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Report on the second WHO integrated meeting on development and clinical trials of influenza vaccines that induce broadly protective and long-lasting immune responses: Geneva, Switzerland, 5–7 May 2014

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Abstract

On 5–7 May 2014, the World Health Organization (WHO) convened the second integrated meeting on “influenza vaccines that induce broadly protective and long-lasting immune responses”. Around 100 invited experts from academia, the vaccine industry, research and development funders, and regulatory and public health agencies attended the meeting. Areas covered included mechanisms of protection in natural influenza-virus infection and vaccine-induced immunity, new approaches to influenza-vaccine design and production, and novel routes of vaccine administration. A timely focus was on how this knowledge could be applied to both seasonal influenza and emerging viruses with pandemic potential such as influenza A (H7N9), currently circulating in China. Special attention was given to the development of possible universal influenza vaccines, given that the Global Vaccine Action Plan calls for at least one licensed universal influenza vaccine by 2020. This report highlights some of the topics discussed and provides an update on studies published since the report of the previous meeting.

Keywords

Influenza; Vaccine; Pandemic influenza vaccine; Universal influenza vaccine; H7N9 vaccine; Seasonal influenza vaccine

1. Introduction

Circulating influenza strains undergo antigenic drift, and occasionally shift, over time. These phenomena, coupled with waning immunity post vaccination, necessitate the annual review and frequent revisions of seasonal influenza vaccines and yearly vaccination. The burden of influenza disease (reviewed by WHO in 2012 [1]) and its socio-economic impact, is likely to increase during influenza pandemics, when antigenically shifted viruses infect susceptible human populations that have little or no virus-specific antibody from prior infection or vaccination. Research is needed to develop influenza vaccines that produce broadly protective and long-lasting immune responses to obviate the need for annual immunization to prevent seasonal influenza and to produce a new vaccine to prevent disease when a pandemic virus emerges.

In May 2014, the World Health Organization (WHO) held its second integrated meeting on “Influenza vaccines that induce broadly protective and long-lasting immune responses”. Around 100 invited experts from academia, the vaccine industry, research and development funders, and regulatory and public health agencies attended the meeting. Areas covered included correlates of protection in natural influenza-virus infection [2] and vaccine-induced immunity, new approaches to influenza-vaccine design and production, and novel routes of vaccine administration. A timely focus was on how this knowledge could be applied to seasonal influenza and also emerging viruses with pandemic potential such as influenza A (H7N9), currently causing outbreaks in humans in China. This report highlights some of the topics discussed and provides an update on studies published since the report of the previous meeting [3].

2. Goals of universal or universal-like influenza vaccines

Since the first WHO meeting on this topic in January 2013, the Global Vaccine Action Plan [4], which includes a target for developing a universal influenza vaccine by 2020, was approved by the World Health Assembly. There remains debate however, about what constitutes a “universal” influenza vaccine. A universal influenza vaccine is generally considered to be one that elicits a broader immune response to protect against a greater range of influenza viruses and for longer than current influenza vaccines, obviating the need for annual updates of vaccine formulations.

At the most optimistic extreme, this would be an entirely new type of influenza vaccine where one dose or course would provide life-long protection against all influenza virus infections, without requiring any intervening boosting doses. At the other extreme, progress may involve incremental improvements over the status quo, whereby a “universal-like” vaccine would produce broader or longer lasting immunity compared to current vaccines, but would still require boosting doses (though not annually) and would not be expected to

protect against all influenza A virus subtypes. For example, existing influenza vaccines could be combined with new approaches to produce vaccines, and/or vaccination strategies that induce broader immunity to protect against more antigenically drifted influenza strains and/or for a longer duration. Some of these approaches could reduce the need for annual re-vaccination and/or increase vaccine effectiveness in years where there is a poor match between vaccine strains and circulating virus. The development of broadly protective (across all or many subtypes of influenza A viruses) and long-acting influenza vaccines was widely agreed to be very important but also very challenging.

