboratories, the risk is not zero (as illustrated by the recent incidents and others during the past decade). According to CDC officials, the risks from accidental exposure or release can never be completely eliminated and even laboratories within sophisticated biological research programs—including those most extensively regulated—has and will continue to have safety failures. Many experts agree that as the number of high-containment laboratories has increased, so the overall risk of an accidental or deliberate release of a dangerous pathogen will also increase. We recommended that CDC and APHIS work with the internal inspectors for Department of Defense and Department of Homeland Security to coordinate inspections and ensure the application of consistent inspection standards." (Testimony of Nancy Kingsbury, July 16, 2014, available at: http://gao.gov/assets/670/664799.pdf)

Occupational Risk

The Report appears to consider that any risk below that of a pandemic has been addressed through other biosafety guidance, such as the 2013 CDC Biosafety Recommendations for Work with Influenza Viruses Containing a Hemagglutinin from the A/goose/Guangdong/1/96 Lineage (MMWR June 28, 2013 / 62(RR06);1-7). However, the Report fails to consider how workers will be affected by enhanced (GOF) pathogens.

The immediate risk is to laboratory workers, who are the only workers addressed in the 2013 CDC Recommendations. If pathogens are successfully engineered to be more virulent, then exposed laboratory employees are at risk of more serious disease, including permanent sequelae or death. If those pathogens are engineered to be more resistant to anti-viral drugs, then employees who contract LAIs are also at greater risk of serious illness. Similarly, infections which might have been prevented through vaccination of employees will occur if employees have unprotected exposures.

California is unique among the states in adopting regulations to address biological risks to laboratory workers (beyond the requirements of the Bloodborne Pathogens standards). In 1994, the California Occupational Safety and Health Standards Board adopted a standard requiring employers to maintain biosafety cabinets in accordance with CDC recommendations, and adopted a laboratory biosafety section as part of the Aerosol Transmissible Diseases Standard in 2009. (This regulation can be found at: http://www.dir.ca.gov/Title8/5199.html.) During the relatively few inspections Cal/OSHA has conducted in chemical, biochemical, biomedical and microbiological laboratories the agency has found significant problems in maintenance of

containment equipment, training, personal protective equipment, ventilation, and other control measures.

Although laboratory employees are at the greatest risk of exposure and may be aware of their risk, they are only one category of employees who may be at risk. It is often the case that specific research projects in a lab, particularly research that may have defense implications, is unknown to other occupants of the building or outside of the specific lab. Although BSL 3 and BSL 4 labs are required to have secondary containment, the minimal level of acceptable negative pressure, and more importantly, the minimal maintenance provided in some facilities, may expose workers outside of the lab to the enhanced pathogens. Other routes of exposure include contact with waste or equipment that has been inadequately decontaminated, contact with co-workers who either have been inadequately decontaminated, are infectious but asymptomatic, or have symptoms that they and others attribute to seasonal influenza, particularly when the pathogen has been enhanced to be more transmissible between people. First responders, such as firefighters, police and paramedics may also be exposed to these pathogens in responding to incidents at these facilities. Those non-GOF workers may be unaware that they have been exposed to an enhanced pathogen, and therefore will not provide that information to medical providers, or even seek prompt medical attention, because they assume they have contracted a wild-type, self-limiting infection.

Nor does the occupational risk stop there. Unless a health care facility is specifically informed about the nature of the enhanced pathogen, health care workers would treat a symptomatic patient as they would any similar patient, unaware that they are being exposed to an enhanced pathogen that may not be susceptible to anti-viral drugs, etc. An influenza patient is not typically housed in airborne infection isolation, for example. Clinical laboratories conduct analyses for various pathogens and do not have BSL3 capacity. (This contributed to decisions to handle EVD samples at state or federal labs). If a pathogen such as SARS or MERS is not currently circulating in the US, absent a positive history such as travel to outbreak areas, it is unlikely that health care providers would suspect that infection. While a laboratory may instruct its employees to contact a specific health care provider if they become ill, when the employee is ill they may not be able to direct their medical care. It is unlikely that employees with secondary or inadvertent exposures as described above will be able to provide information to health care providers. We have seen with SARS in Asia and Canada, with MERS in Saudi Arabia and Korea, and with Ebola, that health care workers are at significantly increased risk from diseases borne by patients. All of these occupational risks would also apply if there were an intentional breach of the type identified in the biosecurity section.

Although these local infections may never rise to the level of an epidemic or pandemic, the risks to workers and their families and other contacts must be addressed in conducting this research. The risk to the community from laboratory exposures is illustrated by the nine cases of SARS in 2004 in Beijing resulting from exposure of two graduate students at China's National Institute of Virology Laboratory. In addition to the two graduate students who became ill, the mother of one student contracted the disease and died, and a nurse who treated the student also became ill. Five other SARS patients were linked to contact with the nurse. The 1978 Sverdlovsk anthrax leak, in which an estimated 100 people died due to the release of anthrax spores from a military facility, is another example of how laboratory incidents may impact the surrounding community.

I do not believe that this Report provides a basis for reinstituting NIH funding for GOF research on influenza or coronaviruses. Given the current state of control measures in “high containment” laboratories, the risks to employees and the community from GOF, such as enhanced virulence, transmissibility, drug resistance and evasion of immunity, are serious enough to warrant continuation of the moratorium. The benefits identified in the report are speculative, and in most cases can be achieved through other, less dangerous means. I refer you to comments by Dr. Raina MacIntyre and the CWG for more thorough discussion of the Report.

I hope that the NSABB decides to extend the period for public comment, as I look forward to providing additional comments on the full document and associated working papers. I also believe that the discussion must go beyond the interested scientific community to reach out to unions and other employee representatives, and members of the public. Thank you for your consideration.

Sincerely,

Deborah Gold, MPH, CIH

From: Lynn Klotz [mailto:lynnklotz@live.com]
Sent: Thursday, December 31, 2015 4:35 AM
To: National Science Advisory Board for Biosecurity (NIH/OD) <NSABB@od.nih.gov>
Subject: Comments on the Gryphon risk-benefit assessment

Dear NSABB,

Attached are my comments on the Gryphon risk-benefit assessment in advance of the January 7 meeting.

Lynn Klotz
Senior Science Fellow
Center for Arms control and Non-proliferation

Attachment

A Commentary and Analysis of Chapter 6 in Gryphon Scientific's Report: Risk and Benefit Analysis of Gain of Function Research

A Commentary and Analysis of Chapter 6 in Gryphon Scientific's Report: Risk and Benefit Analysis of Gain of Function Research

By: Lynn C. Klotz, Ph.D.
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Date: December 30, 2015

With less than a month to analyze and comment on the thousand-page report before the December 31 "soft" deadline for the NABCC January meeting, it would be nearly impossible for anyone to follow in detail Gryphon's analysis and comment on all the chapters. I chose, therefore, to limit my comments and analysis to only Chapter 6, the Biosafety Risk Assessment.

Summary

Based largely on Gryphon's numbers, I estimated the likelihood-weighted fatalities for a pandemic seeded by a laboratory-acquired infection (LAI) from an mtHPAI (a mammal-adapted airborne-transmissible highly pathogenic avian influenza virus). Along the way, comments on aspects of Gryphon's Chapter-6 analysis will be made.

Generally, likelihood-weighted pandemic risk equals probability of a pandemic times consequences of the pandemic. The probability of a pandemic from a lab escape through an LAI for ten labs conducting research on mtHPAI strains for ten years was found to be 1.8×10^{-05} using Gryphon's numbers that an LAI lab escape leads to a pandemic. Ten labs for ten years is my estimate of the "research enterprise" that already is or will be conducting research with these strains.

In my analysis, consequences were restricted to fatalities. The case-fatality rate was chosen to be 5%, which is twelve-fold less than the World Health Organization's accepted case-fatality rate of 60%. For a pandemic infecting 25% of the world's population, the number of fatalities would be 90 million. With these numbers, the Likelihood-weighted fatalities for the research enterprise are

$$\text{likelihood-weighted fatalities} = (1.8 \times 10^{-05}) \times (90 \times 10^{6}) = 1{,}640$$

For a single lab for a single year, the likelihood-weighted fatalities are 10x10-fold less or 16.4, which I call "the fatality burden" for the lab. To put this fatality burden in perspective, no Institutional Review

Board tasked with assessing human subject research would approve a proposed research project with an expected 16.4 fatalities per year.

This 5% case fatality rate is much higher than the small fraction of 1% claimed by Morens and Taubenberger. But airborne-transmissible mtHPAI, a key focus of the NIH deliberative process, are not wild type viruses. They infect lung to lung via the airborne route. We do not know the case-fatality rate for these strains. It could be quite high, perhaps over 60%. Arguments over case fatality rate for wild-type HPAI are likely moot. Since we don't know, and the potential consequences in morbidity and mortality are so high, caution dictates instituting a ban on making and researching live airborne-transmissible mtHPAI. This will be discussed a bit more at the end of my Commentary.

The Gryphon report seems to dismiss gain-of-function studies in SARS and MERS, by assuming that mitigation measures such as quarantine should prevent a large outbreak. SARS has about an eight-day incubation period before an infected person can transmit infection, a fair amount of time to quarantine those exposed to an infected person. Timely and strong mitigation measures may be possible in developed nations, but we need only to look at the Ebola epidemic in the poor and war-torn African nations to understand the potential for large outbreaks. GOF studies in SARS and MERS should be looked at very carefully and perhaps many banned as well.

Details of and rationale for my analysis

In describing my analysis and the rationale for the numbers and estimates used, I will rely on quotes and data from the Gryphon risk assessment. Also, I will reproduce here relevant tables and graphs from the Gryphon RA as a convenience to you.

The three steps to a pandemic are illustrated in Gryphon's Figure 6.2 for seasonal influenza.

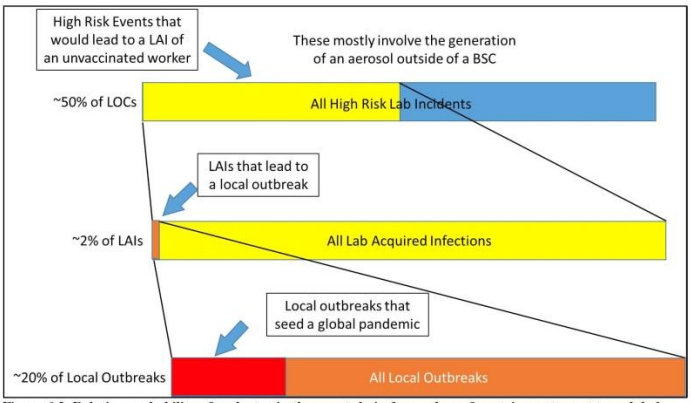

Figure 6.2. Relative probability of each step in the event chain from a loss of containment event to a global pandemic for a loss of containment event involving seasonal influenza.

The absolute probability of an escape from a single lab in a single year though an LAI or other routes, p_1, is not shown in the graph. Making a reasonable guess for this absolute probability is the subject of Section 6-8 of the Gryphon report. For the second and third steps, the probability that the LAI will lead to a local outbreak is estimated by Gryphon to be about 2% for seasonal influenza, and the percentage of local outbreaks that will lead to a pandemic is about 20%. The probability that a single lab in a single year seeds a pandemic, pan_1, is then

$$pan_1 = p_1 \times 0.02 \times 0.2 = 0.004\ p_1 \qquad (1)$$

The 0.004 or 0.4% figure is quoted many times throughout Chapter 6 (sometimes Gryphon uses 0.5%). It is the result of their analysis of risk using branching theory and the HHS-BARDA Interactive Influenza Model.

Gryphon's dividing the path from a lab escape leading to a pandemic into two steps--(1) the escape causes a local outbreak and (2) the local outbreak then causes a pandemic--is not necessary. A single infected researcher can seed a pandemic directly. From Figure 4 in the Lipsitch *et al.* (2003) paper, the probability that the single infected researcher can seed a pandemic is 10% to 30% (for R_0=1.3 and smaller k values). Thus, the 0.4% value is likely 1/0.02 = 50-times higher due to eliminating this intermediate local-outbreak step.

These are two well-established methods; and given Gryphon's high-level mathematical and analytic skills, I will use the 0.4% Gryphon number to stay closer to their analysis. In Gryphon's words,

> "Sufficient biomedical and epidemiological evidence exists to develop robust models of the initiation of an outbreak from the primary to the secondary cases and the expansion of this outbreak within a community to eventually spark a global pandemic."

For a "research enterprise" of 10 x 10 = 100 lab years, the probability that some lab in some year will seed a pandemic is approximately 100 x pan_1 or 100 x 0.004 p_1 = 0.4 p_1. Clearly, p_1 is the key probability to carry out the analysis with high confidence. The two parts of Gryphon's and my analysis that are uncertain are values for the probabilities p_1 and for the case fatality rate.

To obtain an absolute probability for p_1, in Section 6.8 Gryphon basically guesses. In Gryphon's words,

> "…absolute risk estimates are desired. For this reason, the historical rate of laboratory acquired infections could be used to predict a reasonable upper bound for the frequency with which these incidents occur. However, the research team is unaware of any laboratory acquired infections in laboratories that study influenza or coronaviruses and so an absolute risk analysis will have at its foundation a weak estimate of the frequency at which laboratory acquired infections occur. That being said, this historical rate of laboratory infections can then be combined with calculated rates of laboratory acquired infections leading to secondary infections, local outbreaks and global pandemics from this assessment to produce an estimate of absolute risk."

The remarkable observation here is that in 100 mostly seasonal influenza BSL2 research labs over 20 years of research, Gryphon was unable to find any reported LAIs. Gryphon offers the following explanation:

> "The project team knows of no laboratory acquired infections involving any one of these laboratories. This lack of a laboratory acquired infection could be due to the fact that none have occurred in that time frame or that some have occurred but the project team does not have access to the reports or data."

The report neglects additional possible reasons: asymptomatic or subclinical infections, or misattribution of LAI to the community. If a researcher contracts seasonal influenza, it might not be detected, as a high proportion of seasonal influenza is subclinical particularly among individuals with considerable levels of natural immunity or immunity from vaccinations. If it were detected clinically, it would likely be attributed to a community infection, not from the lab. In any case, reporting it as possibly an LAI would lead to time-consuming follow up. It could be unspoken policy in seasonal influenza research labs to not report infections of uncertain origin given that the infected person will be better in few days. I find it difficult to believe that there have been no LAIs in 100 mostly BSL2 labs in 20 years. That would be inconsistent with rates of LAI in other BSL2 labs, even in settings where underreporting is known to be a problem.[1]

In any event, where Gryphon expected to find statistically-useful real data on LAIs in seasonal influenza labs, it found none. I suspect Gryphon then resorted to historical data from other labs researching other pathogens to obtain its range of zero to ten LAIs. Gryphon raises a valid and important point on using accident data from other pathogens and laboratories.

> "very little data exists on human reliability in life science laboratories, which drives the probability that laboratory acquired infections occur in the first place. Fortunately, the accidents that humans cause (or contribute to) in the laboratory are the same regardless of the pathogen manipulated. That is, workers may overfill a centrifuge tube with the same frequency regardless of the pathogen in the tube, or will slip while working with scissors during a necropsy with the same frequency regardless of the pathogen studied. Because the absolute rate at which these accidents happen and cause infections is not supported by robust data, absolute estimates of the rate of laboratory acquired infections cannot be made using the method described in this report."

Lacking real data, Gryphon makes an educated guess that perhaps three LAIs did occur in the hundred mostly seasonal influenza labs over the twenty years. Gryphon calculates

> "Across all 100 laboratories …if the assumption is made that three LAIs have surreptitiously occurred, then …a global pandemic could be triggered once every 750-5,000 years."

Gryphon chooses to report its findings as "return periods" in years, not probabilities. Return periods are the reciprocal of probabilities per year. My problem with return periods is that they can fool you into thinking something is safe when it is not when consequences are considered. It is necessary to stick to the more fundamental probabilities for calculations.

For seasonal influenza, with Gryphon's guess of 3 LAIs in 20 x 100 = 2,000 lab years, the probability of an LAI (escape) per lab per year is $p_1 = 3/2{,}000 = 1.5 \times 10^{-3}$. (Three LAIs in over 2,000 lab years seems conservative to me, there were likely more.) Thus, the return period for one lab in one year is $1/p_1 = 667$ years for an LAI to occur. This may seem like the experiments are safe, as they will be completed in

[1] Marc Lipsitch contributed to this paragraph

perhaps 10 years, well short of the return period. But looked at another way, in 20 years this means that there are three LAIs, where each one has a not-insignificant chance of causing a seasonal influenza pandemic. I would not accept those odds.

What is the probability, $p_{1,HPAI}$, for research on mtHPAI? I assume that research on mtHPAI is conducted in BSL3 labs using the level of biosafety for research on SARS, as SARS has a case-fatality rate of around 10% considerable caution is warranted. Gryphon lists relative probabilities compared to work with seasonal influenza in their Table 6.2, reproduced here.

Table 6.2. Relative probability of a laboratory acquired infection for the various pathogens considered in this study as compared to work with seasonal influenza.

Pathogen	Biosafety Level	Relative Probability of an LAI*
Seasonal influenza virus	BSL2	1 (defined)
Pandemic influenza virus	BSL3	0.10 (0.07-0.15)
Avian influenza virus	BSL3	0.43 (0.21-0.90) (mostly of birds)
SARS-CoV	BSL3	0.03 (0.02-0.04)
MERS-CoV	BSL3	0.01 (0.006-0.02)

These data are generated by comparing the sums of the frequency of infection from all loss of containment pathways for each pathogen. In this case, we use the term laboratory acquired infection to include an infection of wild birds to capture the comparative risk of working with avian influenza viruses. The numbers in the parenthesis are the results from the p5 and p95 outputs of the Monte Carlo analysis.

Before using data from Table 6.2, this is a good place to state what I view as a major shortcoming in the Gryphon report. Sources of data and calculations to obtain it are not referenced throughout Chapter 6. Are the sources not referenced in the Supplementary material? In the published literature? In spreadsheets available from Gryphon? In Table 6.2, for instance, the caption could have provided references. Thus, we don't know how solid or significant various pieces of data are, unless Gryphon chooses to discuss it. I suspect that Gryphon could have used much more time in preparing its report.

Furthermore, Gryphon ignores the frequency of accidents over the years in labs researching Select Agents compiled by the CDC in 2013. Gryphon's analysis also ignores the highly publicized recent accidents in the CDC lab. While none of these accidents involved seasonal influenza, somewhere in Chapter 6 they should have been acknowledged and incorporated into their analysis. It is unclear why guesses well below the empiric rate of LAI should be used for a risk analysis. Nonetheless, in what follows, Gryphon's numbers are accepted for the sake of argument.

From Table 6.2, the probability of an LAI in a SARS lab is a factor about 0.03 times that of seasonal influenza. Specifically, $p_{1,HPAI} = 0.03 \times 1.5 \times 10^{-3} = 4.50 \times 10^{-5}$ for a SARS or mtHPAI lab where $p_{1,HPAI}$ is the probability of an LAI for a single lab for a single year. The probability of a pandemic from a single lab in a single year, pan_1, is

$$pan_1 = 0.004 \times p_{1,HPAI} = 0.004 \times 4.50 \times 10^{-5} = 1.8 \times 10^{-7}$$

As an illustration, I conservatively estimate 10 labs conducting mtHPAI research for 10 years (100 lab years),[2] each with the laboratory safety of a SARS lab. The probability that the research enterprise will seed a pandemic, RE, is approximately

$$RE = 100 \times pan_1 = 1.8 \times 10^{-5}$$

The return period, 1/RE, is 55.6 thousand years, which would seem to make the research very safe if it were not for the potential consequences of millions of fatalities.

The likelihood-weighted pandemic risk, LWR, is given by

LWR = (Probability of a Pandemic) x (Consequences of a Pandemic)

Consequences are restricted to fatalities in this analysis. The case fatality rate was chosen to be 5%, which is twelve-fold less than the World Health Organization's accepted case fatality rate of 60%. For a pandemic infecting 25% of the world's population of 7.3 billion, the number of fatalities, F, would be

$$F = 7.3 \text{ billion} \times 0.25 \times 0.05 = 90 \text{ million.}$$

With these numbers, the likelihood-weighted fatalities, LWF, for the research enterprise is

$$LWF = RE \times F = (1.8 \times 10^{-05}) \times (90 \times 10^{6}) = 1{,}640.$$

The Likelihood-weighted fatalities for a single lab in a single year is 1,640/100 = 16.4, which I call the "fatality burden" for the single lab in a year. As pointed out earlier this fatality burden is likely 1/.02 or 50 times higher. To put this fatality burden in perspective, no Institutional Review Board tasked with assessing human subject research would approve a proposed research project with an expected 16.4 fatalities per year (or 50 x 16.4 = 820 fatalities per year, accounting for the 50-fold error discussed above). There are research approaches not involving live mtHPAI for elucidating the molecular virology of airborne transmission. Such safe research approaches ought to be employed, and research with lab-made, airborne-transmissible, live mtHPAI be banned.

