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Novel universal vaccines to mitigate emerging avian influenza viruses

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Frequent spillovers and recent geospatial expansion of avian influenza virus (AIV) pose significant economic and public health threats. Recent advances in vaccine technologies, bioinformatics, and artificial intelligence will provide newer approaches to target genetically diverse and rapidly evolving AIVs. Here, we review recent advances in, and perspectives on, developing universal vaccines needed for the effective control of AIVs.

Avian influenza virus: diversity, global spread, and challenges

Waterfowl are the natural hosts for AIV; however, spillover of AIV to other avian and mammalian species occurs frequently and results in epizootics and occasional zoonotic infections. AIV is a strain of *Alphainfluenzavirus influenzae* (influenza A virus, IAV). Its genome comprises eight negative-sense RNA segments. AIVs display high antigenic variation, with more than 16 hemagglutinin (HA) subtypes, which are further classified into phylogenetic groups 1 and 2, plus 9 neuraminidase (NA) subtypes. The presence of a multibasic peptide sequence in the HA renders certain AIVs, such as H5N1 and H7N9, highly pathogenic (HPAIV). Given its genetic diversity, rapid evolution, and propensity to reassort, novel AIVs continuously emerge.

The emergence of AIVs resulting in zoonoses has accelerated over the past decade (e.g., H3N8, H9N2, and H10N6) [1]. The recent geospatial expansion of HPAIV H5N1 to North America with associated epizootics in wildlife, poultry and cattle in the USA is of particular concern [2], because there were also more than 60 cases of human infections, with one fatality as of February 2025. A severe case in Canada revealed multiple human-adaptive mutations in the viral genome [3], raising concerns about a possible pandemic.

Mitigating AIV is difficult due to its unique ecology and persistence in the environment. Current vaccines are inadequate to control AIV because of its diversity and rapid evolution. Therefore, there is an urgent need for a novel universal vaccine and corresponding strategic reserve for pandemic preparedness. Here, we provide a brief review of advances in this area and propose new strategies (leveraging next-generation vaccine platforms and advanced biotechnological approaches) to develop a universal vaccine for the prevention and mitigation of AIV.

Antigens for universal AIV vaccines

Cross-reactive immunity can be elicited by vaccines using conserved viral proteins as immunogens. The challenge is to identify the regions that match circulating viruses and are sufficiently immunogenic. Multiple studies have shown the feasibility of this approach (Table 1). For example, a vaccine candidate utilizing conserved stem regions of the HA, NA, and the ectodomain of matrix protein 2 (M2e) as antigens elicited cross-reactive antibodies. In addition, relatively conserved nucleoprotein (NP), polymerase basic protein 1 (PB1), and matrix protein (M1) contain abundant T and B cell epitopes and represent suitable antigens to elicit protective cell-mediated immunity (CMI) [4].

Immunity elicited by a universal AIV vaccine should target multiple viruses. However, simply incorporating multiple antigens in the vaccine may not work, as antigenic competition reduces immunogenicity. To circumvent this, antigens, such as the HA of equine influenza virus (H3N8), which is known to elicit prominent broad-spectrum immunity [5], should be used. Another approach is to elicit cross-reactive CMI by using fusion proteins comprising multiple viral antigens [6].

Use of protein domains or selected peptide epitopes instead of whole viral proteins reduces antigen load to provide more effective and targeted immunity. Therefore, exploiting the conserved regions of HA, NA, and M2 proteins to elicit antibodies against multiple AIV subtypes is appealing (Figure 1A). Vaccines based on the HA stem have shown efficacy against AIVs harboring antigenically different group 1 and group 2 HA proteins [7]. Although the M2 protein has low immunogenicity and elicits mostly non-neutralizing antibodies, vaccine candidates based on M2e monomers, or tandem M2e, do elicit some level of protection. To further enhance the immunogenicity of M2e, a nanovaccine, in which M2e was fused to the capsid protein of porcine circovirus 2, was developed and shown to provide significant protective immunity in pigs [8]. A vaccine candidate in which M2e and H3 HA stem regions were fused into a chimeric M2e-H3 stem immunogen, was shown to provide protective immunity against multiple IAV subtypes in mice [9], indicating that the HA stem increases the immunogenicity of M2e by enhancing the generation of cross-reactive antibodies. Other approaches, including the use of advanced delivery systems, such as nanoparticles, recombinant adenoviruses, and probiotics, have been shown to enhance vaccine immunogenicity [10].

