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Review Article

Universal Vaccines Against Influenza Viruses: Overview of the Past, Present, and Prospective

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Abstract

Influenza is a common disease caused by influenza virus infections. There are an estimated 3 to 5 million annual cases of severe illness and 290 000 to 650 000 respiratory deaths caused by influenza viruses worldwide. Although antiviral drugs are available to treat influenza, vaccination remains the best infection prevention modality. However, current influenza vaccines provide a narrow range of protection and limited efficacy against seasonal and pandemic virus strains. Due to these limitations, novel vaccines that bestow broad protection and demonstrate a high level of efficacy against seasonal and pandemic viruses are desperately needed. The development of several universal influenza vaccines which target conserved epitopes such as the hemagglutinin stalk domain, neuraminidase, and the matrix 2 proton channel have made significant strides in this field. This article provides an overview of promising universal influenza virus vaccine designs, as well as current universal influenza vaccine clinical trials.

Keywords: Influenza virus, Universal vaccine, Seasonal vaccine, Hemagglutinin, Neuraminidase, Matrix protein

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Classification of influenza viruses

Influenza viruses are enveloped RNA viruses that are divided into four different groups: A, B, C, and D, based on their antigenic similarity (1). Both group A and B influenza viruses cause yearly seasonal epidemics. Group A viruses are the only viral class known to have caused pandemics thus far (2). Transmission in humans occurs in three ways: direct contact with an infected person, through fomites, or by inhaling aerosolized infectious particles (3). Group C influenza viruses cause mild infections in humans, but do not contribute to the seasonal epidemics (4). Group D influenza viruses primarily infect cattle, and currently evidence demonstrates that these viruses are not able to infect humans (5). Group A viruses are characterized based on the subtypes of the two major surface proteins expressed: hemagglutinin (HA) and neuraminidase (NA). For example, H3N2 viruses express HA subtype 3 and NA subtype 2. In contrast, group B viruses are divided into two lineages: Yamagata-like and Victoria-like based on their sequence similarity to the ancestral B/Yamagata/16/88 or B/Victoria/2/87 strains, respectively (6). Because of their clinical relevance, the remainder of this review will focus on human influenza A and B viruses.

Influenza viruses have a negative sense, single-stranded RNA genome consisting of 8 segments. These segments encode one or more viral proteins. In influenza A viruses, RNA segment 4 encodes HA, while RNA segment 6 encodes NA (7). Due to the lack of proofreading function by the viral RNA-dependent RNA polymerase, influenza virus genome is prone to mutations, causing the virus to mutate on average one nucleotide per genome per infectious cycle, which requires only 6 hours for completion (8–11). If mutations result in amino acid substitutions in either HA or NA, binding capacity of pre-existing antibodies may be diminished leading to decreased viral recognition by the host. These viruses tend to have a selective advantage and become the dominant circulating strain in a process referred to as “antigenic drift” (12). Due to the segmented nature of the influenza virus genome, co-infection of a single cell with multiple different strains of influenza viruses may cause the emergence of reassortant viruses. These reassortant viruses arise when the segmented genomes of multiple viruses undergo recombination in progeny - a process called “antigenic shift” (13). This reassortment may result in novel viruses that are well-adapted for infection and transmission in humans, but contain significantly altered glycoproteins that the majority of the human population have not previously encountered (13). Thus, antigenic drift typically results in seasonal epidemic strains, while antigenic shift is responsible for pandemic influenza virus strains capable of causing global pandemics (13).

Influenza virus surface proteins and antigens

Influenza virus particles consist of a lipid membrane that is studded with viral surface proteins, including the aforementioned HA and NA. HA is also one of the main antigenic targets for protective antibodies generated against the virus following infection or vaccination. HA is composed of head and stalk domains; the globular head is connected to the viral membrane by the stalk (14). The function of the HA head domain is to bind to sialic acid on host cells and initiate infection, while the HA stalk domain undergoes complex conformational changes to mediate the

viral fusion process within the cell (14) (Figure 1). HA also undergoes enzymatic cleavage by host proteases to reach its active conformation, and thus, allowing progeny virus to become infectious (15). Most antibodies raised by seasonal influenza vaccines are directed towards the HA head domain. Because the HA head domain is distal to the virus particle, this region is easily accessible by the immune system. However, this domain is also prone to mutation and thus is highly variable between different strains of influenza virus (16). In contrast, the HA stalk undergoes fewer genetic changes. This is perhaps due to decreased immunological selective pressure, and the need for sequence conservation to allow for complex conformational changes; necessary for the membrane fusion process (16). Antibodies targeting the head domain are therefore more likely to be strain-specific, whereas anti-stalk antibodies recognize multiple different strains of influenza virus.

Other proteins embedded in the membrane of influenza viruses include NA and the matrix-2 (M2) proton channel. NA is an enzyme that cleaves terminal sialic acid residues from glycoproteins. This process is critical to release nascent virus particles, as HA remains bound to sialic acid residues during budding (Figure 1) (17). Other functions of NA include cleavage of mucins to allow the virus particles to access target cells in the respiratory tract, and binding to receptors on host cells to mediate endocytosis and internalization (17). While the virion is trapped in the endosome following receptor-mediated endocytosis, the M2 proton channel acidifies the interior of the virus capsid (18). The decrease in pH due to endosome acidification, mediates dissociation of the viral genome from the viral capsid, allowing for the release of the ribonucleoproteins into the cytosol after membrane fusion (19) (Figure 1).

Vaccination and antiviral therapies to prevent and treat influenza in Canada

In Canada, antiviral therapy is recommended for individuals belonging to groups with high risk of complications, such as adults 65 years of age and older, pregnant women, and individuals with severe or complicated influenza who require hospital admission or demonstrate severe symptoms. Additionally, it is used to treat or prevent influenza outbreaks in institutional settings (20). NA inhibitors, which primarily prevent influenza virus egress and budding from infected cells, are the only class of antiviral drugs approved for use in Canada (21). NA inhibitors include oral oseltamivir (Tamiflu), inhaled zanamivir (Relenza), and intravenous peramivir (Rapivab). Since 2006, amantadine and rimantadine, which are M2 proton channel antagonists, are no longer recommended due to widespread resistance in clinical isolates (22). A selective cap-dependent endonuclease inhibitor, Baloxivir Marboxil (Xofluza) was recently approved in the United States (but not Canada) for the treatment of acute uncomplicated influenza in individuals 12 years and older, or those with high risk of complications (23,24). NA inhibitors and cap-dependent endonuclease inhibitors are similarly effective at alleviating influenza symptoms approximately 24 hours sooner compared to placebo when administered within 48 hours of symptom onset (23,25–27). Although antivirals are somewhat effective at both treating and preventing influenza, these drugs can cause considerable side effects, such as nausea, vomiting, and diarrhea. Similar to

antibacterial drugs, antiviral medications can also be rendered ineffective by the emergence of resistant strains of influenza virus (21,26).

