Published in final edited form as:

Future Virol. 2015 ; 10(9): 1033–1047. doi:10.2217/fvl.15.69.

Refining the approach to vaccines against influenza A viruses with pandemic potential

Rita Czako¹ and Kanta Subbarao^{*,1}

¹Laboratory of Infectious Diseases, NIAID, NIH, Bethesda, MD, USA

Abstract

Vaccination is the most effective strategy for prevention and control of influenza. Timely production and deployment of seasonal influenza vaccines is based on an understanding of the epidemiology of influenza and on global disease and virologic surveillance. Experience with seasonal influenza vaccines guided the initial development of pandemic influenza vaccines. A large investment in pandemic influenza vaccines in the last decade has resulted in much progress and a body of information that can now be applied to refine the established paradigm. Critical and complementary considerations for pandemic influenza vaccines include improved assessment of the pandemic potential of animal influenza viruses, proactive development and deployment of pandemic influenza vaccines, and application of novel platforms and strategies for vaccine production and administration.

Keywords

antigenic mismatch; avian influenza; pandemic influenza; prepandemic vaccine; prime boost

Influenza viruses are enveloped viruses belonging to the *Orthomyxoviridae* family. Influenza viruses are grouped into three types: A, B and C. Of these, influenza A and B viruses are responsible for epidemic human disease. Influenza A viruses are further divided into subtypes distinguished by antigenic properties of the viral surface proteins: hemagglutinin (HA) and neuraminidase (NA). These proteins are critical for entry into host cells and for release of mature, infectious progeny virus and are the main targets of the human immune response [1]. To date, 16 subtypes of HA and 9 subtypes of NA have been isolated from waterfowl and shorebirds, the natural hosts of influenza A viruses [2]. In addition, sequences of two novel influenza-like viruses have been identified in bats and classified as two novel subtypes: H17N10 and H18N11 [3,4]. A segmented RNA genome, error-prone RNA polymerase and the ability to infect many different species contribute to the substantial diversity of influenza A viruses in nature.

* Author for correspondence: Tel.: +1 301 451 3839; Fax: +1 301 480 4749; ksubbarao@niaid.nih.gov.

For reprint orders, please contact: reprints@futuremedicine.com

Financial & competing interests disclosure

The authors are supported by funding from the Intramural Research Program of the NIH and NIAID. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Two influenza A subtypes, H1N1 and H3N2, currently co-circulate with influenza B viruses in humans. Vaccination is the most effective strategy for prevention and control of influenza and its associated morbidity and mortality [5]. Strain selection, manufacture and deployment of seasonal influenza vaccines for the control of these viruses have become a routine component of national health programs in many countries. Antigenic drift in the HA protein necessitates annual reformulation of seasonal vaccines to maximize vaccine efficacy. Prediction of the influenza variants that will dominate a given influenza season is a challenging task that is based on global surveillance of circulating influenza viruses [6]. The lead time of this 'reactive' approach to control of seasonal influenza is several months. Between 1999 and 2009, four seasonal vaccine formulations selected for implementation in the northern hemisphere failed to adequately match the epidemic strain because a new antigenic variant emerged after the vaccine strain composition decision was made [7]. Mismatch events have occurred at a similar frequency in the southern hemisphere in recent years [8].

The assessment of the pandemic potential of animal influenza viruses is a complex task. There have been four pandemics of influenza in the last century. In addition, interpandemic periods have been punctuated by occasional epidemics caused by viruses with unusual properties, for example, enhanced pathogenicity or transmission in certain subgroups of the population [9]. Furthermore, several avian influenza viruses (AIV) have caused sporadic zoonotic infections in humans [10]. Although human-to-human transmission of these zoonotic viruses has not been efficient, their potential to acquire this property renders them a pandemic threat.

The public health response to the 2009 H1N1 pandemic (H1N1pdm) was rapid and included the development and deployment of monovalent H1N1pdm vaccines. However, production and distribution were not rapid enough to prevent the second wave of the pandemic [11]. Effective control of pandemic influenza may therefore require a different philosophical approach than the established paradigm for control of seasonal influenza viruses. A proactive pandemic vaccination strategy will rely on three critical elements: timely identification of viruses with pandemic potential, proactive development and characterization of vaccines, and development of improved vaccines. This article summarizes new developments and open questions in each of these areas.

Improved identification of avian influenza viruses with pandemic potential

Influenza pandemics occur when novel influenza viruses are introduced into susceptible human populations. When such a virus is capable of efficient human-to-human transmission, lack of pre-existing immunity facilitates rapid spread. Novel influenza viruses may be introduced into humans via reassortment between animal and human viruses, as in the case of the 1957 and 1968 pandemic viruses, or via direct zoonotic transmission, as in the case of the 1918 and 2009 H1N1pdm viruses [12,13]. As the natural hosts of influenza viruses, aquatic birds are the source of novel influenza viruses. Influenza viruses have also become enzootic to domesticated animals, most notably poultry and swine [14]. Molecular analysis of the four known pandemic influenza viruses (1918 H1N1, 1957 H2N2, 1968 H3N2 and 2009 H1N1) has revealed the contribution of AIV genes to pandemic viruses [12,13,15–17].

The continued rise in the number of animal and human cases of avian H5 and H7N9 infections and sporadic cases of infection with H6, H9 and H10 subtype viruses are a direct call to action for improved understanding of the public health threat posed by AIV [10].

Standardized tools for assessment of pandemic potential

Virologic surveillance in livestock, poultry and wild birds is a critical first step in cataloging circulating AIV, but tools for assessing their pandemic potential are needed to guide preparedness efforts. The development of standardized and objective criteria for pandemic risk assessment has been the subject of much debate. In 2012, the US CDC proposed a standardized influenza risk assessment tool (IRAT) for evaluating the pandemic potential of influenza viruses not currently circulating in humans [18,19]. This framework is based on ten key elements, including a spectrum of factors representing molecular and biological attributes of the virus, attributes of the population and the epidemiology of the virus, which are agreed upon by a panel of experts and weighted to reflect their relative importance. However, the IRAT framework must be updated as new understanding of the molecular determinants of transmission and pathogenesis of AIV emerges. One example is the multibasic cleavage site seen in some H5 and H7 avian influenza HAs that distinguishes highly pathogenic from low pathogenic AIV in poultry and is a virulence motif in mammalian models of influenza [20–22]. Another example is the recent identification of the minimum set of adaptive mutations necessary for airborne transmission of avian H5N1 viruses among ferrets [23,24]. These studies confirmed the importance of receptor binding specificity (an existing IRAT element) and polymerase activity, but also identified the acid stability of HA as a novel factor influencing transmissibility.

Some AIV defy simple classification with regard to pandemic risk assessment. For example, avian H9N2 viruses have been associated with only a handful of mild human infections and may be classified as having only intermediate pandemic risk. However, H7N9, H5N1, H6N1 and H10N8 AIV that have caused severe human disease derived their internal protein genes from H9N2 viruses [25–29]. In another example of such gene constellation effects, an H3N2 variant (H3N2v) virus that was the product of reassortment between circulating swine influenza viruses and the H1N1pdm virus caused mild disease in children in the USA between 2011 and 2012 and has only been detected sporadically in humans since 2012 [30]. Finally, virulence in humans is difficult to predict; for example, zoonotically transmitted avian H7N9 viruses cause no apparent disease in wild birds and poultry, but cause severe respiratory disease in humans [31–33]. Similarly, precursors of the H1N1pdm virus circulated for over a decade without causing apparent disease in swine, yet transmission to humans resulted in the first influenza pandemic of the 21st century [34].

AIV continue to evolve in nature and their diversity presents a major challenge in deciding which strains and subtypes should be targeted for vaccine development. The consequences, for humans, of antigenic drift in animal species are not well understood. Veterinary vaccination is a powerful tool that has been used to control AIV outbreaks in poultry [14]. However, large-scale vaccination of livestock is not affordable in some parts of the world. Furthermore, vaccination may confound serological surveillance efforts for early detection of AIV in domestic poultry unless the vaccine design and serologic detection assay are

directed against different viral proteins. This strategy is referred to as a Differentiating Infected from Vaccinated Animals (DIVA) approach [35]. However, the growing availability of AIV sequence data and increasingly widespread laboratory capacity for molecular surveillance has the potential to abrogate this problem. Finally, suboptimal vaccination via poor vaccination coverage or use of mismatched vaccines may result in nonsterilizing immunity, where morbidity and mortality in poultry are reduced but viral replication still occurs. This may drive viral escape and promote endemicity of escape variants. In this manner, suboptimal vaccination may drive antigenic drift and provide more opportunities for reassortment with human viruses, thereby potentially increasing the chances of zoonotic transmission [14,36–38].

Proactive development & deployment of pandemic influenza vaccines

Mathematical modeling of the spread of a pandemic influenza virus suggests that intervention during the 2 weeks following initial recognition of a pandemic has the greatest potential for an effective response in terms of human morbidity and economic cost [39]. The production of licensed seasonal influenza vaccines takes 5–7 months [40]. There are several opportunities in the existing framework for influenza vaccine production for an accelerated response, which will be a critical consideration in the event of a pandemic (Figure 1). First, preclinical and clinical evaluation of the safety and immunogenicity of candidate pandemic influenza vaccines during the interpandemic period can enhance pandemic preparedness. Second, the creation of a library of vaccine seed stocks may substantially reduce the lead time for vaccine production [41,42]. Third, new technologies such as reverse genetics and synthetic biology can facilitate accelerated production of vaccine viruses [43]. Fourth, deployment of a vaccine prior to a pandemic (prepandemic vaccination) has been proposed to circumvent the delays associated with distribution of a new vaccine in the midst of a pandemic [44]. Such proactive approaches are not without precedent. Several H5N1 vaccines have been licensed in the USA and Europe; stockpiling of these vaccines is now a component of the WHO Pandemic Influenza Preparedness framework [45]. However, the safety and immunogenicity of vaccines against other subtypes would have to be established in clinical trials.

Is an exact match between vaccine virus & pandemic virus necessary?

