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Prospects of universal influenza virus vaccine and the current challenges of new antiviral drugs

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Abstract

The profound impact of influenza viruses on human health persists as a significant burden, given their potent capacity to cause morbidity and mortality. Despite efforts to mitigate the annual burden of influenza, the effectiveness of current seasonal vaccines in providing substantial protection falls short, leaving undesirable pandemic of influenza viruses. The challenges posed by the influenza pandemic lies from the constant changes inherent in the virus itself, as well as the limitations of current immunization approaches in achieving sufficient immunogenicity. Influenza viruses exhibit antigenic drift and shift, undergoing antigenic evolution by altering the surface glycoproteins. These changes contribute to the persistent and dynamic nature of the influenza virus, posing formidable challenges to effective prevention and control strategies. Currently, there is growing recognition of unique viral targets that hold promise development of broad-protective vaccines against influenza. These targets are distinct from traditional vaccine targets and offer the potential for more comprehension protection against diverse strains of the virus. Here, we present a review about the novel drugs and vaccines that target the influenza virus would signify the unique immune correlates of protection that need to be initiated to accelerate the vaccine efficacy.

Key words innate immunity, influenza virus, universal vaccine, neutralizing antibodies

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Introduction

Influenza caused by influenza viruses, is a respiratory illness that poses a significant public health concern and has far-reaching economic implications on a global scale (**Figure 1**) [1-3]. Vaccination remains the most effective defense against influenza, offering unparalleled protection. Vulnerable groups, such as young children, the elderly, pregnant women, and those with compromised immune systems, are at an elevated risk of experiencing severe complications during infection. As a result, these individuals constitute priority target groups for influenza vaccination. The elderly population in countries with aging demographics is on the rise due to increased life expectancy. Despite being a disease that can be prevented through vaccination, seasonal influenza vaccines generally provide a temporary and short-lived protective effect. Mutations in the main surface antigenic determinants of influenza can enable the virus to evade neutralizing antibodies induced by vaccination. In addition, studies have shown a reduction in antibody levels induced by vaccination over time [4]. There exists a significant opportunity for enhancing influenza vaccines to offer improved and prolonged protection against the antigenically diverse strains of influenza viruses. This review offers a concise discussion on how understanding the interactions between the influenza virus and its host can contribute to the development of present and future influenza vaccines.

Challenges in vaccination

Vaccines remain essential in controlling human influenza virus infections. They work by promoting the production of neutralizing antibodies, which predominantly target the hemagglutinin (HA) glycoprotein, a major antigenic element of the influenza virus (**Figure 2**). Current trivalent influenza vaccines are designed to generate neutralizing antibodies against the H1 and H3 subtypes of influenza A, as well as one lineage of influenza B (either Yamagata or Victoria), according to the HA phylogenetic grouping [5]. Since the 2013-2014 influenza season, quadrivalent vaccines have been introduced, offering both live-attenuated and inactivated options. These new vaccines are expected to gradually replace the previously used trivalent formulations [6]. The effectiveness of yearly influenza vaccines is primarily driven by neutralizing antibodies that recognize specific antigenic sites on the globular head of the HA surface glycoprotein. These antibodies work best when the HA of the vaccine strain closely matches the circulating seasonal influenza strains. The selection of vaccine strains is informed by global influenza surveillance, which identifies the strains most likely to be prevalent in the upcoming flu season.

Selecting a vaccine strain that closely matches the circulating influenza strain is a complex process and often presents challenges, which may result in less-than-optimal protection. These challenges arise from the ongoing antigenic changes that occur in the HA molecule of the influenza virus, which can cause the virus to evade recognition by the immune response. Additionally, the emergence of novel antigenic variants cannot always be accurately predicted, further complicating the selection of an appropriate vaccine strain. According to the Centers for Disease Control (CDC), it is estimated that annually, the prevention of influenza B virus infections could potentially lead to the avoidance of 40,000 to 275,000 illnesses [7]. Hence, there is a dire need for alternative strategies to effectively control emerging influenza viruses.

