NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY

Written Public Comments (Dec. 13, 2015 – Jan. 8, 2016)

The following are written comments submitted to the National Science Advisory Board for Biosecurity (NSABB) for the period December 13, 2015 – January 8, 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
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.
Executive Director, NSABB
NIH Office of Science Policy

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,

David Fedson

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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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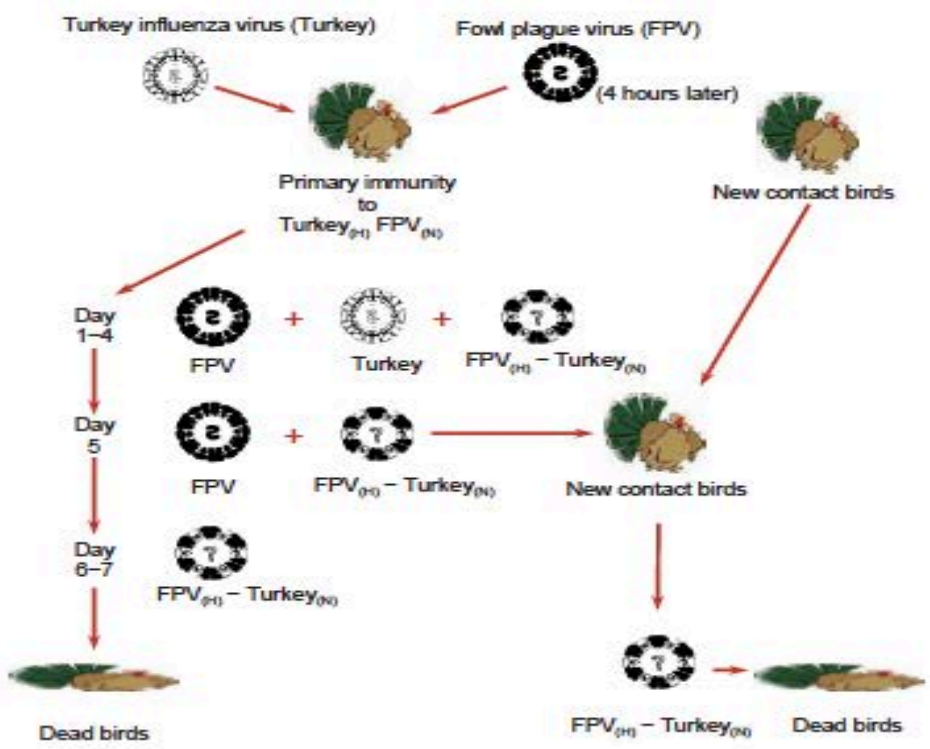

Figure. Genetic reassortment and genesis of a new pandemic influenza virus. This study was designed to determine whether the selection and transmission of a new reassortant influenza A virus could occur under experimental conditions in vivo that mimic what might occur in nature. Reassortment between 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?

David S. Fedson

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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

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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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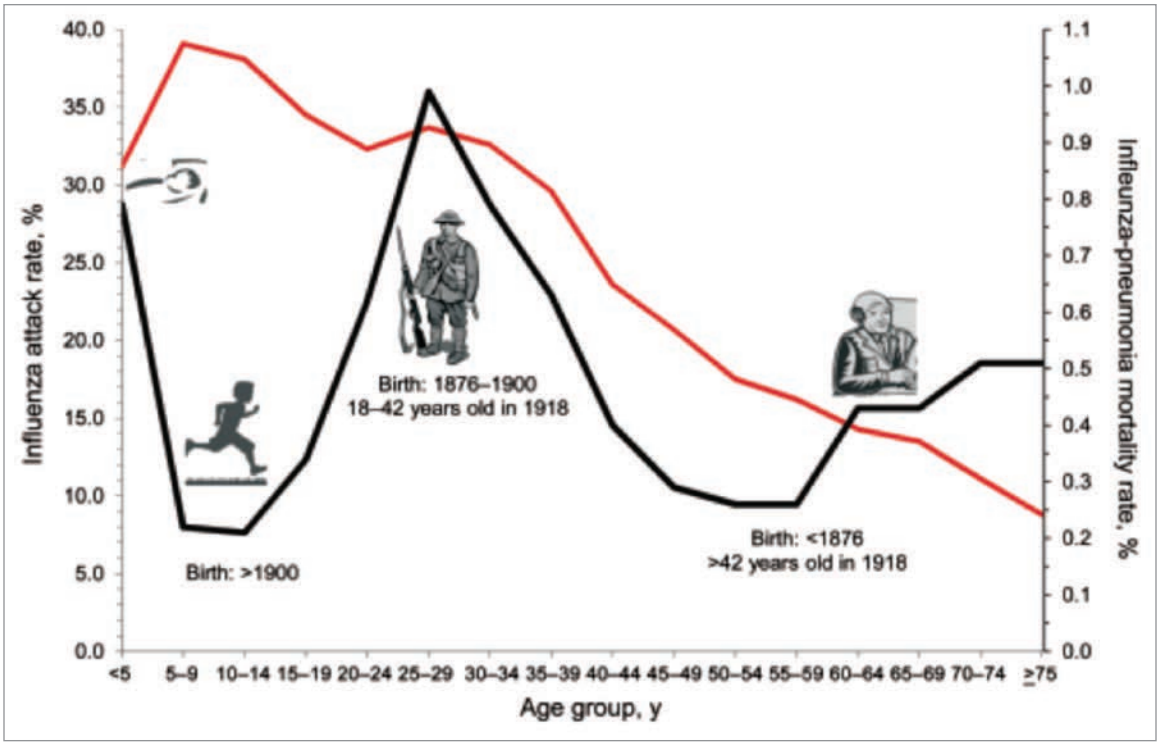

