

How Would You Spend \$100 Million over the Next Five Years to Prevent the Next Pandemic Flu?

WORKING GROUP DESCRIPTION

Background

The 1918 pandemic influenza killed an estimated 30 million people worldwide. More than 80 percent of the deaths in the U.S. armed forces during World War I were due to the flu rather than combat. Subsequent epidemics have occurred on a regular basis as new flu strains have arisen. Experts agree that without further protective measures, it is only a matter of time before a new and deadly pandemic occurs.

There are currently multiple influenza strains for which humans lack immunity that are circulating in wild bird populations. One of the most dangerous is the H5N1 strain, where H5 and N1 denote the particular variants of the viron surface proteins hemagglutinin and neuraminidase, respectively, which our immune system uses to recognize the virus. Primarily through contact with birds, the H5N1 strain has infected more than a hundred people with a high mortality rate but has not yet gained the capacity for efficient transmission from human to human. In previous epidemics such capacity has often been achieved by a process called viral reassortment, in which some of the eight segments of the influenza genome are exchanged in the cells of a pig that is simultaneously infected with a deadly avian strain and a common mammalian strain. Population growth in Asia has greatly increased the number of locations where domesticated birds, pigs, and humans are living in close quarters, providing more opportunities for reassortment than before, and increased worldwide travel has provided better opportunities for a new reassorted flu strain to develop into a pandemic. Hence, the gloomy prognosis by the experts.

The Problem

The challenge to the working group is to come up with a strategy to prevent the next flu pandemic, be it H5N1 or another newer strain. Some possible approaches are:

1. Development and deployment of antivirals, such as oseltamivir phosphate (Tamiflu), which blocks the essential action of neuraminidase, inhibiting the mature viron particles from exiting the infected cell, at least until a resistant flu strain appears
2. Development of rapid means of creating and deploying a new vaccine that is specific to a new flu strain
3. Building an early warning system that is capable of detecting a new potential pandemic flu strain before it has infected so many people that containment is difficult
4. Develop a better understanding of the human host immune system to allow completely new types of intervention

Working group members are encouraged to explore ways genomics can help in these tasks. When considering detection, the group might consider a technology spectrum from simple antibody tests to full sequencing of flu genomes, exploring which systems could be effectively deployed in the field in the third world and which procedures would be centralized. Use of sophisticated research tools, such as reverse genetics, should be considered. Here *E. coli* plasmids containing the eight segments of the flu virus plus some viral proteins to initiate viral replication are transfected into mammalian cell lines so that different virus strains can be created and propagated in cell culture. This method is increasingly used to create live attenuated vaccines. Working group members should also contemplate the dangers of misuse of these technologies.

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WORKING GROUP SUMMARY

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Summary

Working group members had their work cut out for them. With \$100 million in imaginary (virtual) money and a dream, they set out to prevent the next pandemic influenza. In the past several years H5N1, an influenza virus endemic to wild bird populations, has infected more than 100 humans. While the virus has not yet mutated to become transmissible between humans, its potential to do so has scientists scrambling to derive new methods and approaches. This group of scientists had quite a plan.

Simply stated, \$100 million is probably not enough money to prevent a global pandemic. Though advised by conference administrators that more virtual money was available if it would make the proposed challenge more feasible, the group decided that limited funds would encourage a focused and prioritized plan of action. As the group members brainstormed to organize the spending in a way that would complement currently proposed flu preparedness plans, the far-reaching impact of limited but well-placed funding would become clear.

Following eight hours of intense collaboration through roundtable discussions, the money was divided into two broad categories. Approximately half would fund a centralized flu research resource. The remaining half would finance a series of grants and contracts for vaccine and antiviral research, excluding human trials and full-scale vaccine production. Group member Rob Carlson explained how the action plan could be sustained once the \$100 million was exhausted. “This money will make the initial stages go faster,” he said, “and then when things work, it will be obvious what should garner more funding.”

