National Collaborating Centre for Infectious Diseases
Centre de collaboration nationale des maladies infectieuses

Purple Paper

Highlights of the *Options for the Control of Influenza VII* Conference 3-7 September 2010 Hong Kong SAR, China

Part II

Options for the Control of Influenza began as a small scientific symposium in Keystone, Colorado in 1985. Since then, this triennial conference has become the largest international conference devoted exclusively to all facets of influenza, from basic science to health care policy. The ***Options VII*** conference was held in the Hong Kong Special Administrative Region (SAR), China from September 3 to 7, 2010, and was the first in the meeting series to be convened following an influenza pandemic. This is the second of a two-part *Purple Paper* series, which presents some of the conference highlights, with emphasis on the 2009 pandemic response and future pandemic preparedness.

Pandemic Influenza Vaccine

Public health mitigation strategies of large-scale infectious disease outbreaks usually consist of both pharmaceutical and non-pharmaceutical interventions. In the last issue of the *Purple Paper*, the subject of non-pharmaceutical interventions was discussed in conjunction with an overview of Hong Kong's experience in implementing some of those interventions during the 2009 pandemic. This issue will focus on pharmaceutical interventions, namely vaccines and antivirals.

Vaccination is one of the pillars of communicable disease prevention and control in public health, and it played an indispensable role in the global and national response to the 2009 influenza pandemic. What made vaccination unique during this public health emergency was that, in addition to uptake of the pandemic vaccine, the timing of its availability was crucial for the effective control of pH1N1 spread. Drs. Nancy Cox from the Influenza Division

Key Points

- All licensed formulations of the pH1N1 vaccine were uniformly immunogenic, having exceeded all regulatory criteria after a single dose in adults and two doses in children in most cases.
- Vaccine effectiveness data are being reported at present. Available reports suggest that the pH1N1 vaccine was highly effective at preventing laboratory-confirmed infection, with vaccine effectiveness approaching > 90% for most age groups.
- The current influenza vaccine production timelines were the major obstacle to the timely provision of the pH1N1 vaccine during the pandemic. In many countries, the pH1N1 vaccination campaign only began after the second pandemic wave was well underway or had peaked.
- WHO had worked vigorously to ensure the access of low-income countries to the pH1N1 vaccines; however, deployment efforts were slower than hoped and were fraught with logistical, legal and ethical barriers.
- Neuraminidase inhibitors, namely oseltamivir and zanamivir, were the primary treatment options for pH1N1. Optimal effectiveness was achieved when the drug was given within 48 hours of symptom onset; however, late therapy was still beneficial in severely ill pH1N1 patients.
- Oseltamivir-resistant cases remained a rare occurrence, with only 304 cases confirmed globally as of August 18, 2010.
- Many investigational anti-influenza agents are in various stages of clinical development, with peramivir and laninamivir being licensed or close to licensure in some countries.
- Moving forward in improving preparedness efforts, public health authorities need better tools for describing and understanding the severity of a pandemic. Public health authorities must also contend with the anti-science movement that is counter-productive to public health mitigation measures.
- Mathematical modelling can be an important adjunct to the public health armamentarium, but could only be of value when it is explicitly linked to action.

of the National Center for Immunization and Respiratory Diseases at the Centers for Disease Control and Prevention (CDC) and David Wood from the Quality, Safety and Standards Team in the Department of Immunization, Vaccines and Biologicals at the World Health Organization (WHO) highlighted some of the lessons learned during the 2009 pandemic and discussed challenges that need to be overcome for improving preparedness efforts for the next pandemic. The following assessments are based on the personal observations of the presenters and are not official statements from their respective organizations.

What worked: Pandemic influenza vaccine

More than 20 monovalent pH1N1 vaccines were licensed for use globally and they spanned all classes of influenza vaccines, including inactivated whole- or split-virus vaccines, subunit surface antigen vaccines and live attenuated vaccines. The majority of the pH1N1 vaccines were manufactured using the traditional egg-based platform; however, cell-based production platforms (i.e. Baxter's Vero cell system and Novartis' MDCK cell system) were also employed on a large scale for the first time. In addition to Novartis' MF59 adjuvant, two new adjuvant systems – Sanofi Pasteur's AF03 and GlaxoSmithKline's AS03 – were introduced for use with the monovalent vaccine during the 2009 pandemic. All licensed formulations of the monovalent pH1N1 vaccine were uniformly immunogenic, having exceeded all regulatory requirements after a single dose of the vaccine in adults and two doses in children, with the exception of GlaxoSmithKline's AS03-adjuvanted vaccine which was immunogenic in children after only one dose. Effectiveness data are being reported at present. Nevertheless, available reports suggest that the pH1N1 vaccine was highly effective at preventing laboratory-confirmed infection, with vaccine effectiveness approaching > 90% for most age groups [1-4].

The safety profile of the pH1N1 monovalent vaccine was comparable to that of the seasonal influenza vaccine. Local and systemic reactions (e.g. pain and swelling at the injection site, fever, chills, malaise, fatigue, headache and muscle pain) were common. Allergic reactions (e.g. hives, rash, angioedema and anaphylaxis) were noted but these were within expected range for seasonal influenza vaccines. The frequency of Guillain Barré syndrome was at or below the observed rates for the seasonal influenza vaccine. Although gastrointestinal symptoms (e.g. diarrhea, vomiting and nausea) were somewhat higher than expected rates, these were mild and self-limited. Narcolepsy was a new adverse effect associated with the use of one particular pH1N1 vaccine observed in a small number of countries. Dr. Wood pointed out that there are a variety of alternative possible explanations for this observation, and studies are being planned to investigate these issues in the coming few months.

While high media coverage on the pH1N1 vaccine probably had both positive and negative effects on vaccine uptake, the timeliness of scientifically credible information was one of the key issues in communicating with the general public about the vaccine.