One point still needs to be discussed, case fatality rate. The 5% case fatality rate used in this analysis is much higher than the small fraction of 1% claimed by Morens and Taubenberger. There are well-documented studies (for instance, here and here) that claim the case fatality rate is not low but close to the 60% often quoted for wild type H5N1 HPAI. But the airborne-transmissible mtHPAI, a key focus of the NIH deliberative process, are not wild type viruses. They infect lung to lung via the airborne route. We do not know the case-fatality rate for these strains. It could be quite high, perhaps over 60%. So, arguments over case fatality rate for wild-type HPAI are likely moot. Because the potential consequences in morbidity and mortality are potentially high, caution dictates instituting a ban on making and researching live airborne-transmissible mtHPAI.

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[2] Gryphon estimates "approximately 40 research groups in the US because these groups have been performing, or have the capacity to perform, certain types of GOF experiments involving influenza, MERS, and SARS viruses. This maximum number is supported by the case studies examined which showed that a new discovery in virology may proliferate to as few as one and as many as 70 new groups around the world within 10-15 years."

The Gryphon report seems to dismiss gain-of-function studies in SARS and MERS, by assuming that mitigation measures such as quarantine should prevent a large outbreak. SARS has about an eight-day incubation period before an infected person can transmit infection. Timely and strong mitigation measures may be possible in developed nations, but we need only to look at the Ebola epidemic in the poor and war-torn African nations to understand the potential for large outbreaks. GOF studies in SARS and MERS should be looked at very carefully and many perhaps banned as well.

December 31, 2015

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RE: Draft Report By Gryphon Scientific, *Risk and Benefit Analysis of Gain of Function Research*

Dear Dr. Viggiani and Members of NSABB:

Below are my comments on the draft GOF report prepared by Gryphon Scientific. I have spent nearly 35 years as an industrial hygienist on the staff of labor organizations, most recently with the Safety and Health Department of the AFL-CIO (from which I retired in October 2013). During these years, I devoted my efforts at protecting workers from exposures to hazardous substances and infectious agents, some of which are the subject of the referenced report. At the AFL-CIO, I held major responsibility for representing the AFL-CIO at OSHA rulemaking proceedings and public hearings on proposed safety and health standards. I also served on various policy and scientific committees addressing occupational safety and health issues, including two Institute of Medicine committees dealing with respiratory protection for healthcare worker exposed to H1N1 influenza and personal protective equipment for healthcare workers exposed to pandemic influenza and other viral respiratory diseases.

I would like to make the following points on the draft document:

(1) When OSHA issues any major proposed new or revised safety and health standard, the agency typically provides for a minimum 90 day period for submitting written comments and documents to the record, followed by public hearings and a post-hearing opportunity to submit additional comments and documents to the record. In the case of the draft GOF Report, a period of less than 30 days was established for receiving public comments. Additionally, this shortened timeframe included the holiday and New Year period. A comment period of less than 30 days for a report of such importance and magnitude as the draft GOF is absurd. It does not provide for an adequate time period in which to digest, analyze, and respond to the many critical issues raised in the report. Instead, this woefully shortened comment period has all the appearance of nothing more than a superficial attempt at giving the public an opportunity for

comment while the real underlying objective is to move the process to a rapid conclusion. In my view, the comment period needs to be extended considerably.

(2) I have serious concerns that the report fails to address the consequences of the release of highly virulent and drug resistant viruses in the laboratory as well as the general environment. The infection risk posed to laboratory workers who are exposed to these newly designed agents, via whatever protective measure is breached, is hugely problematic. With no effective drugs or vaccine available, a highly virulent virus is likely to cause serious, if not fatal, adverse health effects in an infected lab worker. And healthcare workers who provide care to infected lab workers are even more vulnerable, given the absence of preparedness by our healthcare facilities to protect its workforce from patients infected with dangerous viral agents – one only needs to examine the problems uncovered in Dallas, TX for healthcare workers during the Ebola outbreak. This problem is further magnified enormously by the fact that 49 of the 50 states in the United States do not have a mandatory OSHA standard that requires employers to protect workers from infectious disease (California being the exception). Instead, CDC guidelines are merely recommendations that state and local health departments and healthcare facilities can ignore with impunity (this was rampant during the H1N1 pandemic).

(3) I'm not convinced that the benefits of this research path outweigh the risks. Once the genie is out of the bottle, it will be difficult to put it back in. Developing the technologies to create highly virulent, drug resistant infectious agents represents a security risk that is too dangerous to undertake. For when the technologies are developed in the US, essentially all governments and other forces will at some point be able to utilize the technologies, be they friendly government's or not. In the "wrong hands", we would then have a huge problem to address.

Thank you for the opportunity to submit these comments,

Sincerely,

Bill Kojola

Bill Kojola

Silver Spring, MD 20902

Written comments for NSABB meeting Jan 7-8, 2016

Marc Lipsitch, DPhil

Harvard T.H. Chan School of Public Health

Cofounder, Cambridge Working Group

Contains original written comments submitted December 31, 2015 plus additional comments (on benefits) submitted January 3, 2016. Additional comments added to this version concern the Benefit Assessment and are in dark red font.

Dear Chairman Stanley and Members of the NSABB:

I am pleased to have the opportunity to offer written comments pertinent to the upcoming meeting of the Board, specifically concerning the Risk-Benefit Assessment provided by Gryphon Scientific and the Working Paper Draft dated December 23, 2015 by the NSABB in response to the RBA. I consider these in order and conclude with some comments on the process. My comments are in no sense a complete evaluation of any of these documents, given their enormous length and the short time available. I may choose to submit additional comments at a later date. These are simply my comments on the most important issues I have noticed in the time available.

In these comments I make reference to written comments submitted by other members of the public. I will not reiterate the details of their arguments, but I register my agreement with them in particular cases.

I. Comments on the NSABB working paper (WP)

Comment I.1. Overall, the working paper accurately identifies that the research involving a reasonably anticipated creation of a strain combining high virulence and high transmissibility is the central "Gain of Function of concern" research that should be the focus of scrutiny. That has been apparent since the start of this process, and it was the NSABB that broadened the charge of Gryphon to include many less-risky experiments. The NSABB has now appropriately narrowed the focus to GOF of concern.

Comment I.2. The scope of GOF of concern identified by the NSABB, however, is unduly narrow. It includes as a condition for GOFoc, not only combined virulence and transmissibility, but also the ability to evade countermeasures. This is inappropriate because countermeasure availability for a transmissible, virulent strain produced by GOF is not guaranteed even to the US, and timely countermeasures will be unavailable for the vast majority of the world. Thus even a strain susceptible to antivirals and to immunity produced by a hypothetical vaccine could do tremendous damage. **Resistance to countermeasures should be deleted from the requirements for GoFoc.**

Comment I.3. The WP fundamentally fails to answer the question posed in the NSABB's own Principle 9 to determine "whether there are certain studies that should not be conducted under any circumstances, and if so, articulate the critical characteristics of such studies." Instead, it states "There are life sciences research studies 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" (p. 4). **This is an abdication of responsibility given that the Working Paper is a response to a 1000-page RBA.**

Comment I.4. Given the findings of the RBA, the most important of which is that a single year of BSL3 work on mammalian-transmissible high-path avian influenza has an expected fatality toll of some 50+ lives, **creating mammalian-transmissible avian influenza is GOF of the highest concern and should not be undertaken**. Similarly, creation of novel coronaviruses with transmissibility similar to SARS have, by Gryphon's reasoning, an expected toll of >10 lives per laboratory-year. This also is research that should not be

undertaken, by Gryphon's own reasoning (here I rely heavily on the Public Comments submitted by Lynn Klotz). As noted by Klotz, no Institutional Review Board would approve a research plan with an expected fatality toll in this range. The fact that the expected fatality toll is in this case a low probability of a catastrophic death toll should, if anything, be an even stronger bar to such activities.

Comment I.5. Recommendation 2, that "In general, oversight mechanisms for GOF studies of concern should be incorporated into existing policy frameworks" should be modified or replaced. **There is strong evidence that existing policy frameworks are *inadequate* to regulate GOF of concern.** That evidence includes the following:

- Prior to the Funding Pause in October 2014, HHS had put in place a Framework for review of H5N1 GOF research [1] and later for H7N9 GOF research[2]. These frameworks were inadequate in that (i) no formal risk or benefit assessment (ie nothing quantitative) was done when HHS considered these studies [this I have heard from a participant in the review]; (ii) the review was done in private with no public input; (iii) the same day that the H7N9 framework was published [2], Fouchier and colleagues published a paper describing HHS-sponsored GOF research on H7N9 (see http://comments.sciencemag.org/content/10.1126/science.1244158). This is prima facie evidence of the inadequacy of the Frameworks.
- During the funding pause, Baric and colleagues published a paper [3] describing NIH-funded experiments that by any standard met the terms of the funding pause: "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." While the circumstances surrounding this work (in particular why it was permitted under the funding pause) have not been publicly described, this is clear evidence that even enhanced scrutiny may be circumvented by NIH as funder and/or an investigator.
- These instances, along with common sense, indicate that placing NIH or CDC (both direct funders and in the case of CDC, performers of GOF of concern research) as the judges of what may and may not be performed is a direct conflict of interest and is not a way to arrive at impartial judgments.

Given these considerations, an interagency task force that receives input from HHS but is independent of it seems much preferable to existing mechanisms[4]. Expansion of the Select Agent rule to prohibit GOF of concern without the specific consent of such a board would be a possible policy solution.

Comment I.6. **The suggestion to use existing regulatory approaches for regulating GOF of concern requires that institutional oversight have the capacity to deal with this topic, making fine distinctions that have not yet been defined, much less codified in ways that can be applied at the institutional level.** There is no reason to think that Institutional Biosafety Committees have the requisite expertise to perform risk-benefit evaluations on this scale. As an example, the minutes of the University of Wisconsin IBC obtained by *Nature* for GOF work by Prof. Kawaoka (http://www.nature.com/polopoly_fs/7.18249!/file/WISC_Review.pdf) contain no numerical estimates of risk (that is to say, do not perform risk assessment, although they assert on p. 1 that it includes a risk benefit assessment) and accept uncritically all assertions of the investigator about benefits of the proposed work, including false statements ("The proposed research will determine the likelihood of an influenza virus similar to the 1918 pandemic strain of [sic] emerging naturally." The research has been published, and that likelihood has not been determined. Thus the benefit assessment cannot be considered adequate either. *This further demonstrates the inadequacy of existing regulatory mechanisms to deal with GOF of concern.*

II. Comments on the Gryphon Risk-Benefit Assessment (RBA)

Comments on Biosafety Risk.

Comment II.1. There is a presumption in the RBA, starting with the Executive Summary, that experiments with the pandemic H1N1 strain of 1918 constitute an acceptable level of risk against which other experiments should be compared. Moreover, it is stated (section 1.1) that " No GoF experiment is likely to create a strain riskier than work with wild-type 1918 H1N1." **Both the assumption that this level of risk is acceptable, and the claim that no GOF experiment is likely to create a strain riskier than work with wt 1918 H1N1, are unjustified.** The source of either claim is unclear, and in particular the claim that no more dangerous strain exists is based on a misreading of the literature on H1N1 case-fatality risk (see comment below). The quoted statement also directly contradicts the statement (RBA p. 78-9): "In short, a strain of influenza virus that is as transmissible (or to which the population has as little minimal immunity) as newly emerged pandemic strains WHILE leading to a case fatality rate of more than 5%, would pose more of a risk of a global pandemic than any wild type strain heretofore identified. No experiments that are likely to be conducted under the rubric of GoF research will drive risk more than this combination of traits or significantly increase the risk of a laboratory acquired infection."

Comment II.2. The RBA appropriately identifies creation of novel viruses combining mammalian virulence with mammalian transmissibility as the most risk-enhancing experiments (Figure 6.1). Notably, it does *not* add "resistance to countermeasures" to this category, although it does note that resistance to countermeasures would further enhance the risk of such experiments in the developed world, where countermeasures might be available. **I recommend that the NSABB adhere to this classification, without requiring resistance to countermeasures, when defining GOF of concern.**

Comment II.3. Notwithstanding the serious flaws in the analysis that lead to an underestimate of the risk of such experiments, I draw the NSABB's attention to the fact that: Using Gryphon's own numbers, the expected fatality toll from a lab-year of coronavirus experimentation with enhanced transmissibility in BSL3 is approximately 16 fatalities

(Written comments of Lynn Klotz to the NSABB, December 2015). A corresponding calculation for mammalian-transmissible avian influenza would be around 50 fatalities. **Absent an exceptionally compelling prospect of life-saving, justly distributed benefits, this conclusion from the RBA merits the immediate discontinuation of experiments meeting the definition of GOF of Concern proposed by the NSABB, with the modification suggested above to remove the requirement for escaping countermeasures.**

Comment II.4. The RBA contains a number of erroneous parameter assumptions that lower the estimate of risk of various experiments relative to appropriate estimates. These are shown in a table below.

Table 1: Errors in the Risk Assessment Leading to Underestimate of Risk

Assumption	Source of Error and corrected assumption	Impact on risk estimates
CFR of 1918 influenza is 10-20% of infected persons (Table S7 in supplement http://www.gryphonscientific.com/wp-content/uploads/2015/12/Supplemental-info-disease-course-of-influenza.pdf)	Misreading of a graph in the reference cited, ref 82. Actual values are mainly in the range of 0.5%-3% of those with clinical disease (except for extremes of age). This is therefore a 6-20x overestimate, not accounting for medical improvements and larger denominator of asymptomatic cases)	Allegedly acceptable risk of experiments with 1918 pandemic flu are significantly overstated, raising the bar for what should be permitted to a much higher level and seemingly justifying false statements like that noted in Comment II.1.
CFR of influenza is 0.0001%-0.00043% of those infected (Table S7 in supplement http://www.gryphonscientific.com/wp-content/uploads/2015/12/Supplemental-info-disease-course-of-influenza.pdf)	Error source unclear. Actual estimate from authoritative systematic review [5] is 0.001%-0.010%. Thus this is more than a 10x error.	Suggests manipulations of seasonal influenza have smaller risk than they do.
R0 of SARS is 1.5, may go as low as <1 (http://www.gryphonscientific.com/wp-content/uploads/2015/12/Supplemental-information-R0-of-CoV.pdf).	This seems to result from a combination of not understanding what R0 is (it does not incorporate the later stages of the epidemic or the impact of control measures), especially as used in a branching process. Averaging over different phases of the epidemic is completely inappropriate. Two of the three authoritative estimates of R0 are not cited; with Riley (cited) they all estimated approximately 3.0 [6-8]	Significantly underestimates severity of SARS outbreaks
Control measures (community mitigation) will be effective	There is no evidence of this in modern influenza pandemics	Underestimates severity and probability of pandemic from

		modified influenza strains
Assumes that all event trees for LAI happen in the source lab at the specified biosecurity level	Errors with a probability of leading to a LAI have repeatedly, consistently occurred outside the source lab, usually at a lower BSL. For example, 2014 CDC anthrax exposure occurred in BSL2 after inadequate decontamination; 2014 CDC HPAI exposure occurred outside source lab (though fortunately at BSL3) due to contamination of sample; 2014 CDC Ebola exposure occurred at BSL2 due to falsely assumed decontamination and removal to lower BSL; 2015 DOD anthrax exposures occurred in conditions designed for inactivated anthrax because of lack of proper inactivation.	This leads to neglect of a fault tree that routinely occurs in top US government labs, in which the probability of LAI is higher, the likelihood of its going undetected is higher, the likelihood of having prophylactic measures in place for laboratorians is lower, and thus the risk of outbreak and escaping local control is higher. For more details, see [9].
Probability that a single LAI with a pandemic-capable influenza triggers a pandemic is 0.4%.	Other branching process models, which account for negative-binomial overdispersion, find estimates of 5-60%[6, 10, 11]	Vastly underestimates by 1-2 orders of magnitude all risks.

Comments on biosecurity

These may be supplied at a later date when time allows.

Comments on benefits of GOF

Comment II. 5. A very good feature of the BA is the consideration of alternatives to GOF experiments to either answer the same scientific questions or achieve similar public health benefits in a different way. Had appropriate skepticism been applied to the claims of those performing and sponsoring GOF research, these alternatives would have proven far more

compelling than the Benefit Assessment suggests**. The extreme skew of the experts consulted for the Benefit Assessment (see Section III below), combined with a surprisingly credulous evaluation of their claims, leaves the BA with a number of statements that do not stand up to scrutiny.**

Comment II.6. The vast majority of the public health benefits asserted for GOF experiments are for the development of costly countermeasures, including vaccines and antiviral drugs**. These benefits will be limited to the wealthiest populations, which have access to the newest drugs and vaccines.** This problem is recognized in the BA, for example with respect to antiviral development in the statement (p. 438): "In sum, although U.S. policy supports the donation of influenza antivirals in the event of a pandemic, the relatively small number of doses donated in comparison to the global need in the event of a pandemic means that developing countries would face shortages, which would in turn exacerbate poor usage in-country." In the case of pandemic preparedness benefits, similar statements are made (pp. 442 and 444) In contrast, the risks of GOF research, which are distributed globally and if anything will fall harder on lower-resource populations, [12], As recently as 2009, developing countries had little access to antivirals or vaccines until long after the peak of pandemic risk. **In this sense, GOF experiments unjustly require unconsenting populations to bear pandemic risk while promising them no realistic prospect of benefit. This is a serious and independent ethical objection to such research, which is not adequately addressed in the separated ethical analysis commissioned by NSABB.**

Comment II.7. At multiple points in the BA and in the corresponding section of the Executive Summary (1.4), there are statements that particular types of experiments involving the evasion of novel therapeutics or vaccines involve no human health risk because the countermeasures are not yet extant. This statement is false unless one assumes that the immunity produced by novel vaccines, and the protection by novel treatments, is unrelated to that produced by existing natural exposure or vaccines (for immunity) or antivirals (for resistance). Vaccine-related immunity and natural immunity may involve the same epitopes (especially as vaccine development is often based on observations of naturally acquired immunity), and cross-resistance between novel and existing antivirals within a class is expected, just as cross-resistance within existing classes (eg zanamavir and oseltamivir, or

rimantadine and amantadine) can occur with the same mutation. **In summary, these statements -- that GOF to evade countermeasures not yet available has no human health risk -- are unjustified and tend to underestimate the risk of corresponding GOF experiments.**

Comment II.8. Virtually all of the benefits of GOF experiments described in the Benefit Assessment are characterized as *not* unique to GOF (Table 9.1, 3rd column). This is extremely important, as it means that the Benefit Assessment characterizes nearly all of the claimed benefits as being achievable by alternative means. While some of these alternative means involve localized risk of infection of a few laboratory personnel, these risks are minimal in comparison to pandemic risk. Thus **the BA implies that nearly all of the benefits of GOF (especially of GOF of concern) could be achieved with alternatives that avoid the vast majority of GOF risk. This finding creates a strong presumption in favor of alternative approaches [13]. Indeed, under such circumstances, I would argue it is unethical to perform GOF of concern experiments[14].**

Comment II.9. It is stated (Section 1.4, p. 6) that "GoF approaches that enhance virulence represent the most efficient and effective strategy for discovering novel virulence factors, which may be good targets for new therapeutics." This does not make sense. If the virulence factors found are not present in naturally circulating strains, then finding changes that could result in increased virulence could only facilitate the development of therapeutics for strains that do not exist. **Development of therapeutics for nonexistent strains would be a highly speculative activity with little likelihood of being supported in the absence of a foreseeable market.**

Comment II.10. The most important unique benefit asserted for GOF of concern (enhancement of mammalian transmissibility of avian influenza) is informing pandemic risk assessment and prioritization of countermeasures. The BA asserts these are of particular importance in rapid risk assessment and prioritization: "GoF data play an important role in rapid risk assessments when novel flu viruses first emerge in human populations due to the early availability of sequence data. These risk assessments facilitate more rapid initiation of response activities such as pre-pandemic vaccine development" (p. 244).