Replacing annual influenza vaccination with less-frequent re-vaccination could have important manufacturing and programmatic implications, especially for low-resource countries. An important role of the strengthening of public and private sector influenza vaccine manufacturing capacity has been to increase the surge capacity for pandemic vaccine production [5,6]. In 2011, global production of seasonal influenza vaccines was at least 620 million doses [7]. However, if the annual demand for influenza vaccines was reduced through the development of universal-like vaccines that induce broader and longer lasting immunity against seasonal influenza viruses, this could lead to a reduction in global capacity to respond to an emerging influenza pandemic.

3. Measuring immune responses: correlates of protection

Increasing the breadth of the immune response induced will require vaccines that incorporate novel influenza antigens designed to stimulate multiple immune effector mechanisms. There was much discussion about which antigens should be included, what assays or immune markers could be used to measure vaccine immunogenicity, and how these could be used as surrogate markers to predict protection against influenza virus infection or disease.

3.1. Assays measuring antibody

Neutralizing antibodies against the globular head of haemagglutinin (HA) are the primary mediators of most vaccine-induced protection against influenza; a haemagglutination inhibition (HI) antibody titre of 1:40 is the currently accepted correlate of protection (CoP) for current HA-based vaccines. The HI assay is not appropriate for live attenuated influenza vaccines (LAIVs) however, and studies in ferrets suggest that mucosal IgA production might be a better CoP for LAIVs in previously primed animals [8].

For new influenza vaccines, assays for serum antibodies against other antigens, such as neuraminidase (NA) or the ectodomain of influenza matrix protein 2 (M2e), will be required. Assays for NA-inhibition and conformation-dependent anti-M2 antibodies have been established, but need to be tested to determine whether they can be used as correlates of a protective immune response. Further-more, additional or alternative endpoints to serum antibody titres might be more relevant for assessing the quality and durability of the antibody response to vaccination, such as mucosal antibody titres, antibody affinity, and frequencies of antibody-secreting cells and memory B cells.

There is renewed interest in the role of antibody-dependent cell-mediated cytotoxicity (ADCC) following infection or vaccination. ADCC can destroy virus-infected cells and cause the release of cytokines and chemokines to induce an anti-viral state. Analysis of sera collected before and after the 2009 influenza pandemic (pH1N1) suggest that pre-existing anti-pH1N1 ADCC activity in the sera of older subjects correlated with protection of this age group against pH1N1-associated mortality [9]. The ADCC activity in non-human primates (NHPs) and humans can recognize a broad range of influenza subtypes, but more research is required to determine if vaccines can be designed to stimulate this effector mechanism. In NHPs, a trivalent inactivated influenza vaccine (TIV) was poor at inducing ADCC-activity [10], whereas cross-reactive antibodies capable of mediating ADCC were induced by a modified vaccinia Ankara (MVA) vector expressing HA and nucleoprotein (NP) [11].

Novel assays for influenza antibodies can also facilitate sero-epidemiological studies and zoonotic surveillance. A new high-throughput protein micro-array assay has been developed to measure antibody responses to multiple antigens from many influenza strains [12,13]. It is being validated in several areas of the world [14], using neonatal-screening of dried blood spots. Recent data demonstrate how this technique can be used to track responses to circulating influenza viruses over time in a geographically defined population, comparing responses across countries, and possibly identifying the origins of pandemic strains [15].

In an on-going study of immunological memory, volunteers have donated bone-marrow samples pre- and post-vaccination so that the frequency of influenza-specific plasma cells can be determined and tested for a correlation with influenza-specific serum antibody titres over time (unpublished data, Rafi Ahmed, Emory University, Atlanta, GA, USA).

3.2. Measuring cell-mediated immune responses

Cell-mediated immunity (CMI) frequently targets conserved regions of influenza proteins, and inducing potent CMI responses could be important for mediating protection against multiple influenza virus types or influenza A virus sub-types. Reproducible assays for CMI will be needed to understand better its role in controlling infection and for measuring immune responses to LAIVs and vaccine platforms (such as viral vectors or DNA vaccines) designed to stimulate CMI, but which often do not induce high antibody titres. For example, the frequency of interferon-γ (IFN-γ) secreting T cells appeared to correlate with heterologous protection in naïve ferrets immunized with LAIVs [16].