Advancements in universal vaccines

The discovery of monoclonal antibodies (mAbs) capable of neutralizing a diversity of influenza strains has opened new avenues for antiviral treatments and the strategic development of a universal vaccine driven by these antibodies. These antibodies are classified into three groups based on their reactivity with influenza A viruses: (1) those that cross-react with group 1 HAs, (2) those that cross-react with group 2 HAs, and (3) those that react with both group 1 and group 2 HAs [8]. Recently, Friensen and colleagues made a notable breakthrough by identifying a monoclonal antibody, CR8043, which targets a conserved epitope in the HA stem of group 2 influenza viruses. CR8043 has shown in vitro neutralizing potential against H3 and H10 viruses and has demonstrated protective effects in animal studies, where it safeguarded mice from lethal doses of both H3N2 and H7N7 viruses. This finding represents a promising development in the field of influenza research, as it expands our understanding of antibodies with broad activity against group 2 HAs, and highlights the potential of CR8043 as a therapeutic or vaccine candidate for controlling influenza infections caused by these subtypes [9]. S139/1 a murine mAb has been found to exhibit activity against both group 1 and group 2 HAs of influenza viruses. S139/1 specifically binds to the conserved region and due to this unique characteristic of S139/1 makes it a promising candidate for potentially providing broad-spectrum protection against various influenza virus subtypes, regardless of their group classification [10]. In addition to the previously mentioned antibodies, FI6v3 and CR9114 are two human antibodies with broad-spectrum neutralizing activity against influenza viruses containing both group 1 and group 2 Has [11, 12].

Structural analyses revealed that broadly neutralizing antibodies against influenza viruses, such as CR6261, F10, F16, CR8020, CR8033, and CR8071, target distinct sites on the stem region of the HA molecule. Some antibodies like CR8033 and CR8071 are capable of neutralizing influenza B viruses from both Yamagata and Victoria lineages by recognizing two distinct conserved epitopes on the HA of influenza B viruses. These antibodies inhibit virus progeny release by interfering with viral replication and preventing the release of new virus particles, thereby providing a potential mechanism for their antiviral activity [13]. Broadly neutralizing antibodies against influenza viruses are being developed as monoclonal antibody therapies in different countries [14]. Identifying vulnerabilities in the defense mechanisms of the influenza virus can also pave the way for the development of potential drug candidates, such as proteins and small molecules that mimic the interactions of antibodies, thereby competing for receptor binding [15]. Moreover, the subsequent objective is to develop a universal influenza vaccine that can trigger the production of antibodies targeting the conserved epitopes of the HA protein.

Universal vaccines can be designed by altering vaccine candidates to eliminate strain-specific, immunodominant epitopes located on the head region of the HA protein. Instead, these vaccines can incorporate cross-reactive, non-immunodominant epitopes found in the stem region. A key challenge in this strategy is improving the immune system's ability to access these conserved HA determinants. Although several headless HA immunogens have been developed by removing a significant portion of the HA1 region, the reasons for their failure to elicit a broadly cross-reactive neutralizing antibody feedback in animal models remain unclear [16-18].

For the past 60 years, the development of influenza vaccines has primarily focused on the highly variable HA-head region. However, further research and a deeper insight of the molecular mechanisms underlying neutralization of influenza viruses by both neutralizing and non-neutralizing antibodies are needed to generate an immune response. Recent data have also reported cases of vaccine-associated enhanced respiratory disease in pigs following vaccination with whole inactivated H1N2 (human-like) virus, and subsequent challenge with a different strain of