The assertion of these unique benefits represents an uncritical acceptance of the assertions of GOF proponents that is contrary to the evidence. The assertion has four **fatal flaws**:

1. **Every mutation cited by GOF proponents as having been discovered in GOF experiments and used to prioritize pandemic response [15, 16] has been found (in most cases prior to the GOF studies) in a non-dangerous, non-GOF study and identified as a predictor of pandemic risk.** Thus the claim of uniqueness is unjustified (see Table below). Alt-GOF can, and indeed have, identified mutations and phenotypes of concern.
2. While it is true that GOF-identified mutations have been used to inform surveillance and preparedness strategies, **there is no evidence that the use of such findings has improved the accuracy of these strategies.** Using information is different from using it productively. There is no case in which a pandemic has been anticipated using GOF-derived data. The evidence that decisions are improved is weakened even further by the fact that many GOF mutations have highly context-dependent effects, so that they may or may not be predictive in actual wildtype strains [17, 18].
3. **GOF data may be misleading, resulting in worse not better decisions.** In the one case when a pandemic has emerged during the era of widespread virus sequencing (2009) it lacked the mutation PB2 E627K[17], which has been identified as perhaps the most important single GOF mutation for mammalian adaptation [19]. Surveillance did not identify this virus in swine before it became pandemic, but had it been identified, use of GOF data would have incorrectly classified it as low risk. Ruling out one of the four strains that caused a pandemic in a century as low risk would be a remarkably large error. Incidentally, this story also highlights the uselessness of any genetic information when surveillance does not catch a strain before it emerges. No pandemic strain has ever been discovered in animals before it caused a pandemic.
4. The accuracy of ferrets in predicting human transmissibility is imperfect, though they are the best available model [20]. Indeed, **several GOF researchers and proponents have said in public meetings that they expect the strains isolated from ferret transmission experiments would not be readily transmissible in**

humans. This uncertainty nullifies or even negates the benefit for pandemic preparedness, because mutations identified in these studies, which are being used as *positive predictors of human pandemic potential*, are in fact uncertain predictors and may not indicate human transmissibility. This could mean that strains with little human pandemic potential are tagged for special prevention efforts, and/or that strains with different genetic profiles that are actually high-risk are identified as low-risk and deprioritized. Notably, this uncertainty makes the use of such mutations highly impractical for decision-making, yet it does not nullify the risk presented by these strains. It negates or nullifies the benefit, and yet only reduces the risk, because the statement that the GOF strains would not be pandemic-capable in humans are informed guesses, which may be wrong.

Table 2: Non-uniqueness of benefits for GOF of concern studies for pandemic response

Mutation claimed to be significant based on GOF by Davis [15]or Schultz-Cherry [16]	Prior studies not involving PPP creation that identified these mutations	Counterexamples
H5 &H7N9 HA Q222L HA	[21-23] [18, 24-26]	CONTEXT DEPENDENCE: Changes do not quantitatively shift receptor binding in related H5 strains [18]
H5N1 HA S133A S135N S123P S155N	[23, 27]	
H7N9 HA T156A, Q222L	[28, 29]	
PB2 E627K, D701N	[30]	MISLEADING INFERENCE: Both absent in 2009pdm [17]. Would have led to its misclassification as low risk

Comment II.11. I endorse the critiques submitted as comments to the NSABB by Dr. Stanley Plotkin of the asserted benefits of GOF experiments. These represent further examples of the widespread exaggeration of benefits and downplaying of alt-GOF in the Benefit Assessment. I will not recapitulate these here but simply incorporate them by reference to his remarks.

III. Comments on the NSABB process

On the whole, I would characterize the process of the RBA development as distinctly unwelcoming of public participation, and as heavily weighted in favor of those who do and fund GOF of concern research. Major shortcomings include the following:

- At all in-person meetings of the NSABB including the upcoming one, public comment has been possible only in writing or in person, but not in real time by any electronic medium. This excludes many persons who may wish to comment in real time on the proceedings but do not have the ability to attend in person.
- The development of the RBA included site visits and conversations with many investigators in 14 labs, most of which do GOF research. The benefit assessment in particular received more than 80 percent of its input from scientists who do PPP research or representatives of agencies that fund it (RBA Fig. 9.3). In contrast, only about 10 (12%) of those interviewed for the benefit assessment were persons who have expressed reservations about RBA research.
- The timeline for public comment was extremely short, with the NSABB waiting apparently 2 weeks from the time it saw Gryphon's RBA until it posted it publicly, and then only 1 month (including Christmas and New Year's) before its meeting. There were only 8 days including Christmas from the release of NSABB's draft working paper to the deadline for public comments to be submitted and seen by the NSABB members.
- The unbalanced representation of GOF researchers/funders versus those who have raised concerns is continued in the agenda for the January 7-8 meeting. 3 outspoken critics are on the panels, plus one additional member of the Cambridge Working Group; 9-10 funders or researchers of GOF studies are speaking. This imbalance was raised in plenty of time to the NSABB leadership, which chose not to address the problem.

Overall, it is difficult to see this process as having been designed to maximize public input or to achieve balance between proponents and critics of GOF, or indeed to address the

inherent conflicts of interest of those whose research or funding portfolios are at issue in the discussion.

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January 3, 2016

National Science Advisory Board for Biosecurity
Office of Science Policy, OD
Rockledge 1, Suite 750
6705 Rockledge Drive
Bethesda, MD 20817

Dear Chairman Stanley and Members of the NSABB:

I am writing to express my support for the comments submitted by Marc Lipsitch, Stanley Plotkin, and Lynn Klotz. I am deeply concerned by the potential fatalities that could result from accidental laboratory infections that might occur in a laboratory conducting gain-of-function research on influenza and other infectious diseases. The number of accidental releases of potentially fatal pathogens in recent years has demonstrated unequivocally that human error is inevitable and impossible to completely eliminate from experiments with deadly pathogens. Specifically, I agree with Dr. Lipsitch that resistance to countermeasures should be deleted from the requirements for Gain of Function of concern research. I concur that the benefits of this research are overestimated, and that the risks are being borne by non-consenting members of the public and disproportionately by those in developing nations that would not be able to implement countermeasures.

Thank you for taking these concerns seriously and including the voices of concerned scientists in your deliberations on how to address the potential dangers to the public from GOF research.

Sincerely yours

Carlos S. Moreno, Ph.D.
Associate Professor
Department of Pathology and Laboratory Medicine
Emory University School of Medicine

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615 Michael St.
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From: Nariyoshi Shinomiya [mailto:shinomi@ndmc.ac.jp]
Sent: Monday, January 04, 2016 3:43 AM
To: National Science Advisory Board for Biosecurity (NIH/OD) <NSABB@od.nih.gov>; Viggiani, Christopher (NIH/OD) [E] <christopher.viggiani@nih.gov>
Cc: 'Husbands, Jo' <JHusband@nas.edu>
Subject: Written comment to the NSABB meeting
Importance: High

Christopher Viggiani, Ph.D.
Executive Director, NSABB
NIH Office of Science Policy

Dear Dr. Christopher Viggiani,
(CC to Dr. Jo Husbands)

I am a person who were invited by Dr. Amy Patterson to the 2012's workshop on "Gain-of-Function (GOF) Research on Highly Pathogenic Avian Influenza (HPAI) H5N1 Viruses" as a panelist. Since then I have been having a strong interest in this topic. This time I got the information about the NSABB meeting from Dr. Jo Husbands. Unfortunately, I cannot attend the meeting because of my tight schedule. She suggested me to make some comment to the meeting. Here I send my comment about the issue of GOF studies. I hope it is taken up in the session of Public Comment Period or so.

I hope my comment reaches you in time.

Best regards,
Nariyoshi Shinomiya

****
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****

National Defense Medical College

A comment from the viewpoint of balance between scientific advancement and risks to the society

by Nariyoshi Shinomiya
Professor, Department of Integrative Physiology and Bio-Nano Medicine
National Defense Medical College, Japan

Mr. Chairman, distinguished representatives of the NSABB, and participants in the symposium,

It's my pleasure if I could have a chance to make a comment in such an important meeting about gain-of-function (GOF) studies.

In collaboration with the group of the University of Bradford, UK, our research group has developed a biosecurity education module for scientists which is translated into may languages and used worldwide. In my school the biosecurity education is very successful; the educational programs for undergraduates as well as graduate course students are now dealt with a regular subject and supported by the faculty members. I lead a symposium related to "dual use research of concern (DURC) issues" in the Japan Association for Bioethics every year from 2011, in which many participants have an interest in this issue and join the discussion.

As many of you may know, after we introduced the discussion of this issue several years ago, the Science Council of Japan revised a code of conduct for scientists in which an article has been added as one of the most important standards that the scientists should think about. The article says "Dual use concern of scientific research: The scientists should recognize that their research results might be used for malign destructive purposes against their will, so when they perform research activities and make their results public, they select appropriate measures and methods which are acceptable to the society (*the original sentence is written in Japanese*)." Also, the Center of Research and Development Strategy, Japan Science & Technology Agency released a book for strategic proposal entitled "Preparedness Framework and Its Governance of Dual Use Research of Concern for Promising Progress of Life Sciences". However, those efforts just showed a general instruction and a framework. So a precise explanation and a scenario setting in each case of DURC should be added.

Here, I would like to make a comment about GOF studies from the viewpoint of balance between scientific advancement and risks to the society.

I believe the freedom of research activities should be guaranteed to the maximum within professional ethics, yet the following points should be considered.

1. In the risk-benefit analysis, a way of thinking or a condition that the benefit exceeds the risk should be explained in plain words to lay persons. Sometimes the concept, recognition, or perception of risks is quite different among people, and may change depending on the situation. The same thing can be said about the benefits. So, not abstraction but specific idea in each case should be provided.
2. What are real risks in each GOF study? Possible scenarios should be provided, and the influence of the risks needs to be analyzed with accuracy. Are the risks acceptable to the society? If the benefits are considered to exceed the risks, the researchers should ask the society about their research idea and need to get people's consent.
3. It is important for mass media to inform the society about the facts of GOF studies because mass media is the main source for people to get information of this sort. Some mass media may have their own opinions and off course the freedom of speech should be considered, yet information without a bias/arbitrary expression is a priority matter.
4. Similar to nuclear or chemical weapons there is no going back once we get a thing in our hands. So, before making new infectious agents we should deliberate upon the GOF studies. Not only the control of a new infectious agent itself but also the regulation of the information how to make it should be considered as the subject of this issue.

I hope these points are extensively discussed, and clear conclusions are provided in the NSABB meeting.

Thank you, Mr. Chairman, distinguished representatives of the NSABB, and participants in the symposium.

From: Steven [mailto:steven.salzberg@gmail.com] **On Behalf Of** Steven Salzberg
Sent: Tuesday, January 05, 2016 3:26 PM
To: National Science Advisory Board for Biosecurity (NIH/OD) <NSABB@od.nih.gov>
Cc: Steven Salzberg <salzberg@jhu.edu>
Subject: comments on risks and benefits of gain-of-function research in the life sciences

Dear NSABB,

Please accept the attached letter as my comments on the risk-benefit assessment provided by Gryphon Scientific and the Working Paper Draft of Dec 23 by the NSABB.

My comments are very brief, but given the time constraints I didn't have time to write more. Nonetheless I feel this is such a critical issue that I wanted to at least register my grave concerns about the continuing efforts by a small number of scientists to create highly virulent viruses in their laboratories.

Sincerely,
Steven Salzberg
--

Steven L. Salzberg, Ph.D.
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Blog: genome.fieldofscience.com

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January 5, 2015

Dear Chairman Stanley and Members of the NSABB:
I'm writing to express my strong support for the comments submitted by Dr. Mark Lipitsch, which I have read closely and with which I agree in almost every detail. I am very concerned that the continuing gain-of-function research on influenza viruses, and more recently on other viruses, presents extremely serious risks to the public health. As a former influenza researcher myself, I also concur with Dr. Lipitsch and others that the benefits of gain-of-function research are minimal at best. These minimal benefits could easily and far more safely be obtained through other avenues of research.

In addition to my primary research at Hopkins, I also write a popular science blog at Forbes magazine, where I expressed grave concerns about this topic in August 2013, in an article that had over 50,000 hits (see http://www.forbes.com/sites/stevensalzberg/2013/08/08/scientists-will-create-a-deadly-new-flu-strain-just-to-prove-they-can/). As I wrote then, it seems clear that some of the scientists leading the GOF research on influenza are doing it primarily for the publicity and acclaim (including publication in high-profile journals), while downplaying the risks. Their primary justification for their work–that lab-created influenza strains will teach us how to avoid or treat future pandemics–has no evidence to support it.

I am pleased that the U.S. government has called for a pause in this research, and I strongly urge you to recommend that this pause become permanent. Continuing research that is intended to make influenza or other viruses more infectious, or more deadly, carries great risks and almost no practical benefits.

Sincerely,

Steven Salzberg

Steven Salzberg, Ph.D.
Bloomberg Distinguished Professor of Biomedical Engineering, Computer Science, and Biostatistics
Director, Center for Computational Biology
McKusick-Nathans Institute of Genetic Medicine
Johns Hopkins School of Medicine

From: Charles Stack [mailto:cstack3@uic.edu]
Sent: Wednesday, January 06, 2016 10:04 AM
To: National Science Advisory Board for Biosecurity (NIH/OD) <NSABB@od.nih.gov>
Subject: NSABB Public Comment regarding Gain of Function safety
Importance: High

I am a Public Health Advisor to the FBI through the Chicago "Infragard" Chapter and have this comment regarding your upcoming NSABB meeting.

I have reviewed the "Risk and Benefit Analysis of Gain of Function Research" Draft Final Report, December 2015 and am VERY concerned that the largest, deadliest incident of domestic breach of biosafety, namely the "Amerithrax" incident involving the late Bruce Ivins PhD, was only mentioned once in 1006 pages.

The incident of Dr. Ivins is very troubling because he had a high-level US Government security clearance, worked within the government's secure bioterrorism research infrastructure, had privileged access to dangerous infectious materials, and was able to single-handedly conduct an attack upon the American public that resulted in five deaths and other injuries. Ivin's actions put scores of US government workers, including law enforcement, politicians postal service and others at risk, and this event cost untold millions in remediation and lost business.

Gain of Function research entails a similar risk to the public. I consider the likelihood of a researcher releasing potentially pandemic agents much higher than an armed assault upon university laboratories by terrorists or criminals, but this scenario is downplayed. Motivations could include mental illness, coercion by a foreign power, or self-aggrandizement as seemed to be the case for Ivins.

Thank you for your consideration of my comments for your meeting.

Sincerely,
Charles R. Stack, MPH
DrPH Candidate
Estelle Goldstein Memorial Scholar
UIC School of Public Health

Deputy Sector Chief, Healthcare and Public Health
FBI Infragard

From: Simon.Warne@hse.gsi.gov.uk [mailto:Simon.Warne@hse.gsi.gov.uk]
Sent: Wednesday, January 06, 2016 12:10 PM
To: National Science Advisory Board for Biosecurity (NIH/OD) <NSABB@od.nih.gov>
Cc: m.skinner@imperial.ac.uk; Andrew.Cottam@hse.gsi.gov.uk; Michael.Paton@hse.gsi.gov.uk
Subject: FW: Risk and Benefit Analysis of Gain of Function Research undertaken by Gryphon Scientific

This is a brief response to the public consultation on the above document. I am a Specialist in Biosafety working in the UK for the Health and Safety Executive (HSE). I replying as the Secretary of the UK Scientific Advisory Committee on Genetic Modification (SACGM). Ideally I would have liked to put together a response to reflect the consolidated views of SACGM and other parts of the UK regulatory structure covering genetic modification. However, this has not been possible in the limited time available. I, therefore, hope that there will be a further opportunity to have an input as this Risk and Benefit Analysis covers an important area of science policy and the consequences of 'getting in wrong' are clearly very significant.

In the time available I have not been able to go into all the detail within the Risk and Benefit Analysis. My attention has been primarily focused on section 6 covering 'Risk Assessment of Laboratory Accidents and Natural Disasters'. In my analysis to date there one statement that has particularly caught my attention. On page 164 it is stated that 'a global pandemic caused by research on pandemic influenza viruses is expected every 560-13000 years'. I believe that as part of this exercise it is crucial that this figure is placed in some kind of context. As part of this I would draw your attention to the HSE document 'The Tolerability of Risk from Nuclear Power Stations' that is available at the following link http://www.onr.org.uk/documents/tolerability.pdf . This HSE document identifies what is seen as an acceptable risk for a major accident at a nuclear or chemical plant causing roughly 1500 casualties (see pages 31-33).

I would like to put down this e-mail as a marker that I would be interested in being informed about any further consultation on this issue. As I have said above, it is unfortunate the current consultation period of less than a month (including the Christmas break) has not provided time to prepare a more substantial response. If we were given sufficient time I would hope that the UK would be able to put together a consolidated response to represent the views of the various regulatory and policy making bodies.

Simon Warne PhD
Biotechnology Portfolio Holder / HSE Biological Agents Unit / Desk 41 5S.2 Redgrave Court / Bootle / L20 7HS / United Kingdom

Telephone +44 (0) 151 951 3335

From: Andrew Kilianski
Sent: Thursday, January 07, 2016 1:42 PM
To: National Science Advisory Board for Biosecurity (NIH/OD) <NSABB@od.nih.gov>
Subject: Tangible translational products from GOF research

Some members of the board have asked for clarification and specific examples of basic-to-clinical research products generated from GOF research. The attached article and link below can clarify some of these questions. It was published during the RBA and might not have been available to everyone. Thanks!

Andy

Attachments

1. **Gain-of-Function Research and the Relevance to Clinical Practice -- J Infect Dis. 2015**
   http://jid.oxfordjournals.org/content/early/2015/10/27/infdis.jiv473

2. **When gain-of-function research is not "gain-of-function" research -- EMBO Rep., 2015**
   http://embor.embopress.org/content/early/2015/11/04/embr.201541617

Journal of Infectious Diseases Advance Access published October 28, 2015

REVIEW ARTICLE

Gain-of-Function Research and the Relevance to Clinical Practice

Andy Kilianski,[1] Jennifer B. Nuzzo,[2] and Kayvon Modjarrad[3]

[1]BioDefense Branch, Biosciences Division, Edgewood Chemical Biological Center, Aberdeen Proving Ground, [2]University of Pittsburgh Medical Center – Center for Health Security, Baltimore, and [3]US Military HIV Research Program, Walter Reed Army Institute for Research, Silver Spring, Maryland

The ongoing moratorium on gain-of-function (GOF) research with highly pathogenic avian influenza virus, severe acute respiratory syndrome coronavirus, and Middle East respiratory syndrome coronavirus has drawn attention to the current debate on these research practices and the potential benefits and risks they present. While much of the discussion has been steered by members of the microbiology and policy communities, additional input from medical practitioners will be highly valuable toward developing a broadly inclusive policy that considers the relative value and harm of GOF research. This review attempts to serve as a primer on the topic for the clinical community by providing a historical context for GOF research, summarizing concerns about its risks, and surveying the medical products that it has yielded.

Keywords. gain of function; potential pandemic pathogens; coronavirus; influenza; science policy; health policy.

Gain-of-function (GOF) research typically involves mutations that confer altered functionality of a protein or other molecule. These types of mutations have been used as powerful tools to understand basic bacterial and viral biology and pathogen-host interactions. Despite the recency of a public debate, GOF research has constituted a common, long-standing practice in the discipline of microbiology. In recent years, a public discussion has surfaced, centering on the application of GOF research to highly pathogenic and potentially lethal viruses [1]. Despite the emergence of this public dialogue, much of it has been steered by members of the microbiology and policy communities. There remains room for additional input from clinical and public health practitioners, who are often the end users of the products GOF research yields. As the results from GOF research are salient to both the improved understanding of disease pathogenesis and the development of medical countermeasures to infectious diseases, the debate over its safety and value is of direct relevance to medical and public health practitioners. This review article will provide a historical context for the current debate, describe the potential risks and benefits of this type of experimental study, and present some examples of how GOF research translates into tangible products of use to practicing clinicians.

GOF: AN HISTORICAL PERSPECTIVE

Genetic mutations can be classified in many ways, one of which is by their impact on protein function. In the simplest terms, mutations can result in a protein's loss of function or GOF. The distinction between the 2 phenotypes is not always clear. GOF research, in this context, usually results in the introduction of changes to biological agents that might increase their ability to infect a host and cause disease by enhancing their transmissibility or pathogenicity [2]. In recent years, this class of research has provoked controversy, particularly in the setting of dual use research of concern (DURC). DURC is a subset of microbiological research that, as defined by the US government, "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,

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or national security" [3, p. 1]. Some of the potential consequences of DURC that have been cited include the manipulation of pathogens for use as biological weapons and the development of mechanisms by which pathogens can evade countermeasures. DURC currently pertains to the select agents and toxins defined by the US Centers for Disease Prevention and Control and the US Department of Agriculture [4]. Among these pathogens, highly pathogenic avian influenza virus (HPAI) is of high concern to both public health and agriculture authorities.

Public discourse on the controversies of influenza virus research is about a decade old, beginning in 2005 with the reconstitution of the 1918 influenza A(H1N1) [5–7]. The more recent debates over the safety and merits of GOF research first surfaced in 2010, in the context of studies on the transmission dynamics of HPAI A(H5N1) (Figure 1). Laboratories at the University of Wisconsin (Madison) and Erasmus University Medical Center (EMC; Rotterdam, the Netherlands) performed a series of experiments [8, 9] that involved the mutation of 2 influenza A (H5N1) strains through multiple passaging. The two laboratories identified specific amino acid changes that enhanced airborne transmissibility of the virus between ferrets—a standard animal influenza model that exhibits a natural history and pathology similar to what is observed in humans. The potential translation from ferrets to humans raised concerns among funders (ie, the National Institutes of Health [NIH]) and the broader biosecurity policy community that the research could be used for intentionally harmful purposes or result in an accidental release of pathogens from the laboratory into the general population.