CMI has been studied in a cohort of healthy young adults recruited before the 2009 influenza pandemic and followed for infection with pH1N1. A correlation was found between levels of pre-existing heterosubtype-specific $CD8^+$ T cells and natural resistance to mild-to-moderate influenza symptoms and viral shedding. These cells recognized NP, matrix protein (M1) and the polymerase basic 1 (PB1) protein, produced only IFN-γ, and had a late-effector, lung-homing and cytotoxic-capable phenotype [17]. In contrast, results from human challenge studies suggested a role for pre-existing $CD4^+$ T cells in controlling influenza disease severity [18].

A strong, early $CD4^+$ T-cell response following vaccination has previously been found to be a good predictor of long-term antibody persistence following influenza vaccination [19]. It has now been shown that after a single-dose of a candidate MF59-adjuvanted H5N1 vaccine, blood-derived $CXCR5^-$, $ICOS1^+$, $IL\text{-}21^+$ $CD4^+$ T-follicular helper (TFH) cells provide T-cell help and support differentiation of influenza-specific B cells. Identification of this subset offers the potential of providing a predictive measurement at an early time point of a subsequent response, for influenza and maybe other vaccines.

3.3. Harmonisation of assays

Immunoassays are an essential tool for influenza vaccine development and assessing the performance of vaccines in clinical trials. As such, it is critical that results are comparable between different laboratories. A recent study, performed in collaboration with the European Medicines Agency on pH1N1 clinical-trial sera found high inter-laboratory variation (as much as 5.8-fold differences) in HI and virus neutralization assays. This variability could be decreased by calibration against international standards [20]. The work to standardize antibody assays by the Consortium for the Standardization of Influenza Sero-epidemiology (CONSISE) [21] was reported and proposed as a model to further develop and standardize cellular assays. The European Union-funded UNISEC project [22] also includes harmonisation of cellular assays.

4. New approaches in vaccine design

Renewed interest in influenza vaccines that generate longer lasting and a more broadly cross-protective immune response has resulted in a surge of research efforts in this area in recent years. Several promising candidate vaccines have progressed into clinical evaluation, with many more in preclinical development. Priority efforts include continuing to support the advancement of as many new approaches as possible, the development and availability of standardized non-HA based immunological assays, understanding the value and limitations of the human challenge model in the assessment of candidates for further advancement, defining benchmarks for improvements over existing vaccines, and engaging regulatory agencies in early discussions on how study endpoints for “broadly cross reactive” and “increased duration of protection” can be defined to help guide licensure efforts (Table 1).

4.1. Targeting antibodies to conserved epitopes

Strategies are being developed to target antibody responses away from the immunodominant but variable epitopes of HA, towards non-variable epitopes on this protein. The stalk domain of HA is immunologically subdominant to the head domain and antibodies induced to this domain are conformation-dependent, neutralizing and cross-reactive (as reviewed by WHO previously [3]). In humans, anti-HA-stalk antibodies are generally detectable after infection but generally are not induced by vaccination. Novel strategies will be required to induce anti-HA-stalk antibodies, such as sequential immunization with different chimeric HAs (cHAs) that have the stalk domain of one virus combined with the globular head of another (usually “exotic” avian) virus. H1N1-primed subjects, who were subsequently vaccinated with an H5N1-vaccine, generated high titres of anti-HA-stalk antibodies which protected mice against lethal challenge in passive transfer experiments [23]. Antibodies to the stalk-

region within the HA2 domain have however, been shown to be associated with enhanced respiratory disease in pigs following challenge with a mismatched influenza strain [24]. More studies are needed to determine whether fusion- and disease-enhancement is a common property of anti-stalk antibodies and if this applies to humans. In parallel studies done with NA, a highly conserved, functionally important linear epitope and a single monoclonal antibody that recognizes this region in all nine NA subtypes has been identified. This monoclonal antibody can protect mice against lethal infection [25].

The Computationally Optimized Broadly-Reactive Antigen (COBRA) approach focuses on the globular head rather than the stalk of HA. This strategy aims to identify HA1 consensus sequences that are combined in a rationally engineered HA construct to induce broadly cross-reactive antibodies to the globular head of the HA [26].

COBRA and chimeric haemagglutinins will need to be incorporated into a delivery platform such as a subunit vaccine or virus-like-particle (VLP), or expressed in a LAIV or other live vector. Monovalent formulations based on these approaches might not induce immunity against all types of influenza virus; they may however, in the nearer term, be able to be integrated into seasonal vaccination strategies to provide greater breadth and duration of protection.