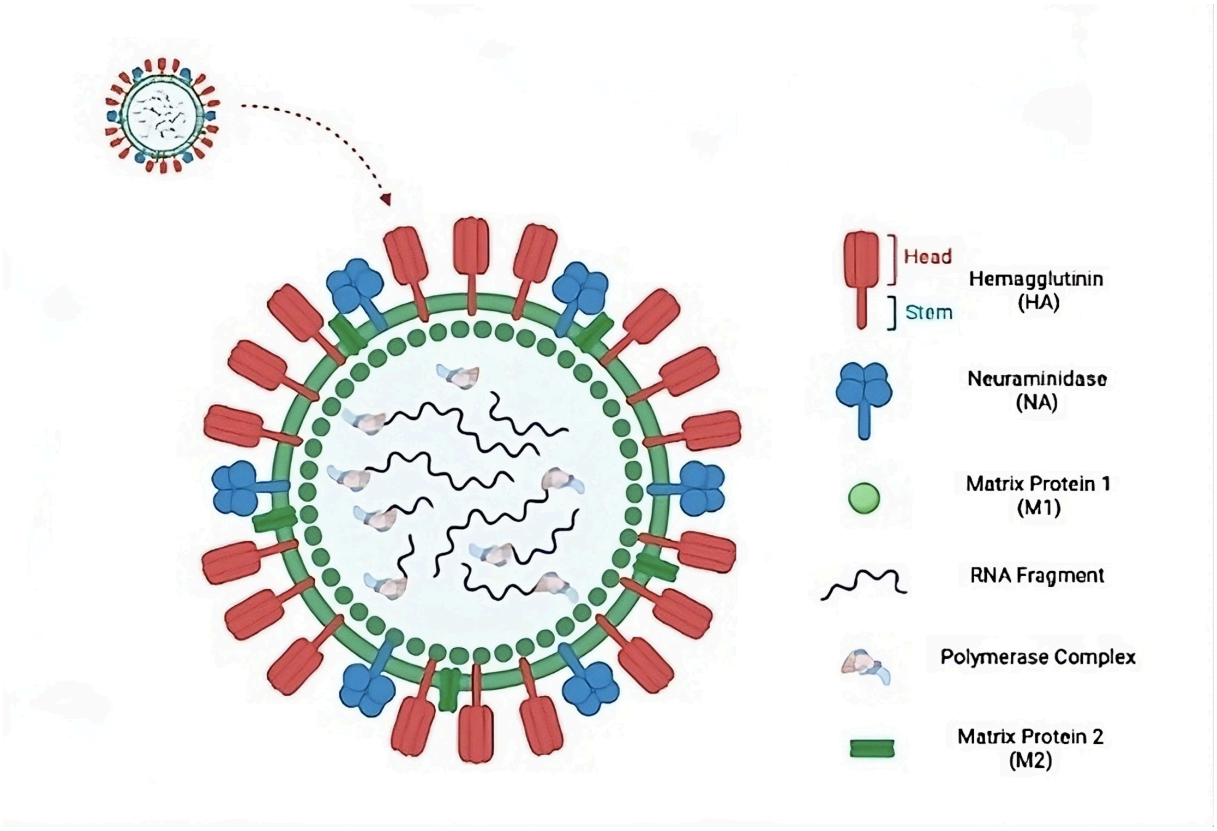

Figure 1. Influenza A virus and its components structural overview. (HA): a glycoprotein on the surface that helps the virus attach to host cells and make entrance easier. (NA): a surface enzyme that facilitates the release of new virions by cleaving the sialic acid present on the host cell. (M1): a structural protein that maintains the structural integrity and aids in viral assembly on the inner surface of the viral membrane. (RNA fragment): viral variety and genetic reassortment are made possible by the segmented RNA genome that codes for viral proteins. (Polymerase complex): a multi-subunit complex that is in charge of the viral RNA genome's transcription and replication. (M2): an ion channel that permits the release of viral RNA by enabling viral uncoating within host cells.

H1N1pdm09 virus [19]. The authors of the study revealed that vaccination with whole inactivated H1N2 virus induced antibodies that cross-reacted with the H1N1pdm09 virus. As a result, this led to emphasized fusion of the H1N1pdm09 virus with target cells, ultimately resulting in increased disease severity. In addition to the mechanism mentioned above, there are two other potential ways in which antibodies may enhance disease: (1) non-neutralizing antibodies that specifically target the HA stem region may bind to the HA protein and direct the virus to cells that express Fc receptors. (2) Antibodies that target HA on the surface of influenza virus-infected cells may bind and trigger antibody-mediated complement activation, leading to inflammation and cell lysis [20]. Consequently, further research is needed to better understand the rational model of safe and effective universal influenza vaccines. This includes investigating ways that can provide durable protection against diverse influenza virus strains.

Challenges in addressing the demand for novel antiviral drugs

Therapeutic interventions can play a pivotal role in defending against influenza, especially in situations where vaccine efficacy is low. The efficacy of antiviral therapies for influenza is restricted by a number of variables. One of the key issues is the restricted availability of anti-influenza medications, with only one class, neuraminidase inhibitors (NAIs), currently approved for use.
NAIs are a class of antiviral drugs that specifically target the enzymatically active site of the neuraminidase (NA) protein in influenza A and B viruses. By binding to this site, NAIs interfere with the protein's function, particularly its role in cleaving terminal sialic acid residues from the host cell surface, which is essential for the release of newly produced virions [21]. Consequently, NAIs prevent the release of budding viral particles from infected host cells, limiting the virus's spread to uninfected cells. Unlike antibiotics, which can effectively eliminate or significantly reduce bacterial presence, NAIs provide temporary relief by stopping the infection of new host cells with influenza viruses. For this reason, administering NAIs within 48 hours of symptom onset is critical for effective influenza treatment, which can be challenging in many regions [22]. However, some evidence indicates that NAIs may still offer benefits if given later in the disease, particularly in underdeveloped areas or for hospitalized patients. Administering NAIs early in the course of the infection can help modulate disease progression, allowing the immune system to clear the virus more rapidly. Despite this, the limited availability of only one class of antiviral drugs and the short therapeutic window for NAIs pose considerable challenges for healthcare providers.