In 2011, the Department of Health and Human Services (DHHS) convened the National Science Advisory Board for Biosecurity (NSABB)—an independent federal advisory committee chartered to provide advice on the biosecurity oversight of dual use research. The NSABB was asked to weigh in on whether the GOF studies should be published in the public domain. After initial review of 2 manuscripts, one submitted to *Science* (by investigators at EMC) and the other to *Nature* (by investigators at the University of Wisconsin), the NSABB requested that study authors and the journals withhold from publication the details about the study methods [10]. Consequently, the influenza research community voluntarily implemented a year-long moratorium on GOF research. In March 2012, the NSABB recommended publication of both studies, with some minor changes to the EMC manuscript [11]. These deliberations led to the creation of a US framework for DURC studies [3, 12] and further stimulated a debate on GOF research within the scientific community [13].

Recently, influenza virus researchers laid out a rationale for GOF experiments in the context of influenza A(H7N9) [14, 15]. These arguments were met with some criticism [16–18], especially with respect to the risks of accidental or intentional release of this HPAI. Given the growing concern over this and other HPAI subtypes, the White House Office of Science and Technology Policy and the DHHS announced a moratorium, on 17 October 2014, on all new funding for GOF research on all influenza viruses, severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV) [2]. Additionally, the US government called for a voluntary moratorium on all such research, irrespective of funding source, while the risks and benefits of such experiments could be assessed. On 15 and 16 December 2014, the National Academy of Sciences, National Research Council, and Institute of Medicine convened experts from the disciplines of infectious diseases, research ethics, and science policy to discuss the potential risks and benefits of GOF research in a public forum to help inform the federal government on how best to proceed in regulating GOF research on potentially dangerous biological agents [19]. Shortly after the meeting, the NIH notified a subset of researchers affected by the research pause that their work could resume [20]. Specifically, 5 research projects on MERS-CoV animal model development and 2 on HPAI were cleared to continue.

The discussion on the merits and risks of GOF research has not been limited to the United States, as the Dutch Court of Appeals recently handed down a verdict concerning EMC's objection to export license rules regarding the publication of HPAI GOF research [21]. Export licenses in the European Union are in place to prevent the proliferation of weapons of mass destruction and, thus, apply to specific biological agents, chemical agents, and technologies. In 2012, the Dutch government ruled that EMC had to apply for an export license to publish their GOF work, which they did to expedite publication. However, EMC later filed an objection, maintaining that GOF research in this context was for "basic scientific research." The Dutch Court of Appeals ruled that EMC had no legal standing to contest the export license regulations but did not address the legality of the export license itself, leaving the issue open for continued debate. Currently, all GOF research within the European Union requires export licenses for publication.

A deliberative review process, headed by the NSABB, is currently underway [22] to evaluate the potential impacts of GOF research and to set criteria for what types of research can be conducted and made available in the public domain. A large part of the risk analysis will likely involve the potential for these pathogens to be misused either intentionally or accidentally. Attempts have been made to anticipate the likelihood of the latter scenario, resulting in wide-ranging estimates [1, 19, 23]. The recent safety lapses at the Centers for Disease Control and Prevention and the NIH that could have resulted in exposure to anthrax and smallpox, respectively, have diminished public confidence in the ability of even high-containment laboratories to mitigate the risk of accidental release of pathogens of potential harm. Though the actual risk of accidental release of highly pathogenic viruses may be low, public tolerance of that

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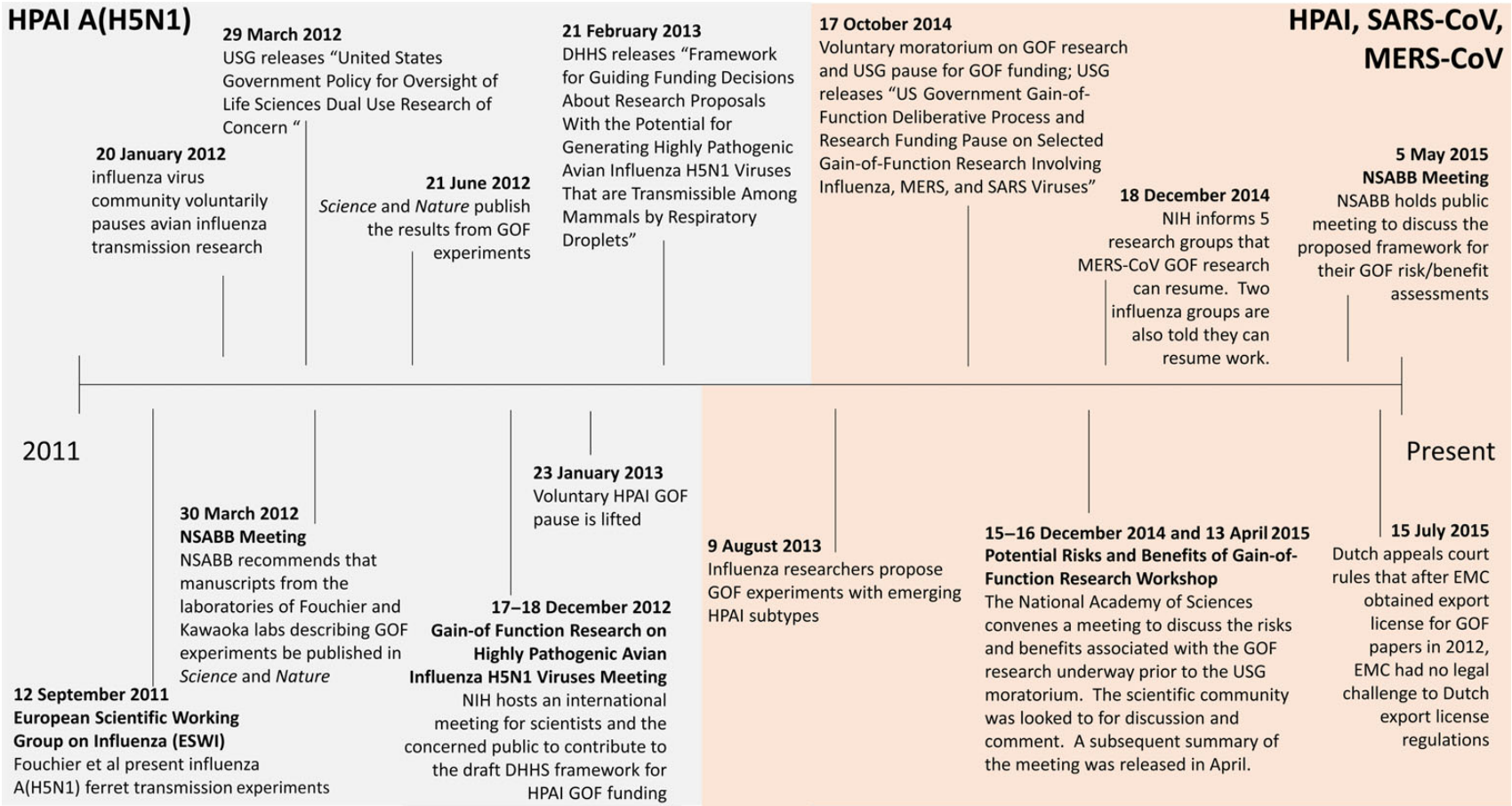

Figure 1. Historical perspective on recent debates associated with gain-of-function (GOF) research. Abbreviations: DHHS, Department of Health and Human Services; EMC, Erasmus University Medical Center; HPAI, highly pathogenic avian influenza virus; MERS-CoV, Middle East respiratory syndrome coronavirus; NIH, National Institutes of Health; NSABB, National Science Advisory Board for Biosecurity; SARS-CoV, severe acute respiratory syndrome coronavirus; USG, US government.

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risk may be the ultimate determinant of what types of research are allowed to proceed.

Increasing attention has been brought to the use of alternative methods of investigation in areas that have historically been studied through GOF research. Some of the alternatives that have been proposed rely heavily on in silico technologies, such as computational modeling and disease forecasting [24–26]. The relevance of these other methods is an important consideration for the scientific community, medical practitioners, and the general public, as the risks and benefits of each approach and the tangible outcomes they yield will vary according to the interests and needs of each sector. All of these factors are being considered by the NSABB, which will decide how to proceed with the current moratorium and the future of GOF research. As the GOF debate has transpired to date, the ramifications of this research for the practicing clinician have not been made clear.

CLINICAL APPLICATIONS OF GOF RESEARCH

Animal Models

The development of novel prophylactic and therapeutic interventions invariably requires evaluation in animal models that, at least partially, recapitulate the disease in infected humans. Many emerging and reemerging zoonotic diseases lack relevant animal models that closely recapitulate human disease [27]. In these instances, GOF experiments are often needed to adapt virus isolates from humans to different, sometimes unnatural, mammalian hosts. Adaptation to a new host inherently involves the alteration of pathogens through mutation. As the development of appropriate animal models can be a rate-limiting step in the evaluation of prophylactic and therapeutic interventions, GOF modifications to viral strains can be an important tool toward accelerating the product development pipeline.

Coronaviruses such as SARS-CoV and MERS-CoV require meaningful small-animal models that elucidate viral pathogenesis and immunity. The human isolates are manipulated either through natural evolution, targeted mutation, or repeated exposure to human factors in nonhuman hosts. One of the more reliable SARS-CoV murine models was developed by modifying a human isolate through 15 serial passages, after which it was lethal to young mice [28]. This mouse-adapted virus strain contained 6 coding mutations that conferred increased virulence, approximating many features of SARS-CoV disease in humans and thus providing a robust and reproducible challenge model for testing vaccines, antivirals, and other interventions [29]. The development of an appropriate animal model for MERS-CoV, on the other hand, provides unique challenges because the viral receptor used for cell entry is radically different in mice. Models thus far have included transient transfection [30] and transgenic mice [31], although it is still unclear whether these models accurately recapitulate human infection. Approximating human disease in these small-animal models might require further passaging in the presence of a humanized receptor, thus creating a potential for the development of GOF phenotypes.

Vaccines

Many live-attenuated vaccines, including some of the most successful vaccines ever developed, have been generated through GOF research. From polio to smallpox to influenza, live-attenuated vaccines elicit immunity against authentic epitopes on whole pathogens without causing disease. The live-attenuated measles vaccine was created by passaging the virus until mutations arose that altered virus tropism—a technique that could be considered, by current definitions, GOF research [32]. New research on highly pathogenic viruses has emphasized the different ways GOF mutations can generate even-more-effective live-attenuated vaccines. Mutations within RNA virus polymerases, for example, modify replication fidelity to generate higher or lower mutation rates during viral replication. These fidelity mutants could potentially alter viral tropism, modify key antigens, and increase resistance against novel therapeutic interventions or antibody responses, but they could also lead to a virus that is less fit [33, 34]. These particular types of experiments have been carried out on a range of viruses, including alphaviruses [35, 36] and picornaviruses [37]. The introduction of GOF mutations not only attenuates the virus but also provides improved understanding of the mechanics of viral replication, thus potentially uncovering new strategies in the development of vaccines against emerging pathogens.

Therapeutic Interventions

The generation of escape mutants in the presence of an investigational agent is common practice for the evaluation of antibiotics, antivirals, and other monoclonal antibodies. GOF experiments with HPAIs and highly pathogenic human influenza viruses, for example, have identified specific mutations that can confer multidrug resistance [38, 39]. GOF experiments are necessary in this context because naturally occurring resistant strains may not yet exist or the complex background of naturally occurring mutations may preclude identification of the amino acid residues that are critical to resistance [40]. These GOF studies are equally important in research on antivirals and antibiotics and can help inform the development of combination therapies. Passive immunotherapy, which often includes a combination of products, is particularly dependent on GOF experiments for evaluating efficacy [41–43], as seen in the current Ebola outbreak that has prompted a robust program to evaluate combination monoclonal antibody therapies [44, 45].

Disease Surveillance

In the past half-century, GOF research has contributed to an improved understanding of the epidemiology of emerging pathogens and has informed efforts to conduct surveillance for

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future outbreaks. In the context of influenza, data, derived from GOF research, on the relative transmissibility of hemagglutinin mutations has aided in the interpretation of molecular surveillance data [46]. Specifically, the initial influenza A(H5N1) [8, 9] and later influenza A(H7N9) experiments identified amino acid changes in influenza virus hemagglutinin or RNA polymerase through viral passaging or site-directed mutagenesis. This research elucidated mechanisms by which naturally occurring influenza virus strains might evolve to replicate more efficiently and transmit more easily within mammalian hosts [47, 48]. The results of these experiments can be used to cross-reference traits found among circulating strains and help predict transmission patterns and pathogenicity [49]. As the field of disease surveillance evolves to accommodate a growing repository of viral sequences, GOF research will also play an important role in assessing the public health significance of genotypic variation. Though current understanding of the relationship between genotypic data and phenotypic expression is suboptimal, the increasing reliance by the clinical community on molecular diagnostic tools may help to reduce that uncertainty. As costs of whole-genome sequencing continue to decrease, data from these techniques are likely to become more central to disease surveillance programs. The results of GOF experimentation can also help inform decisions about countermeasure selection and stockpiling, particularly in the context of influenza surveillance programs [50]. The improved understanding of how HPAIs evolve to transmit more efficiently has also factored into decisions about the creation of prepandemic vaccine stockpiles.

THE ROLE OF CLINICIANS IN THE GOF RESEARCH DEBATE

The world has been witness to a number of emerging infectious disease pandemics over the past several decades. Each time, clinical and public health practitioners were on the front lines, providing care and treatment and finding ways to interrupt transmission, and were ultimately responsible for containing the outbreak. Healthcare providers require effective medical countermeasures and epidemiologic information to assess risk and support decisions about treatment and prevention. Recent outbreaks of infection due to Ebola virus, MERS-CoV, and pandemic influenza virus, however, continue to demonstrate that medical and public health readiness for emerging infections is not always optimal and could benefit from more research and development. As outlined above, GOF research plays a significant role in ensuring that clinicians have the tools they need to respond to infectious disease outbreaks. Therefore, the clinical community is directly affected by policy decisions on what types of research are and are not is allowed to continue. There are also risks associated with GOF research, of which the clinical community will have to be acutely aware. As recent lapses at high-profile laboratories have illustrated, there remains the potential that bacterial and viral strains can escape even the most secure environments. Should a pathogen escape, whether it is naturally occurring or the product of GOF research, the clinical community will have an important role in detecting and responding to such incidents. Because of their unique role as both beneficiaries of the products of GOF research and mitigators of its risks, clinicians have a vital stake in the public debate on how GOF research should proceed.

Notes

Acknowledgment. Information in this report is cleared for public release, and distribution is unlimited.

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Opinion

EMBO reports

When gain-of-function research is not "gain-of-function" research

Andy Kilianski[1] & Randall S Murch[2,3,4]

There is ongoing discussion among the scientific and biosecurity communities over how to address concerns about "gain-of-function" (GOF) research using highly pathogenic agents [1–3]. The discussion has mainly centered on previous work by Yoshihiro Kawaoka's group at the University of Madison-Wisconsin in the USA [4] and Ron Fouchier's group at Rotterdam University in the Netherlands [5]. Both groups introduced mutations into highly pathogenic H5N1 avian influenza (HPAI) that could potentially increase human-to-human transmission of the virus. These mutations are classified as GOF because they increase airborne transmissibility in ferrets—a good model for human transmission. Some in the research and biosecurity communities are concerned that these experiments could result in accidental or intentional releases of the mutated pathogen, or that the now publicly available information about how to increase the human-to-human transmissibility of H5N1 influenza could be abused for developing biological weapons [6,7].

Earlier this year, Kawaoka's group again published the results of GOF research on the PR8 influenza backbone in which they created a high-yield vaccine strain capable of hosting multiple HA/NA antigenic combinations [8]. The high-yield phenotype was observed in diverse host cells in addition to chicken embryos, which are used for influenza vaccine production. This is a potentially major breakthrough for vaccine development and production, as it would greatly reduce the time and cost of rapidly producing influenza vaccines in response to disease surveillance and prediction, as well as to emergent pandemic strains. Nonetheless, and despite the obvious scientific and commercial value of this research, the decision whether to publish GOF-related research such as this, especially in human pathogens like influenza, is not straightforward.

The research performed by the Kawaoka group—which was finished before the current moratorium on GOF research in the USA came into place—resulted in a GOF phenotype. This work would have fallen under the current moratorium [9], but should not be classified as GOF research in our view. It is unlikely that the release of these high-yield strains from the laboratory would have any negative effect on human health because these are vaccine strains of influenza. Neither is this a case of dual-use research of concern (DURC) because the information in the paper has little potential to be applied to pathogenic strains of influenza. The mutations described are unlikely to be broadly applicable to other influenza subtypes or strains: growth-enhancing mutations from other influenza backbones did not necessarily confer a high-yield phenotype in the PR8 backbone. The decision to categorize this work as GOF—meaning that it falls under the current moratorium that has halted such research in the USA—was because of the previous experiments to increase transmissibility of avian H5N1 and HPAI's designation as a "Pathogen with Pandemic Potential (PPP)".

This example illustrates why we need a more appropriately structured classification system of GOF research with sufficient fidelity to consider individual pathogen strains and their features, instead of merely the pathogen being used. As demonstrated by the lack of HPAI human pandemics—and the emergence of other known and unknown pathogens causing severe disease—singling out pathogens as having "pandemic potential" without sufficient supporting evidence is scientifically problematic. Furthermore, determining the "pandemic potential" of pathogens is sometimes only possible with GOF research. For the infectious disease community, the only way to proactively prepare for the next pandemic is to clearly define what constitutes a GOF and/or DURC in a way that is not wholly defined just by the pathogen. While the NIH and National Science Advisory Board for Biosecurity (NSABB) are reviewing the risks and benefits of GOF research, a clearer and more effective definition of what constitutes GOF research—one which circumscribes all infectious disease agents and not just a select list—should be established. The community needs to build this consensus to be able to safely continue GOF research and responsibly keep these experiments in the traditional antibiotic, antiviral, and vaccine development methodology.

The scientific community has always had a great interest in openly and accurately disseminating knowledge, which is now becoming possible with the advent of open access publications and other web-based tools; the research to increase the yield of the PR8 influenza backbone was in fact published in an open access journal. The proliferation of open access journals, preprint servers, and posting of scientific research on the internet is inherently good for science as a whole. However, it provides multiple challenges for DURC and GOF

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research to prevent their dissemination without proper review and management. It is clearly not sufficient to simply perform DURC reviews at the editorial level prior to publication in peer-reviewed journals because, in today's publication landscape, it is possible to publish work without review on pre-print servers or open-review journals. To better evaluate DURC and GOF research as a whole, a more comprehensive "systems" construct is needed. The review process should be initiated earlier, at the proposal step at the funding agency. In addition, it may require regular monitoring after the initial review to avoid "surprises", as occurred with Kawaoka's and Fouchier's original papers.

As the NIH and NSABB determine a course forward how "gain-of-function" research should be evaluated in the USA in the future, it needs to flesh out guidelines that list which pathogens and experiments require review and that standardize the review process itself. We suggest that the review and reporting should encompass the most critical phases of research from the proposal to the publications stage. Draft guidelines should be made available for public comment with meaningful responses considered for incorporation, published, and then formally reviewed on a regular basis and modified if required. These reviewing and reporting structures should be exercised prior to the formal requirement, with participation from outside actors and full transparency.

US government-funded research proposals should require a consistent, comprehensive DURC review prior to funding and to the initiation of the research, and not only at the level of the institution (which has recently been recently enacted [10]) and the publication stage. This review process should be consistent across agencies. A common set of standards and guidelines should guide the review procedures of US public funding entities to determine whether research proposals present GOF and DURC concerns. Such a process will ensure that the research being funded has been cleared of these issues, and any potential dissemination of this work has been vetted. Similar to the definition of GOF research, the NIH and NSABB should establish how this work is to be reviewed, not simply whether the work has tangible merits.

The international scientific community, governments, private funders, overseers, regulators, publishers, and stakeholders should consider designing, testing, implementing, and embracing a consistent end-to-end protocol which promotes safe and valuable research while minimizing uncertainties and risks, including the misuse of science. We recognize that this is not an easy achievement to attain, but we believe that it will be worth the investment and effort and will help to prevent future funding moratoriums being placed on the GOF and DURC research communities.

Conflict of interest

R.S.M. was a former member of the NSABB from December 2009 to April 2012. The conclusions and opinions presented here are those of the authors and are not the official policy of the National Research Council, DTRA, the US Army, ECBC, or the US Government. Information in this report is cleared for public release, and distribution is unlimited.

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From: David Wolinsky []
Sent: Sunday, January 17, 2016 12:21 PM
To: National Science Advisory Board for Biosecurity (NIH/OD) <NSABB@od.nih.gov>
Subject: no pain, no gain of function

Please: NO gain of function research or development on pathogens. In practice, "defensive" capacities will remain limited, while offensive uses will appear as they always do. Meanwhile the risks of accident should be clearly -- for theses cases--unacceptable.