Centralized research and resource facility

Steered unobtrusively by the group's administrator, David Haussler, the group decided the primary goal should be to promote innovative, genome-centered research. The group members agreed that a core research facility dedicated to gathering all available flu information in a centralized location was imperative for rapid and cost-effective vaccine development.

The planned community flu-research resource would run on a \$5 million administrative budget. It would dedicate \$5 million to sequencing full genomes of the bird and human versions of the virus, as well as to monitoring emerging strains. With \$15 million, the plan would also provide access to BSL3+ and BSL4 testing facilities for a broader group of scientists to test for the virulence and transmissibility of threatening mutation combinations, and the effectiveness of new vaccines and antivirals. An international clone library would be established to consolidate vaccine and antiviral research, as well as provide plasmids coding for known gene variants to vaccine development laboratories, discussed below.

If a virus like pandemic flu surfaces, global spread is essentially unavoidable because most people have little to no immunity. Because it would spread like wildfire, detection just one day earlier could have dramatic effects. Monitoring people for viral antibodies, which show up a few days after infection, is often too late. "Antibodies are historical information," quipped Stephen Johnston. "They'll tell you what someone died of."

The group therefore spent another \$5 million, distributed across five laboratories, on the development of new field detection technologies. Ronald Davis, director of the Stanford Genome Technology Center, briefly joined the group to discuss inexpensive and rapid detection devices in the works. He said that while the technology to identify specific flu strains is there, such as with the recently developed flu chip, the readout devices present the greatest obstacle to fast, on-site detection. He seemed certain, however, that increased funding would make the difference. "It's not like building a bomber," he said. "One million dollars can do so much."

Changes in evolutionary genetic and ecological factors of a virus are inextricably linked to its virulence and pathogenicity. Consequently, the group allocated \$10 million to modeling the landscape of flu strains edging toward pandemic by documenting how the virus is changing over time in its wild reservoirs, and how it might mutate or combine with other viruses in the future according to observed patterns.

Myles Axton presented an analogy comparing the evolutionary landscape of a flu virus to a golf course. With the hole signifying a devastating pandemic flu, the green surrounding the hole represents all the versions of that virus that an ideal vaccine should protect against. The ridges and slopes of the golf course are the characteristics of the virus's environment that determine whether it rolls toward or away from pandemic potential. Cataloging and understanding these bioinformatics (ridges and slopes), which would be a key purpose of the community flu resource, is necessary in the creation of an anticipatory and broadly protective vaccine. "We have to think evolutionarily," said Axton. "Otherwise we're just playing catch-up with a virus that can clearly outrun us."

The *Gimish* vaccine competition

Central to the group's plan was the "*Gimish*" vaccine concept: create a vaccine that protects against many known variants of the virus, as well as anticipates possible mutations.

Traditional vaccines consist of attenuated (weakened) or killed viruses that stimulate the immune system's humoral response. The antigens introduced by the vaccine "instruct" B cells to produce antibodies with the help of T cells; the antibodies adhere to the antigens and flag them for destruction by white blood cells. The immune system is then "trained" to deal with the active virus in the future. Annual influenza vaccines are typically killed viruses grown in eggs or cell culture. Because a pandemic flu is both yet unknown and rapidly evolving, an attenuated or killed virus vaccine is insufficient.

Another method of influenza vaccine development involves reverse genetics, a process by which cloned DNA is custom arranged to code for only certain flu antigens that will trigger immune response. Synthetic vaccines range from these expressed protein

subunit vaccines to full gene vaccines in which “naked” DNA plasmids with genes coding for pathogenic proteins are directly injected.

Gene vaccines differ from traditional vaccines in that they trigger both humoral and cellular immune responses. Gene vaccines work by introducing a gene that codes for an antigenic protein directly into the nucleus of dendritic immune cells. When the gene is expressed, the surface of the cell is modified in such a way that cellular immune response is triggered: white blood cells recognize and kill foreign organisms and infected cells as detected by surface proteins. The humoral response is also triggered because antibodies respond to the flu antigen(s) secreted by those altered cells.