What worked: Immunization policies

To aid in the rollout of vaccination campaigns, the WHO Strategic Advisory Group of Experts issued, and was able to achieve, consensus on two sets of immunization policy recommendations. The first set of recommendations was made on July 7, 2009. It listed specific priority groups for receiving the pH1N1 vaccine in an effort to reduce morbidity and mortality. Frontline health care workers were also identified as one of the priority groups in order to maintain health care system infrastructure during the 2009 pandemic. The second set of recommendations was made on October 28, 2009, and was intended to be an update to the July recommendations. The October recommendations concerned the number of administered doses in the context of limited vaccine supplies. Considering the high immunogenicity of the pH1N1 vaccine, WHO supported the use of a single dose of vaccine in individuals aged 10 years and older, provided that this was consistent with indications by national regulatory authorities. Furthermore, in cases where national authorities assigned priority to children, WHO recommended administering one dose of vaccine to as many children as possible. A second

dose could be administered as further supplies became available, if it was recommended by regulatory authorities.

What worked: Data sharing

The level of data sharing among different stakeholders around the world who were involved in the vaccine endeavour was extensive and unprecedented. During the 2009 pandemic, WHO was running a series of parallel teleconferences on a weekly basis on various issues regarding the pH1N1 vaccine. Regulatory and public health agencies were sharing data with WHO and with each other to allow for rapid information exchange on key issues such as vaccine safety. WHO held a second set of teleconferences exclusively with national regulatory bodies to exchange information on the decision-making process and regulatory decisions that were made. WHO was also in constant communication with the vaccine manufacturers to discuss and solve practical problems that arose during vaccine production. All these activities were key to keeping WHO abreast of the global vaccine situation.

The ad hoc mechanism WHO undertook to ensure equitable vaccine access had its limitations.

What didn't work well: Vaccine production timelines

Although the pH1N1 vaccine became available six months after the identification of the causal strain as planned, the influenza vaccine production timelines did not permit vaccines to be made sufficiently quickly enough. For this pandemic, the first doses of the pH1N1 vaccine were not shipped until early October in the U.S. (late October in Canada) when North America was well into the acceleration of the second wave. In Ireland, pH1N1 vaccination campaigns commenced after the pandemic had peaked. Given that it takes about two weeks for immunity to develop, the pH1N1 vaccine probably only started to take effect when pandemic activities had already subsided considerably.

In order for the pandemic vaccine to achieve its full epidemiological effectiveness, production timelines must be shortened by four to eight weeks. There are several solutions that could incrementally improve the timeliness of pandemic vaccine production in the short term. For instance, high-yield influenza vaccine strains need to be developed through a comprehensive research program, and they need to be collected in a production-ready vaccine virus library. In addition, more streamlined methods for vaccine potency evaluation and sterility testing – two major steps that are currently the regulatory bottleneck in the vaccine timeline – should be determined. Some of these initiatives are underway at the global level, but to address this problem in the long term, new vaccine technologies that are ideally independent of virus growth should be developed.

What didn't work well: Vaccine yield

The global pH1N1 vaccine yield was less than expected. WHO had conducted a survey in June 2009, asking vaccine manufacturers worldwide to predict the amount of vaccines they could produce in a week. From the provided estimates, WHO extrapolated that there would be nearly 5 billion doses of the pH1N1 vaccine produced worldwide for the 2009 pandemic. This figure was later revised to 3 billion doses in October 2009. These estimates were based on the initial assumptions that there would be a 1:1 yield of the pH1N1 strain as compared to typical seasonal influenza vaccine strains, that most dose sparing formulations of the vaccine would be used by each manufacturer, and that full manufacture capacity would be immediately switched from seasonal influenza vaccine to pH1N1 vaccine production. In reality, production of the pH1N1 vaccine was about only one-third of the expected yield of the seasonal vaccine. Not all manufacturers were able to use their dose-sparing formulations, and not all of the production capacity could be immediately switched to focus on developing the pH1N1 vaccine. All of these factors, in addition to the collapsed demand for the pH1N1 vaccine in 2010, led to the failure to meet the updated production estimate of 1.3 billion doses from WHO's second survey in January 2010.

What didn't work well: Vaccine uptake

WHO estimated that of the 570 million doses of vaccines distributed worldwide at least 350 million doses were administered. Pandemic vaccine uptake varied among countries and even among regions within the same countries. This resulted in vaccine

shortages in some parts of the world and surpluses in others. While high media coverage on the pH1N1 vaccine probably had both positive and negative effects on vaccine uptake, the timeliness of scientifically credible information was one of the key issues in communicating with the general public about the vaccine. This was a particular challenge when questions were raised about the safety of the vaccine before unequivocal evidence became available. Other barriers to vaccination, such as poor self-recognition of vulnerable status and impression that the pandemic was mild or that the pandemic peak had passed, might have also deterred individuals from receiving the pH1N1 vaccine. These barriers need to be better understood and addressed for future pandemics.

What didn't work well: Equity in access to vaccines

Early on during the 2009 pandemic, WHO Director General Dr. Margaret Chan and UN Secretary General Ban Ki-moon called for international solidarity to meet donation target coverage of 10% of the population of countries in need. WHO was particularly concerned that low-income and low-middle income countries would not have access to the pH1N1 vaccine since much of the vaccine production capacity had already been pre-purchased by wealthier nations. WHO had worked vigorously to gain access to the influenza vaccines for 95 target countries that were classified as international priorities. Consequently, 13 donor governments and five manufacturers pledged support by providing 200 million doses of vaccine, 70 million syringes and $48 million for operation costs. In return, WHO was asked to direct and coordinate the deployment of vaccine donations and provide technical and operational support.