Experience with seasonal influenza vaccination has demonstrated that antigenic drift, or the gradual accumulation of antigenic changes in the influenza virus surface glycoproteins, has a profound effect on vaccine efficacy [46]. The closer the antigenic match between vaccine strain and epidemic strain, the greater the efficacy of the vaccine. It is important to note that antigenic differences are determined using post-infection ferret antisera and there is increasing evidence that an individual's immune history is an important determinant of the specificity of their antibody response. Therefore, the data from ferrets following primary infection may not accurately reflect what would happen in influenza-experienced humans [47–49].

A recent meta-analysis of vaccine efficacy studies estimated that mismatched vaccines can protect against laboratory-confirmed influenza with a vaccine efficacy of 60% (95% CI: 44–71) in the case of live-attenuated influenza vaccines (LAIV) and 56% (95% CI: 43–66) in

the case of trivalent inactivated influenza vaccine (IIV) compared with no vaccine [46]. Seasonal LAIV administered to children was superior to trivalent IIV in its ability to protect against drifted viruses not contained in the vaccine [50–52]. In contrast, the efficacy of seasonal IIV was equal or superior to LAIV in adults, possibly because adults were immunologically primed by previous influenza infections and vaccine [53,54]. However, neither LAIV nor IIV were efficacious against the mismatched circulating H3N2 virus in the 2014–2015 season [55]. Because seasonal influenza vaccines provide variable protection against drift variants, their composition is modified after new antigenic variants with epidemic potential are identified.

Antigenic drift will pose a challenge for novel antigens as well. Phylogenetic analysis of avian H5N1 HAs reveals diversification into multiple distinct clades [56] and similar diversity may occur in other AIV subtypes. In the past 3 years, eight new clades of H5N1 viruses, including fifth order subclades, have emerged [57]; it is not clear whether all clades pose equivalent pandemic risk. In the event of a pandemic, an H5 vaccine from a different clade may not prevent infection but may still prevent severe disease and death. It has been estimated that a pandemic vaccine with efficacy as low as 30% would have a substantial impact on limiting morbidity and mortality and would be more cost effective than a reactive pandemic response [58]. Furthermore, recent characterization of pandemic influenza vaccines and advances in immunology suggest that an exact match between vaccine strain and pandemic strain may not be necessary. These include the discovery of conserved epitopes in the influenza HA, an independent contribution of T-cell-mediated immunity to cross-protective immunity, empirical evidence from Phase I clinical trials of cross-reactive serum antibody responses elicited by pandemic vaccine candidates and improvements in vaccine formulations.

Conserved epitopes can elicit cross-protective immunity—The identification of a conserved epitope on the HA stalk that is the target of neutralizing antibodies supports the concept that a mismatched vaccine may provide some cross-protection [59,60]. Stalk-reactive antibodies have been detected in the sera of individuals following infection with H1N1pdm or immunization with seasonal influenza vaccine [59,61–63] or an adjuvanted H5N1 split virion vaccine [64].

Cell-mediated immunity elicited against conserved T-cell epitopes can also independently contribute to cross-protective immunity. Although cell-mediated immunity cannot prevent infection, T-cell responses enhance viral clearance and can protect from severe disease and death in preclinical models [65]. Syngeneic T-cell transfer and T-cell depletion studies in vaccinated or influenza virus-infected mice established a role for cell-mediated cross-protection from lethal challenge with heterologous influenza viruses [66–69]. In a human challenge study with an H1N1 virus, the presence of pre-existing cross-reactive influenza-specific CD4⁺ T cells correlated with protection from severe illness and decreased shedding [70]. In the 2009 pandemic, the presence of cross-reactive CD8⁺ T cells correlated with protection from influenza virus infection [71]. H1N1pdm and H5N1 vaccines elicited influenza-specific T cells with cross-clade and heterosubtypic reactivity [72,73]. Furthermore, a T-cell response to conserved epitopes in influenza proteins, such as the nucleoprotein, was associated with protection from severe illness [74].

Pandemic influenza vaccines can elicit cross-protective antibody responses—

Pandemic influenza vaccine candidates have been developed on several platforms and have been found to elicit cross-clade immunity in pre-clinical models and clinical studies.

Inactivated and live attenuated H5N1 vaccines were highly effective in ferrets and mice against lethal challenge with homologous and heterologous H5N1 viruses from different clades [75,76]. Similarly, live-attenuated vaccines against H2, H3, H6, H7 and H9 viruses elicited subtype-specific cross-reactive serum antibodies and reduced the replication of homologous and heterologous challenge viruses in mice or ferrets [77–82]. An inactivated monovalent H7N7 vaccine candidate conferred protection from lethal challenge of mice with H7N9 influenza virus [83].

Pandemic influenza vaccines also induce antibody responses against heterologous viruses in humans. An adjuvanted subunit vaccine containing the HA of an avian H5N3 virus elicited serum neutralizing antibody responses against several H5N1 clinical isolates collected over a 7-year period [84]. Adjuvanted inactivated whole virion and split virion H5N1 vaccines elicited neutralizing antibodies against homologous virus and drifted H5N1 viruses representing distinct clades in children, adults and the elderly [85–88]. Furthermore, H1N1pdm vaccine elicited H5N1-reactive memory B-cell responses in the upper respiratory mucosa [89].

Novel formulations & platforms can enhance cross-protective immunity—

Enhancement of cross-protective responses is an ongoing challenge and can be seen as an important step in the development of universal influenza vaccines. Recent studies directly comparing different formulations of pandemic influenza vaccines have demonstrated that the choice of vaccine platform and inclusion of adjuvant can influence the breadth and quality of the immune response [90,91]. Improved vaccine formulations have the potential to elicit protection against a greater breadth of influenza virus subtypes [90,92]. Novel formulations may also elicit protective immunity by other mechanisms, such as antibody-dependent cellular cytotoxicity (ADCC) mediated by non-neutralizing antibodies [93]. As previously stated, there is growing evidence that exposure and immune history can also influence the immune response to influenza vaccines. Serological studies suggest that previous exposure does not constrain B-cell responses to novel antigens in the context of seasonal influenza vaccination [94]. In fact, it has been suggested that administration of a novel antigen preferentially elicits antibodies to conserved epitopes that are shared with existing seasonal influenza viruses [95]; the H1N1pdm vaccine elicited antibodies with broad cross-reactivity in adults [96].

Are pandemic influenza vaccines safe & immunogenic?

Safety—The manufacture of pandemic influenza vaccines poses some unique safety concerns. Manufacturing protocols must be carefully designed and implemented to reduce the risk of exposure to novel viruses. Two strategies proposed to mitigate this risk are the use of less pathogenic viruses of the same subtype and the production of vaccine using reverse genetics-derived viruses bearing a recombinant HA from which known pathogenicity determinants have been removed [97]. Pandemic LAIV (pLAIV) have a number of theoretical advantages over IIVs, including a superior ability to elicit mucosal

and cellular immune responses, needle-free administration and greater yield. Clinical studies in controlled settings have demonstrated that replication of pLAIV candidates was significantly restricted in healthy adults; vaccine virus was shed only at low titer from a subset of vaccine recipients [98–100]. However, because they contain replicating, albeit attenuated virus, there is a possibility that pLAIV virus could reassort with circulating influenza viruses; the outcome of such an event may be a virus with a novel HA and potential for spread in the community [101]. Therefore, pLAIV would only be used when recommended by public health officials and likely only when a pandemic is inevitable. However, proactive generation and evaluation of a library of pLAIV against a range of HA subtypes will allow rapid production of vaccine if needed.

Postmarketing surveillance following the mass vaccination efforts during the 2009 H1N1 pandemic demonstrated that unadjuvanted H1N1pdm vaccines licensed in North America and Europe had comparable safety profiles to seasonal influenza vaccines [102,103]. Several adjuvanted IIV formulations marketed in Europe were also found to be safe. Similarly, pLAIV have demonstrated acceptable safety profiles in small Phase I trials. pLAIVs carrying the HA and NA proteins of avian H5N1, H7N3 and H9N2 viruses elicited only mild, local reactogenicity that was comparable to seasonal LAIVs [98–100,104]. Inactivated H5N1, H7N1, H7N7 and H9N2 vaccines were also demonstrated to be safe in Phase I clinical trials [84,91,105–109]. Together, these data suggest that administration of novel influenza virus vaccines to unprimed hosts is generally safe and well tolerated.

However, rare and unexpected adverse events may not be seen until vaccines are administered to a large population. One formulation of adjuvanted, split-virion, inactivated H1N1pdm vaccine marketed in Europe was associated with an increased incidence of narcolepsy in children and young adults [110–117]. Interestingly, a different formulation of AS03-adjuvanted monovalent H1N1pdm vaccine [118,119] and other monovalent H1N1pdm vaccines were not associated with narcolepsy [102,120,121]. It is possible that differences in antigen processing or adjuvant formulation contributed to the observed differences [119,122]. Host factors could have also influenced the safety outcomes observed with AS03-adjuvanted vaccines; narcolepsy has been associated with genetic loci that influence antigen presentation and T-cell differentiation. Age was also identified as a risk factor for the development of narcolepsy following exposure to H1N1pdm via either immunization or infection [123]. The complexity of the narcolepsy-pandemic influenza issue was recently reviewed [124]; further studies will be necessary to clarify these issues.

Effective control of a pandemic by vaccination will require the rapid development, evaluation and deployment of safe and efficacious products. Novel vaccine targets, platforms and adjuvant formulations offer great promise, but do not have the benefit of the decades of clinical experience of licensed vaccines. Postmarketing surveillance for adverse events provides critical safety data.