--

David Wolinsky

Frederick, MD

From: Francy Williams []
Sent: Tuesday, January 19, 2016 10:03 PM
To: Viggiani, Christopher (NIH/OD) [E] <christopher.viggiani@nih.gov>
Cc: Beth Willis
Subject: GOF Research - concern comment

Comment: Frances Williams RN MS (retired and living in Frederick MD - the location of BSL-3 labs), private citizen and member of the Religious Society of Friends (Quakers)

I am writing in response to a request for public comment regarding the upcoming NAS symposium March 10-11 2016.

With the story of Flint Michigan's polluted water (an unintended consequence of the city's attempt to save money) in the headlines,

I reflect on the possibility of unintended consequences from Gain of Function research gone awry in our community here in Frederick.

My prayer is that science be conducted for the highest good and that we not fall prey to events that occur as the result of conflicts of interest, or as the result of nefarious intentions.

I hold the vision that someday non-violence will become the American Way and resources will no longer be used to support tools designed to destroy life.

Gain of Function (gain of function of microrganisms for the purpose of eliminating humans). Conducting research on pathogens to make them more virulent, transmissible, and resistent to treatment, in my opinion should be illegal.

I endorse the commments made by Beth Willis at the workshop held at NIH on Jan. 7-8 2016.

I send hope that good minds and hearts will develop measures to assure safety for all.

Respectfully,

Frances Williams

(**Note:** The comments delivered by Beth Willis, a panelist at the Jan. 7-8 NSABB meeting, referenced above were copied in the original email but omitted when comments were compiled. Ms. Willis' comments were previously conveyed to the NSABB and can be found as part of the Session IV presentations archived on the Jan 7-8 NSABB meeting webpage.)

From: ROLAN.CLARK@comcast.net []
Sent: Saturday, January 23, 2016 9:05 AM
To: National Science Advisory Board for Biosecurity (NIH/OD) <NSABB@od.nih.gov>; GOF <GOF@nas.edu>; Willis, Beth
Subject: comments nsabb gof

23 January 2016

To: NSABB (National Science Advisory Board for Biosecurity)
From: Rolan O. Clark
Subject: Bio Labs, Gain of Function comments

Dear NSABB and to whom it may concern.

Experience

U.S. Navy 1957-63, 4 years active, 2 years inactive reserve. 6 months ETA Radar school, Treasure Island California, 2 years, 9 months U.S.S. Estes AGC-12 radar tech

15 years calibrate and repair of electronic test equipment to Bureau of Standards specs, now NIST, then 10 years two way radio systems repair, 20 years R&D space tubes/technology and space battery testing and writing computer programs to control data collection equipment, collect and display test data and help write reports.

My lack of formal higher education has not changed the laws of physics nor the meaning of words.

Testing Philosophy and Data Collection

Science is first philosophical, then anecdotal, then data collection and knowing the limits, application, specifications and accuracy of data collection equipment/procedures , the first steps in collecting believable data.

Next, presentation of data in proper reference to properties of test under consideration, such as what is listed/tabled/plotted/displayed against what. There are lies, damn lies and data. Science is only as good as the next peer review.

Gain of Function

Definition. A type of mutation in which the altered gene product
possesses a new molecular function or a new pattern of gene expression.
Gain-of-function mutations are almost always Dominant or Semidominant.
See also: Amorphic Mutation.

The concept of Gain of Function as I try to understand it, by watching the archived video of the NIH meetings, downloading and reading 5 plus web sites dealing with Gain of Function and reading linked comments by scientists, leads me to come to the conclusion that trying to apply Gain of Function to Bio Labs research with its inherent lack of means to detect non recognized/unanticipated

mutations/variations, along with expected results, is not a reliable research methodology while at the same time recognizing at times the possible need for research into to the unknown but possibly Gain of Function as it is used in Bio Labs research should not be considered as an accepted research tool, rather a definition.

I don't know if Gain of Function is supposed to be a guide to amplify or exaggerate disease transmission/reaction characteristics and then see what will mitigate the result but if some method is found to mitigate these amplified or exaggerated results that in itself may not be an indicator that the original disease will react favorably to what may have been a favorable reaction to amplified or exaggerated conditions. But what I referenced in this paragraph may not be a purpose of Gain of Function.

Why would one try to develop more efficient methods of transmitting diseases or make diseases more virulent when there may be no way to mitigate or detect all variations of these 'developments' of such dangerous measures and how can these new mutations be considered typical or representative of diseases being researched.

http://www.livescience.com/53410-stephen-hawking-warns-of-planetary-doom.html?cmpid=NL_LS_weekly_2016-1-19 , it is interesting to note the concern by Mr. Hawking.

The below, between the ********** are from the above URL
****
Stephen Hawking has once again warned that humanity could wipe
itself out before it has a chance to establish far-flung space colonies.
At a recent talk in England, the famed physicist singled out nuclear war,
genetically engineered viruses and global warming as likely culprits.

****

Ferrets

Googling why ferrets were the animal of choice for some studies on spread of diseases I found that ferrets sneeze about as often as humans and putting 'infected' ferrets up stream in a controlled air flow environment simulated studying disease spread by the sneeze route down the air stream. The comments in the article weren't too exciting about the controls of this type of test but I got my ferret use question answered.

Programming, Computers, DNA/RNA

Re:
http://www.ncbi.nlm.nih.gov/books/NBK26887/
I don't understand the info in the immediate above URL.

If a computer program does not 'work' one has to know the source code and programming and the operating system to analyze the problem if a resolution to the problem is not found using other methods.

Gain of Function, or research, as it relates to the topic of diseases requires knowledge of how DNA/RNA 'source code' signals/triggers molecular changes in the disease and other molecules probably not

possible at the present level of research therefore one is relegated to observing results of tests and drawing conclusions based on how data and data collecting procedures function all the while possibly blinded to other DNA /RNA reactions/instructions and results because other detection mechanism for other mutations present in bio mutations work may not be available unlike other spectrum identifying devices such as spectrum analyzers for rf energy and mass spectrometers for molecular activity identification. I believe at present there is no DNA/RNA 'spectrum' identifier to detect unwanted or unanticipated results.
I assume DNA RNA react upon contact with other molecules and the molecular/chemical reactions simulate 'instructions' as per the chemicals in the DNA , RNA and molecules, whether from 'normal' cells or pathogens, bacteria or viruses or anything in the body. I would assume blood flow is the distribution method for these bio entities to make contact with each other.

Patterns

It is my belief that patterning is a very useful tool, if data follows a pattern it denotes some consistency. I believe patterns can be a very useful security tool if used with a computer and possibly this example: use a computer to sample and log all air pressures in labs and entrances, hall way pressure, transition room pressure, lab pressure along with possibly iris and fingerprint info and clock times.

If all data mentioned above is plotted over time then any deviation from 'normal' should immediately signal an alarm. This may also require input from behavioral experts to develop sign in routines to enhance security. As perfection is the enemy of progress too much routine in sign in procedures leads to laxness in security and this pattern can also be put in a computer display to detect any changes in patterns.

Regulations

I have written to our local government entities that the only thing worse than regulations/laws/codes in a democracy is no regulations/laws/codes. Single Source Federal oversight consisting of Regulations/laws/codes are needed for all the biolabs for a one voice oversight function regardless of the inconveniences regulations/laws/codes may bring. We have seen recent failures in governments oversight function as in the Flint Michigan water issue but all proper oversight functions in a properly functioning government rests solely on the integrity of the persons responsible for administrating the rules assuming the guidelines are in place and correct.

Bio Labs not in Residential Areas

Bio Labs should not be in residential areas for at least 2 major reasons:
1. Terrorist's thrive on publicity and a terrorist attack on a biolab in a residential/inhabited would be more desirable to a terrorists goal as compared to a biolab apart from residential/inhabited areas. areas.
2. If a bio lab was 5 miles from a residential/inhabited area and an aerosol type escape of test pathogens occurred there would be dispersion plus the time to react. Distance is time in an aerosol environment plus determining the time the escape happened may be very difficult to determine and time is important. There is also the possibility that the escaped pathogen may be rendered moot in the environment of open air and sunlight plus the concentration would probably continuously decrease, say particles per unit volume.

Rolan O. Clark January 23, 2016

Single Source Bio Labs Oversight Entity

The last 2 or 3 years I have written my U.S. Senators and U.S. Representative and our local State Delegation about the need for a Single Source Bio Labs oversight entity consisting of a single Federal Department for oversight of for ALL biolevel labs and the need and right for the public to know where these labs are.

Not only would there be one voice but there would be defined word/words to describe each issue. Words are important. There would be the advantage that when any issue needs to be addressed all labs could be notified at the same time by the internet, for example, using words defined and understood by all entities.

Communications

Communications and speed of communications is extremely important in emergency conditions. A single source method to distribute and communicate using accepted and approved procedures/wording would be very beneficial.

Conclusions

I believe there should be one Federal oversight entity for all Bio labs with accepted procedures, wording and communications paths.

Gain of Function seems to be a definition instead of a research procedure. I believe that Gain of Function is a very narrow definition, though references a very complicated process, of a type of research.

There is nothing wrong with not knowing, there is something wrong with not asking.

Respectfully submitted as my concerns and what I think I understand, however limited, about a very complicated process.

Rolan O. Clark

From: Beth Willis [] _____
Sent: Tuesday, January 26, 2016 6:21 PM
To: Viggiani, Christopher (NIH/OD) [E] <christopher.viggiani@nih.gov>
Subject: Concepts to inform decision-making about risk: Setting Specfic Safety Goals

Hi Chris,

I hope you've survived the last week safe and warm. As have I.

A colleague asked that I pass the following on to the NSABB and the NAS.

As you may know, DOE and the NRC have established specific public safety goals and mechanisms to determine what public risk is considered acceptable. The concepts in these materials might help to inform a similar effort for GOF Research of Concern, DURC, Select Agent and other risky biological research. I don't believe such safety goals currently exist.

DOE Nuclear Policy Safety and Goals: http://energy.gov/sites/prod/files/2013/12/f5/DOE_P420-1_Final_2-8-11.pdf

And

http://energy.gov/sites/prod/files/2013/12/f5/Technical_Basis_for_DOE_P_420-1.pdf

The Nuclear Regulatory Commission has a similar set of goals:

http://nuclearsafety.gc.ca/eng/pdfs/Presentations/Guest-Speakers/2014/2014-01-13-Safety-Goals-and-Risk-Informed-Regulation-at-the-US-NRC.pdf

with best regards,

Beth Willis
Frederick Citizens for Bio-lab Safety

From: Kim Loll [] _____
Sent: Saturday, February 06, 2016 12:37 PM
To: National Science Advisory Board for Biosecurity (NIH/OD) <NSABB@od.nih.gov>
Subject: Comments from the Containment Laboratories Community Advisory Committee (CLCAC) Frederick, Maryland

I am writing on behalf of the Containment Laboratories Community Advisory Committee (CLCAC) of Frederick, Maryland. The CLCAC was formed as joint committee sponsored by both the City of Frederick, as well as Frederick County, MD.

The purpose of the Committee is to:

- Foster two-way communication between the public and the operators of the high containment laboratories operating at Fort Detrick and elsewhere in Frederick County.
- Seek information about public concerns and ways to address those concerns.
- Advise and make recommendations on behalf of the public to government, containment laboratory and Fort Detrick officials regarding opportunities to improve any laboratory-related operational matters that may potentially impact public safety and health.

The CLCAC has been following the many issues related to the current discussion on Gain of Function research over the last several years. Several members of CLCAC attended the January 7/8, 2016 NSABB Meeting or observed the webcast, and the past Chair of CLCAC, Ms. Beth Willis, was a panel member on the Workshop. The CLCAC would like to take this opportunity to endorse the following papers and presentations provided at the NSABB Meeting:

- Presentation and written comments provided by Ms. Beth Willis
- Presentation and written comments provided by Dr. Marc Lipsitch

Thank you for the opportunity to participate in this important deliberative process. We look forward to future opportunities to provide additional public perspective on biosafety and biosecurity policy issues as they relate to public health concerns.

Local newspaper coverage of the January 13, 2016 CLCAC meeting following the NSABB Meeting can be found at:

http://www.fredericknewspost.com/news/health/treatment_and_diseases/frederick-committee-addresses-pathogen-research-debate/article_10e694b8-2a98-59ba-85dd-674337980152.html

Additional information about the CLCAC and its activities can be found at:

https://www.cityoffrederick.com/index.aspx?nid=127

Sincerely,

Kim R. Loll, Vice-Chair
Containment Laboratories Community Advisory Committee

From: Frank, Gregory [mailto:gfrank@idsociety.org]
Sent: Tuesday, February 23, 2016 12:46 PM
To: National Science Advisory Board for Biosecurity (NIH/OD) <NSABB@od.nih.gov>
Cc: Viggiani, Christopher (NIH/OD) [E] <christopher.viggiani@nih.gov>; Chang, Shion <schang@idsociety.org>
Subject: IDSA comments to the NSABB

Dr. Stanley

Please accept the attached comments to the NSABB concerning its draft findings and recommendations on gain of function research of concern on behalf of IDSA. I'd be happy to answer any questions.

Warm Regards,

Greg

Greg Frank, PhD
Program Officer for Science & Research Policy
Public Policy and Governmental Relations
Infectious Diseases Society of America (IDSA)
Direct: 703-299-1216
Mobile: 412-527-7535
gfrank@idsociety.org

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IDSA
Infectious Diseases Society of America

February 23, 2016

[Submitted electronically to nsabb@od.nih.gov]

Samuel L. Stanley, MD
Chairman of the NSABB
Office of Science Policy
National Institutes of Health

IDSA Comments to the NSABB Working Paper on Evaluating the Risks and Benefits of Gain-of-Function Studies to Formulate Policy Recommendations

Dear Dr. Stanley,

The Infectious Diseases Society of America (IDSA) has closely followed the National Science Advisory Board for Biosecurity (NSABB) as it develops formal recommendations on how to assess the risks and benefits of gain-of-function (GOF) research of concern on pathogens with pandemic potential. IDSA members will be among the first responders to care for affected individuals in any disease outbreak, and will also lead research efforts to counter these disease threats. Accordingly, they are well positioned to understand the risks and benefits of these potentially dangerous experiments. Last summer, our society submitted recommendations for the NSABB as it worked with its contractor, Gryphon Scientific, to undertake a risk-benefit assessment (RBA) of the paused GOF research projects of concern, and then release its initial findings and recommendations.

IDSA has limited our comments today to those that apply to the NSABB's working paper, as it will shape the U.S. Government (USG) policy on the oversight of GOF research of concern. We applaud the NSABB's efforts to address IDSA's recommendations in the working paper, including its focus back to only the research of highest concern and its exclusion of seasonal influenza vaccine manufacturing and development. On the other hand, we are unified in our conclusion that the NSABB's draft findings and recommendations will not provide the appropriate guidance needed to develop a streamlined mechanism that provides appropriate oversight of the risk and benefits of GOF research of concern.

Below, IDSA offers specific recommendations to improve the areas of the working paper of greatest concern:

1. **Remove resistance to public health control measures as an attribute of GOF studies of concern**

IDSA strongly supports the NSABB's "key finding 1," that only a small subset of GOF research has risk that warrants an additional level of oversight." As IDSA stated in its earlier comments, a narrow focus only on GOF research of concern will

avoid an inadvertent regulatory capture of low risk research, which was not mentioned in the original White House description of research to be included in this deliberative process.

Consequently, IDSA believes the NSABB's proposed scope of GOF of concern, research that generates a pathogen that is highly transmissible, highly virulent, and resistant to public health control measures, may be unduly narrow. The limitations set forth on research in the NSABB document may fail to identify any GOF research for review and regulatory oversight, notably the types of experiments that sparked our current deliberation over the risk of GOF of research on pathogens with pandemic potential. Moreover both Gryphon Scientific and a number of panelist speakers at the January NSABB meeting concluded that public health control measures would have little ability to control a widespread outbreak of a highly virulent and transmissible pathogen. As stated in our earlier comments, IDSA again recommends that the NSABB focus oversight on GOF research that would be anticipated to combine both high pathogenicity and transmissibility in a pathogen; while escape from medical countermeasures is a concern, it is secondary to the above characteristics. This definition would capture the GOF experiments of greatest concern, and ensure that they are reviewed appropriately to assess their risk and benefits.

2. Exempt routine, responsible vaccine manufacturing from GOF oversight

The NSABB explicitly identifies the development and manufacture of seasonal influenza vaccines as not GOF research of concern. IDSA strongly agrees with this conclusion, understanding the critical importance of adapting and manipulating wild type influenza virus for improved growth in eggs and mammalian cell lines for vaccine manufacturing. However, our society believes that this explicit exclusion can be expanded to include all routine, responsible vaccine manufacturing activities. For example, the development of pre-pandemic and pandemic influenza vaccines uses standard methods and safety procedures that are widespread in the field. IDSA affirms that these routine activities pose little risk to the public, and play a critical role in public health preparedness.

3. Institute an independent standing board to review GOF of concern

The NSABB working paper concludes that "the U.S. government has effective policy frameworks in place for managing risks associated with life sciences research." IDSA strongly disagrees that the current policy frameworks, the USG Policy for Federal Oversight of DURC and the Department of Health and Human Services (HHS) GOF framework for H7N9 and H5N1 influenza, are sufficient to oversee GOF research of concern. For example, the USG DURC policy requires institutions to provide initial oversight of a GOF research project. As raised on several occasions by panelists at the January NSABB meeting, institutional biosafety committees (IBCs) vary widely in their expertise on assessing GOF research and lack transparent, easily accessible guidance to aid in these efforts. Often GOF research may reach a final line of review during submission for publication, where journal editors must take on the task of assessing the risk of publishing the findings; again they lack accessible guidance to ensure they provide appropriate review. In addition, the multiple frameworks of oversight for DURC, select agent research, recombinant DNA research, research that poses biosafety risks to human health or agriculture, research activities involving the shipment or export of infectious agents, and GOF research of concern create an often confusing regulatory environment that can impede scientific research, public health responses, and product development that are in the public interest.

Instead of building upon current oversight efforts, IDSA recommends the NSABB examine the formation of a standing advisory board for GOF research of concern. This board should be independent of GOF funding bodies and of those units within the government that may perform GOF research of concern, and could review GOF research of concern while also providing advice to investigators, IBCs, and journal editors. IDSA believes this board should include stakeholders with expertise in biosecurity, public health, and other relevant perspectives, and also have full access to the security information needed to appropriately assess GOF research. Given the security risks of the GOF research reviewed, it is likely that much of this board's activities may not be made publically available. Therefore, it is critical that the review process itself be as transparent as possible, with aspects that do not involve biosecurity being open to the public. While IDSA proposes that this board initially focus only on GOF research of concern, we do believe it could provide the template -or be expanded in scope-to replace current oversight frameworks in providing a streamlined and appropriate oversight of all DURC.

4. Develop recommendations to address biosecurity information risks

IDSA has noted that the NSABB working paper largely accepts Gryphon Scientific's conclusion that the information risk of GOF research of concern was minimal, stating that "most of the information of interest is already published, or non-GOF information relating to pathogens that are more attractive agents of harm is already available." IDSA asserts that while current GOF research information is already publically available, it is almost certain new research approaches, sequence information, and other data will be generated in the future that would pose novel, additional biosecurity information risks. IDSA strongly recommends that the NSABB reassess these risks, and either develop new recommendations that appropriately address them, and/or request input from other external science advisory groups that currently serve the Intelligence Community, with expertise in the life sciences and access to relevant classified information.

5. Strengthen working relationships with international GOF stakeholders

While the NSABB working report discusses the importance of global engagement and how U.S. policy will likely impact other global efforts, it does not make any specific recommendations on how to better engage international GOF stakeholders. IDSA understands that GOF research is proceeding in a relatively unimpeded manner in many countries outside of the US, but strongly believes that any USG activity would likely play a key role in the establishment of any international consensus on GOF oversight. We urge the NSABB to consider recommendations on how the USG can build strong working relationships with the international GOF stakeholder community. A robust global dialogue would allow the USG to observe the effectiveness of other GOF oversight efforts to better inform domestic USG policy; these stronger relationships will also be critical in making any progress towards international GOF oversight.

IDSA remains committed to ensuring that the broader scientific and science policy communities participates in efforts to guide GOF research appropriately. We hope the March National Academies of Science meeting on the NSABB's draft recommendations will include the perspectives of scientists, healthcare workers, policy-makers, ethicists, and representatives from the public that our society believes are critical in developing an appropriate oversight of GOF research of concern.

IDSA thanks the NSABB for this opportunity to comment, and looks forward to continuing to work with the U.S. Government and those who advise it to clarify the decision-making process on how and whether to undertake high-risk life science experiments. Should you have any questions or concerns about these comments, please feel free to contact Greg Frank, PhD, IDSA Program Officer for Science and Research Policy, at gfrank@idsociety.org or 703-299-1216.