Figure 1. Discrepancy between clinical influenza attack rates and influenza pneumonia mortality rates in the 1918 influenza pandemic (adapted from ref. 38).

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
Sent: Monday, December 21, 2015 12:26 PM
To: National Science Advisory Board for Biosecurity
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

Emeritus Professor of Pediatrics
University of Pennsylvania
Vaxconsult
Doylestown, PA

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
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
Bethesda, Maryland 20892
Via email: 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
Sent: Thursday, December 31, 2015 4:35 AM
To: National Science Advisory Board for Biosecurity
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.
Senior Science Fellow
Center for Arms Control and Non-proliferation
322 4th St., NE, Washington, D.C. 20002

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.

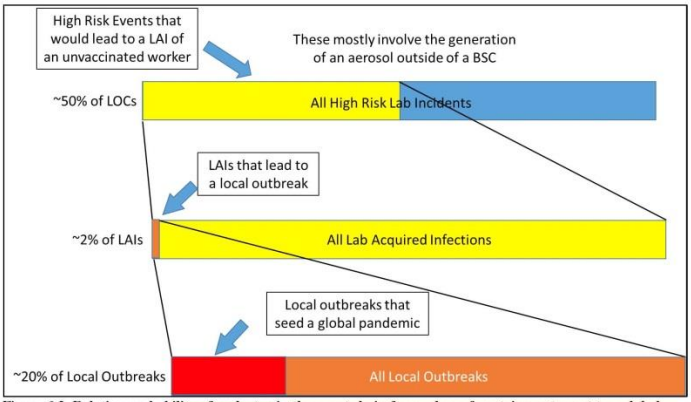

Figure 6.2. Relative probability of each step in the event chain from a loss of containment event to a global pandemic for a loss of containment event involving seasonal influenza.

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

Christopher Viggiani, Ph.D.
Executive Director, NSABB
NIH Office of Science Policy
6705 Rockledge Drive, Suite 750
Bethesda, MD 20892

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
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

From: Nariyoshi Shinomiya
Sent: Monday, January 04, 2016 3:43 AM
To: National Science Advisory Board for Biosecurity; Viggiani, Christopher (NIH/OD) [E]
Cc: 'Husbands, Jo'
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

****

Nariyoshi Shinomiya, M.D., Ph.D.
Professor
Department of Integrative Physiology and Bio-Nano Medicine
National Defense Medical College
3-2 Namiki, Tokorozawa, Saitama 359-8513
Japan

****

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 Salzberg
Sent: Tuesday, January 05, 2016 3:26 PM
To: National Science Advisory Board for Biosecurity
Cc: Steven Salzberg
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.
Bloomberg Distinguished Professor of Biomedical Engineering, Computer Science, and Biostatistics
Director, Center for Computational Biology
McKusick-Nathans Institute of Genetic Medicine
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1900 E. Monument St.
Baltimore, MD 21205
http://salzberg-lab.org

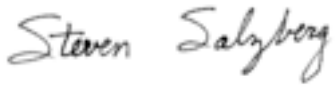
January 5, 2015

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, 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
Sent: Wednesday, January 06, 2016 10:04 AM
To: National Science Advisory Board for Biosecurity
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
Sent: Wednesday, January 06, 2016 12:10 PM
To: National Science Advisory Board for Biosecurity

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 / United Kingdom

From: Andrew Kilianski
Sent: Thursday, January 07, 2016 1:42 PM
To: National Science Advisory Board for Biosecurity
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,

Received 21 July 2015; accepted 2 September 2015.