RNA-based and other next-generation vaccine platforms

mRNA vaccines are both versatile and effective, and have been utilized to develop

Table 1. Properties of preclinically tested IAV vaccine candidates[a]

Antigens	Antigen design	Vaccine platform	Model	Targeted IAV subtypes	Observed efficacy	Refs
Whole virus	H1N9, H3N8, H5N1, H7N3	Inactivated	Mouse, ferret	H1N1, H5N8, H6N1, H7N1, H7N9, H10N7	100% survival for H1N1, H6N1, H7N1, H7N9, H10N7 70–90% survival for H5N8	DOI: 10.1126/scitranslmed.abo2167
	Equine H3N8	Attenuated	Mouse	H1N1, H3N2	100% survival for H1N1 80% survival for H3N2	[5]
HA	Recombinant HA of equine H3N8	Protein subunit	Mouse	H1N1, H3N2	80–100% survival for H1N1 50% survival for H3N2	[5]
	Recombinant HA covering CD4+ T helper cell epitopes	Protein subunit	Mouse	H1N1, H3N2	60% survival for H1N1 60% survival for H3N2	DOI: 10.3390/vaccines12091008
HA stem	Stable H1 HA stem	Protein subunit	Mouse, nonhuman primate	H1N1, H5N1	100% survival in mouse Reduced fever in nonhuman primates	DOI: 10.1126/science.aac7263
	H1 HA stem	Protein Nanoparticle	Mouse, ferret	H5N1	100% survival	DOI: 10.1038/nm.3927
	Glycan repositioning of H1 HA stem	Protein nanoparticle	Mouse	H1N1, H5N1, H7N9	90% survival for H1N1 0% survival for H5N1 45% survival for H7N9	DOI: 10.1038/s41467-020-14579-4
	H1 + H10 HA stem	Protein nanoparticle	Mouse, ferret, nonhuman primate	H1N1, H3N2, H5N1, H7N9, H10N8	Broadly neutralizing serum antibody responses	DOI: 10.1016/j.immuni.2022.10.015
Multiple HAs	'Mosaic' HA	Inactivated	Mouse	Different H3N2 viruses	cHA vaccine groups: 75%–80% survival	DOI: 10.1038/s41541-019-0126-4
	20 HAs	mRNA-LNP	Mouse, ferret	Group 1 HAs and Group 2 HAs	High levels of cross-reactive and subtype-specific antibodies	DOI: 10.1126/science.abm0271
	6 HAs	mRNA-LNP	Mouse	H1N1, H3N2, H5N1, H7N9, IBV	Complete protection	DOI: 10.1128/mbio.00668-24
NA	Quadrivalent NA	RNA particle	Pig	H1N1, H1N2, H3N2	Reduce titers of SIV	DOI: 10.1016/j.vaccine.2023.10.005
	Multi-subtype NA	CircRNA-LNP	Mouse	H1N1, H3N2	100% survival	[11]
M2e	Five tandem repeats of M2e	Bacterial vector	Mouse	H1N1, H3N2, H6N6	100% survival	[10]
	Three tandem repeats of M2e	Self-assembling protein nanoparticle	Mouse	H1N1, H3N2	90% protection against H1N1	DOI: 10.1021/acsnano.3c06526
	Capsid-M2e	Protein nanoparticle	Mouse, pig	H1N1, H3N2	Mouse: 60-100% survival Pig: reduce SIV titers	[8]
NP	Trimeric NP	Recombinant adenovirus	Mouse	H1N1, H3N2, H5N1	100% survival for H1N1, H3N2 40% survival for H5N1	DOI: 10.1080/22221751.2024.2427792
HA+NA	A mixture of four NA-F2A-HA (fused full-length proteins expressed using F2A linker) mRNA-LNPs	mRNA-LNP	Mouse	H1N1, H3N2	100% survival	DOI: 10.1038/s41467-024-52940-z
HA+M2e	The long alpha helix of the HA stem and three tandem repeats of the M2e	Ring nanoplatform	Mouse, chicken	H1N1, H5N8	Mouse: 100% survival Chicken: 0% survival	DOI: 10.3389/fimmu.2021.772550
	H5 HA+ M2e	Recombinant adenovirus	Mouse	H1N1, H5N2	Passive immunization: 100% survival	DOI: 10.3390/v9080234
	H5 HA + four tandem copies of M2e	Recombinant adenovirus	Mouse	H1N1, H5N2	100% survival	DOI: 10.1128/JVI.00823-14

Table 1. (continued)