Immunogenicity—A clear understanding of the determinants of a protective immune response facilitates the rational development and evaluation of influenza vaccines. The immunogenicity of candidate pandemic influenza vaccines is currently evaluated according to criteria developed by the US FDA and the Committee for Medicinal Products for Human

Use in Europe. The Committee for Medicinal Products for Human Use guidelines recommend that candidate vaccines elicit a serum hemagglutination inhibition (HAI) titer of 1:40 in 70% of vaccinated individuals [125]. Although it is clear that serum antibody correlates with protection from influenza, sole reliance on serum HAI titers for the evaluation of vaccines is problematic for a number of reasons. First, this correlate is only relevant for influenza vaccines that contain the globular head of the HA protein, which mediates hemagglutination activity. HAI assays fail to detect potentially functional antibodies that bind HA stem epitopes or other viral proteins. In addition, serum antibody titers ignore the contributions of cell-mediated immunity, which is required for clearance of virus, and innate immune responses, which have been proposed to play a role in influenza pathogenicity. Finally, the HAI titer is not a robust correlate of protection for LAIVs [126,127].

It is not known whether the licensing criteria applied to seasonal influenza vaccines are appropriate for pandemic influenza vaccines. Different criteria may be appropriate for different populations [128]. Furthermore, there is no known correlate for immunologic priming; this would be important for novel pandemic vaccines, which may not meet the classical criteria of HAI for immunogenicity, but prime for robust responses following administration of a booster vaccine. A critical research priority is therefore the identification and validation of additional correlates of protection for influenza. An attractive approach for the identification of potential correlates is a large-scale, high-throughput analysis of antibody repertoires. Convalescent serum from survivors of AIV infection could be used to probe a library of peptides representing the viral genome (antigenic fingerprinting) in order to identify key viral antigenic epitopes [129]. Furthermore, the development and application of ADCC [93] and cellular immunity assays will also enhance understanding of vaccine-mediated protection.

The H1N1pdm LAIV vaccines developed in the USA and Russia elicited comparable serologic responses to seasonal LAIVs. In Phase I/II clinical trials in the USA, monovalent H1N1pdm LAIV elicited seroconversion rates of 14.9% in adults aged 18–49 after two doses of vaccine [130]. Phase I/II clinical trials conducted with the Russian formulation of monovalent H1N1pdm LAIV elicited similar serum HAI antibody responses. A modest increase in the frequency of virus-specific CD4⁺ and CD8⁺ memory T cells was reported in the peripheral circulation of healthy adults [131]. With both formulations, immunogenicity was slightly higher in children below 18 years of age compared with adults. Several adjuvanted and unadjuvanted IIV were also characterized in the USA and Europe and found to be robustly immunogenic. A single dose of unadjuvanted H1N1pdm IIV was immunogenic in all age groups over 3 years of age. Inclusion of an adjuvant in H1N1pdm IIV formulations permitted dose sparing; the equivalent of a quarter dose of HA antigen was comparable in immunogenicity to unadjuvanted formulations [11].

pLAIV for H5, H7 and H9 AIV have shown promise in preclinical studies. The first target of a pLAIV was an H9N2 virus. The vaccine was immunogenic in healthy, seronegative adults; two doses elicited seroconversion in >90% of vaccine recipients [98]. Similarly, LAIVs on two different backbones targeting H7N3 and H5N2 AIV were immunogenic in healthy seronegative adults, eliciting seroconversion rates of >45% in those who received

two doses of vaccine [100,132]. These Phase I clinical trials suggest that pLAIV-bearing novel antigens derived from AIV can elicit serum antibody responses. However, all pLAIV were not equally immunogenic. Two H5N1 pLAIV representing distinct viruses, which had demonstrated great promise in pre-clinical studies, failed to elicit robust serum neutralizing antibody responses in Phase I clinical trials [99]. Recombinant H5 HA and unadjuvanted H5N1 IIV were also poorly immunogenic in humans [133,134]. Notably, however, both LAIV and recombinant HA vaccines primed for a robust response to subsequent IIV [135,136].

The way forward: improved vaccine formulations & platforms

Novel vaccine formulations and combinations of existing vaccine platforms offer great promise for enhanced immunogenicity and expanded breadth of immunity, critical characteristics of an ideal pandemic influenza vaccine.

‘Second generation’ prime-boost strategies

Mass vaccination in response to the ‘swine flu’ outbreak of H1N1 in 1976 provided a clear demonstration of the impact of priming on the immunogenicity of a novel vaccine. A single dose of split or subunit vaccine was immunogenic among older individuals, who had been primed by previous exposure to related H1N1 viruses during their lifetime. However, a higher dose of antigen (administered either as additional doses of vaccine or a single dose of vaccine formulated with more antigen) was necessary to elicit comparable responses in immunologically naive, younger individuals [137–141].

Prime-boost schedules for pandemic vaccines have been evaluated in numerous clinical studies. Two doses of a live attenuated H9N2 prototype vaccine were highly immunogenic in seronegative individuals [98], but this was not typical for other candidate pLAIV [101]. However, the combination of different vaccine platforms demonstrates promise for improving the immunogenicity of prototype pandemic vaccines. This has been illustrated clearly in the evaluation of H5N1 pandemic vaccines. Sequential use of inactivated vaccine following priming with DNA [142,143], recombinant HA [134,136], vectored HA [144], LAIV [135] or inactivated H5N1 vaccine [145–149] elicited robust neutralizing antibody responses. Booster vaccination with an H5N1 IIV could rapidly elicit serum antibody responses, even when the interval between primary and secondary vaccination was >5 years [135,136]. Similar results were found with H7N7 influenza vaccines with an interval of 18–22 months between pLAIV and pIIV [150].

Furthermore, these prime-boost strategies elicited cross-reactive serum antibodies. Primary vaccination with pLAIV, DNA or vectored H5N1 vaccine followed by a homologous H5N1 IIV elicited antibodies with cross-clade neutralization activity [135,142–144,150]. Similarly, a booster dose of H5N3-inactivated vaccine delivered 16 months after primary vaccination elicited cross-clade antibody responses [151,152]. Sequential vaccination with antigenically mismatched vaccines also elicited serum antibody with cross-clade neutralizing activity. In individuals primed with an H5N3-inactivated vaccine, boosting with an inactivated H5N1 vaccine elicited high titers of cross-clade neutralizing serum antibody within 7 days [153]. Administration of a recombinant, baculovirus-expressed HA subunit vaccine primed for

robust cross-clade recall responses upon boosting with an antigenically distinct H5N1 inactivated subvirion vaccine up to 8 years later [134,136].

Finally, sequential administration of antigenically mismatched vaccines may be used to modulate antibody responses to the HA stalk. It has been suggested that sequential administration of antigenically distinct HA antigens can selectively elicit a more robust antibody response to the more conserved, but less immunodominant, HA stalk or stem [96]. These 'stem antibodies' often have potent and broad cross-neutralization activity, but are poorly elicited by vaccination with monovalent inactivated vaccines [154]. In contrast, priming with pLAIV or DNA vaccines prior to vaccination with pandemic IIV efficiently elicited high affinity stem antibodies [142,155,156].

These studies suggest that a proactive prime-boost approach to pandemic preparedness should be considered. A booster vaccine could be administered to a vaccine-primed population when a pandemic threat materializes. Prepandemic vaccine priming could potentially be coupled with seasonal influenza vaccination. It has been suggested that while concurrent administration of H1N1pdm and seasonal trivalent IIV did not negatively affect the safety or immunogenicity of either vaccine, sequential administration could slightly attenuate the immunogenicity of inactivated H1N1pdm vaccine [157,158]. The underlying immunological mechanism for this, and whether it would apply to pandemic vaccines containing completely novel HA subtypes, is not known. However, the results of these studies, as well as the Phase I/II evaluations of prototype pandemic vaccines described above, suggest that robust serum antibody responses are achievable despite prior seasonal influenza vaccination. Prime-boost strategies for delivery of pandemic influenza vaccines show promise even in older age groups, in which effectiveness of seasonal influenza vaccines is suboptimal. At least two formulations of inactivated H5N1 vaccines have been shown to prime for a rapid and robust serum-neutralizing antibody response to heterologous inactivated booster vaccine in elderly cohorts [86,159].

New production methods & formulations

Production of an adequate supply of vaccine in a short period of time is a major challenge for a number of reasons, including the need for facilities that meet good manufacturing practice (GMP) standards, a dependence on eggs for production and the lead time required to generate vaccines on existing platforms. The first step is the selection and characterization of a 'vaccine seed virus.' This is amplified in embryonated eggs or cell culture to produce antigen. Dependence on chicken eggs for amplification is a critical weakness if the seed virus is pathogenic to and/or grows poorly in eggs. Finally, the vaccine must be formulated, purified and prepared for distribution. Egg-based amplification of vaccine seed stocks can be improved by introduction of mutations that enhance amplification in embryonated eggs without compromising antigenicity [160]. Indeed, there is growing evidence that some of these substitutions, particularly those that influence the stability of the HA, can enhance the immunogenicity of live attenuated avian influenza candidate vaccines in preclinical models [161]. However, adaptive mutations introduced through amplification of influenza viruses in eggs or mammalian cell lines may impair the immunogenicity of the vaccine; therefore thorough characterization of vaccine seed stocks is necessary [162].

Novel platforms that are not dependent on infectious virus can circumvent the need for egg-based amplification and mitigate risk to humans during vaccine production. Proof of principle for cell-based amplification of virus for LAIV and IIV has been established. A vaccine for seasonal influenza produced in a mammalian cell line, Flucelvax[®], was licensed by the FDA in 2014 [5]. Mammalian cell culture derived H5N1 vaccines have also been shown to be well-tolerated and immunogenic [163–166]. Other candidates, including an H7N9 vaccine, show promise in preclinical models [167]. These vaccines are likely to be safe for individuals who are allergic to eggs. Furthermore, recombinant influenza virus proteins have been expressed in a variety of cell lines, including insect cells and plant cells, as soluble protein or noninfectious virus-like particles. This approach offers the opportunity to modulate immune responses by genetic engineering of the influenza virus antigens.