Correspondence: Andy Kilianski, PhD, BioDefense Branch, BioSciences Division, Edgewood Chemical Biological Center, 5183 Blackhawk Rd, Aberdeen Proving Ground, MD 21010 (andrew.kilianski.ctr@mail.mil).

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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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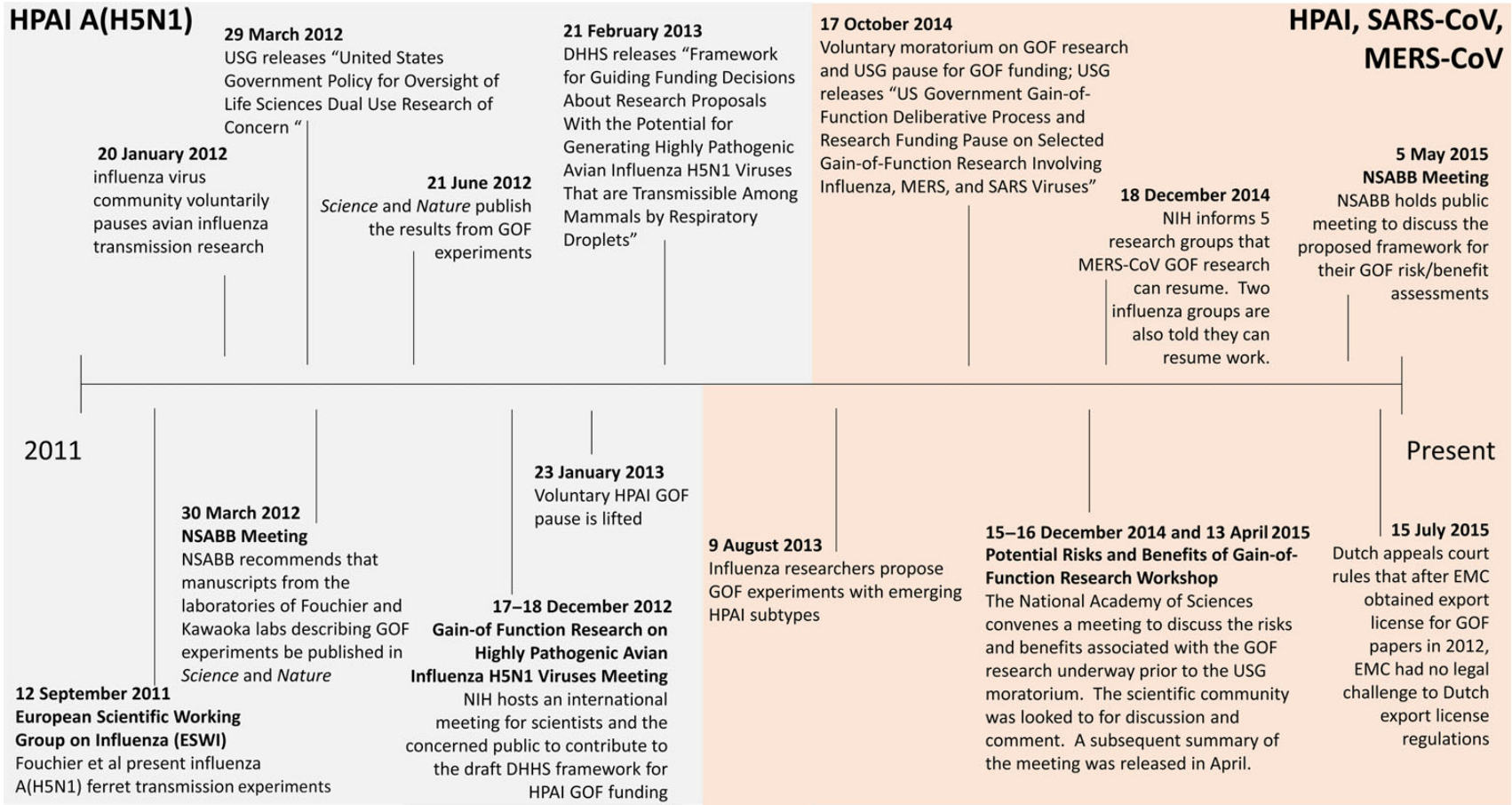

Figure 1. Historical perspective on recent debates associated with gain-of-function (GOF) research. Abbreviations: DHHS, Department of Health and Human Services; EMC, Erasmus University Medical Center; HPAI, highly pathogenic avian influenza virus; MERS-CoV, Middle East respiratory syndrome coronavirus; 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.

Disclaimer. The conclusions and opinions presented here are those of the authors and are not the official policy of the National Research Council, the Defense Threat Reduction Agency (DTRA), the US Army, the Edgewood Chemical Biological Center, or the US government. The authors declare no conflicts of interest.

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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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