4.2. Viral vectors and induction of cellular responses

Surface and internal influenza antigens are also being expressed in viral vectors, such as adenovirus type 4 (Ad4) [27] and AdHu5 [28], both for oral use, and ChAdOx1 [29] and MVA [30], both for parenteral use. Some of the clinical studies with these vectors indicate that co-administration or a boost with an inactivated influenza vaccine is needed to induce strong antibody responses. Additional candidates, including lactobacillus vector-based vaccines are also in pre-clinical development for mucosal administration [31].

Researchers are developing live, replication-incompetent influenza vaccines as a means to mimic natural influenza infection and stimulate immune responses without concomitant virus replication and transmission. Examples include M2 (Flugen, Madison, WI, USA, [32]) and PB2 protein knock-out viruses [33]. Another group (BiondVax Pharmaceuticals, Ness Ziona, Israel) is developing a candidate vaccine consisting of a string of conserved B- and T-cell epitopes expressed as a single recombinant protein to induce humoral and cellular immune responses [34].

5. New vaccine production and administration approaches

New production platforms, adjuvants, and delivery routes are being explored either to avoid use of eggs, to speed up vaccine production in the event of a pandemic, or to increase immunogenicity. These approaches include LAIVs, cell-based vaccine manufacturing, and the use of vaccine adjuvants (Table 1).

5.1. Live-attenuated influenza vaccines

LAIVs offer a number of advantages compared with inactivated influenza vaccines including more rapid production, high manufacturing yield, and also ease of administration via the

intranasal route [35]. Recent data suggest that an apparently poorly immunogenic pre-pandemic H5N1 LAIV was an effective prime for subsequent boosting with a homologous inactivated subvirion vaccine, resulting in antibodies that cross-reacted across several H5N1 clades [36]. LAIVs are regarded as a promising and useful technology, and their further development was recommended by the meeting participants.

5.2. Adjuvants

Inactivated vaccines of some influenza subtypes, especially H5 and H7, need adjuvants to induce protective antibody titres. Effects vary between adjuvant formulations, depending on whether the adjuvant is used for priming or boosting doses or both, and between target populations. The association of some AS03-adjuvanted pH1N1 influenza vaccines with narcolepsy in children in Europe, at very low but important incidences, was only identified after licensure [37]. Risk/benefit ratios change during a pandemic, when there is usually a higher mortality than during seasonal epidemics. Whether the additional risks of adding an adjuvant will be acceptable for seasonal influenza vaccines will continue to be discussed, especially by national regulators.

5.3. Cell-based flu-vaccine manufacturing

Cell-based manufacturing of influenza vaccines has several theoretical advantages compared with egg-dependent production [38]. For example, there is no selective pressure during manufacturing for mutations that favour growth in eggs, which in turn might alter the antigenicity and potentially lower the effectiveness of vaccines [39]. As part of its pandemic preparedness strategy, the USA has invested over US$1 billion in cell-based influenza-vaccine production [40]. Despite this, several companies seem to be withdrawing from cell-based manufacture of influenza vaccines. The vast majority of on-going clinical trials for seasonal and pandemic influenza vaccines, live and inactivated, use egg-grown rather than cell culture produced vaccine [41], suggesting that although cell-based production offers theoretical advantages over egg-based production, the technical, regulatory and financial barriers are significant.

5.4. Recombinant protein-based manufacturing processes

Several influenza vaccines are being manufactured as recombinant antigens. One baculovirus-produced, HA-only, unadjuvanted, seasonal influenza vaccine (FluBlok, Protein Sciences Corporation) has been licensed, [42]. This vaccine has the same mode of action and CoPs as “traditional” seasonal influenza vaccines; nevertheless, it took 17 years to develop and obtain FDA approval. Highly novel influenza vaccines might therefore take even longer.

A number of VLP vaccines are also in development including H1 and H5 [43], and seasonal quadrivalent [44] HA VLPs produced in tobacco plants. Baculovirus-produced VLPs consisting of HA, N and M1 proteins are also being evaluated [45]. Two aims of these approaches are to shorten the interval from initial isolation of a pandemic strain to vaccine release and to avoid the dependency on eggs, which might not be available during an avian influenza pandemic.