A *Gimish* vaccine for pandemic flu, using the virus landscape model as systematized by the community research center, would incorporate as many potential variants of the virus as possible using gene vaccine technology. In this case, researchers would also engineer site-directed mutations in potentially pandemic flu viruses (also through reverse genetics) and evaluate how virulence and pathogenicity are affected. By genetically engineering what one group member called “our own nasty version of the virus,” as informed by the bioinformatics models, the central research center could test all vaccines created by the competing labs and gauge how the flu variants respond.

Fifty million dollars was earmarked to subsidize parallel research efforts to produce a *Gimish* gene vaccine. For the *Gimish* vaccine competition, which one group member called the “elimination jamboree,” 10 laboratories would be selected to receive \$1 million, one-year grants to work toward a fundamentally new, broadly protective gene vaccine. All labs would have access to the established community research resources, and by interfacing with that infrastructure, could save considerable time and money.

At the end of one year, two standout labs would each be granted \$20 million, two-year contracts to create a *Gimish* vaccine. The two chosen labs would have to demonstrate the capacity and capability to meet the following criteria: 100 million vaccine doses costing less than a dollar each produced in two months; broad protection for two animal species; suitable for distributed global production; and broad protection against many virus variants and, ideally, anticipate future changes as well.

The development of adjuvants to optimize a *Gimish* vaccine would also be encouraged. Adjuvants are substances that enhance the immunogenicity of antigens,

meaning that when they are administered in combination with another treatment, they improve the body's immune response. In this case, adjuvants could promote broader protection and vaccination in one dose.

In addition to grants for a *Gimish* vaccine, \$5 million was allocated for seed grants for antiviral research. One new antiviral approach halts viral proliferation by capitalizing on a natural cellular process called RNA interference (RNAi). When a virus enters a cell and begins replicating, a nuclear enzyme known as Dicer cleaves the viral RNA into short interfering segments (siRNA). These siRNA segments are picked up and unwound by a protein complex, and then essentially re-adhere to viral RNA strands and prevent their replication. Antiviral therapy involves introducing artificial siRNA into potential host cells, essentially tricking the cells into activating the RNAi pathway before the virus is actually present. This is promising for reducing influenza pathogenicity and transmission for two reasons: it requires only a synthesized segment of the viral genome (which could be selected from those already sequenced) and it does not require waiting for the virus to appear in the population. The primary problem with RNAi in flu prevention is its requirement for prepositioning of the siRNA in a cell before the virus infiltrates the body. Because pandemic flu strains like H5N1 do not target specific cells, delivery is highly problematic.

The group decided to award ten \$500,000, one-year grants to laboratories committed to ongoing siRNA research, with a deserving laboratory receiving \$5 million upon demonstrating a potentially effective RNAi therapy.

Perhaps the most crucial characteristic of the group's comprehensive plan was the feedback loop. Ongoing sequencing of flu strains would not only drive vaccine production but also allow for constant updates of the detection devices. As vaccines from the competing labs were to be tested against the constructed viruses, the efficacy or inefficacy of those vaccines would inform both the landscape model and ongoing research. Similarly, advances in RNAi therapy could shift the research center's focus from the *Gimish* to methods of siRNA introduction. The research findings of each laboratory would become commonly available through the centralized facility, with an understanding that thwarting the next pandemic flu requires greater openness in research while still meeting the biosecurity needs of participating governments.

At the conclusion of eight hours of lively conversation and planning, this brain conglomerate had contributed its fair share. Many of the energized group members seemed genuinely disappointed that they had been dealing with virtual money all along. While the \$100 million challenge may have seemed broad and amorphous at the start, it had been whittled into a sharply focused vision. With the momentum of the collaboration pushing them along, several group members refused to let the plan evaporate on the last day. Rumor has it that a grant proposal may be in the works to help bring them together once again.