DNA vaccines are another alternative to traditional influenza vaccine platforms; they are delivered subcutaneously or intramuscularly and rely on host cells at the injection site to express and present viral proteins to the immune system [168]. DNA vaccines can be produced in large quantities and are very stable, facilitating the stockpiling of such vaccines. Alternatively, vaccine technologies that deliver viral antigens via replication-defective vectors such as adeno-virus or modified vaccinia virus Ankara have been demonstrated to be safe and immunogenic in humans [144,169]. These platforms share the advantages of a pLAIV in that they can stimulate robust cell-mediated and antibody-mediated immune responses without the risk of introduction of novel pandemic influenza genes into seasonal influenza viruses. Influenza vaccine candidates using other vectors, including adeno-associated viruses [170], alphaviruses [171], Newcastle disease virus [172] and vesicular stomatitis virus [173] demonstrate promise for delivery and expression of influenza antigens in a variety of preclinical models, but have not yet been evaluated in humans.

Finally, inclusion of adjuvants in vaccine formulations has great potential to enhance the magnitude of serum antibody responses, facilitate the stimulation of mucosal and cell-mediated immunity, and to enhance the longevity of serum antibody responses following vaccination [174]. Inclusion of MF59 in subunit H5N1 vaccine formulations enhanced the quality and breadth of the serum antibody responses [92]. Furthermore, inclusion of adjuvant permits dose sparing, where a lower antigen dose can elicit a response sufficient to meet immunogenicity criteria, thereby increasing the number of doses of available vaccine in the event of a pandemic. Two formulations of pandemic vaccine that were deployed in efforts to control the 2009 H1N1 pandemic were adjuvanted with AS03, an oil-in-water emulsion, and were found to permit dose sparing and to enhance the magnitude and persistence of serum HAI titers relative to those elicited by unadjuvanted vaccine. However, the association with narcolepsy in some countries illustrates the safety and regulatory challenges of novel vaccine formulations and platforms for pandemic influenza vaccines.

Conclusion

A proactive approach to pandemic influenza vaccination requires international collaboration and a shared commitment to surveillance, research and characterization of pandemic vaccines. The foundation has already been laid for such an approach and much progress has been made over the past decade. The establishment of a formal pandemic influenza pre-

paredness plan in the 1970s illustrates a shift toward proactive pandemic preparedness in the USA. This plan called for increased support for influenza research, established a framework for interagency collaboration, and recommended increased surveillance of influenza viruses in animal populations and has since undergone periodic refinement [175]. An increasing recognition of the fundamental overlap between veterinary and human public health is embodied in the 'One Health' initiative, formally launched in 2011, and is illustrated by a growing number of countries with influenza surveillance programs in animal populations [176]. International collaboration of experts has resulted in the refinement of pandemic preparedness plans; notable among these is the WHO's Pandemic Influenza Preparedness framework, which has provided a framework for sharing of influenza virus sequence data and access to diagnostic tests and vaccines since 2011 [45]. New tools for pandemic risk assessment and novel platforms and strategies for vaccination are paving the way for improved pandemic preparedness.

Future perspective

In the next 5–10 years, it is likely that AIV that are already enzootic in Asia will become established in avian species in other parts of the world. This will prompt a re-examination of agricultural practices, a greater emphasis on biosecurity and development of vaccines for veterinary and human use. In the near term, discussions will focus on the pros and cons of novel vaccine formulations, especially adjuvanted and vectored vaccines, and schedules with sequential use of vaccines based on different platforms. In the longer term, newer technologies and approaches to develop vaccines that induce broader immunity will progress, with the ultimate goal of a universal influenza vaccine. However, the regulatory path to licensure of vaccines that do not mediate protection through induction of antibodies to the HA head is not yet clear. Successful implementation of novel or, ultimately, universal influenza vaccines will require the development of assays to measure correlates of protection for such vaccines, with parallel advances in regulatory science.

References

1. Wright, PF.; Neumann, G.; Kawaoka, Y. Orthomyxoviruses. In: David, M.; Knipe, PMH., editors. *Fields Virology*. Lippincott Williams & Wilkins; PA, USA: 2013. p. 1186–1243.
2. Yoon S-W, Webby RJ, Webster RG. Evolution and ecology of influenza A viruses. *Curr Top Microbiol Immunol*. 2014; 385:359–375. [PubMed: 24990620]
3. Tong S, Li Y, Rivailler P, et al. A distinct lineage of influenza A virus from bats. *Proc Natl Acad Sci USA*. 2012; 109(11):4269–4274. [PubMed: 22371588]
4. Tong S, Zhu X, Li Y, et al. New world bats harbor diverse influenza A viruses. *PLoS Pathog*. 2013; 9(10):e1003657–e1003657. [PubMed: 24130481]
5. Grohskopf LA, Olsen SJ, Sokolow LZ, et al. Prevention and control of seasonal influenza with vaccines: recommendations of the advisory committee on immunization practices (ACIP) – United States, 2014–15 influenza season. *MMWR Morb Mortal Wkly Rep*. 2014; 63(32):691–697. [PubMed: 25121712]
6. Ampofo WK, Baylor N, Cobey S, et al. Improving influenza vaccine virus selection: report of a WHO informal consultation held at WHO headquarters, Geneva, Switzerland, 14–16 June 2010. *Influenza Other Respir Viruses*. 2012; 6(2):e141–145.
7. Jackson LA. Using surveillance to evaluate influenza vaccine effectiveness. *J Infect Dis*. 2009; 199(2):155–158. [PubMed: 19086916]

8. Richard SA, Viboud C, Miller MA. Evaluation of southern hemisphere influenza vaccine recommendations. *Vaccine*. 2010; 28(15):2693–2699. [PubMed: 20153352]
9. Kilbourne ED. Influenza pandemics of the 20th century. *Emerg Infect Dis*. 2006; 12(1):9–14. [PubMed: 16494710]
10. World Health Organization. Cumulative number of confirmed human cases for avian influenza A (H5N1) reported to WHO. 2003–2015. www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/
11. Broadbent AJ, Subbarao K. Influenza virus vaccines: lessons from the 2009 H1N1 pandemic. *Curr Opin Virol*. 2011; 1(4):254–262. [PubMed: 22125588]
12. Dawood FS, Jain S, Finelli L, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med*. 2009; 360(25):2605–2615. [PubMed: 19423869]
13. Kawaoka Y, Krauss S, Webster RG. Avian-to-human transmission of the *PBI* gene of influenza A viruses in the 1957 and 1968 pandemics. *J Virol*. 1989; 63(11):4603–4608. [PubMed: 2795713]
14. Swayne DE. Impact of vaccines and vaccination on global control of avian influenza. *Avian Dis*. 2012; 56(4 Suppl):818–828. [PubMed: 23402099]
15. Laver WG, Webster RG. Studies on the origin of pandemic influenza. 3 Evidence implicating duck and equine influenza viruses as possible progenitors of the Hong Kong strain of human influenza. *Virology*. 1973; 51(2):383–391. [PubMed: 4632653]
16. Scholtissek C, Rohde W, Von Hoyningen V, Rott R. On the origin of the human influenza virus subtypes H2N2 and H3N2. *Virology*. 1978; 87(1):13–20. [PubMed: 664248]
17. Wendel I, Rubbenstroth D, Doedt J, et al. The avian-origin *PBI* gene segment facilitated replication and transmissibility of the H3N2/1968 pandemic influenza virus. *J Virol*. 2015; 89(8):4170–4179. [PubMed: 25631088]
18. Cox NJ, Trock SC, Burke SA. Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Curr Top Microbiol Immunol*. 2014; 385:119–136. [PubMed: 25085014]
19. Trock SC, Burke SA, Cox NJ. Development of an influenza virologic risk assessment tool. *Avian Dis*. 2012; 56(4 Suppl):1058–1061. [PubMed: 23402136]
20. Schrauwen EJA, Herfst S, Leijten LM, et al. The multibasic cleavage site in H5N1 virus is critical for systemic spread along the olfactory and hematogenous routes in ferrets. *J Virol*. 2012; 86(7):3975–3984. [PubMed: 22278228]
21. Suguitan AL, Matsuoka Y, Lau Y-F, et al. The multibasic cleavage site of the hemagglutinin of highly pathogenic A/Vietnam/1203/2004 (H5N1) avian influenza virus acts as a virulence factor in a host-specific manner in mammals. *J Virol*. 2012; 86(5):2706–2714. [PubMed: 22205751]
22. Yoon S-W, Kayali G, Ali MA, Webster RG, Webby RJ, Ducatez MF. A single amino acid at the hemagglutinin cleavage site contributes to the pathogenicity but not the transmission of Egyptian highly pathogenic H5N1 influenza virus in chickens. *J Virol*. 2013; 87(8):4786–4788. [PubMed: 23408622]
23. Herfst S, Schrauwen EJA, Linster M, et al. Airborne transmission of influenza A/H5N1 virus between ferrets. *Science*. 2012; 336(6088):1534–1541. [PubMed: 22723413]
24. Imai M, Watanabe T, Hatta M, et al. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature*. 2012; 486(7403):420–428. [PubMed: 22722205]
25. Guan Y, Shortridge KF, Krauss S, Webster RG. Molecular characterization of H9N2 influenza viruses: were they the donors of the ‘internal’ genes of H5N1 viruses in Hong Kong? *Proc Natl Acad Sci USA*. 1999; 96(16):9363–9367. [PubMed: 10430948]
26. Li X, Shi J, Guo J, et al. Genetics, receptor binding property, and transmissibility in mammals of naturally isolated H9N2 avian influenza viruses. *PLoS Pathog*. 2014; 10(11):e1004508. [PubMed: 25411973]
27. Pu J, Wang S, Yin Y, et al. Evolution of the H9N2 influenza genotype that facilitated the genesis of the novel H7N9 virus. *Proc Natl Acad Sci USA*. 2015; 112(2):548–553. [PubMed: 25548189]
28. Hoffmann E, Stech J, Leneva I, et al. Characterization of the influenza A virus gene pool in avian species in southern China: was H6N1 a derivative or a precursor of H5N1? *J Virol*. 2000; 74(14):6309–6315. [PubMed: 10864640]