5.5. Novel formulations for novel administration routes

Alternative routes of vaccine administration are being investigated. LAIVs are given intranasally as are the M2 and PB2 knock-out, replication-incompetent vaccines [32,33]. Investigators are using influenza as a model antigen to evaluate various formats of microneedle array skin patches [46]. Some of these skin patches might be antigen-sparing compared with intramuscular injection, which could be important in a pandemic when antigen-demand is high. The first microneedle arrays are likely to start phase I clinical trials in 2015.

Whole inactivated H5 influenza vaccine has been formulated into spray-dried, thermo-stable sublingual tablets [47], which might be attractive for pre-pandemic stockpiling. Aerosolised dry-vaccine particles for pulmonary delivery are also being developed [48]. Use of these administration routes aims to induce mucosal immune responses, similar to natural infection. Because influenza replicates locally and rapidly following infection, local immune responses might be more timely and effective at moderating symptoms compared with a systemic memory immune response.

5.6. Novel immunization schedules

Heterologous prime-boost regimens are being explored as vaccination strategies against the most intractable infectious diseases, especially where CMI is important for protection. Several groups propose developing prime-boost approaches for universal-like influenza vaccines [49]. Recent clinical studies priming with LAIVs (H5 or H7), followed by boosting with inactivated vaccines are just one example of this approach [36]. A DNA-vaccine prime (H5) has also been shown to improve the response to an H5 monovalent inactivated-vaccine boost [50]. These regimens might have utility in pandemic preparedness, and can also be used experimentally to probe for memory cells established by a priming dose to better understand responses to vaccination.

5.7. Animal and human models

A mouse model of influenza transmission has been established and used to demonstrate both protection from lethal disease and reduced transmission with a candidate (NP and M2) universal vaccine [51]. In general however, animal models of influenza virus infection, particularly in small rodents, are regarded as having limited predictive value for clinical effectiveness. A key issue is the difficulty simulating multiple exposures to influenza virus infections (and vaccines) experienced by humans over a lifetime.

The human challenge model provides an alternative experimental system for studying immune responses after infection and vaccination. The model attempts to approximate natural influenza virus infection; however it is limited by the route of challenge (intranasal only), the small number of influenza viruses qualified for human challenge studies and the number of facilities capable of conducting influenza challenge studies. It was agreed at the meeting that this model could be a useful tool, provided that well-validated protocols and well-characterized, recently circulating influenza viruses were available.

6. Programmatic issues and demand in low-resource countries

Recent epidemiological studies [52] have illustrated the complex seasonality of influenza in some sub-tropical and tropical countries. Some countries have no distinct seasonal peaks and other countries exhibit a variety of influenza-activity patterns. The twice-yearly production of seasonal influenza vaccine, which is typically purchased by high-resource, temperate countries, might not serve low-resource countries with sub-tropical and tropical cli-mates well. A longer-lasting vaccine offering broader protection might be more practical if used year-round.

6.1. Increasing vaccine demand in high-burden countries

Epidemiologic studies have shown that the burden of seasonal influenza is high in low-resource countries [53], but it can be challenging to increase use of influenza vaccines in these countries.

LAIVs are expected to be more effective than inactivated vaccines in influenza sero-negative children, and theoretically they would have a greater impact on severe influenza disease if they were used in children, possibly synchronized with receipt of routine vaccines. But at present they are not licensed for use in children younger than 2 years of age. PATH has sponsored studies in Senegal and Bangladesh [54–56], which will inform on safety and efficacy in children (aged 2–6 years) of a single dose of trivalent seasonal LAIV (based on the Leningrad backbone from the Serum Institute of India, Ltd.). Initial results from the trials are due in 2015, and could lead to further age de-escalation trials.

LAIV strains and production technology are being transferred from the Institute of Experimental Medicine (St Petersburg, Russia) to manufacturers in low-resource countries, and LAIVs have been licensed in India as a monovalent 2009 pandemic influenza vaccine [57] and now a trivalent seasonal influenza vaccine [58].