Despite WHO's attempt to ensure that low-income countries had access to the pH1N1 vaccine, initial available vaccines were dispatched mainly to developed countries. As a result, the vaccine rollout to low-income countries was slower than anticipated, with first donated doses arriving in Azerbaijan and Mongolia in January 2010. The deployment effort continued over the course of 2010. As of August 24, 2010, 72 million doses were distributed to a total of 69 countries.

Aside from issues related to the initial availability of the pH1N1 vaccine, donation negotiations and deployment programs were fraught with legal, logistical and ethical complexities. Legally, there was a need for new approaches such as liability agreements by recipient countries. Logistically, some recipient countries struggled with developing their deployment plans partly because they did not know what type of vaccine and how many doses they would receive, nor when the vaccines would be delivered. This was compounded by the inability of some countries to secure sufficient resources to fund critical in-country operational deployment activities due to competing resource needs to carry out other essential public health services. Moreover, many countries did not use the seasonal influenza vaccine. While many poor-resourced countries have national programs to deliver childhood immunizations, planning and executing vaccination campaigns for adult cohorts to receive the pH1N1 vaccine presented unforeseen challenges. Lastly, on the part of donor countries, balancing the retention of vaccines for domestic use and donations to support global solidarity was an ethical dilemma with no easy answer. Clearly, the ad hoc mechanism WHO undertook to ensure equitable vaccine access had its limitations. What is needed for the future will be a systematic framework for equitable access that is negotiated in advance of a public health emergency. This, according to Dr. Wood, will alleviate some of the unnecessary procedural complications to make vaccine deployment efforts more seamless and will be an essential component of better preparedness.

M2 proteins play a crucial role in uncoating the virus once it is inside the cell, and are a potential target for a universal influenza A vaccine that matches multiple strains and subtypes.

Towards ensuring global access

Moving forward, WHO has been working diligently to increase global vaccine production capacity by expanding the number of countries that have viable influenza vaccine production facilities. Countries with new or planned influenza vaccine production capacity since 2006 include Mexico, Brazil, Serbia, Egypt, Iran, India, Thailand, South Korea, and

Indonesia. WHO also facilitated the transfer of new technology by providing three developing country vaccine manufacturers – Serum Institute of India (SII), Government Pharmaceutical Organization of Thailand (GPO Thailand), and Zhejiang Tianyuan Bio-pharmaceutical Company in China – with a license for the Russian live attenuated influenza vaccine (LAIV) technology. The LAIV from SII has been licensed, while that from GPO Thailand is in clinical trials. In its endeavour to increase vaccine production capacity in more countries, major difficulties encountered by WHO were finding a technology provider and the limited human resources at new manufacturer sites. In response, WHO established a technology hub at the Netherlands Vaccine Institute, with the support of the Government of the Netherlands to serve as a technology provider and platform for transferring optimized and documented production and quality control processes to interested developing country vaccine manufacturers without the intellectual property rights hurdles. Equally importantly, WHO is working with international regulatory authorities in these developing countries to strengthen their capacity for independent regulatory oversight.

[Adjuvants] have a dose-sparing effect, such that an adjuvanted influenza vaccine requires a smaller amount of antigen than an unadjuvanted vaccine to achieve the same or higher level of immunogenicity.

Innovations in influenza vaccinology

Drs. Cox and Wood provided an overview on some of the ongoing national and global developments that can improve the current vaccine production protocol. However, innovations in the fundamental influenza vaccine theories and technologies would be required to completely transform how the influenza vaccine is made in the long term. Dr. Albert Osterhaus from the Department of Virology at the Erasmus Medical Centre in Rotterdam, The Netherlands, presented some novel strategies that could lead to new influenza vaccine formulations.

Many new approaches to enhancing the influenza vaccines are under investigation, the majority of which revolves around four major themes: 1) identification of new influenza targets; 2) identification of new correlates of protection; and 3) improving efficacy through the use of adjuvants; or 4) through the use of novel vector delivery systems. The traditional vaccine approach focuses on inducing antibody responses in the host as the primary correlate of protection by inactivating the invading pathogen and blocking infection events (i.e. sterile immunity). In the case of the influenza vaccine, protection is afforded by targeting the antibody response to the hemagglutinin (HA) surface proteins. A major shortfall of this approach is that the emergence of drift variants necessitates a new vaccine composition for each influenza season. One way to avoid the perpetual influenza vaccine update is to target antibody responses to other surface proteins – neuraminidase (NA) and matrix protein 2 (M2) – that are less prone to mutations. M2 proteins play a crucial role in uncoating the virus once it is inside the cell, and are a potential target for a universal influenza A vaccine that matches multiple strains and subtypes.

Humoral and cellular immunity recognize and respond to pathogens using very different mechanisms. Although cellular immunity does not prevent infection, it plays an important role in viral clearance and can reduce the duration of influenza disease (i.e. clinical protection). By targeting conserved internal proteins, cellular immunity can provide cross-protection against different influenza subtypes and even against the highly pathogenic influenza A/H5N1. This observation has been confirmed by numerous studies involving the use of mouse and human immune cells in *in vitro* experiments. Thus influenza vaccines that can appropriately stimulate cellular immunity should be further explored as options for therapeutic vaccines.

Adjuvants are compounds that are added to a vaccine to augment the induced immune response, but they do not themselves confer immunity. Adjuvants are not a new concept. Alum (aluminum hydroxide) was the first adjuvant to be approved in the 1920s and it has since been used widely. Besides alum, a number of newer adjuvants are recently licensed for use globally. These are MF59, AS03, AF03, virosomes (i.e. liposomes) and polyoxidonium

(i.e. poly-electrolyte). A long list of others are in Phase 1 and 2 of clinical trials.