29. Liu M, Li X, Yuan H, et al. Genetic diversity of avian influenza A (H10N8) virus in live poultry markets and its association with human infections in China. *Sci Rep*. 2015; 5:7632–7632. [PubMed: 25591167]
30. Centers for Disease Control and Prevention (CDC). . Update: influenza A (H3N2)v transmission and guidelines – five states, 2011. *MMWR Morb Mortal Wkly Rep*. 2012; 60(51–52):1741–1744. [PubMed: 22217624]
31. Li Q, Zhou L, Zhou M, et al. Epidemiology of human infections with avian influenza A(H7N9) virus in China. *N Engl J Med*. 2014; 370(6):520–532. [PubMed: 23614499]
32. Gao R, Cao B, Hu Y, et al. Human infection with a novel avian-origin influenza A (H7N9) virus. *N Engl J Med*. 2013; 368(20):1888–1897. [PubMed: 23577628]
33. Zhang Q, Shi J, Deng G, et al. H7N9 influenza viruses are transmissible in ferrets by respiratory droplet. *Science*. 2013; 341(6144):410–414. [PubMed: 23868922]
34. Smith GJD, Vijaykrishna D, Bahl J, et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza a epidemic. *Nature*. 2009; 459(7250):1122–1125. [PubMed: 19516283]
35. Capua I, Terregino C, Cattoli G, Mutinelli F, Rodriguez JF. Development of a DIVA (Differentiating Infected from Vaccinated Animals) strategy using a vaccine containing a heterologous neuraminidase for the control of avian influenza. *Avian Pathol*. 2003; 32(1):47–55. [PubMed: 12745380]
36. Cattoli G, Fusaro A, Monne I, et al. Evidence for differing evolutionary dynamics of A/H5N1 viruses among countries applying or not applying avian influenza vaccination in poultry. *Vaccine*. 2011; 29(50):9368–9375. [PubMed: 22001877]
37. Bertran K, Moresco K, Swayne DE. Impact of vaccination on infection with Vietnam H5N1 high pathogenicity avian influenza virus in hens and the eggs they lay. *Vaccine*. 2015; 33(11):1324–1330. [PubMed: 25657093]
38. Lee C-W, Senne DA, Suarez DL. Effect of vaccine use in the evolution of Mexican lineage H5N2 avian influenza virus. *J Virol*. 2004; 78(15):8372–8381. [PubMed: 15254209]
39. Ferguson NM, Cummings DaT, Fraser C, Cajka JC, Cooley PC, Burke DS. Strategies for mitigating an influenza pandemic. *Nature*. 2006; 442(7101):448–452. [PubMed: 16642006]
40. World Health Organization. Pandemic influenza vaccine manufacturing process and timeline. Pandemic (H1N1) 2009 briefing note 7. 2009. www.who.int/csr/disease/swineflu/notes/h1n1_vaccine_20090806/en/
41. Jennings LC, Monto AS, Chan PKS, Szucs TD, Nicholson KG. Stockpiling prepandemic influenza vaccines: a new cornerstone of pandemic preparedness plans. *Lancet Infect Dis*. 2008; 8(10):650–658. [PubMed: 18922487]
42. Luke CJ, Subbarao K. Vaccines for pandemic influenza. *Emerg Infect Dis*. 2006; 12(1):66–72. [PubMed: 16494720]
43. Bart SA, Hohenboken M, Della Cioppa G, Narasimhan V, Dormitzer PR, Kanesa-Thasan N. A cell culture-derived MF59-adjuvanted pandemic A/H7N9 vaccine is immunogenic in adults. *Sci Transl Med*. 2014; 6(234):234ra255.
44. Stohr K. Vaccinate before the next pandemic? *Nature*. 2010; 465(7295):161. [PubMed: 20463719]
45. World Health Organization. Pandemic influenza preparedness framework for the sharing of influenza viruses and access to vaccines and other benefits. 2011. www.who.int/influenza/resources/pip_framework/en/
46. Tricco AC, Chit A, Soobiah C, et al. Comparing influenza vaccine efficacy against mismatched and matched strains: a systematic review and meta-analysis. *BMC Med*. 2013; 11:153–153. [PubMed: 23800265]
47. Hensley SE. Challenges of selecting seasonal influenza vaccine strains for humans with diverse pre-exposure histories. *Curr Opin Virol*. 2014; 8:85–89. [PubMed: 25108824]
48. Li Y, Myers JL, Bostick DL, et al. Immune history shapes specificity of pandemic H1N1 influenza antibody responses. *J Exp Med*. 2013; 210(8):1493–1500. [PubMed: 23857983]
49. Linderman SL, Chambers BS, Zost SJ, et al. Potential antigenic explanation for atypical H1N1 infections among middle-aged adults during the 2013–2014 influenza season. *Proc Natl Acad Sci USA*. 2014; 111(44):15798–15803. [PubMed: 25331901]

50. Belshe RB, Gruber WC, Mendelman PM, et al. Efficacy of vaccination with live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine against a variant (A/Sydney) not contained in the vaccine. *J Pediatr.* 2000; 136(2):168–175. [PubMed: 10657821]
51. Halloran ME, Piedra PA, Longini IM Jr, et al. Efficacy of trivalent, cold-adapted, influenza virus vaccine against influenza A (Fujian), a drift variant, during 2003–2004. *Vaccine.* 2007; 25(20): 4038–4045. [PubMed: 17395338]
52. Mendelman PM, Rappaport R, Cho I, et al. Live attenuated influenza vaccine induces cross-reactive antibody responses in children against an a/Fujian/411/2002-like H3N2 antigenic variant strain. *Pediatr Infect Dis J.* 2004; 23(11):1053–1055. [PubMed: 15545863]
53. Monto AS, Ohmit SE, Petrie JG, et al. Comparative efficacy of inactivated and live attenuated influenza vaccines. *N Engl J Med.* 2009; 361(13):1260–1267. [PubMed: 19776407]
54. Ohmit SE, Victor JC, Rotthoff JR, et al. Prevention of antigenically drifted influenza by inactivated and live attenuated vaccines. *N Engl J Med.* 2006; 355(24):2513–2522. [PubMed: 17167134]
55. Flannery B, Clippard J, Zimmerman RK, et al. Early estimates of seasonal influenza vaccine effectiveness – United States, January 2015. *MMWR Morb Mortal Wkly Rep.* 2015; 64(1):10–15. [PubMed: 25590680]
56. Abdel-Ghaffar A-N, Chotpitayasunondh T, Gao Z, et al. Update on avian influenza A (H5N1) virus infection in humans. *N Engl J Med.* 2008; 358(3):261–273. [PubMed: 18199865]
57. Antigenic and genetic characteristics of zoonotic influenza viruses and development of candidate vaccine viruses for pandemic preparedness. *Wkly Epidemiol Rec.* 2014; 89(42):457–464. [PubMed: 25337626]
58. Halder N, Kelso JK, Milne GJ. A model-based economic analysis of pre-pandemic influenza vaccination cost–effectiveness. *BMC Infect Dis.* 2014; 14:266–266. [PubMed: 24884470]
59. Ekiert DC, Bhabha G, Elsliger M-A, et al. Antibody recognition of a highly conserved influenza virus epitope. *Science.* 2009; 324(5924):246–251. [PubMed: 19251591]
60. Xu R, Ekiert DC, Krause JC, Hai R, Crowe JE, Wilson IA. Structural basis of preexisting immunity to the 2009 H1N1 pandemic influenza virus. *Science.* 2010; 328(5976):357–360. [PubMed: 20339031]
61. Sangster MY, Baer J, Santiago FW, et al. B cell response and hemagglutinin stalk-reactive antibody production in different age cohorts following 2009 H1N1 influenza virus vaccination. *Clin Vaccine Immunol.* 2013; 20(6):867–876. [PubMed: 23576673]
62. Wrammert J, Koutsouanos D, Li GM, et al. Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection. *J Exp Med.* 2011; 208(1): 181–193. [PubMed: 21220454]
63. Corti D, Voss J, Gamblin SJ, et al. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. *Science.* 2011; 333(6044):850–856. [PubMed: 21798894]
64. Nachbagauer R, Wohlbold TJ, Hirsh A, et al. Induction of broadly reactive anti-hemagglutinin stalk antibodies by an H5N1 vaccine in humans. *J Virol.* 2014; 88(22):13260–13268. [PubMed: 25210189]
65. Cobbin JC, Zeng W, Jackson DC, Brown LE. Different arms of the adaptive immune system induced by a combination vaccine work in concert to provide enhanced clearance of influenza. *PLoS ONE.* 2014; 9(12):e115356. [PubMed: 25522323]
66. Taylor PM, Askonas BA. Influenza nucleoprotein-specific cytotoxic T-cell clones are protective *in vivo*. *Immunology.* 1986; 58(3):417–420. [PubMed: 2426185]
67. Li J, Arevalo MT, Chen Y, Chen S, Zeng M. T-cell-mediated cross-strain protective immunity elicited by prime-boost vaccination with a live attenuated influenza vaccine. *Int J Infect Dis.* 2014; 27:37–43. [PubMed: 25172265]
68. Guo H, Santiago F, Lambert K, Takimoto T, Topham DJ. T cell-mediated protection against lethal 2009 pandemic H1N1 influenza virus infection in a mouse model. *J Virol.* 2010; 85(1):448–455. [PubMed: 20980523]
69. Epstein SL, Lo CY, Misplon JA, Bennink JR. Mechanism of protective immunity against influenza virus infection in mice without antibodies. *J Immunol.* 1998; 160(1):322–327. [PubMed: 9551987]