7. Vaccines against influenza a (H7N9)

In 2013, influenza A (H7N9) viruses emerged as a new pandemic threat affecting all ages in China, but especially older adults [59]. Unfortunately, H7N9 virus infections are asymptomatic in birds, making stamping out strategies in domestic birds impossible; however, H7N9 disease is often severe in humans, especially in those aged over 60 years. This pattern of asymptomatic infection in birds but severe illness in humans is different from H7 strains found previously in humans and animals [60]. Some cases have a lung-focused “cytokine storm” pathology, and most cases require intensive care support [61]. In common with many other potentially pandemic strains, H7N9 is intrinsically poorly immunogenic.

An H7N9 LAIV candidate (Ann Arbor backbone) was derived by reverse genetics and optimized to have a high-growth phenotype. This candidate is now in preclinical development with positive immunogenicity data seen in a ferret model [62]. An H7N3 LAIV (Leningrad backbone) has completed a phase 1 trial and was found to generate cross-reactive antibodies with H7N9 [63,64].

Several inactivated H7N9 vaccines have now been evaluated in clinical trials, and these studies indicate that, as with H5N1 vaccines, adjuvants are required to generate high HI titres. New approaches are being explored for rapid production and improved immunogenicity of influenza vaccines. One uses synthetic biology, cell-based production and MF59 adjuvant [65,66]. Another employs baculovirus-produced HA with ISCOMATRIX adjuvant [45].

The H7 HA stalk shares conserved epitopes with the H3 HA stalk, to which most humans have been primed. H7 vaccination (or infection) might therefore, boost anti-stalk antibodies, providing information on whether these antibodies can play a role in protection [67]. Initial data show that H7N1 vaccination of humans induced antibodies to the HA head and conserved stalk domains. These antibodies improved clearance of H7N9 virus in a mouse challenge model [68].

8. Issues facing development of broadly protective influenza vaccines

Several international agencies are funding or plan to fund research to develop, or support the development of, broadly protective influenza vaccines. The cost of taking a seasonal vaccine from preclinical development in 2011 to licensure (in 2022) has been estimated to be $337–570 M Canadian dollars [69], and typically requires 1–5 phase I trials, 1–12 phase II and 2–4 phase III studies.

The overall costs of developing novel universal vaccines are likely to be even greater, but the costs associated with the early stages of development, including preclinical and clinical evaluation are significantly less compared to later stage or “advanced development”, and numerous funders are actively engaged in supporting these efforts. Improved assays and animal models are needed to “down select” those candidates that show the most promise, before they enter the more expensive later stages of development.

There are commercial challenges facing the development of new influenza vaccines, including defining how much better they need to be in order to justify investment. For clinical testing of novel influenza vaccines, there are issues surrounding choice of trial sites and comparator vaccines. Trials with an unadjuvanted, quadrivalent inactivated vaccine in children from eight countries [70], have shown that it is possible to perform large phase III clinical studies in resource-limited settings. However, for scientific and ethical reasons, such studies might have to be conducted only in countries where there is no recommendation for seasonal influenza vaccine use.

Since 2009, there have been changes in the regulation of influenza vaccines in the European Union, and long-standing guidelines for the approval of seasonal vaccines are being withdrawn. New guidance for quality aspects of traditional influenza vaccines has just been issued [71] whilst draft guidelines for non-clinical and clinical aspects were due to be available for comment later in 2014. There are regulatory challenges facing the development of novel, universal and universal-like influenza vaccines, but in general they are similar to those of any new vaccines. The willingness of regulators to support development and testing of completely novel influenza vaccines, at least to phase I, was emphasized.

9. Conclusions

This meeting report describes the presentations and discussion from the second WHO integrated meeting on development and clinical trials of influenza vaccines that induce broadly protective and long-lasting immune responses. The final versions of meeting agenda, list of participants, and presentations are available via the WHO website [72].

Several different HA-based vaccines that are safe and moderately effective against influenza are available worldwide, but they require annual revaccination. Universal or universal-like influenza vaccines that elicit broader cross-protective antibody responses and longer-lasting protective responses would obviate the need for annual updates of vaccine formulations.

At this meeting, there was much discussion, but no resolution, regarding whether incremental changes were preferable to “game-changer” strategies as approaches to developing universal or universal-like influenza vaccines. Some participants felt that it was only worth investing in approaches that change completely how we think about and administer influenza vaccines. Others would invest in technologies that “simply” provide longer and/or broader protection than current vaccines.