Neuraminidase inhibitors

Of the four existing neuraminidase inhibitors, two have been widely used globally since the 1999-2000 flu season: oral oseltamivir (Tamiflu®, Roche) and inhaled zanamivir (Relenza®,

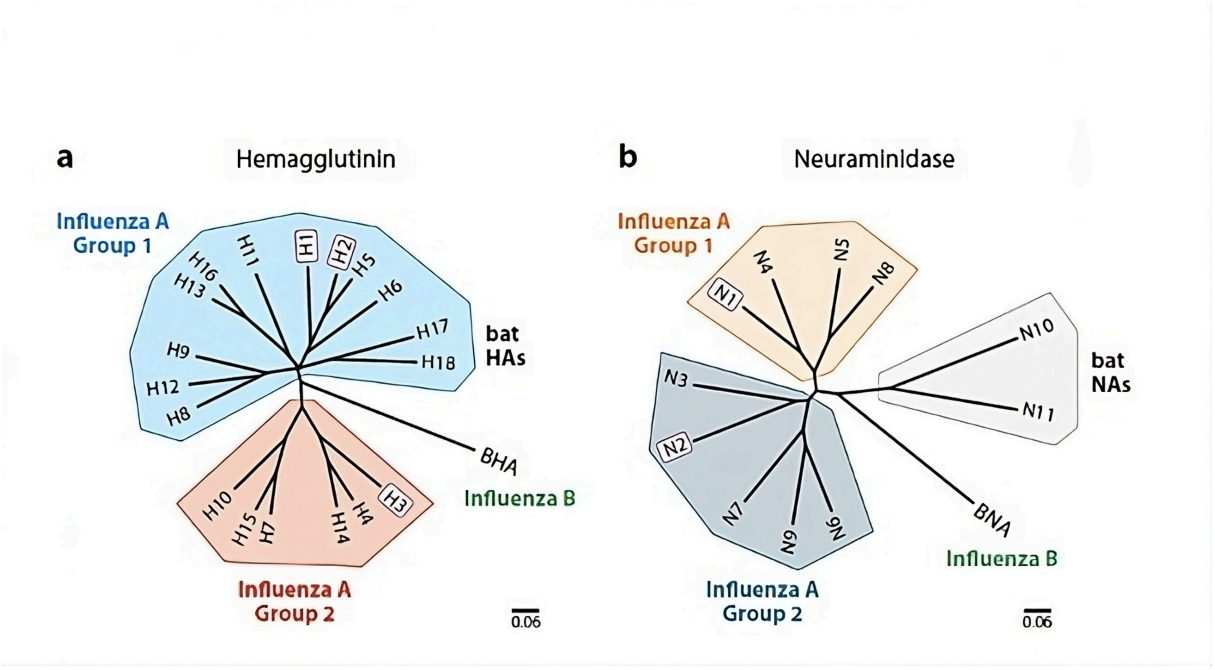

Figure 2. Phylogenetic trees illustrating the relationships of (a) hemagglutinin (HA) and (b) neuraminidase (NA) reveal the classification of these major surface glycoproteins of influenza viruses into distinct groups and subtypes. Notably, only those viruses featuring H1, H2, or H3 hemagglutinins and N1 or N2 neuraminidases—such as H1N1, H2N2, or H3N2 (highlighted in purple)—have circulated globally within the human population over the past century. The scale bars on the trees represent a 6% variation at the amino acid level, providing insight into the genetic diversity among these influenza virus strains.