Seasonal influenza vaccination is currently the best way to prevent influenza viral infections. Several formulations of influenza virus vaccines are clinically approved for use in Canada. These formulations differ based on the following four variables: number of strains, effective dosage, method of virus inactivation, and the inclusion of an adjuvant (29). The seasonal influenza vaccine includes three or four strains of influenza viruses as recommended by the World Health Organization (WHO) approximately 6 months prior to the beginning of the flu season. In the 2019-2020 season, trivalent vaccines contain an H1N1 (A/Brisbane/02/2018-like), H3N2 (A/Kansas/14/2017-like), and a Victoria-like (B/Colorado/06/2017-like) strain, while quadrivalent vaccines contain the aforementioned trivalent strains along with an additional Yamagata-like (B/Phuket/3073/2013-like) strain (30). Quadrivalent vaccines are generally recommended over trivalent vaccines, if both are available, due to the broader range of protection offered (29). High dose vaccines contain four times the amount of antigen compared to their standard dose counterparts, and are reserved for those who are above the age of (29,31).

The viruses found within vaccines can be inactivated or attenuated in one of three ways. In split vaccines, viruses are disrupted by a detergent, while subunit vaccines are further processed to purify the antigens of interest, largely HA and NA (32). Live attenuated vaccines are composed of viruses that are adapted to replicate at a lower temperature (25˚C) and therefore have decreased virulence in humans. Live attenuated vaccines are reserved for children 2-17 years in the form of a nasal spray, while all other vaccines are delivered intramuscularly (29). Adjuvanted vaccines are made with MF59, a proprietary adjuvant that uses squalene, a long hydrophobic molecule, to form an oil-in-water emulsion. This allows for the elicitation of stronger immune responses to vaccination (33). Adjuvanted vaccines are available for children 6-23 months and adults 65 years and older; as a means to improve immunogenicity in those who typically respond poorly to vaccination attributing to an immature immune system or immunosenescence (29).

The majority of seasonal influenza viral vaccines are manufactured using embryonated chicken eggs (34). Although this method has been used for over 50 years there are several major drawbacks. For example, in order to produce high titers of the candidate vaccine strain, viruses used in seasonal vaccines must be adapted to grow in chicken eggs (35). This process lengthens the production time of influenza virus vaccines relative to cell-based vaccine production (35). In turn, longer production times reduce the flexibility of manufacturing, necessitating that vaccine production begins long before the influenza season commences (34). Therefore, vaccine developers cannot alter vaccine formulations in response to the most recent mutations in circulating strains that occur after WHO recommendations have been made (34). This inability to adapt virus strains can result in further delays in vaccine production due to the low yield (36). Additionally, some virus strains, especially H3N2, grow poorly in eggs (37). Furthermore, some egg-based adaptations that occur during the manufacturing process may cause epitope mutations, resulting in poor vaccine efficacy due to inadequate congruency between the circulating and vaccine strains (36). With that being said, several shortcomings of egg-based vaccine production

can be rectified through cell-based vaccine production. Cell-based vaccine production can be scaled up more quickly. In addition the viruses produced with this method are more similar to the seed strain, reducing the emergence of antigenic variants that can arise as a result of egg-based adaptations (36). Despite the advantages of cell-based production compared to egg-based production, the latter continues to represent the majority of the influenza vaccine market due its cost effectiveness (36).

While seasonal influenza vaccination is the best preventative measure against influenza virus infections currently, the protection provided is often transient and ineffective in subsequent influenza seasons. This is largely due to the aforementioned antigenic shift and antigenic drift (38). As a result, seasonal influenza virus vaccines must be reformulated and re-administered yearly for optimal protection against "drifted" strains. In addition, pandemic strains that arise due to antigenic shift render any seasonal vaccines ineffective (38). The strain-specific nature of antibodies raised in seasonal vaccines highlights the need to develop vaccine platforms that offer both prolonged and more broad protection. Recent developments in the field of universal influenza vaccines show great potential in addressing these needs. These universal formulas do not require yearly reformulation due to their ability to elicit antibodies that target numerous influenza viral strains. One especially promising formulation involves generating immune responses targeting the HA stalk domain.

Universal influenza vaccines targeting the HA stalk domain

Seasonal influenza virus vaccination elicits the production of HA stalk binding antibodies that can neutralize multiple strains of influenza virus (39,40). These antibodies are termed broadly neutralizing antibodies (bNAbs) (40). bNAbs provide protection *in vivo* largely by activating immune effector cell functions such as antibody dependent cellular cytotoxicity (ADCC) and antibody dependent cellular phagocytosis (ADCP) (41,42). Additionally, bNAbs are capable of providing protection by blocking fusion of the viral envelope with the endosomal membrane, and preventing proteolytic activation of the HA protein (43). By targeting conserved epitopes found within the HA stalk domain, bNAbs elicited by universal influenza vaccines are capable of providing protection against diverse influenza virus strains and subtypes (44).

Although the broad range of protection granted by bNAbs is clearly superior to the narrow range of protection offered by seasonal vaccines, bNAbs have notable limitations. For example, bNAbs have reduced neutralization potency compared to HA head binding antibodies (45). In microneutralization assays, multi-log differences in neutralization potency in favour of HA head binding antibodies over bNAbs were present when both were compared in a monoclonal context (45). However, when neutralization potency was measured in a polyclonal context, the difference in potency was reduced from a multi-log difference to only a 3-fold difference in favour of HA head binding antibodies. This ultimately suggests that the interactions between antibodies present in polyclonal serum of influenza-exposed adults boosts the neutralization efficacy of bNAbs (45).