70. Wilkinson TM, Li CKF, Chui CSC, et al. Preexisting influenza-specific CD4⁺ T cells correlate with disease protection against influenza challenge in humans. *Nat Med.* 2012; 18(2):274–280. [PubMed: 22286307]
71. Sridhar S, Begom S, Bermingham A, et al. Cellular immune correlates of protection against symptomatic pandemic influenza. *Nat Med.* 2013; 19(10):1305–1312. [PubMed: 24056771]
72. Crowe BA, Brühl P, Gerencer M, et al. Evaluation of the cellular immune responses induced by a non-adjuvanted inactivated whole virus A/H5N1/VN/1203 pandemic influenza vaccine in humans. *Vaccine.* 2010; 29(2):166–173. [PubMed: 21055500]
73. Kreijtz JH, De Mutsert G, Van Baalen CA, Fouchier RA, Osterhaus AD, Rimmelzwaan GF. Cross-recognition of avian H5N1 influenza virus by human cytotoxic T-lymphocyte populations directed to human influenza A virus. *J Virol.* 2008; 82(11):5161–5166. [PubMed: 18353950]
74. Hayward AC, Wang L, Goonetilleke N, et al. Natural T cell mediated protection against seasonal and pandemic influenza: results of the flu watch cohort study. *Am J Respir Crit Care Med.* 2015; 191(12):1422–1431. [PubMed: 25844934]
75. Baras B, Stittelaar KJ, Simon JH, et al. Cross-protection against lethal H5N1 challenge in ferrets with an adjuvanted pandemic influenza vaccine. *PLoS ONE.* 2008; 3(1):e1401–e1401. [PubMed: 18167560]
76. Suguitan AL, Mcauliffe J, Mills KL, et al. Live, attenuated influenza a H5N1 candidate vaccines provide broad cross-protection in mice and ferrets. *PLoS Med.* 2006; 3(9):e360–e360. [PubMed: 16968127]
77. Chen Z, Baz M, Lu J, et al. Development of a high-yield live attenuated H7N9 influenza virus vaccine that provides protection against homologous and heterologous H7 wild-type viruses in ferrets. *J Virol.* 2014; 88(12):7016–7023. [PubMed: 24719414]
78. Baz M, Paskel M, Matsuoka Y, et al. A live attenuated equine H3N8 influenza vaccine is highly immunogenic and efficacious in mice and ferrets. *J Virol.* 2015; 89(3):1652–1659. [PubMed: 25410860]
79. Chen H, Matsuoka Y, Swayne D, et al. Generation and characterization of a cold-adapted influenza a H9N2 reassortant as a live pandemic influenza virus vaccine candidate. *Vaccine.* 2003; 21(27–30):4430–4436. [PubMed: 14505926]
80. Carter DM, Bloom CE, Kirchenbaum GA, et al. Cross-protection against H7N9 influenza strains using a live-attenuated H7N3 virus vaccine. *Vaccine.* 2015; 33(1):108–116. [PubMed: 25448100]
81. Chen Z, Santos C, Aspelund A, et al. Evaluation of live attenuated influenza A virus H6 vaccines in mice and ferrets. *J Virol.* 2009; 83(1):65–72. [PubMed: 18945773]
82. Min JY, Vogel L, Matsuoka Y, et al. A live attenuated H7N7 candidate vaccine virus induces neutralizing antibody that confers protection from challenge in mice, ferrets, and monkeys. *J Virol.* 2010; 84(22):11950–11960. [PubMed: 20810733]
83. Tang LH, Lim JH, Kuah LF, Lau YF. Complete protection against lethal challenge of novel H7N9 virus with heterologous inactivated H7 vaccine in mice. *Vaccine.* 2014; 32(42):5375–5378. [PubMed: 25130540]
84. Stephenson I, Bugarini R, Nicholson KG, et al. Cross-reactivity to highly pathogenic avian influenza H5N1 viruses after vaccination with nonadjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a potential priming strategy. *J Infect Dis.* 2005; 191(8):1210–1215. [PubMed: 15776364]
85. Leroux-Roels I, Borkowski A, Vanwolleghem T, et al. Antigen sparing and cross-reactive immunity with an adjuvanted rH5N1 prototype pandemic influenza vaccine: a randomised controlled trial. *Lancet.* 2007; 370(9587):580–589. [PubMed: 17707753]
86. Fazekas G, Martosne-Mendi R, Jankovics I, Szilvasy I, Vajo Z. Cross-reactive immunity to clade 2 strains of influenza virus A subtype H5N1 induced in adults and elderly patients by Fluval, a prototype pandemic influenza virus vaccine derived by reverse genetics, formulated with a phosphate adjuvant, and directed to clade 1 strains. *Clin Vaccine Immunol.* 2009; 16(4):437–443. [PubMed: 19020111]
87. Moris P, Van Der Most R, Leroux-Roels I, et al. H5N1 influenza vaccine formulated with AS03 A induces strong cross-reactive and polyfunctional CD4 T-cell responses. *J Clin Immunol.* 2011; 31(3):443–454. [PubMed: 21174144]

88. Nolan T, Richmond PC, Formica NT, et al. Safety and immunogenicity of a prototype adjuvanted inactivated split-virus influenza A (H5N1) vaccine in infants and children. *Vaccine*. 2008; 26(50): 6383–6391. [PubMed: 18801398]
89. Mahallawi WH, Kasbekar AV, McCormick MS, et al. Infection with 2009 H1N1 influenza virus primes for immunological memory in human nose-associated lymphoid tissue, offering cross-reactive immunity to H1N1 and avian H5N1 viruses. *J Virol*. 2013; 87(10):5331–5339. [PubMed: 23468498]
90. Nicholson KG, Abrams KR, Batham S, et al. Immunogenicity and safety of a two-dose schedule of whole-virion and AS03A-adjuvanted 2009 influenza A (H1N1) vaccines: a randomised, multicentre, age-stratified, head-to-head trial. *Lancet Infect Dis*. 2011; 11(2):91–101. [PubMed: 21168369]
91. Nicholson KG, Thompson CI, Klap JM, et al. Safety and immunogenicity of whole-virus, alum-adjuvanted whole-virus, virosomal, and whole-virus intradermal influenza A/H9N2 vaccine formulations. *Vaccine*. 2009; 28(1):171–178. [PubMed: 19799843]
92. Khurana S, Verma N, Yewdell JW, et al. Mf59 adjuvant enhances diversity and affinity of antibody-mediated immune response to pandemic influenza vaccines. *Sci Transl Med*. 2011; 3(85):85ra48.
93. Jegaskanda S, Reading PC, Kent SJ. Influenza-specific antibody-dependent cellular cytotoxicity: toward a universal influenza vaccine. *J Immunol*. 2014; 193(2):469–475. [PubMed: 24994909]
94. Andrews SF, Kaur K, Pauli NT, Huang M, Huang Y, Wilson PC. High preexisting serological antibody levels correlate with diversification of the influenza vaccine response. *J Virol*. 2015; 89(6):3308–3317. [PubMed: 25589639]
95. Palese P, Wang TT. Why do influenza virus subtypes die out? A hypothesis. *MBio*. 2011; 2(5) pii: e00150-11.
96. Li G-M, Chiu C, Wrammert J, et al. Pandemic H1N1 influenza vaccine induces a recall response in humans that favors broadly cross-reactive memory B cells. *Proc Natl Acad Sci USA*. 2012; 109(23):9047–9052. [PubMed: 22615367]
97. Baz M, Luke CJ, Cheng X, Jin H, Subbarao K. H5N1 vaccines in humans. *Virus Res*. 2013; 178(1):78–98. [PubMed: 23726847]
98. Karron RA, Callahan K, Luke C, et al. A live attenuated H9N2 influenza vaccine is well tolerated and immunogenic in healthy adults. *J Infect Dis*. 2009; 199(5):711–716. [PubMed: 19210163]
99. Karron RA, Talaat K, Luke C, et al. Evaluation of two live attenuated cold-adapted H5N1 influenza virus vaccines in healthy adults. *Vaccine*. 2009; 27(36):4953–4960. [PubMed: 19540952]
100. Talaat KR, Karron RA, Callahan KA, et al. A live attenuated H7N3 influenza virus vaccine is well tolerated and immunogenic in a Phase I trial in healthy adults. *Vaccine*. 2009; 27(28):3744–3753. [PubMed: 19464558]
101. Coelingh KL, Luke CJ, Jin H, Talaat KR. Development of live attenuated influenza vaccines against pandemic influenza strains. *Expert Rev Vaccines*. 2014; 13(7):855–871. [PubMed: 24867587]
102. Centers for Disease Control and Prevention (CDC). . Safety of influenza A (H1N1) 2009 monovalent vaccines – United States, October 1–November 24, 2009. *MMWR Morb Mortal Wkly Rep*. 2009; 58(48):1351–1356. [PubMed: 20010511]
103. Rudenko L, Isakova-Sivak I. Pandemic preparedness with live attenuated influenza vaccines based on A/Leningrad/134/17/57 master donor virus. *Expert Rev Vaccines*. 2015; 14(3):395–412. [PubMed: 25555687]
104. Rudenko L, Kiseleva I, Naykhin AN, et al. Assessment of human immune responses to H7 avian influenza virus of pandemic potential: results from a placebo-controlled, randomized double-blind Phase I study of live attenuated H7N3 influenza vaccine. *PLoS ONE*. 2014; 9(2):e87962. [PubMed: 24533064]
105. Ehrlich HJ, Muller M, Oh HM, et al. A clinical trial of a whole-virus H5N1 vaccine derived from cell culture. *N Engl J Med*. 2008; 358(24):2573–2584. [PubMed: 18550874]
106. Couch RB, Patel SM, Wade-Bowers CL, Nino D. A randomized clinical trial of an inactivated avian influenza A (H7N7) vaccine. *PLoS ONE*. 2012; 7(12):e49704. [PubMed: 23239968]