Sincerely,

Johan S. Bakken, MD, PhD, FIDSA
IDSA President

About IDSA

IDSA represents over 10,000 infectious diseases physicians and scientists devoted to patient care, disease prevention, public health, education, and research in the area of infectious diseases. Our members care for patients of all ages with serious infections, including meningitis, pneumonia, tuberculosis, HIV/AIDS, antibiotic-resistant bacterial infections such as those caused by methicillin-resistant *Staphylococcus aureus* (MRSA) vancomycin-resistant enterococci (VRE), and Gram-negative bacterial infections such as *Acinetobacter baumannii, Klebsiella pneumoni*ae, and *Pseudomonas aeruginosa*, and, finally, emerging infectious syndromes such as Ebola virus fever, enterovirus D68 infection, Zika virus disease, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and infections caused by bacteria containing the New Delhi metallo-beta-lactamase (NDM) enzyme that makes them resistant to a broad range of antibacterial drugs.

From: Lynn Klotz [mailto:lynnklotz@live.com]
Sent: Tuesday, February 23, 2016 6:45 PM
To: National Science Advisory Board for Biosecurity (NIH/OD) <NSABB@od.nih.gov>; GOF@nas.edu
Subject: Commentary on Gryphon RBA to the NSABB and NAS

Dear NSABB and NAS,

The attached Commentary shows that an absolute probability of escape from a lab of an mammalian transmissible HPAI may be calculated, contrary to Gryphon's claim. I also do the calculation.

Lynn Klotz, PhD
Senior Science Fellow
Center for Arms Control and Non-proliferation

>>

To: National Science Advisory Board for Biosecurity (nsabb@od.nih.gov)
National Academy of Sciences (GOF@nas.edu)

From: Lynn C. Klotz, Ph.D.
Senior Science Fellow
Center for Arms Control and Non-proliferation
322 4th St., NE, Washington, D.C. 20002

Home: 5 Duley Street
Gloucester MA 01930
E-mail: lynnklotz@live.com

Date: February 23, 2016

Commentary for the March NAS meeting on GOF: Toward absolute probabilities for escape from a laboratory

Summary and conclusion

This Commentary presents a calculation of "direct" or "absolute" probability[1] of escape from a laboratory of a potential pandemic pathogen, specifically mammalian-airborne-transmissible, highly-pathogenic avian influenza viruses (matHPAI). Absolute probabilities are necessary to calculate the probability of a laboratory escape and subsequently the likelihood of a pandemic from an escape, a key goal of Gryphon Scientific's risk-benefit (RBA) analysis.

Gryphon employed a relative probability approach that in the end failed to arrive at an absolute probability of an escape. Thus, this key part of their analysis ended up where it started, not accomplishing its goal of estimating the risk of the research (risk = likelihood x consequence). Gryphon acknowledges this failure.

Here, I will argue that Gryphon went down a wrong path by pursuing a relative probability approach. I will further show that it is possible to estimate absolute probability of escape by actually carrying out the calculation using laboratory incident data reported under the NIH reporting guidelines for BSL3 or BSL4 laboratories. Since all steps of my analysis are explicit and transparent to the reader, it provides a basis for focused discussion and assessment of each step.

In comparison, Gryphon's analysis does not explicitly provide the exact data employed or direct references to it, and Gryphon often provides little detail of the steps in its various analyses. This lack of transparency makes it difficult to verify Gryphon's conclusions. Furthermore, Gryphon fails to define the meanings of or labels for various variables. For instance, if they report a value for a lab-related accident probability, they fail to say if the probability represents one lab for one year, one lab for many years, etc. This failure to define precisely key variables adds to the lack of transparency and the ability to assess their RBA.

My analysis concludes that the probability of escape and likelihood of a potential pandemic is much too high, with an expected "fatality burden" of 512 fatalities per year for each lab conducting this research. To put this fatality burden in perspective, no Institutional Review Board tasked with assessing human subject research would approve a proposed research project with an expected 512 fatalities per year.

Dr. Marc Lipsitch, in his presentation at the January 2016 National Science Advisory Board for Biosecurity (NSABB) meeting, described published research to understand how HPAI may become airborne transmissible in humans that does not require live matHPAI viruses. Many mutations that contribute to airborne transmission have already been identified by this research without employing live virus. Thus, there is little to be lost by banning the live virus research.

I conclude that NIH should not fund this specific matHPAI research and should also not fund any other research with comparable risk. Since the NSABB mandate is very narrow, only whether NIH should fund the research, the NSABB should strongly recommend that the U.S. ban the research regardless of funding source, and recommend that the State Department make a serious effort at an international agreement to ban the research.

Two approaches for estimating absolute probabilities of a lab escape and subsequent pandemic

To estimate the likelihood (probability) of a pandemic beginning with a laboratory escape of a matHPAI, there are two general approaches:

(1) A "bottom-up" approach where probabilities are obtained for significant mechanical/equipment failures or for human error that can lead to laboratory acquired infections (LAIs) and other escape paths into the community. Then, add them all up. This appears to be Gryphon's approach. The approach here is bottom-up as well, but it starts with laboratory incident data reported under the NIH reporting guidelines for BSL3 or BSL4 laboratories, a starting point and path forward different from Gryphon's.

(2) The "top-down" or "real-data" approach. A number of us have been arguing that Gryphon should have taken into account real data as well (for instance, the probability of escape into the community of undetected or unreported LAIs calculated from the 2013 CDC report). Gryphon's valid criticism of the CDC data is that the LAIs were for bacterial pathogens, and certainly not for matHPAI viruses.

Gryphon could have carried out a "control" calculation to demonstrate that its approach can produce probabilities of escape through LAIs comparable to those calculated from the 2013 CDC data. If the two calculations end up with greater than one or two orders-of-magnitude difference, there is a problem with their data used in the bottom-up approach. In a conversation with Gryphon's Managing Director, Rocco Casagrande, he pointed out the data they have collected is not relevant to bacterial select agents, so the control calculation could not be done. But they could and should have collected the missing data as part of their risk-benefit analysis (RBA) to gain confidence in their bottom-up approach data.

In its RBA, Gryphon notes that human error far exceeds mechanical failure. This is borne out by NIH reported incident data (see below) and by the highly publicized recent incidents of human errors leading to escapes into the community.

It is a hypothesis of this Commentary that likelihood of human error will be similar in laboratories researching matHPAI and in laboratories researching other less dangerous select agents. A further hypothesis is that absolute probabilities of escape can be estimated from data already publically available and can be supplemented by data gathered easily. This is a more useful and different approach from Gryphon's approach that employs relative probabilities.

Toward absolute probabilities: A flow chart analysis of paths for escape from a laboratory

To determine the absolute probability of escape for a matHPAI virus from a BSL3 laboratory, a number of events must occur, beginning with an incident that can involve mechanical or equipment failure or human error. The flow chart in Figure 1 describes the events and connections among events, and it lists symbols for probabilities[2] that would eventually lead to an escape. For a matHPAI virus, an escape could lead to a pandemic.

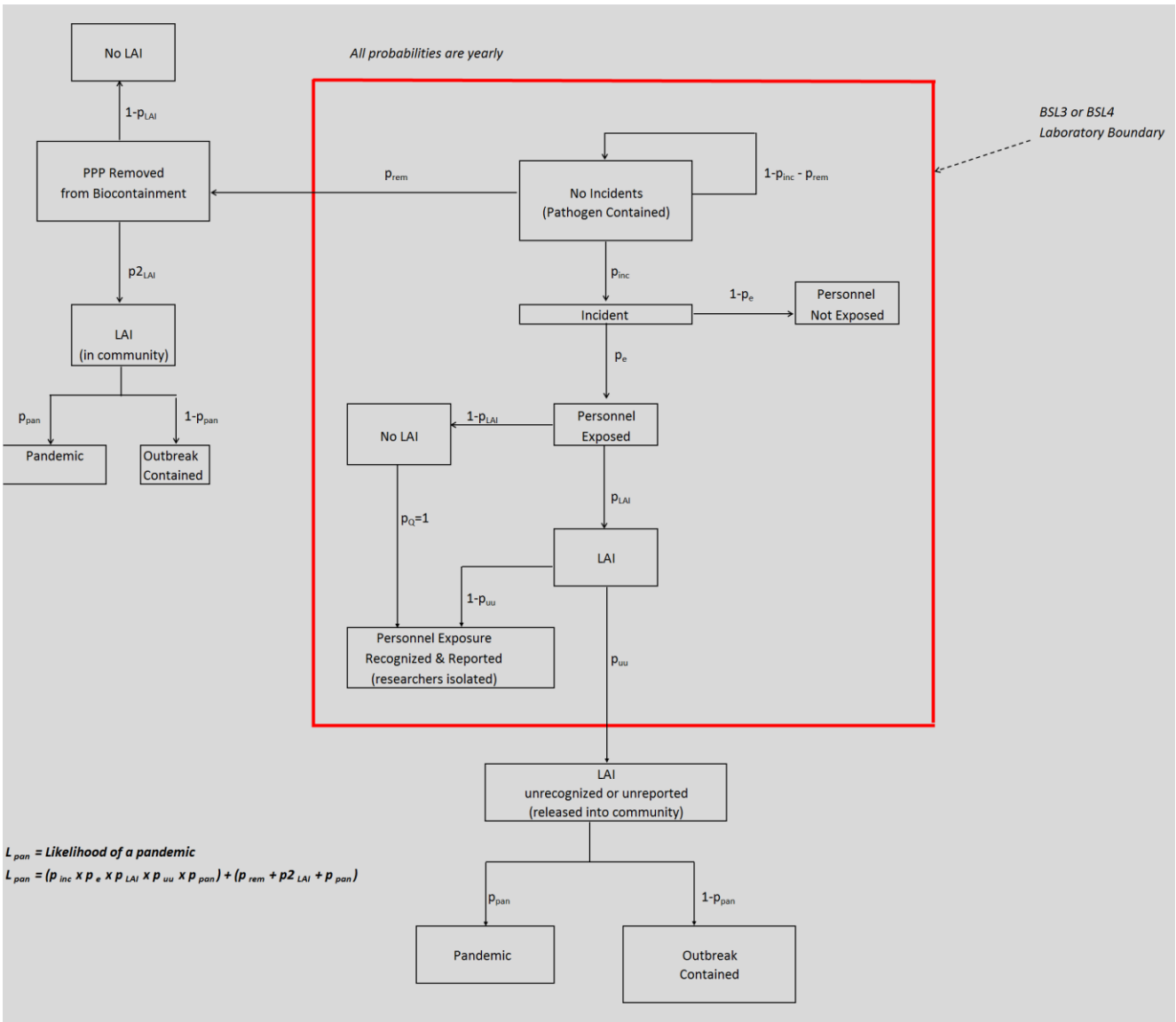

Figure 1. Flow chart of events leading to a lab escape and a pandemic.

In the Figure 1 rendering, there are two independent paths for escape: (1) the undetected or unreported LAI path (top to bottom) and (2) the purposeful removal from containment path (to the left).

For path (1), the likelihood (probability) of a pandemic is $L1_{pan} = p_{inc} \times p_e \times p_{LAI} \times p_{uu} \times p_{pan}$. Here, p_{inc} is the probability that there is an incident is the first place. p_e is the probability that the incident involves exposure of one or more lab personnel. p_{LAI} is the ratio of LAIs to exposures (not strictly a probability because it includes multiple LAI from each exposure). p_{uu} is the probability that the LAIs are undetected or unreported, so infected persons leave the laboratory into the community. In the flow chart, the undetected or unreported LAI moves outside the red laboratory boundary into the community. Finally, p_{pan} is the probability that a pandemic results.

For path (2), the likelihood of a pandemic is $L2_{pan} = p_{rem} \times p2_{LAI} \times p_{pan}$. Here, p_{rem} is the probability that a matHPAI is purposely removed from the laboratory. This could happen for a number of reasons, a common reason being that a researcher has mistakenly believed that the pathogen has been made inactive and is removed for research in a BSL2 lab or removed for transport to another facility.

The overall rate at which pandemics occur (effectively, the probability of generating a pandemic per calendar year) is

$$L_{pan} = (p_{inc} \times p_e \times p_{LAI} \times p_{uu} \times p_{pan}) + (p_{rem} \times p2_{LAI} \times p_{pan})$$

All probabilities in this analysis should be estimated for one year and one lab, as this is the basic probability from which many-lab, many-year escape probabilities can be readily calculated.

Determining values for the probabilities

For path (1), start with p_{inc}. It is a probability that should be obtainable with reasonable accuracy from incident data for many labs over many years. Gryphon should already have this data. I would guess that it is possible that every lab would experience some reportable incident each year, for instance a spill. So, p_{inc} might be 50% or greater. To be a bit more conservative, I will assume that p_{inc} =0.2, which assumes a lab will experience on average one incident every five years (1/0.2). This is likely a generous probability reduction.

In a telephone conversation with Rocco Casagrande, he commented that only 2% of incidents result in personnel being exposed. In analyzing incidents that result in LAIs[3] (Table 1), clearly exposure has occurred.

Thus, the probability that an incident escapes containment and a lab worker is exposed is p_e = 2% = 0.02. So 98% of the time incidents involve no personnel exposure ($1-p_e$ = 98%) and no LAI could occur. Gryphon should be able to comment on the accuracy of the 2% number--that is, how much data supports it. This is a key number.

To estimate the other probabilities, I turn to a table of reported lab incidents collected for the *Final Supplementary Risk Assessment for the Boston University National Emerging Infectious Diseases Laboratories (NEIDL).* (http://www.bu.edu/neidl/files/2013/01/SFEIR-Volume-III.pdf) This 2,716 page risk assessment is abbreviated as the SFEIR (Supplemental Final Environmental Impact Report).

An informative table in the SFEIR is Table D-7, "Recent Reported Incidents Involving U.S. BSL-3 laboratory Facilities." The table is 27 pages long and lists and summarizes 118 incidents, with 23 incidents involving viruses. The table does not report the number of laboratories reporting and their years of operation, so probabilities for each of the different kinds of incidents cannot be ascertained (the frequently encountered "denominator" problem). However, it does provide a way that allows the probabilities downstream of p_e in Figure 1 to be estimated, using as denominator the 118 incidents.

The table covers 1984 through 2010, with most reported incidents after the year 2000. I sorted the table to collect all the LAIs together. The sorted table, including only confirmed LAIs, with a few columns deleted and a few non-substantive changes, is presented in Table 1 below.

LAI Category	Research agent	Description	Results	Action
Detected	West Nile virus (WNV)	A microbiologist working under BSL-3 conditions suffered a finger puncture from a hypodermic needle harboring WNV being harvested from infected mouse brain (Centers for Disease Control and Prevention 2002).	The wound was cleansed and bandaged. Serologic testing showed evidence of acute WNV infection. Mild symptoms developed and resolved.	CDC determined that applicable handling and biocontainment protocols were followed.
Undetected or unreported	Sabia virus‹	A research virologist discovered a leaking vessel upon opening a sealed aerosol biocontainment centrifuge rotor outside of a BSC. Personal respiratory protective equipment consisted of a surgical mask. The incident was not reported (Altman 1994).	Symptoms began 8 days afterward. Two days later the infection was correctly diagnosed.	Antiviral therapy cured the nearly fatal infection. Two external committees strongly criticized the researcher and institution. The university agreed to implement all recommendations. No secondary infections were found among the 142 subsequent human contacts (ref needed)
Undetected or unreported	*Neisseria meningitidis*	A microbiology researcher at BU sought medical attention for laboratory-acquired bacteremia and meningitis. Molecular typing determined the infecting strain was the same strain he had been working with. Work with *N. meningitis* is conducted at BSL-2 using BSL-3 precautions (respiratory protection provided by Class II BSC) (Boston University 2009; Smith 2009, 2009)	Intravenous antibiotics were administered and the researcher recovered fully.	University experts determined the researcher did not consistently wear appropriate personal protective equipment, and did not consistently follow appropriate safe microbiological practices. It was surmised that the researcher touched his gloved hand to his face while working with the bacterium
Undetected or unreported	*Coxiella burnetii*	Previously undiagnosed exposures to *C. burnetii* are diagnosed in three laboratory workers by serologic testing (Centers for Disease Control and Prevention 2007, 2007). As many as ten workers might have been infected (further information is unavailable (Subcommittee on Oversight and Investigations 2007).	Responsible officials did not report these infections to federal authorities as required by federal law.	CDC issued a cease and desist order to TAMU on April 20, 2007 that was expanded on June 30 to include work with all Select Agents. Other serious violations were found during a site visit inspection in July 2007.
Undetected or unreported	*Brucella melitensis*	A researcher contracted undiagnosed brucellosis during improper disinfection of aerosolization chamber. She later required prolonged administration of intravenous and oral antibiotics (Centers for Disease Control and Prevention 2007, 2007; Subcommittee on Oversight and Investigations 2007).	Responsible officials did not report this infection to federal authorities, as required by federal law, until April 11, 2007 in response to an inquiry from the Sunshine Project. (Texas A&M University 2007)	CDC issued a cease and desist order to TAMU on April 20, 2007 that was expanded on June 30 to include work with all Select Agents. Other serious violations were found during a site visit inspection in July 2007.
Undetected or unreported	*Francisella tularensis*	Researchers were working under BSL-2 biocontainment protocol with what was believed to be a non-infectious vaccine strain of the bacterium. Later, it was determined the bacterial culture also contained the infectious wild-type strain that requires BSL-3 biocontainment precautions. Investigation was unable to determine the cause for the mixed culture .(Anonymous 2005; Barry 2005; Lawler 2005; Dalton 2005).	Two researchers became infected with *Francisella tularensis* in May and were not correctly diagnosed until a third scientist became infected with the bacterium in September.	An investigation revealed that researchers had failed to follow proper BSL-2 biocontainment protocol, and that the University failed to identify work- related illness in laboratory staff and failed to immediately report suspicious work-related illness to local and state health departments. Biosafety policies and SOPs were revised accordingly. The Chief of Infectious Diseases was replaced.
Undetected or unreported	*Brucella* species	A laboratory worker became feverish months after handling a culture of *Brucella* sp (The Associated Press 2009).	Infection was confirmed in July by laboratory testing.	It was determined that employee had handled the culture without using proper biocontainment precautions. The employee eventually returned to work.
Detected	West Nile virus (WNV)	A microbiologist, working under BSL-2 conditions using a Class II BSC, lacerated a thumb with a scalpel during necropsy of a bird infected with WNV (Centers for Disease Control and Prevention 2002).	The superficial wound was cleansed and bandaged. Symptoms began 4 days post injury; medical attention was sought 7 days after injury. Infection was self- limiting and was confirmed by serologic testing.	CDC recommends BSL- 3 biocontainment measures for WNV. However, CDC does accept BSL-2 biocontainment facilities that incorporate certain elements of BSL-3 biocontainment measures.
Uundetected or unreported	*Bacillus anthracis*	A lab worker used an incorrect disinfectant; failed to wear disposable gloves; and failed to cover a pre-existing skin defect (facial cut from shaving) (Centers for Disease Control and Prevention 2002).	Cutaneous anthrax resulted following skin exposure to a contaminated surface.	Patient was successfully treated using antibiotics. CDC reviewed proper biosafety measures with laboratory personnel.
Uundetected or unreported	*Burkholderia mallei*	A research microbiologist routinely failed to wear disposable gloves, and became infected. A primary care physician prescribed antibiotics without knowledge of the specific etiology (Srinivasan et al. 2001; Centers for Disease and Prevention 2000).	The patient improved but relapsed to a life- threatening condition. Culture revealed specific etiology and appropriate antibiotics resulted in cure.	A review of laboratory procedures was conducted but no further information is available.
Uundetected or unreported	*Mycobacterium tuberculosis*	PPD skin test conversion was noted for a laboratory technician (Johnson 2009).	Source of infection suspected to be from samples sent by outside laboratories. Samples were to have been inactivated prior to receipt, but validation was uncertain.	Policies and SOPs for sampling handling were revised to assume that samples could be infectious. HVAC systems were upgraded. Air flow alarms were added to BSCs. Aerosol-containment centrifuge was added.
Uundetected or unreported	*Chlamydia trachomatis*	Researcher was diagnosed with a lung infection soon after working with the pathogen (Johnson 2009).	Policies and SOPs for safe handling of the pathogen were found to be inadequate.	New requirements for PPE (respiratory protection), use of a BSC to open centrifuge rotors/buckets, and correct use of BSC were instituted.
Uundetected or unreported	*Mycobacterium tuberculosis*	A retrospective survey was sent to 56 state and territorial public health laboratories to determine, by skin tests results, the frequency of probable laboratory-acquired tuberculosis.	Seven laboratory workers were determined to have laboratory-acquired infections (Kao et al. 1997).	CDC guidelines for preventing LAI tuberculosis, and recommendations for regular skin testing of laboratory employees, were re-emphasized.
Uundetected or unreported	*Brucella melitensis*	A laboratory worker thawed a frozen vial of bacterial suspension and inoculated a plate culture on the open bench top instead of within a BSC (Staszkiewicz et al. 1991)	Eight laboratory workers became infected, one being asymptomatic. The outbreak was most consistent with airborne spread.	The 7 symptomatic workers were given antibiotic therapy. One relapsed and required alternative therapy. Enhancements to laboratory SOPs were recommended by the Department of Epidemiology and the Infectious Diseases Division.
Uundetected or unreported	*Mycobacterium tuberculosis*	Three researchers became skin-test positive for tuberculosis after using a newly acquired aerosolization chamber for experimental infection of animals (Washington Department of Labor and Industries 2004).	Infections were sub- clinical. Prophylactic treatment typically is employed in such cases	Investigation revealed multiple faulty seals in the device, and researchers were not fully familiar with proper operation of the device.
Uncertain soure of infection	Venezuelan equine encephalitis virus.	A laboratory worker was found to have a high rise in anti-VEE virus titer. No occupational exposure was confirmed (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	No further information available
Uundetected or unreported	H1N1 influenza A virus (swine)	Two people, working in separate ABSL-3 rooms, each became symptomatic and were diagnosed with influenza 1.5 days after collecting nasal specimens from experimentally infected pigs (Wentworth et al. 1997).	Genetic analyses determined the workers had become infected with the same virus used to infect the pigs.	Investigation determined that an incorrect mask had been supplied to the workers for 1 day, and it is possible this error facilitated infection of personnel.
Uncertain whether detected or not	Various pathogens: U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland	A retrospective review of institute records showed that 67 people were evaluated for likely or highly likely exposure to infectious agents (Rusnak, Kortepeter, et al. 2004)	3 LAI from BSL-3 pathogens were confirmed in 3 cases: (Chikungunya virus, Venezuelan equine encephalitis virus, and *Coxiella burnetii*); LAI was likely in a 4th case (*Yersinia pestis*); post-flooding contamination of a lab with *B. anthracis* was detected.	NA (retrospective review)
Uundetected or unreported	*Francisella tularensis*	A USAMRIID military scientist was reported to have been diagnosed with tularemia, as a result of her work with *F. tularensis* (USAMRIID United States Army Medical Research Institute of Infectious Diseases 2009, 2009; Bhattacharjee 2009).	Oral antibiotics were started on an outpatient basis, followed by inpatient administration of intravenous antibiotics. Recovery was expected	No further information available

Table 1. Excerpts from Table D-7 from the Supplemental Final Environmental Impact Report

For the 118 reported incidents in Table 1, 19 involved LAIs in laboratory personnel, some incidents with multiple infected persons. These 19 are shown in the table. In my reading of the table descriptions, 15 of the 19 incidents involved undetected and unreported LAIs, where presumably the infected persons left the lab and entered the community before they were later diagnosed with infection; that is, the pathogen escaped the laboratory. *This is contrary to Gryphon's claim that most exposures would be detected, the infected persons would be quarantined until found to be not infected or until the infection cleared.*

A direct estimate of the probability that an LAI is undetected or unreported, p_{uu}, from these data would be 15/19= 79%. A very cautious matHPAI research lab might quarantine those who thought that they *may have been* exposed. For calculation purposes, p_{uu} = 0.20 or 20% will be used. This may be a generous reduction, as laboratory management and researchers may be reluctant to be quarantined based only on a thought.