The use of adjuvants, namely MF59, AS03 and AF03, in the pH1N1 vaccine has been a subject of debate and controversy during the 2009 pandemic, mainly because these adjuvants have not yet been extensively used and are not familiar to the general public. There are many benefits to adjuvants. They have a dose-sparing effect, such that an adjuvanted influenza vaccine requires a smaller amount of antigen than an unadjuvanted vaccine to achieve the same or a higher level of immunogenicity. Adjuvants can promote a higher peak primary immune response and a longer-lasting memory response. In the presence of an adjuvant, the memory phase following vaccination plateaus at a higher level after the primary immune response wanes.

Alterations in oseltamivir dosing regimens were recommended and were likely required for high-risk patients including premature infants, neonates, patients who were on renal replacement therapies and those who weighed over 200 kg.

In ferret studies, an AS03-adjuvanted H5N1 vaccine, compared to the unadjuvanted version, effects a lower viral load in the lungs and better survival outcomes following challenge with the same influenza vaccine strain. An adjuvanted H5N1 vaccine can also induce a broad immune response that provides protection against H5N1 viruses of a clade different from the vaccine strain. This effect was commonly seen with MF59-, AS03- and AF03-adjuvanted H5N1 influenza vaccines tested in animals.

Lastly, the use of naked DNA constructs and recombinant viral vectors are another option for the development of influenza vaccine. Through this approach, instead of exposing the host to the actual antigen proteins, the antigens are encoded on a DNA construct and delivered to the host as is or by a viral vehicle. A viral vehicle is a highly-attenuated, replication-deficient virus that is emptied of its virulence factors, with tropism for specific human cells. One major advantage of adapting the recombinant viral vector and DNA construct approach to the pandemic influenza vaccine is its safety during production because it does not rely on the mass growth of a potentially dangerous influenza virus. The second advantage is the flexibility of vaccine design. Using genetic recombination techniques, the target antigens encoded on the DNA construct can be manipulated in such a way that permits the stimulation of a specific correlate of protection. Viral vehicles with the capacity to serve as vaccine vectors are retroviruses, poxviruses, adenoviruses, adeno-associated viruses, herpesviruses, and alphaviruses.

In one animal study, macaques were immunized with a poxvirus-vector vaccine carrying a H5N1 HA gene and then challenged with either a homologous or heterologous H5N1 virus strain. Compared to macaques that were vaccinated with a placebo, no virus was detected in the lungs of animals immunized with the poxvirus-HA vaccine four days after infection with either the homologous or heterologous H5N1 strain. There was also no or little coalescing consolidation in the lungs of the vaccinated animals following infection, in contrast to their placebo counterparts whose lung tissue was affected in the range of 45% to 90% [5].

During the pandemic preparedness phase, it was anticipated that the contemporary vaccine production process would be a major obstacle to the public health efforts to mitigate the impact of an influenza pandemic. Nevertheless the extent of the problem was not fully realized until the arrival of the 2009 pandemic. This pandemic has taught us that the pandemic influenza vaccine production timeline has to be shortened by four to eight weeks in order for the vaccine to be epidemiologically effective. While the timely availability of, and access to, the pandemic influenza vaccine is important for all nations, it is particularly important for low-income countries with limited health care capacity. Therefore, in addition to revolutionizing the current influenza production technologies, which some of the novel vaccine approaches presented at the *Options VII* meeting would have the potential to improve, the larger issue of equity cannot be ignored. It is an inevitable reality that nations would

secure the well-being of their own inhabitants before that of others, but politicians and decision-makers in public health should strive to think beyond their own countries, just as infectious diseases do not respect geographical borders. By improving the preparedness and pandemic response efforts of low-resourced countries, the well-being of everyone in this 'One World' would be assured.

Antivirals

The pandemic H1N1 viruses are resistant to adamantanes including amantadine and rimantadine. For this reason, pH1N1 therapy has been based largely on treatment with neuraminidase inhibitors (NAIs). Although oseltamivir and zanamivir, along with the adamantanes, are the only anti-influenza drugs available in Canada, a number of new pharmaceutical agents are in various stages of clinical development, with some agents already licensed for use in other countries. Dr. Frederick Hayden from the School of Medicine at the University of Virginia (Charlottesville, VA) and Wellcome Trust (London, UK) discussed the contemporary challenges of influenza antivirals and future directions in the field.

Effectiveness against pH1N1

Retrospective analyses have shown that the effect of oseltamivir (Tamiflu®) on pH1N1 was similar to that on seasonal influenza. To ensure optimal effectiveness, oseltamivir should be administered to pH1N1 patients within 48 hours of symptom onset. Compared to pH1N1 patients who received oseltamivir treatment more than 48 hours after the onset of symptoms, early treatment was associated with a shorter duration of viral detection, shorter duration of fever and other symptoms, decreased risk of pneumonia and other influenza-associated complications, a lower risk of death among severely ill patients, and a lower risk of death and admission to intensive care unit (ICU) among those who were hospitalized. High-risk groups, such as pregnant women and solid organ transplant patients, could also benefit from timely oseltamivir treatment. Early treatment in these two groups was associated with a lower risk of ICU admission and death. Therefore, delayed access to oseltamivir was a predictive indicator of severe outcomes (e.g. hospitalization, ICU admission and death) among vulnerable pH1N1 patient groups.

New observations on treatment and usage

Despite the critical 48-hour window for the initiation of oseltamivir treatment, clinical experiences during the 2009 pandemic suggested that delayed oseltamivir therapy was still beneficial to patients with severe pH1N1 disease. Observational studies have shown that pH1N1 patients who were administered oseltamivir soon after hospital admission (approximately three to four days after symptom onset) had a lower risk of death and ICU admission than hospitalized patients who received therapy at yet later timepoints. Benefits of delayed oseltamivir therapy were still evident among severely ill patients because they often exhibited a protracted course of viral replication. While there was no apparent added benefit in doubling the oseltamivir dosage from 75 mg BID to 150 mg BID, it would be more effective to prolong oseltamivir treatment beyond the standard course of five days in order to achieve viral clearance.