Seasonal vaccines and infections do not typically elicit high titers of stalk-binding antibodies (46). This is because the HA head domain sterically shields the stalk from being

immunologically accessible, making it more challenging for bNAbs to be produced (47). Fewer antibodies are also produced against the stalk domain due to high affinity interactions restricted to select few immunoglobulin genes (specifically, VH1-69 and VH1-18) (47). This lowers the frequency of precursor B cells capable of making stalk antibodies. Lastly, bNAbs tend to be more autoreactive, potentially causing peripheral tolerance mechanisms to inhibit the expansion of stalk-specific B cells (48). Fortunately, it has been well documented that vaccination with or exposure to pandemic-like influenza virus strains can lead to an immune response that preferentially produces bNAbs over HA head binding antibodies (Figure 2) (49–52). This phenomenon has been recapitulated using sequential vaccination with viruses expressing chimeric HA (cHA) (53,54). Researchers have shown that sequential immunization of mice with cHA vaccine constructs induced bNAbs conferring protection against both group 1 and group 2 influenza A viruses (53,54). cHA constructs feature an HA head domain derived from an exotic influenza subtype not previously exposed to the human population and the HA stalk domain of a circulating influenza virus subtype (49,55). cHA vaccines result in primary immune responses towards both the HA head and stalk (49). Subsequent vaccinations use different cHA constructs with a homologous stalk domain from the first vaccination, but utilize radically different HA head domains (55). In doing so, the immune response shifts from the previously immunodominant HA head domain towards the stalk domain. cHA vaccines represent an effective method in redirecting immune responses towards the HA stalk domain to boost bNAb titers.

An alternative method to induce high titers of stalk binding bNAbs is to use recombinant HA proteins that lack the head domain (56). Unfortunately, removal of the HA head domain destabilizes the protein and damages the neutralizing epitopes on the stalk domain (56). To improve stability of the constructs, two research groups experimented with the addition of leucine zipper motifs onto the HA stalk domains (57,58). Yassine *et al*. designed their recombinant HA stalk protein around the HA ectodomain of the virus A/New Caledonia/20/1999 and used a ferritin nanoparticle antigen-display platform to create the vaccine (58). This vaccine stimulated both anti-stalk antibody production and provided protection against lethal influenza virus challenge in mice and ferrets (58). Similarly, Impagliazzo *et al*. based their “mini-HA” recombinant stalk domain around the HA sequence of A/Brisbane/59/2007 (57). This soluble protein stimulated high titers of broadly-reactive anti-HA antibodies in non-human primates, and protected mice from lethal influenza virus challenge (57).

Additional strategies to create universal influenza vaccines

The other major surface glycoprotein, NA, represents another promising candidate for universal influenza vaccines (59). Naturally acquired NA inhibiting (NAI+) antibodies protect against influenza infection, and NAI+ antibody titers positively correlate with vaccine effectiveness in both live attenuated and inactivated vaccines (60). NAI+ antibodies can function during the later stages of the viral life cycle relative to HA-neutralizing antibodies by mitigating viral infection through the prevention of viral budding from infected cells (Figure 1) (59). Lastly, NA contains contiguous antigenic domains; monoclonal antibodies against NA can recognize antigenic

domains conserved between virus strains. This allows for cross reactivity of antibodies between viruses possessing the same NA subtype (61). Identification of these conserved epitopes in NA make it an intriguing target as a prospective universal influenza vaccine.

M2 is a transmembrane, homotetrameric proton ion channel involved in viral uncoating following cell entry and in the formation and budding of virus progeny (40,62,63). The extracellular domain of the M2 protein, M2e, is a highly conserved region in all influenza A viruses and, therefore, is a potential target for a universal influenza vaccine (63). The conservation found in M2e is due to its low immune reactivity, which translates to low selective pressure (63). M2e-specific antibodies, such as 14C2, provide protection by reducing the expression level of M2, which in turn inhibits formation of new viral particles and limits viral spread (63). Despite this, M2 itself is a very poor immunogen due to its small extracellular domain, membrane proximity, and relatively low abundance on the viral surface compared to HA and NA (64). Despite its theoretical ability to offer protection against many influenza A viruses, the aforementioned reasons pose significant barriers that any potential M2-based vaccines would have to overcome.

Targeting T cell immunity serves as another potential method of universal influenza virus protection. Studies have shown that T cells mitigate the severity of influenza related illnesses and reduce viral shedding (65). Once infection has taken place, influenza virus-specific CD4+ T helper cells and CD8+ cytotoxic T cells are activated through the recognition of highly conserved epitopes found across influenza virus subtypes encoded in the viral nucleoprotein (NP) and matrix 1 protein (M1) (40). While CD8+ T cell mediated immunity is short lived, a vaccine capable of boosting the cross-reactive T cell responses towards these conserved antigens possesses the potential to provide a broad range of protection against influenza (65). A modified vaccinia virus Ankara (MVA) vector, MVA-NP+M1, expresses the conserved influenza antigens NP and M1 and may serve as a candidate universal influenza vaccine that boosts existing cross-reactive T cell response to these conserved internal antigens (66). T cell mediated immunity should provide a broader range of protection in comparison to antibodies that target the highly variable external glycoproteins (66).

Current universal vaccine clinical trials

Several universal influenza vaccine candidates are currently being evaluated in clinical trials. Peptide vaccines have shown some success, with the “Multimeric-001” universal vaccine entering phase III in August 2018 (67). This vaccine is composed of a recombinant protein containing 9 conserved linear epitopes of influenza virus proteins: 6 from HA, 3 from nucleoprotein (NP), and 1 from matrix protein (M1) (68). The Multimeric-001 vaccine induces influenza-specific cellular responses, such as IL-2 and IFN-γ secretion by T cells (67–69). Other peptide-based vaccines include Flu-v, which is composed of four equimolar mixtures of four polypeptides in the M1, M2, NP, and PB1 regions of influenza virus, and FP-01.1, which is comprised of six peptide chains of NP, M1, PB1 and PB2 linked to an inert fluorocarbon chain to increase *in vivo* half-life (70,71). Peptide vaccines are a relatively new and targeted approach towards eliciting a lasting immune response against specific epitopes. Peptides are readily altered and manufactured, making it

possible for vaccine manufacturers to quickly adapt the formulation to match the circulating influenza virus strains. However, disadvantages including poor immunogenicity and stability must be overcome before these vaccines are viable alternative to conventional interventions.