107. Krammer F, Jul-Larsen A, Margine I, et al. An H7N1 influenza virus vaccine induces broadly reactive antibody responses against H7N9 in humans. *Clin Vaccine Immunol.* 2014; 21(8):1153–1163. [PubMed: 24943383]
108. Patel SM, Atmar RL, El Sahly HM, Cate TR, Keitel WA. A Phase I evaluation of inactivated influenza A/H5N1 vaccine administered by the intradermal or the intramuscular route. *Vaccine.* 2010; 28(17):3025–3029. [PubMed: 19931380]
109. Stephenson I, Nicholson KG, Hoschler K, et al. Antigenically distinct MF59-adjuvanted vaccine to boost immunity to H5N1. *N Engl J Med.* 2008; 359(15):1631–1633. [PubMed: 18843132]
110. Dauvilliers Y, Arnulf I, Lecendreux M, et al. Increased risk of narcolepsy in children and adults after pandemic H1N1 vaccination in France. *Brain.* 2013; 136(Pt 8):2486–2496. [PubMed: 23884811]
111. Nohynek H, Jokinen J, Partinen M, et al. AS03 adjuvanted AH1N1 vaccine associated with an abrupt increase in the incidence of childhood narcolepsy in Finland. *PLoS ONE.* 2012; 7(3):e33536. [PubMed: 22470453]
112. Partinen M, Saarenpää-Heikkilä O, Ilveskoski I, et al. Increased incidence and clinical picture of childhood narcolepsy following the 2009 H1N1 pandemic vaccination campaign in Finland. *PLoS ONE.* 2012; 7(3):e33723. [PubMed: 22470463]
113. Heier MS, Gautvik KM, Wannag E, et al. Incidence of narcolepsy in Norwegian children and adolescents after vaccination against H1N1 influenza A. *Sleep Med.* 2013; 14(9):867–871. [PubMed: 23773727]
114. Miller E, Andrews N, Stellitano L, et al. Risk of narcolepsy in children and young people receiving AS03 adjuvanted pandemic A/H1N1 2009 influenza vaccine: retrospective analysis. *BMJ.* 2013; 346:f794. [PubMed: 23444425]
115. O'Flanagan D, Barret AS, Foley M, et al. Investigation of an association between onset of narcolepsy and vaccination with pandemic influenza vaccine, Ireland April 2009–December 2010. *Euro Surveill.* 2014; 19(17):15–25. [PubMed: 24821121]
116. Persson I, Granath F, Askling J, Ludvigsson JF, Olsson T, Feltelius N. Risks of neurological and immune-related diseases, including narcolepsy, after vaccination with pandemrix: a population- and registry-based cohort study with over 2 years of follow-up. *J Intern Med.* 2014; 275(2):172–190. [PubMed: 24134219]
117. Dauvilliers Y, Montplaisir J, Cochen V, et al. Post-H1N1 narcolepsy-cataplexy. *Sleep.* 2010; 33(11):1428–1430. [PubMed: 21102981]
118. Harris T, Wong K, Stanford L, Fediurek J, Crowcroft N, Deeks S. Did narcolepsy occur following administration of AS03-adjuvanted A(H1N1) pandemic vaccine in Ontario, Canada? A review of post-marketing safety surveillance data. *Euro Surveill.* 2014; 19(36) pii: 20900.
119. Jacob L, Leib R, Ollila HM, Bonvalet M, Adams CM, Mignot E. Comparison of Pandemrix and Arepanrix, two pH1N1 AS03-adjuvanted vaccines differentially associated with narcolepsy development. *Brain Behav Immun.* 2014; 47:44–57. [PubMed: 25452148]
120. Duffy J, Weintraub E, Vellozzi C, Destefano F. Narcolepsy and influenza A(H1N1) pandemic 2009 vaccination in the United States. *Neurology.* 2014; 83(20):1823–1830. [PubMed: 25320099]
121. Yih WK, Lee GM, Lieu TA, et al. Surveillance for adverse events following receipt of pandemic 2009 H1N1 vaccine in the Post-Licensure Rapid Immunization Safety Monitoring (PRISM) system, 2009–2010. *Am J Epidemiol.* 2012; 175(11):1120–1128. [PubMed: 22582207]
122. Vaarala O, Vuorela A, Partinen M, et al. Antigenic differences between AS03 adjuvanted influenza A (H1N1) pandemic vaccines: implications for pandemrix-associated narcolepsy risk. *PLoS ONE.* 2014; 9(12):e114361–e114361. [PubMed: 25501681]
123. Singh AK, Mahlios J, Mignot E. Genetic association, seasonal infections and autoimmune basis of narcolepsy. *J Autoimmun.* 2013; 43:26–31. [PubMed: 23497937]
124. Sturkenboom MC. The narcolepsy-pandemic influenza story: can the truth ever be unraveled? *Vaccine.* 2015; 33(Suppl 2):B6–B13. [PubMed: 26022571]
125. Committee for Proprietary Medicinal Products. Note for guidance on harmonisation of requirements for influenza vaccines. 1997. CPMP/BWP/214/96 www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf

126. Bandell A, Woo J, Coelingh K. Protective efficacy of live-attenuated influenza vaccine (multivalent, ann arbor strain): a literature review addressing interference. *Expert Rev Vaccines*. 2011; 10(8):1131–1141. [PubMed: 21854309]
127. Edwards KM, Dupont WD, Westrich MK, Plummer WD, Palmer PS, Wright PF. A randomized controlled trial of cold-adapted and inactivated vaccines for the prevention of influenza A disease. *J Infect Dis*. 1994; 169(1):68–76. [PubMed: 8277200]
128. Coudeville L, Andre P, Bailleux F, Weber F, Plotkin S. A new approach to estimate vaccine efficacy based on immunogenicity data applied to influenza vaccines administered by the intradermal or intramuscular routes. *Hum Vaccin*. 2010; 6(10):841–848. [PubMed: 20930559]
129. Khurana S, Suguitan AL, Rivera Y, et al. Antigenic fingerprinting of H5N1 avian influenza using convalescent sera and monoclonal antibodies reveals potential vaccine and diagnostic targets. *PLoS Med*. 2009; 6(4):e1000049–e1000049. [PubMed: 19381279]
130. Mallory RM, Malkin E, Ambrose CS, et al. Safety and immunogenicity following administration of a live, attenuated monovalent 2009 H1N1 influenza vaccine to children and adults in two randomized controlled trials. *PLoS ONE*. 2010; 5(10):e13755–e13755. [PubMed: 21060780]
131. Rudenko L, Van Den Bosch H, Kiseleva I, et al. Live attenuated pandemic influenza vaccine: clinical studies on A/17/California/2009/38 (H1N1) and licensing of the Russian-developed technology to who for pandemic influenza preparedness in developing countries. *Vaccine*. 2011; 29(Suppl 1):A40–44. [PubMed: 21684428]
132. Rudenko L, Desheva J, Korovkin S, et al. Safety and immunogenicity of live attenuated influenza reassortant H5 vaccine (Phase I–II clinical trials). *Influenza Other Respir Viruses*. 2008; 2(6):203–209. [PubMed: 19453396]
133. Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. *N Engl J Med*. 2006; 354(13):1343–1351. [PubMed: 16571878]
134. Treanor JJ, Wilkinson BE, Masseoud F, et al. Safety and immunogenicity of a recombinant hemagglutinin vaccine for H5 influenza in humans. *Vaccine*. 2001; 19(13–14):1732–1737. [PubMed: 11166898]
135. Talaat KR, Luke CJ, Khurana S, et al. A live attenuated influenza A(H5N1) vaccine induces long-term immunity in the absence of a primary antibody response. *J Infect Dis*. 2014; 209(12):1860–1869. [PubMed: 24604819]
136. Goji NA, Nolan C, Hill H, et al. Immune responses of healthy subjects to a single dose of intramuscular inactivated influenza A/Vietnam/1203/2004 (H5N1) vaccine after priming with an antigenic variant. *J Infect Dis*. 2008; 198(5):635–641. [PubMed: 18694338]
137. Cate TR, Couch RB, Kasel JA, Six HR. Clinical trials of monovalent influenza A/New Jersey/76 virus vaccines in adults: reactogenicity, antibody response, and antibody persistence. *J Infect Dis*. 1977; 136 (Suppl):S450–455. [PubMed: 342620]
138. Meiklejohn G, Eickhoff TC, Graves P. Antibody response of young adults to experimental influenza A/New Jersey/76 virus vaccines. *J Infect Dis*. 1977; 136(Suppl):S456–S459. [PubMed: 606766]
139. Wise TG, Dolin R, Mazur MH, Ennis FA. Serologic responses after two sequential doses of influenza A/New Jersey/76 virus vaccine in normal young adults. *J Infect Dis*. 1977; 136(Suppl):S496–499. [PubMed: 606771]
140. Edelman R, Scheidt KB. Sequential immunization of laboratory personnel with influenza A/New Jersey/76 split- and whole-virus vaccines. *J Infect Dis*. 1977; 136(Suppl):S500–506. [PubMed: 606772]
141. Pandemic working group of the Medical Research Council (UK) Committee on influenza and other respiratory virus vaccines. Antibody responses and reactogenicity of graded doses of inactivated influenza A/New Jersey/76 whole-virus vaccine in humans. *J Infect Dis*. 1977; 136(Suppl):S475–483. [PubMed: 342621]
142. Ledgerwood JE, Wei CJ, Hu Z, et al. DNA priming and influenza vaccine immunogenicity: two Phase 1 open label randomised clinical trials. *Lancet Infect Dis*. 2011; 11(12):916–924. [PubMed: 21975270]