Of the 304 cases of oseltamivir resistance reported around the world, 28% occurred in severely immunocompromised patients, the majority of whom received oseltamivir therapy.

Other observations related to the therapeutic use of NAIs were also made during the 2009 pandemic. For instance, enteric absorption of oseltamivir (reconstituted with water) was adequate in most critically ill patients who were administered the drug extemporaneously via a nasogastric or nasojejunal tube. Moreover, alterations in oseltamivir dosing regimens were recommended and were likely required for high-risk patients including premature infants, neonates, patients who were on renal replacement therapies and those who weighed over 200 kg.

Zanamivir, commercially available as RELENZA®, is the other major NAI in use. RELENZA® is an inhalational powder mixture of the zanamivir active drug and lactose drug carrier. This antiviral is contraindicated in individuals with underlying airways disease, such as asthma or chronic obstructive pulmonary disease. The use of RELENZA® for pH1N1 was further constrained as bronchospasm among severely ill pH1N1 patients, and virologic failure among immunocompromised pH1N1 patients were reported following the inhalation administration. Dr. Hayden speculated that these adverse side effects may be generic problems pertinent to the delivery of the drug to the site of infection – an issue that was particularly worrisome for pH1N1 patients with pneumonic disease. As such, Dr. Hayden maintained that the safety and effectiveness of nebulized zanamivir among severely ill patients are a persistent concern requiring further study.

… double combinations of zanamivir-oseltamivir and zanamivir-peramivir did not show an additive effect; in some instances depending on the dosage, these NAIs might even be antagonistic when used in combination.

Another specific problem related to the extemporaneous use of RELENZA® was also identified. Delivery of zanamivir reconstituted in saline by means of mechanical ventilation had been a widespread practice for administering the drug to intubated patients in Thailand. Unbeknownst to the physicians at the time, the lactose component RELENZA® could cause blockage of the ventilator filter, and this led to fatal outcomes.

Antiviral resistance

Emergence of resistance is a threat to the antivirals' effectiveness against influenza. The H274Y mutation (an amino acid change from histidine to tyrosine at position 274 in the neuraminidase surface protein) is responsible for oseltamivir resistance in influenza viruses and has been extensively characterized in seasonal influenza A/H1N1 isolates. While the H274Y mutation was rampant, approaching 100% of all recent seasonal A/H1N1 isolates in Japan and parts of Europe, the majority of 2009 pH1N1 virus isolates tested worldwide continued to be sensitive to oseltamivir. Among the pH1N1 isolates tested globally and reported to WHO, 304 have been found to be resistant to oseltamivir as of August 18, 2010 [6]. Since August 30, 2009, 12 cases of oseltamivir-resistant pH1N1 have been reported in Canada [7]. In the U.S., approximately 1% of pH1N1 viruses tested by the CDC since September 1, 2009, were resistant to oseltamivir [8]. Of the 304 cases of oseltamivir resistance reported around the world, 28% occurred in severely immunocompromised patients, the majority of whom received oseltamivir therapy. The remaining cases occurred during or after oseltamivir treatment (33%), were associated with post-exposure prophylaxis (6%), or were among patients who had not used antiviral drugs prior to isolation of the resistant virus (9%). The remaining cases consisted of patients with insufficient clinical information to make inferences about the cause of resistance (24%) [6]. In addition to the H274Y mutation, the I223R mutation (an amino acid change from isoleucine to arginine at position 223 in neuraminidase) has also been found to confer moderate oseltamivir resistance and a low level of zanamivir resistance in pH1N1 isolates harbouring this mutation. The emergence of the I223R mutation remained sporadic.

Although the risk of emergence of oseltamivir-resistant pH1N1 viruses among non-immunocompromised patients with mild-to-moderate disease appeared to be lower than that observed in seasonal A/H1N1 viruses, Dr. Hayden cautioned that public health and health care practitioners should remain vigilant. Both murine and ferret models showed that the replication capacity of and illness caused by oseltamivir-resistant pH1N1 viruses were comparable to wildtype viruses, suggesting that the competitive fitness of the variant viruses was not compromised. These resistant viruses could be readily transmitted by direct contact, and via the respiratory route depending on the virus isolate, in ferrets and guinea pigs. Furthermore, in human studies, the emergence of oseltamivir resistance could occur as early as within two to four days of treatment, and immunocompromised hosts could shed oseltamivir-

resistant viruses for weeks (and sometimes months) irrespective of continued selective drug pressure. Equally concerning was that oseltamivir-resistant pH1N1 viruses had been recovered from persons with no known drug exposure, with well-documented clusters in both community and health care settings.

Combination therapy

Combining various antiviral agents in a single treatment course has the advantage of disrupting the viral life cycle simultaneously at multiple critical control points and preventing the emergence of escape variants. While the concept of combination therapy has been used for the treatment of HIV for quite some time, its application is just beginning to be explored for influenza.

Much of our knowledge about combination therapy for influenza is based on preclinical studies. For example, the combined regimen of amantadine and oseltamivir or ribavirin was synergistic against amantadine-sensitive influenza A viruses in cell cultures and in mice. However, the benefit of dual combinations was lost when the infecting virus was resistant to amantadine. Other double combinations that also had a synergistic effect against influenza A viruses in cell cultures and in the murine model included oseltamivir with ribavirin or favipiravir (a new investigational antiviral targeting the influenza polymerase enzyme; see below).

A recent study has additionally demonstrated that the triple combination of amantadine, ribavirin and oseltamivir was highly synergistic against amantadine- and oseltamivir-resistant influenza A viruses in cultured cells, and its additive effect was significantly greater than that of any double combinations tested [9]. Unpublished data also suggested that this triple combination therapy was effective against the adamantane-resistant pH1N1 virus in the murine model. This triple influenza antiviral combination will be studied in a trial, compared to monotherapies, among high-risk, ambulatory patients in the U.S. later this year.