GlaxoSmithKline). Additionally, intravenous peramivir (Rapiacta®, Peramiflu®, BioCryst Pharmaceuticals) has received approval for treating influenza in adults and children in Japan, South Korea, and China. Inhaled laninamivir octanoate (Inavir®, Daiichi Sankyo) is also approved in Japan for the treatment of influenza in both adults and children [23]. Peramivir has distinct structural features compared to oseltamivir and zanamivir, showing prolonged binding to the neuraminidase site for over 24 hours and slower dissociation. On the other hand, laninamivir octanoate has been proven to have clinical efficacy against seasonal influenza A and B viruses, as well as the H1N1pdm09 virus [24]. The active form of laninamivir persists at a high concentration in the lungs for at least 5 days [25]. As a result, a single dose of laninamivir octanoate can effectively treat influenza by providing a significant impact.

Resistance to neuraminidase inhibitors

The emergence of influenza viruses resistant to neuraminidase inhibitors poses a serious challenge to the effectiveness of antiviral treatments. Amino acid changes linked to NAI resistance can weaken the binding interaction between the neuraminidase enzyme and the inhibitor, allowing the virus to evade NAI treatment [23]. Molecular markers of resistance have been found at both the catalytic and framework residues of the neuraminidase glycoprotein, varying for oseltamivir, zanamivir, and peramivir. However, to date, no resistance-related substitutions have been reported for laninamivir. Oseltamivir, being a hydrophobic drug, requires rearrangements in the influenza NA protein to enable its binding. The E276 residue in NA must rotate and interact with R224 to create a receptive pocket for oseltamivir binding [26]. Any mutation that inhibits this rearrangement, such as preventing the rotation of E276 residue and the formation of the pocket, leading to a decrease in its efficacy. Amino acid substitutions associated with oseltamivir resistance in NA can vary depending on the NA types and subtypes of influenza A and B viruses. These amino acid changes can impair the binding of oseltamivir to the NA enzyme, leading to reduced efficacy of the drug against these resistant variants of influenza viruses [27].

NAI-resistant viruses can develop either due to drug-selection pressure or through natural processes without drug exposure. Before 2007, the rate of oseltamivir resistance in clinical trial samples was reported as 0.3% in adults and 4% in children [28, 29]. However, during the 2007-2009 influenza seasons, oseltamivir-resistant A/Brisbane/59/2007 (H1N1)-like viruses (subclade 2B) with the NA H274Y resistance marker became widespread globally. Despite this, epidemiological data did not link the emergence of resistance directly to oseltamivir usage. This suggests that the H274Y mutation in the NA protein arose naturally, leading to viruses with enhanced transmissibility and fitness compared to their drug-sensitive counterparts [30-32].

Different changes in the NA protein can mitigate the negative effects of the H274Y mutation, such as the D344N, Q222R, and V234M substitutions, which help restore NA affinity and surface accumulation [33-35]. Such changes allow the H274Y mutation to persist without compromising viral function. Since the 2009 pandemic's first wave and the subsequent disappearance of the A/Brisbane/59/2007 (H1N1) strain, oseltamivir resistance in H1N1pdm09 variants has remained low, around 1% [36, 37]. Nonetheless, Vietnam, Australia, and the UK have reported clusters of oseltamivir-resistant infections that are not associated with therapy and most likely involve the spread of H1N1pdm09 mutant strains [38-40]. The intricacy of NAI-resistance patterns draws attention to the difficulties in conducting antiviral monitoring, which is essential for determining the hazards associated with newly emerging viruses, particularly those

originating from avian or human origins.

Development of novel antiviral drugs with multiple pathway targeting

The heavy reliance on NAIs carries the same risk as any monotherapy: the potential for drug resistance. The rise and global spread of naturally occurring NAI-resistant H1N1 influenza viruses during the 2007-2009 seasons underscore the pressing need for new antiviral options and combination therapies. Future treatments could focus on directly inhibiting viral proteins or essential cellular components for viral replication, or by modulating the host's immune response. Several drugs in development are aimed at targets like the viral polymerase (e.g., Favipiravir), hemagglutinin (e.g., nitazoxanide), or sialic acid receptors (e.g., Fludase). Additionally, anti-inflammatory compounds that do not promote antiviral resistance could play a crucial role in improving outcomes in severe influenza cases, either as standalone treatments or part of a combination strategy. Other promising approaches include enhancing the host's innate immune response or employing immunotherapy using antibodies targeting conserved regions on the hemagglutinin protein. Diversifying treatment options and exploring combination therapies can be essential in mitigating the risk of drug resistance and improving outcomes in the management of influenza infections.