Backing up on the flow chart to p_{LAI}, of the 118 reported incidents 17 resulted in LAIs. Taking into account that some incidents involve more than one LAI, the total number of LAIs was 38 (red-highlighted in Table 1). No fatalities were reported, which likely would not be the case with matHPAI. Thus, the probability or rate of LAIs per incident is p_{LAI} = 38/118 = 0.32 or 32%.

The probability values are summarized in Table 2, along with their source and rationale for values used in the analysis.

Parameter Symbol Value Used in Analysis	Definition	Direct Estimate & Source	Rationale for Value Used in Analysis
p_{inc} = 0.2 or 20%	probability there is a reportable incident	likely that every lab would experience some incident each year (e.g. a spill with or without a potential exposure)	assumes, conservatively, one incident every five years, years = 1/0.2 per lab
p_e = 0.02 or 2%	probability a lab worker is exposed in incident	probability is 2% according to Rocco Casagrande comment	2% value used in the analysis implies one exposure every 50 years = 1/0.02
p_{LAI} = 0.32 or 32%	rate of LAIs per incident	118 reported incidents with 38 total LAIs; 38/118 rate or LAIs per incident	32% value used in the analysis
p_{uu} = 0.2 or 20%	probability that an LAI is undetected or unrported	from the LAI data 15 of 19 LAIs were undetected or unreported (uu), implies p_{uu} = 15/19 = 79%	cautious lab might quarantine those *who thought* they may have been exposed, so p_{uu} reduced from 79% to 20%
p_{rem} = ?	probability that an matHPAI is purposely removed from the laboratory	difficult to obtain	not used in the analysis
$p2_{LAI}$ = ?	probability that removed matHPAI will result in an LAI	different from and greater than p_{LAI}	not used in the analysis

Table 2. Summary of probabilities used in the analysis.
(http://osp.od.nih.gov/office-biotechnology-activities/biosafety/institutional-biosafety-committees/incident-reporting)

Although not a large data set, there is enough data here to carry out a preliminary estimate of the likelihood or probability of escape from a lab, L_{esc}.

$$L_{esc} = p_{inc} \times p_e \times p_{LAI} \times p_{uu} = 0.2 \times 0.02 \times 0.32 \times 0.2 = 0.000256 \text{ or } 0.025\%$$

In addition, the 0.025% does not include escapes from purposeful removal from a laboratory. For purposeful removal, probability data might be obtainable from a larger number of incident reports than those collected for Table 1. There is one example of purposeful removal in Table 1, and we know of several more from past human errors and for recent human errors at the CDC and Dugway.

The flow chart and the analysis here should identify explicitly those probabilities where more data might be sought. Even though the probabilities can be made better with more data, those used in the analysis here are likely good enough to provide a fair estimate for the absolute probability of laboratory escape and subsequently the likelihood of a pandemic.

It has been argued that labs working with matPAI are designed to be safer mechanically than other BSL3 and BSL3+ labs. I agree. But human errors dominate. Table D-7 in the SFEIR risk assessment bears this out:

- 82 likely human errors
- 19 likely mechanical or equipment failures
- 3 non applicable incidents
- 14 incidents where it was unclear if human error was involved.

So of the 118 incidents, 82 errors or 69% are human errors, not mechanical or equipment failure. In the bulleted list, I say likely because in a few of the incidents, the descriptions are not clear enough to classify them definitely. Nevertheless, my conclusion holds that many more incidents involve human error than mechanical or equipment failure. Comments in the Gryphon RBA also agree that human errors dominate.

In many of the 118 incidents reported in Table D-7, for example needle sticks, animal bites and other clearly direct exposures “no further information was available.” These are not shown in Table 1, but some may have resulted in LAIs. Most pathogens were not highly contagious or deadly and easily treatable, so I expect the worker could go home.

All that remains is to determine the likelihood of a pandemic from a lab escape from an LAI in the community. For this probability, I consulted Figure 4 in the Lipsitch *et al.* (2003) paper (http://science.sciencemag.org/content/sci/300/5627/1966.full.pdf). The figure is reproduced below for convenience to the reader.

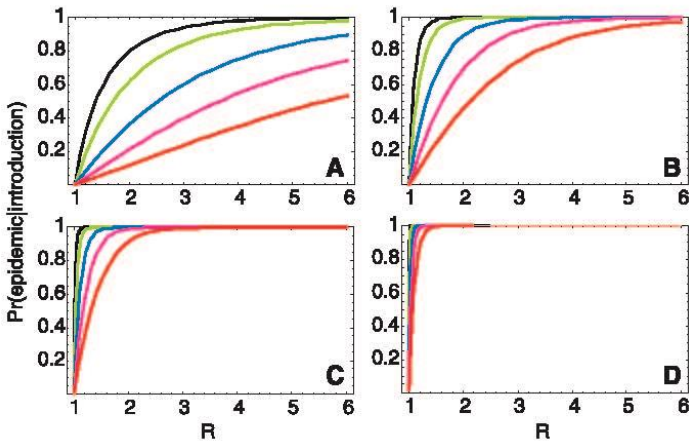

Fig. 4. The probability of an outbreak of SARS in a susceptible population for a range of values of *R*, approximated by the probability of nonextinction of a branching process (*22*) in which the number of secondary cases is given by a negative binomial distribution with a mean of *R* and a variance-to-mean ratio ranging from 1 (for which the negative binomial reduces to the Poisson distribution) to 20 [from left to right: 1 (black), 2 (green), 4 (blue), 10 (magenta), 20 (red)] after the introduction of (**A**) a single infectious case, (**B**) 5 infectious cases, (**C**) 20 infectious cases, and (**D**) 100 infectious cases.

The graphs were generated using branching theory, a pure mathematical construct, which requires only two parameters, the mean R_0 (the reproductive number or the average number of people an infected person infects) and the variance to mean ratio k, which measures the variation in number of people each infected person infects. For instance, some people infected with SARS will infect many other people (super spreaders) and others will infect no one; this implies SARS has a large variance to mean ratio k. I assume for mtHPAI, the subject of this analysis, k will be smaller, perhaps 1 to 2.

Estimating $R_0 = 2$ and k = 2 and a single LAI, the probability of a pandemic, p_{pan}, is about 50% from the green curve in Figure 4a. For more than one LAI entering the community, the probability rises steeply (e.g, Figure 4B for 5LAIs).

Gryphon claims that the probability would not be so high because of public-health efforts to mitigate the spread of community infections. Those of us who watched the 2009 H1N1 pandemic unfold know that such mitigation efforts are likely futile for fast spreading pandemic influenza viruses.

Thus the likelihood or probability of a pandemic for path (1) is estimated to be

$$L1_{pan} = L1_{esc} \times p_{pan} = 000256 \times 0.5 = 0.000128$$

This is the likelihood for a single lab for a single year.

Fatality burden for a single lab in a single year

Assuming the number of fatalities is 4 million, one-tenth of those from the 1918 pandemic flu, the fatality burden for a single lab in a single year is

Fatality burden = 0.000128 x 4 million = 512 fatalities

To put this fatality burden in perspective, no Institutional Review Board tasked with assessing human subject research would approve a proposed research project with an expected 512 fatalities per year.

It should be noted this fatality burden is considerably more than that calculated by me based largely on Gryphon's numbers in my commentary for the January 2016 NSABB meeting. In that calculation, I questioned that their pandemic likelihood was 50% too low, because of an additional 2% probability of unknown origin in the Gryphon analysis. I argue that my calculation using the probabilities estimated here is closer to the true probability of escape. I welcome a response from Gryphon to see if we can reconcile our differences.

For a research enterprise of ten labs conducting this research for ten years, the likelihood of a pandemic is about 100-times greater or 1.28%. I find it very worrisome that laboratory research which could spawn 4 million fatalities has a 1.28% chance of happening in the near future. The assumptions in this analysis are conservative; one reason being that labs in other parts of the world may be much less safe than labs in developed nations.

This live virus research is just too risky to carry out, especially since other means of identifying mutations that lead to airborne transmission in mammals are available. Thus, there is very little to be lost by banning this live virus research.

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[1] "Absolute probability" is the term used by Gryphon Scientific in its risk-benefit analysis (RBA). It seems like a contradiction in terms, since "probability" implies uncertainly, not something absolute. I prefer "direct" probability as it implies leading directly toward a goal. Nevertheless, I will stick with the Gryphon term throughout this analysis.

[2] Each variable p with a subscript is a conditional probability of the event in the chain leading to an accident, given that the previous event in the chain occurred, with two exceptions. p_{inc} is an annual probability (effectively a rate) that an incident occurs. p_{LAI} is a ratio of LAI to exposure, taking into account multiple LAIs in the same exposure event.

[3] Many incidents that must be reported to the NIH involve spills that did not lead to LAIs. The NIH reporting guidelines state "spills or accidents occurring in high containment (BL3 or BL4) laboratories resulting in an overt or potential exposure must be immediately reported." (http://osp.od.nih.gov/office-biotechnology-activities/biosafety/institutional-biosafety-committees/incident-reporting) Potential exposures imply loss of containment to me.

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Date: March 6, 2016

Addendum to my February 23 Commentary for the March NAS meeting on GOF: Toward absolute probabilities for escape from a laboratory

My February 23 Commentary presents a calculation of "direct" or "absolute" probability of escape from a laboratory of a potential pandemic pathogen, specifically mammalian-airborne-transmissible, highly-pathogenic avian influenza viruses (matHPAI). Absolute probabilities are necessary to calculate the probability of a laboratory escape and subsequently the likelihood of a pandemic from an escape, a key goal of Gryphon Scientific's risk-benefit (RBA) analysis.

To obtain data for my calculation, I employed Table D-7 of reported lab incidents collected for the *Final Supplementary Risk Assessment for the Boston University National Emerging Infectious Diseases Laboratories (NEIDL).* (http://www.bu.edu/neidl/files/2013/01/SFEIR-Volume-III.pdf) This 2,716 page risk assessment is abbreviated as the SFEIR (Supplemental Final Environmental Impact Report).

Table D-7 lists and summarizes 118 exposure or potential exposure incidents in BSL3 labs, up to the year 2010. Although not a large data set, there was enough data in Table D-7 to carry out a preliminary estimate of the likelihood or probability of escape from a lab, which I did in my February 23 Commentary.

This small data set can be considerably strengthened in several ways:

(1) It can be brought up to date by including data from 2011 through 2015.

(2) The original incident reports to NIH should be read to clarify the few cases where summaries were confusing. I assume Table D-7 was prepared by the group carrying out the SFEIR analysis, so it is a secondary source.

(3) Similar data should be available from the European Union, and should be included.

Gryphon Scientific should be well positioned to carry out these three tasks quickly. They may already have much of the data. The original reports to the NIH (and the EU) should be made publically available by Gryphon, with names redacted of course, so we can make our own assessments.

Since the absolute or direct probability of escape for a matHPAI is the most important probability in the risk analysis, every attempt should be made to find a reasonable estimate of it. The method I demonstrated in my preliminary analysis seems to me to be a good way of finding a reasonable estimate.

THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS

Comments of The American Association of Immunologists (AAI) to the National Science Advisory Board for Biosecurity on Gain-of-Function Studies

Submitted on behalf of AAI by Lauren G. Gross, J.D.,
Director of Public Policy and Government Affairs
The American Association of Immunologists (AAI)
March 8, 2016

The American Association of Immunologists (AAI), the largest professional association of immunologists in the world, representing more than 7,700 basic and clinical immunologists, appreciates the opportunity to provide comments to the National Science Advisory Board for Biosecurity (NSABB) Working Group on Gain-of-Function (GOF) Studies.

AAI appreciates the careful and thorough investigation of the risks, benefits, and public health considerations associated with select GOF research studies. The resulting working paper is a well-thought out document that provides an excellent foundation for the final ruling on this topic.

AAI is largely in favor of the draft recommendations that have been provided by the Working Group. There are, however, some concerns that have not yet been fully addressed. Importantly, the steps for implementation of these recommendations are not clearly laid out. AAI strongly recommends that these recommendations be implemented very cautiously to avoid potential burdens, including:

1) negatively affecting beneficial research perceived as GOF, but posing little real danger to public health, and
2) increasing the administrative burden on investigators and/or grant reviewers, taking away time and effort from important experimental research.

To avoid these unintended consequences, AAI believes that Recommendation 2 (to utilize existing policy frameworks) is the most crucial aspect of these new guidelines.

AAI believes that, very unfortunately, an individual intent on using biomedical research for nefarious purposes would not be prevented from doing so by these recommendations, and that instead, restriction of GOF research studies could actually impede advances in discovering the function and transmission of, as well as potential countermeasures against, natural and man-made biological threats. Because the risk profile of GOF studies is similar to studies using select agents, it may, in many cases, be more appropriate to apply current Dual Use Research of Concern (DURC) policies to these studies.

Comments on the May 6 NSABB Working Group Draft Report - Recommendations for the Evaluation and Oversight of Proposed Gain-of-Function Research 5-6-2016

Marc Lipsitch
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Overall I congratulate the NSABB and its working group on incorporating a number of issues raised at prior meetings and at the NAS Symposium into this revised draft. This draft addresses key issues to a significantly better degree than prior versions.

The following comments are limited to the Findings and Recommendations.

Finding 1 is exactly correct.

Finding 2 is overly optimistic. It makes no reference to the problems of conflict of interest, real or perceived, that arise when those performing oversight are employed by, or funded by, those who benefit from performing or sponsoring the research. It also acknowledges, but does not sufficiently emphasize, that these decisions are made without adequate quantitative data on the risks, and that this lack of data is a direct consequence of the secrecy requirements as interpreted by CDC and other agencies that both regulate and perform GOFROC, and that oversee biosafety and DURC issues more generally, including Select Agents. The recent *USA Today* article http://www.usatoday.com/story/news/2016/05/10/cdc-lab-secret-sanctions/84163590/ clarifies this point further, that as both regulator and subject of regulation, CDC continues to evade public scrutiny of repeated laboratory errors, including three more examples of improperly killed high-containment pathogens being transported out of high containment, thereby circumventing all the mechanical and biological protections specific to high-containment labs.

Finding 3 is correct but is too limited. The fact is that even during the period of highest scrutiny, the current funding pause, there have been NIH-funded GOF studies performed on coronaviruses that violate the spirit, and I believe the letter, of the funding pause, with very unclear explanations given (1). There has been federal funding cited for what is clearly influenza GOF as well (2), also during the funding pause. These are only the examples I have become aware of, and it is very likely that there are others. If even the funding pause ordered by the White House cannot for a short period stop federally-funded GOF research of concern, it is unclear why we should expect that those systems in place before the pause should be adequate.

Finding 4 is unclear as I am not sure what an “adaptive” policy is or what the alternative option would be.

The bold text of **Finding 5** is correct, but the explanatory text is confusing. None of the examples of unjustifiable research is an example of GOFROC, nor even are they all clearly examples of risks outweighing benefits (human subjects not giving consent is a concern for other reasons, not always to do with risks). In line 1159 the text “or that entail benefits that are

unjustifiable in the light of the risks“ appears to misstate what is meant “…entail risks that are unjustifiable in light of the benefits.” Risks must be justified; benefits are the justification.

I also disagree with the statements on lines 1161-2: “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.” Just as there are clear lines of unethical behavior in research involving human subjects, it should be considered unethical (for example) to conduct a study which imposes a risk of starting a large-scale outbreak or pandemic of a virulent pathogen, in order to gain scientific knowledge where similar scientific goals could be met or equivalent public health benefit could be gained through alternative approaches not involving pandemic or outbreak risk. This claim has not been generally accepted to date, and I would not argue that GOFROC to date has been unethical, but I would argue (and have argued in a peer-reviewed publication) that the same principles that lead us to accept restrictions on human subjects research – demanding humanitarian benefit when risks are significant, and only permitting significant risks to humans when alternatives are unavailable – should also restrict GOFROC (3).

Finding 6 seems correct, subject to the concerns about the inadequacy of current mechanisms noted above.

Finding 7 is correct but needs a corresponding recommendation for how to create international oversight, and this is lacking.

Recommendation 1 and supporting text are improved from prior drafts. The “resistance to countermeasures” criterion has been appropriately removed, but it is to some extent retained in the language:

> 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.

The idea that medical countermeasures alone would be sufficient to reduce the risk of spread of a novel infection is untenable, as recent events dramatically illustrate. Even the basic countermeasures of hygiene and safe burial, routinely available in the US, were not “available” enough to prevent the West African Ebola outbreak from infecting tens of thousands. The current Yellow Fever outbreak represents uncontrolled spread of a virus for which a nearly perfect vaccine has been available for decades. While the further spread of this virus will likely be exacerbated by vaccine shortages, the main problem leading to the current amount of spread is not a vaccine shortage but the fact that the vaccine has not been used in advance of

the epidemic in many places. For most anti-flu countermeasures global availability is extremely poor (4). At best, the "unavailable globally in sufficient quantities" proviso essentially is so universally true that the "'limited options" clause would apply to every infectious agent. At worst, it complicates interpretation. The "uncontrollable spread" aspect should be removed from the first criterion for clarity and brevity.

The *Yersinia* experiment of engineering greater pneumonic tropism for plague in an antibiotic resistant strain described in Appendix C is a good example of how the "limited options" proviso complicates the situation unnecessarily. Surely the same experiment to enhance transmissibility, performed in an antibiotic-susceptible strain, would create substantial risk of uncontrolled spread, given that (a) it might not be recognized and properly treated, even in places with good health infrastructure and (b) there are many parts of the world where treatment is not available on a widespread basis for pneumonic plague. This is exactly the sort of project where the "lack of countermeasures" criterion could create a false sense of security.

The paragraph at line 1274 and following is an important addition reflecting discussions at NAS.

The principles for consideration of GOFROC numbered i through viii are also improved. Principle iv speaks of "the same scientific question" while the explanatory text describes "provide the same or very similar information [as a GOFROC approach]." The two should be harmonized to "the same or similar," as one can always define a scientific question that can only be answered in one way, such as "what is the result of performing manipulation X on strain Y?" which can only be answered with one experiment. There should be no opportunity to circumvent this essential criterion by semantics.

I remain concerned that department-level review (which in practice currently means HHS) cannot be independent given the real conflicts of interest between funding and regulating such research. At a minimum, such a panel should include a substantial membership from non-government employees and/or other departments.