Perhaps the most surprising finding from the triple combination preclinical study was that double combinations of zanamivir-oseltamivir and zanamivir-peramivir did not show an additive effect; in some instances depending on the dosage, these NAIs might even be antagonistic when used in combination [9]. This novel observation is corroborated by findings of a recent randomized placebo-controlled trial conducted in France during the 2008-2009 influenza season, comparing the short-term virological efficacy of oseltamivir-zanamivir combination *versus* each monotherapy plus placebo [10]. Among enrolled adult subjects with seasonal influenza A infection (of whom 85% were tested positive for an A/H3N2 virus), the combination of oseltamivir and zanamivir was less effective than oseltamivir monotherapy and not significantly more effective than zanamivir monotherapy, at reducing nasal viral load and alleviating clinical symptoms. This highlights the importance of conducting careful and in-depth preclinical studies before embarking on human clinical trials of some of these influenza antiviral combination therapies.

New anti-influenza agents

The following table is not an exhaustive list of all investigational anti-influenza agents, but it summarizes many agents that are being developed, either in animal models or in human trials.

Category	Agent
Viral targets	
NAIs	Oseltamivir (IV) Zanamivir (IV) Peramivir (IV) A-315675 (oral)
Long-acting NAIs	Laninamivir (topical) Zanamivir dimers (topical)
Hemagglutinin inhibitors	Cyanovirin-N (topical) Sialylglycopolymer (topical) Peptide entry blocker (topical) FP Arbidol (oral)
Polymerase inhibitors	Ribavirin (oral, IV, inhaled) Favipiravir (oral) Viramidine (oral) siRNA (IV, topical)
Nucleoprotein inhibitors	Nucleozin
Nonstructural Protein 1 inhibitor	JJ3297
Antibodies	Anti-HA Anti-NA Anti-M2
Cellular targets	
Conjugated sialidase	DAS181 (topical)
Protease inhibitor	Aprotinin (topical)
Immunomodulators	IFN inducers RIG-I (5'PPP-RNA)
Cationic airway lining modulators	iCALM (topical)

All of the currently licensed anti-influenza agents and many of the agents that are under development target viral components as their chief mechanism of action. Among the investigational agents in the list above, IV oseltamivir, IV zanamivir, peramivir, laninamivir, favipiravir and DAS181 are in advanced stages of clinical development. Of these, peramivir is the furthest along the development track, having already been licensed for use in Japan and South Korea. Peramivir was also extensively utilized in the U.S. under an emergency use authorization during the 2009 pandemic. Peramivir is closely followed by laninamivir, which is under regulatory review and will soon complete its licensure process in Japan. Laninamivir is in phase 3 clinical trials in other parts of the world. Favipiravir and DAS181 are a new generation of influenza drugs with a mechanism of action different from the adamantanes and NAIs. Given their spectrum of activities, favipiravir and DAS181 will provide a new avenue of combating influenza strains that are resistant to contemporary classes of antivirals.

Perhaps the most important advantage of targeting signalling pathways is that no emergence of resistant variants has yet been detected under experimental conditions – a stark contrast to the ready development of oseltamivir-resistant influenza viruses when cultured serially under selective drug pressure.

One of the advantages of the IV NAIs is that they can provide high levels of drugs rapidly and reliably to seriously ill patients. The maximum plasma concentrations achieved by IV administration of zanamivir and peramivir was found to be over 50-fold higher than those of a double dose of oseltamivir administered orally. Whether this will translate to a higher threshold for the emergence of drug resistance, and greater antiviral effects and better clinical outcomes among severely ill patients awaits further examination.

Findings from some of the recent clinical trials on the effects of peramivir and laninamivir against uncomplicated and severe seasonal influenza disease in comparison to oseltamivir were encouraging. A single IV dose (300 mg or 600 mg) of peramivir was found to be superior to placebo and comparable to a five-day regimen of oseltamivir in adults with uncomplicated influenza. Similar results were also obtained among hospitalized adults with severe seasonal influenza disease where multiple doses (200 mg or 400 mg) of IV peramivir were comparable to oseltamivir in terms of its virologic and clinical effects. Nonetheless, peramivir was not superior to oseltamivir against seasonal variants with the H274Y mutation. This observation was consistent with findings from *in vitro* neuraminidase inhibition assays that the H274Y mutation responsible for oseltamivir resistance also conferred resistance to peramivir in the influenza virus variants. This begs the question whether peramivir would be an effective alternative to oseltamivir in treating infection by resistant strains. In the case of laninamivir, a single inhaled dose (20 mg or 40 mg) was comparable to a five-day course of oseltamivir in both adults and children with uncomplicated influenza. Even though laninamivir was superior to oseltamivir in treating children infected with the H274Y mutant virus variant, this finding was not reproduced among the adult subjects for unclear reasons.

Viruses are obligate intracellular parasites that must rely on the host machinery for replication and propagation. They must overcome multiple membrane barriers imposed by the structure of the host cell and interact with cellular components to proceed through their life cycle. Thus interfaces between the virus and the host cell present new opportune targets for antivirals to interrupt viral transmission, and this approach revolutionizes the current thinking in the field of anti-influenza agents. Dr. Stephan Ludwig from the Institute of Molecular Virology at Westfälische Wilhelms-Universität Münster in Germany gave an overview of new cellular targets for the development of anti-influenza agents.

To date, all investigational anti-influenza agents targeting cellular function can be generally divided into two categories: 1) modulators of immune responses, and 2) inhibitors of cellular factors or pathways that regulate the virus life cycle.