The immune response to influenza virus infection

One of the key tasks in mounting an effective immune defense against the influenza virus is the rapid progression of the infection. Meta-analyses of human studies involving controlled influenza exposure reveal that clinical symptoms typically peak around the second day following infection and generally subside within 10 days, while the virus is shed for an average of 4.8 days. This brief window of infection complicates the design and evaluation of strategies aimed at modulating the immune response [41]. The innate immune system plays a crucial role in controlling and eliminating the virus, particularly because of the short duration of the infection. In humans, innate immunity against influenza is activated through several mechanisms. This activation triggers interferon-based antiviral feedback and the release of proinflammatory cytokines, which help limit viral replication. This early innate immune reaction is essential for immediate defense against the virus and paves the way for the adaptive immune feedback, which includes the development of virus-specific antibodies and stimulation of T cells, essential for long-term immunity [42, 43]. Both CD4+ helper T cells and CD8+ cytotoxic T lymphocytes (CTLs) are key to clearing the virus, as demonstrated in animal models. These findings suggest that similar mechanisms may also play a crucial role in human infections, though additional evidence is still being gathered [44].

CD8+ CTLs are capable of recognizing a wide array of influenza virus antigens, including conserved internal viral proteins presented by HLA class molecules [45, 46]. This ability allows the cellular immune system to mount cross-reactive responses against various influenza strains due to its recognition of these conserved proteins [47-49]. Conversely, though the humoral immune feedback primarily functions by producing antibodies that target viral surface proteins like HA, NA, and to a lesser extent, matrix protein 2 (M2), which is less prevalent on the virus surface. HA, the main antigenic protein, plays a key role in viral attachment and membrane fusion. Therefore, antibodies directed against HA can neutralize the virus and prevent infection.

HA is a homotrimer; each monomer is made up of the two domains, HA1 and HA2, which are joined by a disulfide link. Whereas HA1 creates the distal globular head, which houses the majority of the antigenic areas and receptor-binding sites, HA2 forms the membrane anchor and the "stem" region. As mentioned earlier, antigenic drift in circulating strains primarily results from mutations in the antigenic sites of the HA1 globular head. Between 1999 and 2010, it was estimated that human seasonal H3 and H1 influenza viruses experienced amino acid changes in the range of 2.1% to 3% per drift variant [50]. In contrast, the HA2 region remained highly conserved, with only three amino acid changes observed in both H1 and H3 strains during the same period. Despite its conservation, HA2 is immunogenic, and antibodies targeting this region have been detected in humans following infection and vaccination, though they are produced at much lower levels than those targeting the globular head [51, 52].

The second primary surface glycoprotein of the influenza virus, neuraminidase (NA), is an enzyme that helps release freshly produced viruses by cleaving sialic acid residues from the surface of the host cell. While antibodies targeting NA do not prevent initial infection, they can help limit the spread of the virus. Similarly, the viral proteins M2 and nucleoprotein (NP) are critical to the virus replication process. Although antibodies against M2 and NP do not neutralize the virus directly, studies in mice have shown that they can reduce viral replication and contribute to controlling the infection [53].

Hemagglutination inhibition or neutralization assays are commonly used to detect the induction of HA-specific antibodies in the blood, which is a crucial indicator of recent influenza exposure or vaccination. For inactivated influenza vaccines in adults, a titer of ≥40 is often considered an immune correlate of protection based on findings from vaccine trials and clinical studies. However, for other vaccine platforms, established immune correlates of protection are lacking, which has been recognized as a significant barrier to advancing new influenza vaccine strategies [54]. Assessing the direct protective efficacy of a vaccine its ability to prevent infection in real-world settings presents additional challenges. These include the need for large study populations and the capacity to track participants across at least one flu season, making empirical evaluation difficult [55]. As a result, the process of developing a clinically validated influenza vaccine is both time-consuming and expensive.

Conclusion

Influenza viruses have the potential to cause pandemics in different ways, and it is crucial to analyze their pandemic potential and develop effective therapeutics to combat possible influenza pandemics. In recent years, novel targets being explored, unique vaccination approaches being promoted, and diverse vaccines advancing to human clinical trials. However, several challenges persist, including the identification of novel correlates of protection against influenza, which may vary depending on age group, characteristics of individuals, population, and vaccine type. To improve current vaccination approaches, more immunogenic, broadly cross-reactive, and highly effective universal influenza virus vaccines need to develop. Future research will focus on understanding the immune mechanisms of protection against influenza viruses, which will aid in the design of novel vaccine strategies to better immunize populations against influenza.

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Data availability

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Authors' contribution

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Competing interests

None.

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