Another class of vaccines currently being tested include M2e-based vaccines. Many M2e-based vaccines have been tested in the past, including VAX-102, which is composed of four M2e peptides linked to a toll like receptor 5 (TLR5) agonist to enhance the immune response (72). Although the vaccine induces high antibody levels against the M2e protein, it has been shown to cause considerable side effects such as fever, diarrhea, and fatigue at high doses (73,74). Currently, "Uniflu", another M2e-based vaccine, comprised of the M2e protein fused to the hepatitis B viral core antigen, is being evaluated in a phase I clinical trial (75). The high degree of conservation of the M2e across influenza A virus subtypes makes this an attractive target for a universal influenza vaccine (74). However, antibodies against M2e are unable to neutralize virus directly, and therefore largely rely on immune effector cell mediated cytotoxicity to protect against infection (76).

Additionally, MVA-NP+M1 vaccines have been, and are, currently being evaluated in clinical trials (66). As previously mentioned, MVA-NP+M1 vaccines utilize modified vaccinia virus Ankara to express a fusion protein of M1 and NP, which is used to boost the T cell response to conserved epitopes in these antigens (77). Phase I clinical trials have been conducted comparing the co-administration of seasonal influenza vaccine with the MVA-NP+M1 vaccine to administration of seasonal influenza vaccine solely in patients aged 50 and up (77). Results have indicated that co-administration was safe and tolerated in patients. In addition the T cell response to the conserved epitopes found on the internal antigens were boosted significantly in the group that received the MVA-NP+M1 vaccine when compared to the group that received the seasonal influenza vaccine alone (78). Currently, MVA-NP+M1 is undergoing a phase II clinical trial to assess its efficacy and immunogenicity as an adjunct to a standard, licensed dose of quadrivalent influenza vaccine in adults aged 18 and up (79).

Recently, the first HA stalk based universal vaccine was developed and tested. This vaccine strategy is based around sequential vaccination with inactivated viruses expressing cHA, where the stalk domains are from conserved H1 or H3, and the head domains are from influenza viruses not yet exposed to humans. In this clinical trial, H8 and H5 head domains were used with an H1 stalk to create chimeric H8/1 and H5/1 viruses (80). Interim results of the phase 1 clinical trials showed that H1 stalk-specific IgG antibodies were boosted approximately 5-fold over baseline after two doses of the chimeric H8/1 and H5/1 vaccines with AS03, which is another squalene based adjuvant (80). These anti-stalk antibodies were boosted approximately 2-fold in groups receiving a series of vaccines with the adjuvant when compared to unadjuvanted formulations (80). As expected, the antibodies induced by the vaccine were similarly reactive against the stalk domains of H2, H9, and H18 (80). Stalk-based universal vaccines provide great promise at inducing high levels of stalk-reactive antibodies. However, it remains unknown if antibodies at these titers are protective against infection, and if the auto-reactive tendency of HA stalk-binding antibodies will cause any adverse reactions, especially in those with autoimmune diseases.

Conclusion

Although seasonal vaccination is the current gold standard for protection against influenza, influenza related illnesses and mortality rates remain high, placing a significant strain on today's global healthcare systems. Drawbacks to seasonal vaccines include occasional ineffectiveness against yearly influenza epidemics, inability to provide protection against pandemic strains, cost-intensive and time-consuming yearly vaccine manufacturing process, and the propensity for egg-based adaptations to occur. The development of several universal influenza vaccines that target conserved influenza virus epitopes provide promise for a more effective and reliable method of influenza prevention. This would eliminate the need for yearly vaccine reformulation and potentially prevent future influenza pandemics. While great strides have been made in the field of universal influenza vaccines, further animal studies and validation of immunogenicity and efficacy in humans through clinical trials are warranted.

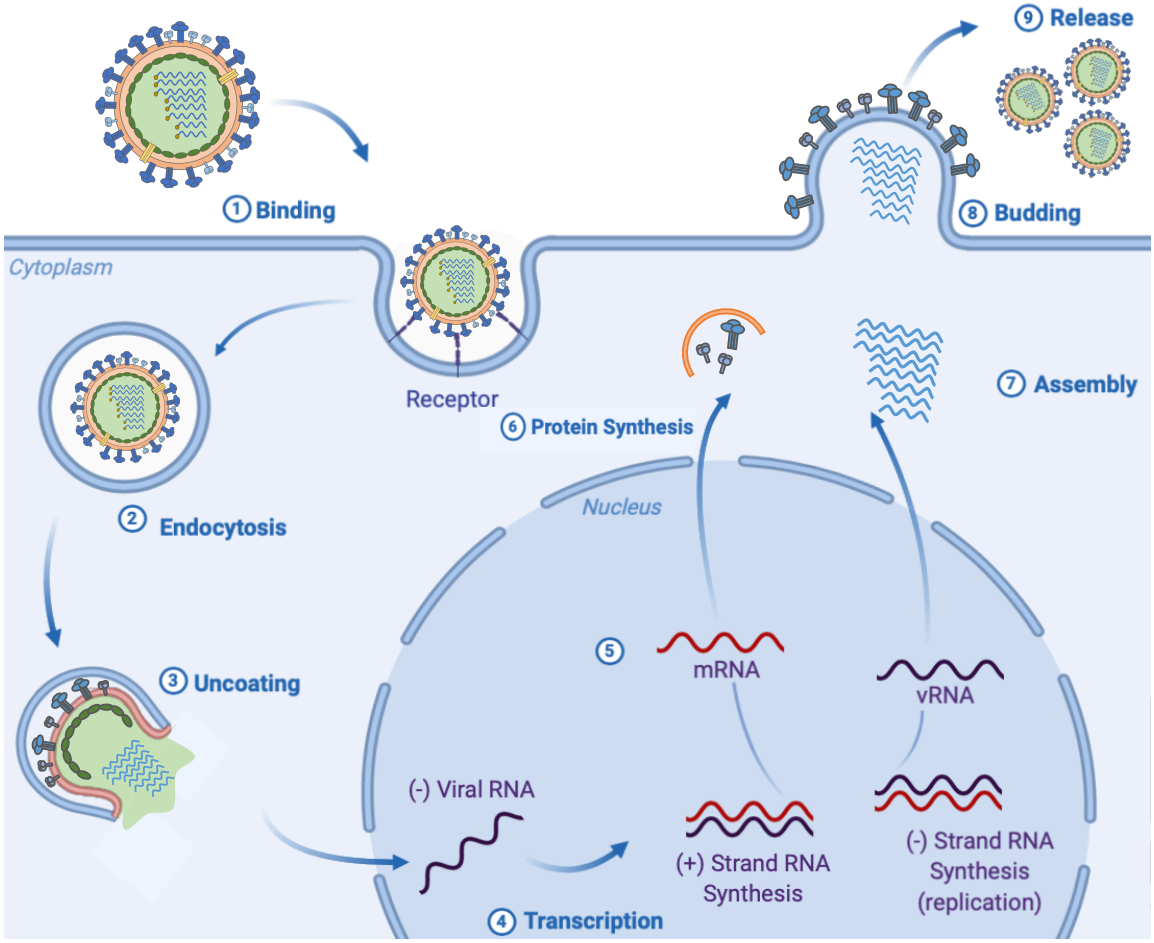

Figure 1 | The influenza virus life cycle begins with the attachment to host cells and ends with the release of viral progeny. The first stage of the viral life cycle, binding, is mediated by HA.