Immunomodulators can be further broken down into two separate arms. The first group consists of compounds that restore or induce interferon (IFN) response. Some investigational agents belonging to this group are ASN2, PS-341 (Velcade®, a drug that is approved for the treatment of multiple myeloma and for the adjunct treatment of relapsed mantle cell lymphoma) and protease-activated receptor-2 agonists. Low dose IFN regimen may also be a favourable prophylaxis option for influenza. The second group of immunomodulators abate the strong inflammatory response induced by highly pathogenic viruses, often comprising inhibitors of inflammatory cytokines. COX-2 inhibitors and existing immunomodulatory drugs, such as statins, glycyrrhizin, and glitazones, are some examples of this group.

Potential life lost [due to pH1N1] in the U.S. was estimated to be between 334,000 and 1,973,000 years, compared with 594,000 years of life lost for an average A/H3N2 season.

Aside from modulating immune responses of the host, cellular complements or pathways that are involved in the viral life cycle can also serve as targets for anti-influenza agents. While genome-wide screening techniques have allowed the identification of many candidate cellular factors that may take part in the influenza life cycle, it is unknown at this time how these cellular factors interact in the overall infection process. Therefore, selecting the correct and the most suitable host function from these screens as targets for the development of antiviral compounds continues to be a challenge. Nevertheless, Dr. Ludwig suggested that one should focus on membrane-crossing events during the influenza life cycle, because they are the prerequisites for subsequent viral interaction or interference with cellular functions. There are several barrier-crossing events during the influenza life cycle: entry, fusion with and release from endosomes, import of viral genetic material into the nucleus, export of the ribonucleoprotein (RNP) from the nucleus, and finally budding of the new viral particles from the infected cell. A number of cellular factors have been recognized as potential players in each of these steps and they may represent windows of opportunity for blocking viral replication. At the present time, however, DAS181 appears to be the most promising compound within its class and it has progressed the furthest along the development track. By removing sialic acid molecules (i.e. HA ligands) from the surface membrane, DAS181 prevents the adsorption of influenza viruses onto the host cell, thereby blocking viral entry at the very early stages of the influenza life cycle.

Signal transduction is a cascade of intracellular events whereby a stimulus generated from the binding of a ligand to a receptor on the cell surface is converted into a specific cellular response or a change in cell function. There is evidence that viral penetration of cellular membrane barriers is controlled by signalling cascades, hence cellular components that are part of the signalling transduction pathways may also be good targets for anti-influenza agents. One signalling protein complex that has received much attention is NF-κB. NF-κB is a transcriptional regulator of the cellular inflammatory response and is additionally a regulator of RNP export. Its dual role thus makes it a suitable target for anti-influenza drugs. Preliminary experiments in cultured cells have demonstrated that NF-κB inhibitors, such as SC75741, can efficiently block RNP export from the cell nucleus and replication of influenza viruses in the absence of cytotoxic side effects at therapeutic concentrations. The immunomodulatory effect of SC75741 was also confirmed in mice infected with the highly pathogenic influenza A/H5N1 virus. If left untreated, mice with H5N1 experienced severe disease, almost always resulting in death. Not only did administration of SC75741 dramatically improve survival among mice with H5N1, it also significantly reduced the expression of inflammatory cytokines, ameliorating the pathogenic effects of H5N1.

As Dr. Ludwig explained, there are many advantages to targeting signal transduction pathways as an antiviral approach. One advantage is its broad antiviral activity. Because signal transduction pathways likely underlie viral replication events common to many viruses and some pathways also regulate the host inflammatory response, a single drug may be effective against different viruses and

their pathogenesis. Perhaps the most important advantage of targeting signalling pathways is that no emergence of resistant variants has yet been detected under experimental conditions – a stark contrast to the ready development of oseltamivir-resistant influenza viruses when cultured serially under selective drug pressure.

A number of new and exciting developments are on the horizon for influenza therapy. Many antiviral agents targeting specific components of the influenza virus are in various stages of clinical testing. Anti-influenza agents targeting cellular factors form a new paradigm in the approaches to antivirals and have produced promising results in preclinical studies. Influenza therapies combining a number of different antivirals are synergistic, and they are effective even against resistant variants depending on the drug combinations. Inclusion of the newer antivirals in combination therapies would further expand treatment options for influenza.

The Public Health Vision

The unexpected arrival of the influenza A/H1N1 pandemic in spring 2009 and its subsequent rapid spread prompted countries around the globe to activate their pandemic preparedness plans for the first time. Preparedness significantly improved the 2009 response overall compared with past pandemics. Yet this pandemic revealed many gaps in our preparedness plans and it left behind a trail of unanswered questions. Looking ahead to tackle some of these issues, Dr. Michael Osterholm, Director of the Center of Infectious Disease Research and Policy (CIDRAP) at the University of Minnesota, presented his vision for future public health efforts on pandemic preparedness.

Describing and understanding influenza pandemics

First and foremost, public health authorities and stakeholders should have better tools for describing and understanding the severity of a pandemic. Echoing some of the concerns shared by Dr. Daniel Jernigan from the Centers for Disease Control and Prevention (CDC) [See *Purple Paper* Issue No. 20], Dr. Osterholm emphasized that severity assessment based on mortality figures alone did not reflect the full impact of the 2009 pandemic and is outdated. Considering CDC's mortality estimates in isolation, the current severity measurement system would suggest that the 2009 pandemic was mild because the number of pH1N1-associated deaths of 12,000 in the U.S. was substantially lower than the expected number of deaths of 47,800 for an average A/H3N2 influenza season. These figures, however, do not take into account that 90% of deaths occurred among people aged <65 years for the 2009 pandemic, whereas 90% of deaths were generally among those aged >65 years for a regular influenza season. Dr. Osterholm suggested that "a death from influenza in an 85-year-old individual with advanced Alzheimer's [disease] is not the same death in an otherwise healthy 26-year-old pregnant woman." He further commented that the reported pH1N1-associated deaths were underestimated, because for the first time in a modern pandemic we had access to intensive care medicine, extracorporeal membrane oxygenation (ECMO) and antivirals. He believed that the number of pH1N1 deaths in the U.S. could be at least a 20% underestimate had the same experience occurred in 1957 and 1968 before either intensive care medicine or antivirals were available.