The development of either truly universal or universal-like vaccines against influenza is challenging, and the meeting participants were not optimistic that the Global Vaccine Action plan goal of at least one licensed universal influenza vaccine by 2020 [4] will be achieved. Nevertheless, participants were encouraged that the current pipeline has more than 25 vaccine candidates, many in or advancing to clinical evaluation. These near-term efforts coupled with continuing scientific advances in optimizing vaccine antigens, delivery platforms, manufacturing, and the development of tools to measure the diversity of immune responses is likely to lead to improvements in seasonal influenza vaccines in the near term.

The sub-optimal performance of the seasonal H3N2 vaccine during the 2014–2015 influenza season due to antigenic drift highlights the need for seasonal vaccines with a greater breadth of protection. The recent zoonotic human infections by avian influenza viruses such as H5N6, H5N1, H10N8 and most notably H7N9 are also reminders of the ever-present threat of pandemics and the need for more effective vaccines to counter them. Thus supporting research towards the development of increasingly universal influenza vaccines is of great importance for public health.

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Abbreviations:

ADCC	antibody-dependent cell-mediated cytotoxicity
CMI	cell-mediated immunity
COBRA	computationally optimized broadly reactive antigen

CoP	immune correlate of protection
HA	influenza haemagglutinin protein
HI	haemagglutination inhibition
IFN	interferon
IgA	immunoglobulin A
LAIV	live attenuated influenza vaccines
M1	influenza matrix protein 1
M2e	ectodomain of influenza matrix protein 2
MVA	modified vaccinia Ankara
NA	influenza neuraminidase protein
NHP	non-human primates
NP	influenza nucleoprotein
PB1	polymerase basic 1 protein
PH1N1	2009 influenza pandemic virus
TFH	T-follicular helper cells
TIV	trivalent influenza vaccines
VLP	virus-like-particle
WHO	World Health Organization

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Table 1

Research and development approaches to produce influenza vaccines that generate longer lasting and a more broadly cross-protective immune response.

Design approach	Example (article section)	Description
New approaches in vaccine design	Targeting antibodies to conserved epitopes (4.1)	• Vaccines that induce anti-HA-stalk antibodies via sequential immunization with different chimeric HAs. • Vaccines against a highly conserved linear NA epitope • Rationally engineered accines targeting consensus sequences in the HA globular head
	Viral vectors and induction of cellular responses (4.2)	• Vaccines using viral vectors that express surface and internal influenza antigens • Live, replication-incompetent influenza vaccines to mimic natural influenza infection • Vaccines consisting of a string of conserved B- and T-cell epitopes expressed as a single recombinant protein to induce humoral and cellular immune responses
New approaches in vaccine production	Live-Attenuated Influenza Vaccines (5.1)	• LAIVs used alone or as part of prime-boost strategies with inactivated influenza vaccines may produce cross-reactive anti-influenza antibodies
	Adjuvants (5.2)	• Inactivated vaccines of some influenza subtypes need adjuvants to induce protective antibody titres
	Cell-based flu-vaccine manufacturing (5.3)	• Cell-based manufacturing of influenza vaccines has several theoretical advantages compared with egg-dependent production, however the technical, regulatory and financial barriers remain
	Recombinant protein-based manufacturing processes (5.4)	• Recombinant antigen and virus-like particle influenza vaccines can shorten the interval from initial isolation of virus strain to vaccine release and avoid the dependency on eggs for culture
	Novel formulations for novel administration routes (5.5)	• Influenza vaccines administered via microneedle array skin patches might be antigen sparing • Mucosal immune responses may be induced via spray-dried, thermo-stable sublingual tablet vaccines as well as by aerosolised dry-vaccine particles for pulmonary delivery
New approaches in vaccine administration	Novel immunization schedules (5.6) Animal and human models (5.7)	• Heterologous prime-boost approaches for universal-like influenza vaccines might have utility in pandemic preparedness • Animal and human challenge models could be a useful tools for influenza vaccine evaluation, provided that well-validated protocols and well-characterized, recently circulating influenza viruses were available