HA binds to α 2-6 sialic acids on host cells in the upper respiratory tract. Interaction with this glycan initiates fusion with the host cell plasma membrane. The virus enters the cell via endocytosis where the endosome containing the viral particle becomes acidified leading to the uncoating and release of the viral ribonucleoproteins (RNP) segments. Following RNP transport into the nucleus, replication of the viral genome begins. As influenza viruses are negative sense RNA viruses, positive-sense mRNA must first be transcribed for the generation of more viral RNPs. The replication of the viral RNA genome also occurs in the nucleus. The viral genome and proteins come together in the cytosol for assembly, which is followed by budding at the plasma membrane. The final stage of the viral life cycle, release, is facilitated by NA. NA cleaves the HA:sialic acid interactions to allow for the release of mature virions which in turn, infect other cells.

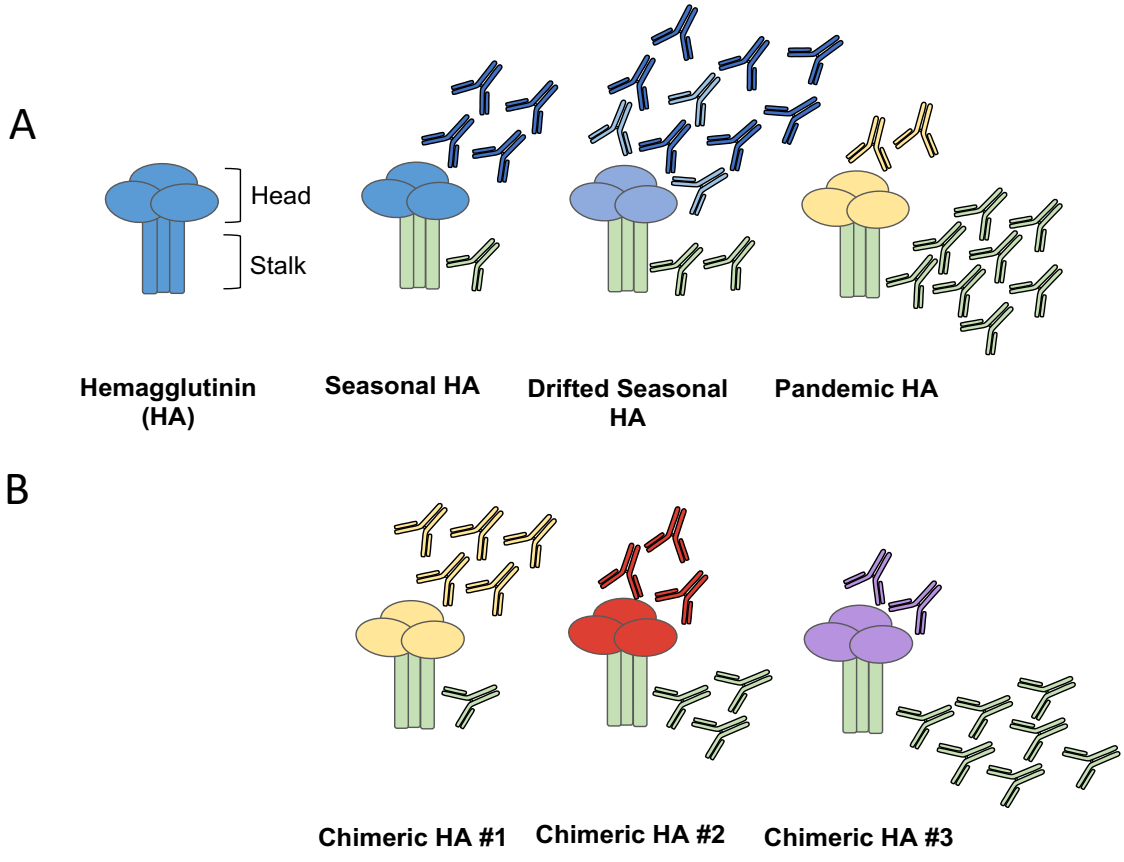

Figure 2 | The immune response to hemagglutinin can be redirected to the subdominant HA stalk following exposure to pandemic strains or sequential exposure to chimeric HA proteins. **(A)** HA is a surface glycoprotein composed of a highly variable head domain and a conserved,

membrane proximal stalk domain. When an individual is exposed to an influenza virus, either through infection or vaccination, the immune system generates an antibody response largely targeting the HA head domain. Antibodies directed to the stalk domain are also generated but to a lesser extent. Upon exposure to a drifted virus strain, antibodies targeting novel head epitopes are produced and antibodies that target conserved epitopes between the previous strain and new drifted variant are boosted. However, upon exposure to a divergent HA, as was the case in 2009 during the Swine flu pandemic, researchers observed that individuals who had been exposed to this pandemic strain had high titers of stalk-binding antibodies. This was attributed to the conserved nature of the HA stalk domain. **(B)** Using this principle, recombinant chimeric HA (cHA) proteins were generated and a sequential vaccination strategy was employed to boost stalk antibody titers. These cHA proteins contained conserved HA stalk domains, but HA heads from avian influenza virus strains to which the human population has no pre-existing immune memory. Following repeat exposures, the response was directed against the sub-dominant stalk domain and only a weak primary immune response against the HA head was observed, generating small titers of head-specific antibodies. This sequential vaccination strategy has been shown to effectively induce high titers of broadly-neutralizing stalk antibodies in a variety of animal models.

References

1. Jang YH, Seong BL. The Quest for a Truly Universal Influenza Vaccine. Front Cell Infect Microbiol. 2019 Oct 10;9:344.

2. Saunders-Hastings P, Krewski D. Reviewing the History of Pandemic Influenza: Understanding Patterns of Emergence and Transmission. Pathogens. 2016 Dec 6;5(4):66.