Overall, while the principles laid out in this recommendation have many strengths, I am concerned that the institutional arrangement may be essentially indistinguishable from that established by the 2014 HHS Frameworks, which were not judged adequate.

Recommendation 3 is excellent, as is Recommendation 3.1 in particular. It should be made explicit that the secrecy barriers currently in place should be reconsidered in light of the strong evidence that secrecy prevents effective learning from mistakes and accountability. Again the recent USA Today story on CDC lapses is very much on point http://www.usatoday.com/story/news/2016/05/10/cdc-lab-secret-sanctions/84163590/.

Recommendation 4 is appropriate, and I would suggest that specific types of experiments be added to the list of prohibited experiments under the Select Agent Rule, as has been suggested

previously http://www.cidrap.umn.edu/news-perspective/2016/03/commentary-six-policy-options-conducting-gain-function-research .

Recommendation 5 is very important and excellent, and 6 and 7 are very good as well. However more specific ideas for international oversight would be most welcome.

1. **Menachery VD**, **Yount BL**, **Debbink K**, **Agnihothram S**, **Gralinski LE**, **Plante JA**, **Graham RL**, **Scobey T**, **Ge X-Y**, **Donaldson EF**, **Randell SH**, **Lanzavecchia A**, **Marasco WA**, **Shi Z-L**, **Baric RS**. 2015. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nature Medicine.

2. **Williams GD**, **Pinto AK**, **Doll B**, **Boon ACM**. 2016. A North American H7N3 Influenza Virus Supports Reassortment with 2009 Pandemic H1N1 and Induces Disease in Mice without Prior Adaptation. J Virol **90**:4796–4806.

3. **Evans NG**, **Lipsitch M**, **Levinson M**. 2015. The ethics of biosafety considerations in gain-of-function research resulting in the creation of potential pandemic pathogens: Table 1. J Med Ethics medethics–2014–102619.

4. **Fedson DS**. 2009. Meeting the challenge of influenza pandemic preparedness in developing countries. Emerging Infect Dis **15**:365–371.

****DRAFT****

A Proposed Oversight and Decision Mechanism for Creating and/or Researching Potential Pandemic Pathogens

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Introduction

About two years ago, the White House ordered (here and here) a "deliberative process and research funding pause" for gain-of-function studies on viruses that "would have enhanced pathogenicity [virulence] and/or transmissibility in mammals via the respiratory route." This White-House-ordered activity is now near completion; the National Advisory Board on Biosecurity has just issued its Draft Final Report

The viruses that are the subject of the White House order include highly pathogenic Asian influenza viruses that can transmit disease from mammal to mammal by the respiratory route (airborne transmission). Such viruses have already been created in the laboratory, in particular but not limited to the laboratories of Ron Fouchier and Yoshihiro Kawaoka. If one of these viruses escaped a laboratory, it could seed a pandemic with thousands to millions of human fatalities. These are called GOF studies of concern by the National Science Advisory Board for Biosecurity (NSABB), or simply studies of concern.

Any review mechanism for studies of concern must take into account risk-benefit, biosafety, biosecurity and other international consequences such as demands for reparations for morbidity and mortality from a laboratory escape. Allowing the most dangerous research to proceed sends a message to other nations that such research is acceptable; and it may send the wrong message that the U.S. is embarking on the most-dangerous-imaginable biological weapons development.

A proactive and on-going review process for studies of concern that involves several committees is proposed here:

- A means of identifying which studies could seed a pandemic in humans if a laboratory-created pathogen escaped.
- A Committee of Outside Experts (COE) to review such research to supplement the current Institutional Biosafety Committee (IBC) review and Federal review, presumably NIH internal review.[1]
- A White-House Committee (WHC) charged with making decisions when there is disagreement among the three committees whether the studies should or should not be conducted (banned) in the U.S.

The WHC could include members from the National Security Council, the Office of Science and Technology Policy, the Department of State, the Department of Health and Human Services (HHS) and perhaps others. This committee composition would help ensure that dual-use security concerns, biosafety risk to the community, and international ramifications are addressed. The WHC would recommend to the President to ban a particular study of concern.

The just released NSABB Draft Final Report in its Findings and Recommendations has come to some of the same conclusions as the proposal here; for instance, the possibility of banning some studies of concern:

> **"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."

Summaries of the current state of affairs, criticisms of the NSABB rules, and discussion of this Proposal follows:

Problems with the NSABB rules for identifying "studies of concern"

In the Gain-of-Function Research Symposium held at the National Academy of Sciences (March 10-11, 2016), the NSABB gave a presentation (Slides 12 and 13) summarizing its conclusions on funding and oversight for GOF studies of concern. The NSABB concluded:

> "Research proposals involving GOF studies of concern…should be reviewed carefully for biosafety and biosecurity implications, as well as potential benefits, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the NIH and institutional levels."

GOF studies of concern needed to be defined. The NSABB offered the following three rules:

> "A GOF study of concern is one that could generate a pathogen with all of the following attributes:
> 1. The pathogen generated is highly transmissible in a relevant mammalian model.
> 2. The pathogen generated is highly virulent in a relevant mammalian model.
> 3. The pathogen generated is more likely capable of being spread among human populations than currently circulating strains of the pathogen."

In its presentation, the NSABB emphasizes that all three rules must apply by underlining the word "all". The White House called for a "deliberative process and research funding pause" for GOF studies on viruses that "would have enhanced pathogenicity [virulence] and/or transmissibility in mammals via the respiratory route." The "and/or" was usually interpreted as "or". The NSABB changing now to the word "all" fundamentally changes the discussion, and could allow dangerous virus strains to escape their studies-of-concern designation.

In the Draft Final Report, the NSABB has dropped Rule 3, but still insists that both Rules 1 and 2 must be met to be a GOF study of concern. In slightly different language:

> "To be considered [Gain of function research of concern] 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.
> ii. The pathogen generated is likely highly virulent and likely to cause significant morbidity and/or mortality in humans."

To make the discussion more real, let's concentrate on one type of pathogen: mammalian-airborne-transmissible, highly-pathogenic avian influenza viruses (matHPAI). Some of these dangerous matHPAI strains created in Fouchier's and Kawaoka's laboratories might not qualify as studies of concern under the NSABB rules. For instance, a strain that is highly transmissible and only modestly virulent in ferrets might not be captured as a study of concern. We would certainly not like to see such a strain escape from a laboratory. The problem here is that both rules must apply to qualify according to the NSABB.

What exactly is meant by "highly virulent" or "highly transmissible" in Rules i and ii? Higher or lower virulence and airborne transmissibility of pathogens in ferrets cannot reliably be extrapolated to humans. We must take a careful approach by assuming many of these pathogen strains might seed an uncontrollable outbreak (pandemic), unless they are deemed not dangerous after careful analysis.

Proposed new rule for Identifying studies (research) of concern

Many of us active in the deliberative process use the expression "potential pandemic pathogens" to better identify pathogens of concern, which would focus disagreements on pandemic potential, not on the vague word "highly."

Pathogens that exhibit, or reasonably could be expected to exhibit, pandemic potential are abbreviated PPPs, obviously standing for potential pandemic pathogens.

"Reasonably could be expected to exhibit" is an important phrase here, as pathogens of concern are laboratory-created and are novel, so their pandemic potential has not been observed in nature. With this definition of a PPP, the two NSABB rules might be rewritten simply as a single rule:

A study of concern is one that creates in the laboratory or studies a live laboratory-created PPP not present in nature that reasonably could be expected to be virulent in humans or transmissible in humans by aerosol-droplets or other means of efficient transmission not requiring direct physical contact.

The focus for this proposal is narrowly defined to humans. The NSABB's "relevant mammalian model" is not necessary as part of the definition, although demonstration of mammalian airborne transmission of HPAI in ferrets was the original trigger for widespread concern and will remain a trigger for concern.

Ebola is an example of efficient (non-airborne) transmission with and without direct physical contact. "Not present in nature" excludes pathogens already in the community prepared from plasmids, as is common today for influenza viruses. It also excludes natural strains of pathogens (not laboratory-created) already in the community, such as MERS.

This rule is an attempt to find a rule(s) that is not too narrow so as to exclude some studies of concern, and not too broad so as to include safe studies. From the many discussions leading to this rule, it is clear that drafting a perfect rule is likely not possible. The Committees described here will sometimes have to make decisions to include or exclude particular studies based on their assessment of virulence, transmissibility, and other factors. With experience, the rule may well be modified.

A Committee of Outside Experts to supplement IBC and NIH review

An NSABB quote in this article refers to "NIH and institutional" review. History tells us that institutional review followed by NIH review has been ineffective.

Review by institutional biosafety committees (IBCs) has been incompetent to non-existent. See, for example, the discussion in Chapter 7, "Who's Minding the Store," in Breeding Bio Insecurity where it is suggested why IBC's do not effectively carry out their duties:

> "The root of these failures probably lies in the free-spirit culture of scientists unaccustomed to regulations and suspicious of them, and the inability of the already-dysfunctional Institutional Biosafety Committees to deal with the new era of security regulations."

The review and oversight process cannot begin unless IBCs contact NIH about questionable research project proposals. There should be stiff and enforced penalties for failure to report to the NIH.

The history of NIH review is concerning as well. Again, from Breeding Bio Insecurity:

> "[M]ost of the law's oversight provisions are guidelines and not legally enforceable…the NIH can withhold funding from those violating the guidelines. But the agency doesn't and won't: too much vital research might be impeded. Even prestigious universities pay only lip service to the guidelines, many not even that."

Recent NIH grant awards for the studies that created and researched live matHPAI viruses do not inspire confidence in that particular NIH review. It appears that these studies were funded with little questioning of their risk, certainly without public discussion.

IBC and NIH review should be supplemented by a Committee of Outside Experts (COE) review. From the scientists, ethicists, lawyers, and international policy experts who have participated in the deliberative process, it should be possible to put together a committee that represents all facets and views.

The NSABB Draft Final Report agrees that a third committee is needed:

> "**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."

and

> "**Recommendation 1.** Research proposals involving GOF research of concern entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding."

Final decisions about proposed studies of concern

The kinds of decisions that might be made range from:

- Outright banning a particular study in the U.S.
- Allowing a study to proceed and be funded at an appropriate biocontainment level BSL3, BSL4 or BSL4+[2]

When the three committees (the IBC, NIH, and the COE) all agree on a decision that does not call for banning the study, the NIH can notify the researchers' Institution of the decision. If one or more of the three committees recommends banning the proposed research, the Final Decision will be made by the President from the advice of the WHC.

The obvious reason for high-level WHC review is that a lab escape of a live pathogen could cause an uncontrolled outbreak, with thousands to millions of fatalities. Even the relatively mild 2009 H1N1 pandemic flu killed over 200,000 people around the world.

But there are other reasons as well for Executive-branch review. Casualties outside the U.S. could make the U.S. liable for reparations, and certainly international condemnation. Also failure to ban the most dangerous research sends a message to the rest of the world saying that such research is acceptable; and it may send the wrong message that the U.S. is embarking on the most-dangerous-imaginable biological-weapons development.

There is already a framework in place to guide funding decisions for matHPAI research. The 2013 framework outlines the criteria for funding.

> 'Such proposals will undergo additional funding agency review as well as [HHS] Department-level review in order to determine its acceptability for funding by HHS…the funding agency will determine whether the proposed research is in accord with the following criteria:
> 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."

Presumably, this framework allowed funding of the Kawaoka and Fouchier matHPAI studies before the 2014 funding pause and deliberative process. A Committee of Experts could well decide that these studies should not be conducted. And the many scientists who signed the Cambridge Working Group statement feel that studies such as these should be "curtailed" until they are reviewed again.

> "For any experiment, the expected net benefits should outweigh the risks. Experiments involving the creation of potential pandemic pathogens should be curtailed until there has been a quantitative, objective and credible assessment of the risks, potential benefits, and opportunities for risk mitigation, as well as comparison against safer experimental approaches."

To be kept informed of decisions, an appropriate Congressional Committee or Caucus will be notified of the Final Decision, along with the three committee's decisions and explanations. The Congressional Biomedical Research Caucus[3] is perhaps the best congressional group to keep informed.

Conclusions

Completion of the NSABB deliberative process should not mean the funding pause should be lifted. All studies subject to the funding pause should remain unfunded by the NIH until a new review process, such as that proposed here, is put in place and new reviews are carried out for all existing studies of concern. The U.S. government should also consider stopping all studies of concern regardless of funding source until they are reviewed again.

This proposal does not address the dual-use concern that someone will use the research for hostile purposes. How to decide what is dual-use research of concern and decisions about its publication might follow a procedure similar to that outlined here.

I thank Richard Ebright and an anonymous reviewer with considerable expertise in controversial science/technology issues for many rounds of comments on this Opinion article, particularly on definitions, the rules, and whether the rules are too narrow or too broad.

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[1] Called Federal review by the National Science Advisory Biosecurity Board. Federal review is likely review by the NIH Recombinant DNA Advisory Committee (RAC) or the NIH Office of Biotechnology Activities (OBA). It may also include review by the Department of Health and Human Services (HHS).

[2] An additional level of biosafety -- call it BSL-4-plus -- that adds special protections for laboratory work with dangerous PPP research. BSL4+ differences from BSL4 include (1) Train full-time technical staff who are dedicated to working with highly dangerous pathogens. These staffers would carry out experiments directed by scientists who would never need to be present in the BSL-4+ laboratory. With modern audio-video technology, research scientists can remotely monitor lab work as if they were present. (2) Require lab staffers to follow up extended work shifts with periods of quarantine before they leave the biocontainment area. Such procedures would assure that no potential pandemic pathogen escapes from a BSL-4+ lab through a laboratory-acquired infection; anyone accidentally infected would show symptoms while still in quarantine.

[3] The Congressional Biomedical Research Caucus (CBRC)…is a bipartisan, bicameral Caucus…Seventy five Members of the House of Representatives and nine Members of the Senate comprise the Caucus Membership...
The Caucus seeks to support the excellent efforts of the congressional committees and Members of Congress with jurisdiction over the National Institutes of Health (NIH), the National Science Foundation (NSF), science research, and health issues.

Comments on NSABB May 6, 2016 Draft Report "Recommendations for the Evaluation and Oversight of Proposed Gain of Function Research"

Submitted by:
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May 20, 2016

Finding 1: Agree with all

Finding 2: Main points unclear as written. In principle, yes I agree that there are places in the research cycle where risks could be managed – if the right policies and effective implementation were in place. But as written it implies that the correct US policies are already in place. It cites a range of guidelines and policies already in place and suggests that these policies together aim to manage and oversee GOFROC. But of those policies cited, only the HHS framework for guiding funding for GOFROC research directly relates to this work, and that framework only applies to H5N1 and H7N9 influenza, not for other influenza or for other respiratory viruses. All but the HHS framework were in place before the GOFROC concerns arose in 2012 and did not stop these experiments (or even flag them as of concern.) This finding also implies that federal advisory committees are responsible for oversight or managing risks, and it is unclear what this is referring to. It also implies that journal editors are responsible for oversight or managing risks – prominent journal editors have said clearly they do not agree that they should bear that responsibility and aren't constituted to implement that. It is correct to note that GOFROC research not federally funded does not currently appear to be subject to oversight. It is true that institutional oversight will vary widely, depending on local expertise and culture. It is true that data is limited regarding the rate and extent of laboratory accidents and near-misses and that no single entity collects all relevant accident data.

Finding 3: Agree with some of this, but it does not go far enough. Agree that current policies are not sufficient for all GOFROC. However, the Finding implies that research subject to Select Agent rule would be receiving oversight for GOFROC issues, and this is not true. It's also the case that DURC policies have not appeared to flag GOFROC research for additional oversight, so we shouldn't expect that policy to identify GOFROC. It is good to point out that GOFROC not funded by USG is currently outside of oversight processes, and that should change. Good to point out that other countries fund GOFROC and that the US policy has nothing to do with this, but the DRAFT recommendations should say more about what the US should do to try to reach international consensus in line with some of the major findings of these recommendations. It is important to point out that there are gaps in oversight in US, and that there are substantial implementation issues.

Figure 4: Unclear what "Adaptive Policy Approach" means. Would more clearly define this term. I do agree with the sentiment expressed that new information and data should influence the policies that are established for GOFROC as knowledge and experience gained. Publishing the series of HHS/NIH (and other federal agencies) reviews of proposed GOFROC research would be valuable to the research community in that it could assess more clearly how decisions are made. These reviews could be anonymized as needed and the particulars of new research ideas removed so that intellectual property protected. It will be important for the oversight and risk management process to get smarter with learning as it evolves.

Finding 5: Agree with bolded text. However I do think it is possible to identify GOFROC research as being unethical – i.e. proposed GOFROC research would be unethical if it exposes large numbers of the public to significant risk without the possibility of substantial public health gain, and if that gain cannot be made using any other safer approach.

Finding 6: Agree. But would be clear about what additional oversight and containment mechanisms are appropriate for GOFROC, either with definitive recommendations or at least illustrations of what additional mechanisms are needed.

Finding 7: Agree. Though draft recommendations should be more specific about what should be done internationally.

Recommendation 1: This recommendation is stronger and clearer than in earlier NSABB drafts, but some ambiguity remains. The two criteria to identify GOFROC are correct. However the first attribute could be clearer. If a newly created pathogen is highly transmissible, it is by definition capable of wide and uncontrollable spread in human populations -- these are not separate criteria. Having them listed as distinct can confuse understanding. The existence of a countermeasure for a given highly transmissible disease should have no impact on whether it is classified capable of wide and uncontrollable spread unless it is vaccine that is used nearly universally around the world routinely. In the example of GOFROC influenza, it should not matter that there exists a vaccine or therapeutic that is effective because the majority of the world will not be able to get such a vaccine or therapeutic. In addition, it will not be able to tell in advance of the GOFROC research whether a newly created GOFROC strain would still be protected against with existing vaccines or therapeutics. Appendix C is a good example of the kinds of teaching and guidance materials that will be useful to give to the research community. As noted above, I think a living catalogue of actual experiments that have gone through the GOFROC oversight process that is established, with details regarding how decisions were made, would be quite valuable to the community.

Principles for guiding review and funding decisions: I think the principles are good. However, these principles should dictate not just whether a project should be funded, but also whether it should be allowed to go forward even if not funded by the US government. Recommend that bolded text for criteria *iv* says "the same or very similar" question because while it may not be possible for an alternative approach to answer exactly the same question, it may be possible for an alternative approach to answer a very similar research question that provides equally or nearly equally valuable information.

Review Process for Proposals Involving GOFROC: **Step 1** – it will be important to assess whether most (all?) institutions receiving federal funding have review committees that are deemed (by themselves and HHS) capable of making these determinations. If not, then institutions should get help in getting ready to do this. **Step 2** – this step seems to leave decisions about whether research is GOFROC to the funding agency program managers. This doesn't seem to be a change from the current status quo which had did not seem to have stopped any GOFROC experiments prior to the Deliberative Pause. It is not clear that program managers who funded the experiments that have now been determined to have been GOFROC (by these new NSABB definitions) would agree that these experiments should be named GOFROC. **Step 3** - A Department level review with a federal panel with diverse views from biosafety, biosecurity, ethics public health etc is an appropriate step, but it appears it will not be triggered unless program managers within the funding agency determine that something is GOFROC, which as noted above, may not occur. The language noted in this step about avoiding real

and apparent conflicts of interest should be applied to Step 2 as well. **Step 4 –** Agree risk management is appropriate step. Not clear who determines what is appropriate risk management. There have been arguments that GOFROC work should only be done in BL4, but NSABB does not take a position on that. And while it lists biocontainment in the text, it is not listed as measure in Box 4. **Step 5 –** Agree.

Recommendation 2: It is good to plan for the continued engagement of external advisory body on these issues, for the reasons articulated. For the committee to be available to all agencies and to be free of funding agency constraints, it should sit outside any one particular federal agency. Agree that the committee should be engaged with the research and public health communities that care about these issues, and it should be transparent and independent.

Recommendation 3: See comments above on need to define "adaptive policy approach. Also see above comment regarding how availability of a countermeasure will not something from being highly transmissible and easily spread. Even if a countermeasure exists, it will not be available for all or most in the world. (unless it is a universally available vaccine, such as a routine childhood vaccine, but it is hard to imagine something qualifying as GOFROC that is protected against by a childhood vaccine.)

Recommendation 4: I agree with this in principle - better to have fewer more comprehensive policies than fragmented ones. However existing policy frameworks have not been effective for GOFROC so far, so would need to ensure the proposals in this report are fully embraced into an existing framework if that is going to be the vehicle to make these changes.

Recommendation 5: Agree.

Recommendation 6: Agree

Recommendation 7: Agree with the recommendation and the text, and the goals stated around international engagement are very important. But it would be useful and important for this recommendation to provide additional concrete proposals for how to engage the international community. The international engagement efforts to date have not been highly attended by the international scientific and relevant policy communities and have mostly been limited to US and European representatives. It is important to expand those dialogues and to consider concretely what norms and international agreements might be established that address GOFROC.