The anti-science movement has led the Council of Europe to accuse pharmaceutical companies of influencing scientists and official agencies responsible for public health standards, including WHO and its advisors, to instigate a "fake pandemic" and to alarm governments worldwide into purchasing unnecessary stockpiles of vaccines.

Examining the severity of the 2009 pandemic from a perspective that accounts for the disparities in the affected age groups, potential life lost in the U.S. was estimated to be between 334,000 and 1,973,000 years, compared with 594,000 years of life lost for an average A/H3N2 season. The years of life lost in the U.S. for the 1918 pandemic was estimated to be 63,718,000 after adjustment to the 2000 population age structure [11]. Presenting the severity data in another way, Dr. Osterholm

compared the life expectancy and mean age of death for past influenza pandemics. The life expectancy in the U.S. in 1918 was 56.4 years and the mean age of death for the 1918 pandemic was 27.2 years; the difference in years was 29.2. The life expectancy in the U.S. in 2009 was 78.2 years and the mean age of death for the 2009 pandemic was 41.0 years; the difference in years was 37.2. The corresponding figures for the 1957 and 1968 pandemics were 4.5 and 8.1 years respectively. Each influenza pandemic was very different. Dr. Osterholm suggested that while the 1957 and 1968 pandemics likened to seasonal influenza years that had gone awry, both the 1918 and 2009 pandemics affected fundamentally different age populations and risk groups. He maintained that influenza is not simple, hence public health cannot respond with "one-size-fits-all" solutions.

Pandemic preparedness plans should be adequately flexible so that they can be adapted to specific features peculiar to a pandemic, matched with operational options.

Public health under attack

Public health is operating under the counter-efforts of the rapidly growing "anti-science" movement. Anti-science refers to ideological attacks on the teaching of evolutionary theory, global climate change, various medical and public health measures and other sciences. This is particularly true when there is conflict with political or religious pseudo-scientific positions. The anti-science position generally holds that in cases where science and ideology come into conflict, science itself must be flawed. Anti-science based efforts are often well-orchestrated and well-financed. They also focus on attacking the science as well as the individual researchers, practitioners and policy leaders. Some public health issues that have become controversial in recent years are vaccinations and their role in autism, raw milk, and radiation. In relation to the 2009 pandemic, the anti-science movement has led the Council of Europe to accuse pharmaceutical companies of influencing scientists and official agencies responsible for public health standards, including WHO and its advisors, to instigate a "fake pandemic" and to alarm governments worldwide into purchasing unnecessary stockpiles of vaccines. This conspiracy theory only got further escalated when health officials and experts came to the defence of WHO, such that affirmation of WHO's pandemic efforts, supported by scientific data, was futile and might even have been counter-productive. Dr. Margaret Chan welcomed openly the review of WHO's pandemic response; nonetheless, Dr. Osterholm was not optimistic that such a review could extinguish doubts and circulating conspiracy theories.

Influenza vaccine and its role in future pandemics

Influenza vaccines will continue to be a mainstay of public health measures to prevent and control the spread of influenza viruses during a pandemic. Dr. Osterholm reverberated many of the concerns related to influenza vaccine efficacy and effectiveness, availability and perceived safety, shared by Drs. Cox and Wood. He also stressed the importance of investing in better adjuvant technologies as discussed by Dr. Osterhaus. To advance the influenza vaccine agenda, Dr. Osterholm urged influenza research and public health communities to work together to confront issues with regard to the relative lack of efficacy and effectiveness data, the urgent need for a game-changing approach to influenza vaccine production, and the anti-science movement against the use of influenza vaccines.

Mathematical modelling as a tool

Mathematical modelling can be a useful adjunct to the public health armamentarium. However, Dr. Osterholm cautioned that models must be based on credible data sources and realistic assumptions to have relevance and applicability.

Referring to a presentation at the *Options VII* conference by Dr. Angus Nicoll from European Centre for Disease Prevention and Control, mathematical models, in simple terms, can explain complex dynamics, quantify uncertainties, generate and sometimes even test hypotheses, but they always need to be validated. Policy-makers should be educated to understand the limitations of models and should challenge the thinking and assumptions of modellers. On the other hand,

modellers should decide and take a stance on when it is inappropriate to provide estimates because a certain situation is plagued with too much uncertainty. In essence, to be of value to public health pandemic preparedness planning and response, information generated by modelling should be linked to action. As such, pandemic preparedness plans should be adequately flexible so that they can be adapted to specific features peculiar to a pandemic, matched with operational options. This is information that can be generated through modelling as a pandemic unravels.

The next pandemic landfall

In the wake of the 2009 pandemic, Dr. Osterholm warned that public health authorities should not let down their guard on preparedness efforts. There is no telling when the next pandemic would come. With the highly pathogenic influenza A/H5N1 virus still circulating in parts of the world, the threat of another pandemic in the near future is tangible. In fact, it was just reported that the H5N1 virus had resurfaced in Hong Kong's human population after a 7-year absence [12]. Hong Kong was the place of origin of the first reported H5N1 human cases in 1997. Whether H5N1 or another influenza virus strain would be the causal agent for the next pandemic is not known, but we must have the same urgency today about the next pandemic as we had in 2008. To be better prepared next time around, we need improved methodologies for understanding and describing the severity of a pandemic, novel approaches to dealing with anti-science sentiments, innovative influenza vaccines and vaccine production technologies, and a new framework for guiding the use of mathematical modelling in public health decision-making.

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