3. Bridges CB, Kuehnert MJ, Hall CB. Transmission of influenza: implications for control in health care settings. Clin Infect Dis Off Publ Infect Dis Soc Am. 2003 Oct 15;37(8):1094–101.

4. CDC. Types of Influenza Viruses [Internet]. Centers for Disease Control and Prevention. 2019 [cited 2019 Dec 18]. Available from: https://www.cdc.gov/flu/about/viruses/types.htm

5. Asha K, Kumar B. Emerging Influenza D Virus Threat: What We Know so Far! J Clin Med [Internet]. 2019 Feb 5 [cited 2019 Dec 18];8(2). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6406440/

6. Rota PA, Wallis TR, Harmon MW, Rota JS, Kendal AP, Nerome K. Cocirculation of two distinct evolutionary lineages of influenza type B virus since 1983. Virology. 1990 Mar;175(1):59–68.

7. Bouvier NM, Palese P. The Biology of Influenza Viruses. Vaccine. 2008 Sep 12;26(Suppl 4):D49–53.

8. Choi KH. Viral Polymerases. Adv Exp Med Biol. 2012;726:267–304.

9. Shao W, Li X, Goraya MU, Wang S, Chen J-L. Evolution of Influenza A Virus by Mutation and Re-Assortment. Int J Mol Sci [Internet]. 2017 Aug 7 [cited 2019 Dec 19];18(8). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5578040/

10. Parvin JD, Moscona A, Pan WT, Leider JM, Palese P. Measurement of the mutation rates of animal viruses: influenza A virus and poliovirus type 1. J Virol. 1986 Aug;59(2):377–83.

11. WHO/Europe. Virology of human influenza [Internet]. WHO/Europe. [cited 2020 Apr 13]. Available from: http://www.euro.who.int/en/health-topics/communicable-diseases/influenza/data-and-statistics/virology-of-human-influenza

12. Boni MF. Vaccination and antigenic drift in influenza. Vaccine. 2008 Jul;26:C8–14.

13. Webster RG, Govorkova EA. Continuing challenges in influenza. Ann N Y Acad Sci. 2014 Sep;1323(1):115–39.

14. Skehel JJ, Wiley DC. Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. Annu Rev Biochem. 2000;69:531–69.

15. Hamilton BS, Gludish DWJ, Whittaker GR. Cleavage Activation of the Human-Adapted Influenza Virus Subtypes by Matriptase Reveals both Subtype and Strain Specificities. J Virol. 2012 Oct;86(19):10579–86.

16. Kirkpatrick E, Qiu X, Wilson PC, Bahl J, Krammer F. The influenza virus hemagglutinin head evolves faster than the stalk domain. Sci Rep [Internet]. 2018 Jul 11 [cited 2019 Dec 25];8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6041311/

17. McAuley JL, Gilbertson BP, Trifkovic S, Brown LE, McKimm-Breschkin JL. Influenza Virus Neuraminidase Structure and Functions. Front Microbiol [Internet]. 2019 Jan 29 [cited 2019 Dec 21];10. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6362415/

18. Pielak RM, Chou JJ. Influenza M2 proton channels. Biochim Biophys Acta. 2011 Feb;1808(2):522–9.

19. Helenius A. Unpacking the incoming influenza virus. Cell. 1992 May 15;69(4):577–8.

20. Antiviral Medications for Seasonal Influenza: Information for Health Care Providers, 2019 [Internet]. Queen's Printer for Ontario; 2019. Available from: https://www.publichealthontario.ca/-/media/documents/qa-antiviral-medication-influenza.pdf?la=en

21. Moscona A. Neuraminidase Inhibitors for Influenza. N Engl J Med. 2005 Sep 29;353(13):1363–73.

22. Canada PHA of. Recommendation for Use of Amantadine for Treatment and Prevention of Influenza [Internet]. gcnws. 2006 [cited 2019 Dec 15]. Available from: https://www.canada.ca/en/news/archive/2006/11/recommendation-use-amantadine-treatment-prevention-influenza.html

23. Hayden FG, Sugaya N, Hirotsu N, Lee N, de Jong MD, Hurt AC, et al. Baloxavir Marboxil for Uncomplicated Influenza in Adults and Adolescents. N Engl J Med. 2018 06;379(10):913–23.

24. Influenza Antiviral Medications: Summary for Clinicians | CDC [Internet]. 2019 [cited 2019 Dec 15]. Available from: https://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm

25. Jefferson T, Jones M, Doshi P, Spencer EA, Onakpoya I, Heneghan CJ. Oseltamivir for influenza in adults and children: systematic review of clinical study reports and summary of regulatory comments. BMJ [Internet]. 2014 Apr 9 [cited 2019 Dec 15];348. Available from: https://www.bmj.com/content/348/bmj.g2545

26. Heneghan CJ, Onakpoya I, Thompson M, Spencer EA, Jones M, Jefferson T. Zanamivir for influenza in adults and children: systematic review of clinical study reports and summary of regulatory comments. BMJ [Internet]. 2014 Apr 9 [cited 2019 Dec 15];348. Available from: https://www.bmj.com/content/348/bmj.g2547

27. Kohno S, Kida H, Mizuguchi M, Shimada J, S-021812 Clinical Study Group. Efficacy and safety of intravenous peramivir for treatment of seasonal influenza virus infection. Antimicrob Agents Chemother. 2010 Nov;54(11):4568–74.

28. Baz M, Abed Y, Papenburg J, Bouhy X, Hamelin M-È, Boivin G. Emergence of Oseltamivir-Resistant Pandemic H1N1 Virus during Prophylaxis. N Engl J Med. 2009 Dec 3;361(23):2296–7.

29. Canada PHA of. Canadian Immunization Guide Chapter on Influenza and Statement on Seasonal Influenza Vaccine for 2019–2020 [Internet]. aem. 2019 [cited 2019 Dec 25]. Available from: https://www.canada.ca/en/public-health/services/publications/vaccines-immunization/canadian-immunization-guide-statement-seasonal-influenza-vaccine-2019-2020.html

30. WHO | Recommended composition of influenza virus vaccines for use in the 2019-2020 northern hemisphere influenza season [Internet]. WHO. [cited 2019 Dec 25]. Available from: http://www.who.int/influenza/vaccines/virus/recommendations/2019_20_north/en/

31. Keitel WA, Atmar RL, Cate TR, Petersen NJ, Greenberg SB, Ruben F, et al. Safety of high doses of influenza vaccine and effect on antibody responses in elderly persons. Arch Intern Med. 2006 May 22;166(10):1121–7.