143. Ledgerwood JE, Zephir K, Hu Z, et al. Prime-boost interval matters: a randomized Phase 1 study to identify the minimum interval necessary to observe the H5 DNA influenza vaccine priming effect. *J Infect Dis.* 2013; 208(3):418–422. [PubMed: 23633407]
144. Gurwith M, Lock M, Taylor EM, et al. Safety and immunogenicity of an oral, replicating adenovirus serotype 4 vector vaccine for H5N1 influenza: a randomised, double-blind, placebo-controlled, Phase 1 study. *Lancet Infect Dis.* 2013; 13(3):238–250. [PubMed: 23369412]
145. Ehrlich HJ, Muller M, Fritsch S, et al. A cell culture (vero)-derived H5N1 whole-virus vaccine induces cross-reactive memory responses. *J Infect Dis.* 2009; 200(7):1113–1118. [PubMed: 19712040]
146. Lin JT, Li CG, Wang X, et al. Antibody persistence after 2-dose priming and booster response to a third dose of an inactivated, adjuvanted, whole-virion H5N1 vaccine. *J Infect Dis.* 2009; 199(2):184–187. [PubMed: 19067606]
147. Zangwill KM, Treanor JJ, Campbell JD, Noah DL, Ryea J. Evaluation of the safety and immunogenicity of a booster (third) dose of inactivated subvirion H5N1 influenza vaccine in humans. *J Infect Dis.* 2008; 197(4):580–583. [PubMed: 18237269]
148. Belshe RB, Frey SE, Graham IL, et al. Immunogenicity of avian influenza A/Anhui/01/2005 (H5N1) vaccine with MF59 adjuvant: a randomized clinical trial. *JAMA.* 2014; 312(14):1420–1428. [PubMed: 25291578]
149. Winokur PL, Patel SM, Brady R, et al. Safety and immunogenicity of a single low dose or high dose of clade 2 influenza A(H5N1) inactivated vaccine in adults previously primed with clade 1 influenza A(H5N1) vaccine. *J Infect Dis.* 2015; 212(4):525–530. [PubMed: 25712967]
150. Babu TM, Levine M, Fitzgerald T, et al. Live attenuated H7N7 influenza vaccine primes for a vigorous antibody response to inactivated H7N7 influenza vaccine. *Vaccine.* 2014; 32(50):6798–6804. [PubMed: 25446831]
151. Nicholson KG, Colegate AE, Podda A, et al. Safety and antigenicity of non-adjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a randomised trial of two potential vaccines against H5N1 influenza. *Lancet.* 2001; 357(9272):1937–1943. [PubMed: 11425416]
152. Stephenson I, Nicholson KG, Colegate A, et al. Boosting immunity to influenza H5N1 with MF59-adjuvanted H5N3 A/Duck/Singapore/97 vaccine in a primed human population. *Vaccine.* 2003; 21(15):1687–1693. [PubMed: 12639491]
153. Galli G, Hancock K, Hoschler K, et al. Fast rise of broadly cross-reactive antibodies after boosting long-lived human memory B cells primed by an MF59 adjuvanted prepandemic vaccine. *Proc Natl Acad Sci USA.* 2009; 106(19):7962–7967. [PubMed: 19416838]
154. Krammer F, Palese P. Influenza virus hemagglutinin stalk-based antibodies and vaccines. *Curr Opin Virol.* 2013; 3(5):521–530. [PubMed: 23978327]
155. Halliley JL, Khurana S, Krammer F, et al. High affinity H7 head and stalk domain-specific antibody responses to an inactivated influenza H7N7 vaccine after priming with live attenuated influenza vaccine. *J Infect Dis.* 2015 (Epub ahead of print). 10.1093/infdis/jiv210
156. Crank MC, Gordon IJ, Yamshchikov GV, et al. Phase 1 study of pandemic H1 DNA vaccine in healthy adults. *PLoS ONE.* 2015; 10(4):e0123969. [PubMed: 25884189]
157. Frey SE, Bernstein DI, Brady RC, et al. Phase II trial in adults of concurrent or sequential 2009 pandemic H1N1 and 2009–2010 seasonal trivalent influenza vaccinations. *Vaccine.* 2015; 33(1):163–173. [PubMed: 25444805]
158. Uno S, Kimachi K, Kei J, et al. Effect of prior vaccination with a seasonal trivalent influenza vaccine on the antibody response to the influenza pandemic H1N1 2009 vaccine: a randomized controlled trial. *Microbiol Immunol.* 2011; 55(11):783–789. [PubMed: 21895745]
159. Fragapane E, Gasparini R, Schioppa F, Laghi-Pasini F, Montomoli E, Banzhoff A. A heterologous MF59-adjuvanted H5N1 prepandemic influenza booster vaccine induces a robust, cross-reactive immune response in adults and the elderly. *Clin Vaccine Immunol.* 2010; 17(11):1817–1819. [PubMed: 20810680]
160. Wang W, Lu J, Cotter CR, Wen K, Jin H, Chen Z. Identification of critical residues in the hemagglutinin and neuraminidase of influenza virus H1N1pdm for vaccine virus replication in embryonated chicken eggs. *J Virol.* 2013; 87(8):4642–4649. [PubMed: 23408613]

161. Krenn BM, Egorov A, Romanovskaya-Romanko E, et al. Single HA2 mutation increases the infectivity and immunogenicity of a live attenuated H5N1 intranasal influenza vaccine candidate lacking NS1. *PLoS ONE*. 2011; 6(4):e18577–e18577. [PubMed: 21490925]
162. Nakowitsch S, Wolschek M, Morokutti A, et al. Mutations affecting the stability of the haemagglutinin molecule impair the immunogenicity of live attenuated H3N2 intranasal influenza vaccine candidates lacking NS1. *Vaccine*. 2011; 29(19):3517–3524. [PubMed: 21406268]
163. Van Der Velden MVW, Fritz R, Pöllabauer EM, et al. Safety and immunogenicity of a vero cell culture-derived whole-virus influenza A(H5N1) vaccine in a pediatric population. *J Infect Dis*. 2014; 209(1):12–23. [PubMed: 24041789]
164. Aichinger G, Grohmann-Izay B, Van Der Velden MVW, et al. Phase I/II randomized double-blind study of the safety and immunogenicity of a nonadjuvanted vero cell culture-derived whole-virus H9N2 influenza vaccine in healthy adults. *Clin Vaccine Immunol*. 2015; 22(1):46–55. [PubMed: 25355797]
165. Ehrlich HJ, Müller M, Kollaritsch H, et al. Pre-vaccination immunity and immune responses to a cell culture-derived whole-virus H1N1 vaccine are similar to a seasonal influenza vaccine. *Vaccine*. 2012; 30(30):4543–4551. [PubMed: 22475864]
166. Van Der Velden MVW, Geisberger A, Dvorak T, et al. Safety and immunogenicity of a vero cell culture-derived whole-virus H5N1 influenza vaccine in chronically ill and immunocompromised patients. *Clin Vaccine Immunol*. 2014; 21(6):867–876. [PubMed: 24739978]
167. Chia M-Y, Hu AY-C, Tseng Y-F, et al. Evaluation of MDCK cell-derived influenza H7N9 vaccine candidates in ferrets. *PLoS ONE*. 2015; 10(3):e0120793–e0120793. [PubMed: 25799397]
168. Kim JH, Jacob J. DNA vaccines against influenza viruses. *Curr Top Microbiol Immunol*. 2009; 333:197–210. [PubMed: 19768407]
169. Kreijtz JH, Goeijenbier M, Moesker FM, et al. Safety and immunogenicity of a modified-vaccinia-virus-Ankara-based influenza A H5N1 vaccine: a randomised, double-blind Phase 1/2a clinical trial. *Lancet Infect Dis*. 2014; 14(12):1196–1207. [PubMed: 25455987]
170. Lin J, Calcedo R, Vandenberghe LH, Bell P, Somanathan S, Wilson JM. A new genetic vaccine platform based on an adeno-associated virus isolated from a rhesus macaque. *J Virol*. 2009; 83(24):12738–12750. [PubMed: 19812149]
171. Sheahan T, Whitmore A, Long K, et al. Successful vaccination strategies that protect aged mice from lethal challenge from influenza virus and heterologous severe acute respiratory syndrome coronavirus. *J Virol*. 2011; 85(1):217–230. [PubMed: 20980507]
172. Dinapoli JM, Nayak B, Yang L, et al. Newcastle disease virus-vectored vaccines expressing the hemagglutinin or neuraminidase protein of H5N1 highly pathogenic avian influenza virus protect against virus challenge in monkeys. *J Virol*. 2010; 84(3):1489–1503. [PubMed: 19923177]
173. Schwartz JA, Buonocore L, Suguitan A Jr, et al. Vesicular stomatitis virus-based H5N1 avian influenza vaccines induce potent cross-clade neutralizing antibodies in rhesus macaques. *J Virol*. 2011; 85(9):4602–4605. [PubMed: 21325423]
174. Even-Or O, Samira S, Ellis R, Kedar E, Barenholz Y. Adjuvanted influenza vaccines. *Expert Rev Vaccines*. 2013; 12(9):1095–1108. [PubMed: 24053401]
175. Holloway R, Rasmussen SA, Zaza S, Cox NJ, Jernigan DB. Updated preparedness and response framework for influenza pandemics. *MMWR Recomm Rep*. 2014; 63(RR-06):1–18. [PubMed: 25254666]
176. Lerner H, Berg C. The concept of health in one health and some practical implications for research and education: what is one health? *Infect Ecol Epidemiol*. 2015; 5:25300. [PubMed: 25660757]

EXECUTIVE SUMMARY

Timely pandemic response requires a philosophical shift from reactive to proactive pandemic preparedness

- The established paradigm for seasonal influenza vaccination may not be appropriate for pandemic influenza vaccination.
- Pandemic preparedness plans have been established by national and international agencies.
- Pandemic preparedness will require a combination of surveillance in wildlife and livestock animal populations, a new approach to development of vaccine and an enhanced understanding of the mechanisms by which vaccines protect from influenza.

Emerging frameworks permit risk assessment of the pandemic potential of avian influenza viruses

- Influenza viruses with pandemic potential are derived from animal influenza viruses.
- The discovery of molecular signatures associated with human transmission and pathogenicity of avian influenza viruses has permitted the development of standardized tools for assessment of pandemic risk.

Prepandemic development & deployment of vaccines is a promising approach

- A proactive pandemic preparedness strategy relies on development and characterization of pandemic vaccines.
- Pandemic vaccines could be either stockpiled for rapid deployment in the event of a pandemic or deployed during the prepandemic period.
- Pandemic vaccines developed on a number of platforms are safe and immunogenic in humans and elicit broad cross-protection in preclinical models.
- Some pandemic vaccines have been shown to elicit broadly cross-reactive serum antibody in humans.

Novel vaccination platforms & deployment strategies facilitate the development of promising pandemic influenza vaccines

- Prime-boost strategies combining different vaccine platforms and formulations can enhance immunogenicity of pandemic vaccines.
- Emerging vaccine technologies have the potential to facilitate rapid and safe production of pandemic vaccine.

Figure 1. Timeline for influenza vaccine development and production, identifying steps where pandemic influenza vaccine development can be accelerated

Seasonal influenza vaccines are formulated using already licensed technology and processes. The timeline for this existing framework, from strain selection to seasonal vaccine deployment, is 5–7 months. Steps in this process at which development and deployment of pandemic vaccines may be accelerated are indicated by arrows.