32. Wong S-S, Webby RJ. Traditional and New Influenza Vaccines. Clin Microbiol Rev. 2013 Jul 1;26(3):476–92.

33. O'Hagan DT. MF59 is a safe and potent vaccine adjuvant that enhances protection against influenza virus infection. Expert Rev Vaccines. 2007 Oct;6(5):699–710.

34. Harding AT, Heaton NS. Efforts to improve the seasonal influenza vaccine. Vaccines. 2018;6(2).

35. Ping J, Lopes TJS, Neumann G, Kawaoka Y. Development of high-yield influenza B virus vaccine viruses. Proc Natl Acad Sci U S A. 2016;113(51):E8296–305.

36. Ping J, Lopes TJS, Nidom CA, Ghedin E, MacKen CA, Fitch A, et al. Development of high-yield influenza A virus vaccine viruses. Nat Commun. 2015;6:1–15.

37. Skowronski DM, Janjua NZ, De Serres G, Sabaiduc S, Eshaghi A, Dickinson JA, et al. Low 2012-13 influenza vaccine effectiveness associated with mutation in the egg-adapted H3N2 vaccine strain not antigenic drift in circulating viruses. PLoS ONE. 2014;9(3).

38. Wong SS, Webby RJ. Traditional and new influenza vaccines. Clin Microbiol Rev. 2013;26(3):476–92.

39. Sautto GA, Kirchenbaum GA, Ross TM. Towards a universal influenza vaccine: different approaches for one goal. Virol J. 2018 19;15(1):17.

40. Pica N, Palese P. Toward a universal influenza virus vaccine: prospects and challenges. Annu Rev Med. 2013;64:189–202.

41. DiLillo DJ, Palese P, Wilson PC, Ravetch JV. Broadly neutralizing anti-influenza antibodies require Fc receptor engagement for in vivo protection. J Clin Invest. 2016 Feb;126(2):605–10.

42. He W, Chen C-J, Mullarkey CE, Hamilton JR, Wong CK, Leon PE, et al. Alveolar macrophages are critical for broadly-reactive antibody-mediated protection against influenza A virus in mice. Nat Commun. 2017 10;8(1):846.

43. Brandenburg B, Koudstaal W, Goudsmit J, Klaren V, Tang C, Bujny M V., et al. Mechanisms of hemagglutinin targeted influenza virus neutralization. PLoS ONE. 2013;8(12).

44. Coughlan L, Palese P. Overcoming Barriers in the Path to a Universal Influenza Virus Vaccine. Cell Host Microbe. 2018;24(1):18–24.

45. He W, Mullarkey CE, Duty JA, Moran TM, Palese P, Miller MS. Broadly Neutralizing Anti-Influenza Virus Antibodies: Enhancement of Neutralizing Potency in Polyclonal Mixtures and IgA Backbones. J Virol. 2015;89(7):3610–8.

46. Krammer F, Palese P. Influenza virus hemagglutinin stalk-based antibodies and vaccines. Curr Opin Virol. 2013 Oct;3(5):521–30.

47. Neu KE, Henry Dunand CJ, Wilson PC. Heads, stalks and everything else: how can antibodies eradicate influenza as a human disease? Curr Opin Immunol. 2016;42:48–55.

48. Andrews SF, Huang Y, Kaur K, Popova LI, Ho IY, Pauli NT, et al. Immune history profoundly affects broadly protective B cell responses to influenza. Sci Transl Med. 2015 Dec 2;7(316):316ra192.

49. Pica N, Hai R, Krammer F, Wang TT, Maamary J, Eggink D, et al. Hemagglutinin stalk antibodies elicited by the 2009 pandemic influenza virus as a mechanism for the extinction of seasonal H1N1 viruses. Proc Natl Acad Sci U S A. 2012 Feb 14;109(7):2573–8.

50. Nachbagauer R, Salaun B, Stadlbauer D, Behzadi MA, Friel D, Rajabhathor A, et al. Pandemic influenza virus vaccines boost hemagglutinin stalk-specific antibody responses in primed adult and pediatric cohorts. Npj Vaccines. 2019 Dec;4(1):51.

51. Nachbagauer R, Wohlbold TJ, Hirsh A, Hai R, Sjursen H, Palese P, et al. Induction of Broadly Reactive Anti-Hemagglutinin Stalk Antibodies by an H5N1 Vaccine in Humans. J Virol. 2014 Nov 15;88(22):13260–8.

52. Ellebedy AH, Krammer F, Li G-M, Miller MS, Chiu C, Wrammert J, et al. Induction of broadly cross-reactive antibody responses to the influenza HA stem region following H5N1 vaccination in humans. Proc Natl Acad Sci. 2014 Sep 9;111(36):13133–8.

53. Krammer F, Pica N, Hai R, Margine I, Palese P. Chimeric Hemagglutinin Influenza Virus Vaccine Constructs Elicit Broadly Protective Stalk-Specific Antibodies. J Virol. 2013 Jun 15;87(12):6542–50.

54. Krammer F, Margine I, Hai R, Flood A, Hirsh A, Tsvetnitsky V, et al. H3 Stalk-Based Chimeric Hemagglutinin Influenza Virus Constructs Protect Mice from H7N9 Challenge. J Virol. 2014 Feb 15;88(4):2340–3.

55. Ermler ME, Kirkpatrick E, Sun W, Hai R, Amanat F, Chromikova V, et al. Chimeric Hemagglutinin Constructs Induce Broad Protection against Influenza B Virus Challenge in the Mouse Model. J Virol [Internet]. 2017 May 26 [cited 2019 Dec 25];91(12). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5446656/

56. Krammer F. The Quest for a Universal Flu Vaccine: Headless HA 2.0. Cell Host Microbe. 2015;18(4):395–7.

57. Impagliazzo A, Milder F, Kuipers H, Wagner MV, Zhu X, Hoffman RMB, et al. A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. Science. 2015 Sep 18;349(6254):1301–6.

58. Yassine HM, Boyington JC, McTamney PM, Wei C-J, Kanekiyo M, Kong W-P, et al. Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection. Nat Med. 2015 Sep;21(9):1065–70.

59. Krammer F, Fouchier RAM, Eichelberger MC, Webby RJ, Shaw-Saliba K, Wan H, et al. NAction! how can neuraminidase-based immunity contribute to better influenza virus vaccines? mBio. 2018;9(2):1–12.

60. Eichelberger MC, Morens DM, Taubenberger JK. Neuraminidase as an influenza vaccine antigen: a low hanging fruit, ready for picking to improve vaccine effectiveness. Curr Opin Immunol. 2018;53:38–44.

61. Wohlbold TJ, Podolsky KA, Chromikova V, Kirkpatrick E, Falconieri V, Meade P, et al. Broadly protective murine monoclonal antibodies against influenza B virus target highly conserved neuraminidase epitopes. Nat Microbiol. 2017 Oct;2(10):1415–24.

62. Fiers W, De Filette M, El Bakkouri K, Schepens B, Roose K, Schotsaert M, et al. M2e-based universal influenza A vaccine. Vaccine. 2009 Oct 23;27(45):6280–3.

63. Deng L, Cho KJ, Fiers W, Saelens X. M2e-Based Universal Influenza A Vaccines. Vaccines. 2015 Feb 13;3(1):105–36.

64. Kim K-H, Kwon Y-M, Lee Y-T, Kim M-C, Hwang H, Ko E-J, et al. Virus-Like Particles Are a Superior Platform for Presenting M2e Epitopes to Prime Humoral and Cellular Immunity against Influenza Virus. Vaccines. 2018 Sep 20;6(4):66.

65. Mullarkey CE, Boyd A, van Laarhoven A, Lefevre EA, Veronica Carr B, Baratelli M, et al. Improved adjuvanting of seasonal influenza vaccines: Preclinical studies of MVA-NP+M1

coadministration with inactivated influenza vaccine: Clinical immunology. Eur J Immunol. 2013 Jul;43(7):1940–52.

66. Lillie PJ, Berthoud TK, Powell TJ, Lambe T, Mullarkey C, Spencer AJ, et al. Preliminary Assessment of the Efficacy of a T-Cell–Based Influenza Vaccine, MVA-NP+M1, in Humans. Clin Infect Dis. 2012 Jul 1;55(1):19–25.

67. A Pivotal Trial to Assess the Safety and Clinical Efficacy of the M-001 as a Standalone Universal Flu Vaccine - Full Text View - ClinicalTrials.gov [Internet]. [cited 2019 Dec 23]. Available from: https://clinicaltrials.gov/ct2/show/NCT03450915

68. Atsmon J, Kate-Ilovitz E, Shaikevich D, Singer Y, Volokhov I, Haim KY, et al. Safety and immunogenicity of multimeric-001--a novel universal influenza vaccine. J Clin Immunol. 2012 Jun;32(3):595–603.

69. Evaluating the immunogenicity and safety of a BiondVax-developed universal influenza vaccine (Multimeric-001) either as a standalone vaccine or as a primer to H5N1 influenza vaccine [Internet]. [cited 2019 Dec 24]. Available from: https://www-ncbi-nlm-nih-gov.libaccess.lib.mcmaster.ca/pmc/articles/PMC5369918/

70. Pleguezuelos O, Robinson S, Stoloff GA, Caparrós-Wanderley W. Synthetic Influenza vaccine (FLU-v) stimulates cell mediated immunity in a double-blind, randomised, placebo-controlled Phase I trial. Vaccine. 2012 Jun 29;30(31):4655–60.

71. Francis JN, Bunce CJ, Horlock C, Watson JM, Warrington SJ, Georges B, et al. A novel peptide-based pan-influenza A vaccine: A double blind, randomised clinical trial of immunogenicity and safety. Vaccine. 2015 Jan 3;33(2):396–402.

72. Talbot HK, Rock MT, Johnson C, Tussey L, Kavita U, Shanker A, et al. Immunopotentiation of Trivalent Influenza Vaccine When Given with VAX102, a Recombinant Influenza M2e Vaccine Fused to the TLR5 Ligand Flagellin. PLOS ONE. 2010 Dec 28;5(12):e14442.

73. Turley CB, Rupp RE, Johnson C, Taylor DN, Wolfson J, Tussey L, et al. Safety and immunogenicity of a recombinant M2e-flagellin influenza vaccine (STF2.4xM2e) in healthy adults. Vaccine. 2011 Jul 18;29(32):5145–52.

74. Mezhenskaya D, Isakova-Sivak I, Rudenko L. M2e-based universal influenza vaccines: a historical overview and new approaches to development. J Biomed Sci. 2019 Oct 19;26(1):76.

75. Tsybalova LM, Stepanova LA, Kuprianov VV, Blokhina EA, Potapchuk MV, Korotkov AV, et al. Development of a candidate influenza vaccine based on virus-like particles displaying influenza M2e peptide into the immunodominant region of hepatitis B core antigen: Broad protective efficacy of particles carrying four copies of M2e. Vaccine. 2015 Jun 26;33(29):3398–406.

76. Saelens X. The Role of Matrix Protein 2 Ectodomain in the Development of Universal Influenza Vaccines. J Infect Dis. 2019 Apr 8;219(Supplement_1):S68–74.

77. Antrobus RD, Berthoud TK, Mullarkey CE, Hoschler K, Coughlan L, Zambon M, et al. Coadministration of Seasonal Influenza Vaccine and MVA-NP+M1 Simultaneously Achieves Potent Humoral and Cell-Mediated Responses. Mol Ther. 2014 Jan;22(1):233–8.

78. Antrobus RD, Lillie PJ, Berthoud TK, Spencer AJ, McLaren JE, Ladell K, et al. A T Cell-Inducing Influenza Vaccine for the Elderly: Safety and Immunogenicity of MVA-NP+M1 in Adults Aged over 50 Years. Doherty TM, editor. PLoS ONE. 2012 Oct 31;7(10):e48322.

79. Efficacy of Candidate Influenza Vaccine MVA-NP+M1 in Adults [Internet]. [cited 2019 Dec 31]. Available from: https://clinicaltrials.gov/ct2/show/NCT03880474

80. Bernstein DI, Guptill J, Naficy A, Nachbagauer R, Berlanda-Scorza F, Feser J, et al. Immunogenicity of chimeric haemagglutinin-based, universal influenza virus vaccine candidates: interim results of a randomised, placebo-controlled, phase 1 clinical trial. Lancet Infect Dis. 2019 Oct 17;