Antigens	Antigen design	Vaccine platform	Model	Targeted IAV subtypes	Observed efficacy	Refs
NA+M2e	Multi-NA + five tandem copies of M2e	Virus-like particles	Mouse	H1N1, H1N2, H3N2, H5N1, H7N9, H9N2	100% survival	DOI: 10.1371/journal.ppat.1010755
HA+M2e+NP	The long alpha helix of the HA stem, M2e, and NP	mRNA-LNP	Mouse	H1N1, H3N2, H9N2	100% survival	DOI: 10.1080/22221751.2023.2256422
M1+NP+PB1	M1, NP, PB1 based on H1N1 1918 influenza	Recombinant cytomegalovirus	Nonhuman primate	H5N1	54.5% survival	[4]
HA stem	H1 HA stem[b]	Protein nanoparticle	Human/clinical trial	Group 1 HAs	Durable neutralizing antibodies	DOI: 10.1126/scitranslmed.ade4790

[a]Abbreviations: cHA, chimeric hemagglutinin; HA, hemagglutinin; M1, matrix protein 1; M2e, the ectodomain of matrix protein 2; NA, neuraminidase; NP, nucleoprotein; PB1, polymerase basic protein 1; SIV, swine influenza virus.
[b]Phase I clinical trials have been completed.

promising H5 HA mRNA-lipid nanoparticle (LNP) vaccine candidates. Coupling of mRNA vaccine technology with the use of conserved antigenic sequences is expected to extend the range of protective immunity and increased efficacy.

Other novel RNA-based vaccine platforms (Figure 1B), such as alphavirus-based 'self-amplifying RNA' (saRNA) and 'trans-amplifying RNA' (taRNA) systems, circular (circ) RNA and endogenous antigen–RNA bacterium (EARB) technologies have also been explored. These platforms are expected to require lower vaccination doses. Studies in animal models reported alphavirus-based AIV vaccine candidates to be effective. However, different alphaviruses express antigens at different levels and, thus, exhibit different immunogenicity. Therefore, further studies are needed to identify the optimal alphavirus vectors for these saRNAs- or taRNA-based vaccines.

CircRNA is an RNA molecule with a closed circular structure containing an internal ribosome entry site (IRES) for translation of the antigen. A trivalent circRNA vaccine developed by using NA genes from H1N1 and H3N2 IAVs and NA from influenza B virus elicited cross-reactive antibodies against all three of these viruses [11]. A novel mRNA vaccine candidate based on EARB technology [12] was shown to outperform conventional mRNA or nanoparticle vaccines by eliciting higher neutralizing antibody responses and with potent CMI. These novel RNA vaccine technologies, coupled with the use of conserved epitopes or viral antigens, are promising pathways for the development of highly effective universal AIV vaccine.

Use of artificial intelligence (AI) with the above-described novel vaccine technologies has been reported. Specifically, the AI-based LinearDesign algorithm was used to optimize the coronavirus disease 2019 (COVID-19) mRNA vaccine with improvement in the expression and stability of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein, resulting in better protective immunity [13]. Similar approaches should also be applied to the design of a more robust universal AIV vaccine. The application of other promising state-of-the-art methods, such as synthetic biology and reverse vaccinology, and a focus on conserved sequences, to design and develop advanced next-generation universal AIV vaccine is also worthy of consideration. In addition, emerging technologies, such as AlphaFold, along with high-resolution imaging techniques, including cryo-electron microscopy, should be explored. These methodologies have been used to predict and validate the structures of engineered proteins or peptides, thereby facilitating the rational design of universal vaccines.

Immunization protocols, utilization, and stockpiling of a universal AIV vaccine

To protect against antigenically different viruses, vaccines should elicit both cross-reactive antibodies and T cell responses. A 'one-shot' universal vaccine is ideal but challenging to achieve, potentially requiring the optimization of novel delivery vectors and adjuvants to more effectively activate the immune response. The effectiveness of vaccines that require multiple inoculations greatly depends on the immunization schedule. Therefore, optimization of the immunization protocols is required. For example, sequential immunization with different COVID-19 vaccines comprising slightly different antigens (i.e., variants of the spike protein) resulted in significantly broader cellular and humoral immune responses [14]. A similar approach has been applied for IAV vaccines. Sequential immunization with a chimeric hemagglutinin ΔNS1 live-attenuated influenza vaccine broadened the immune response and resulted in immunity against IAVs harboring group 1 and group 2 HA proteins [15].

Considering the diverse landscape of current vaccine development, optimizing the combination of antigens and immunization regimens (including optimization of dose and route) becomes especially crucial in developing a universal AIV vaccine. Given the potential respiratory transmission of

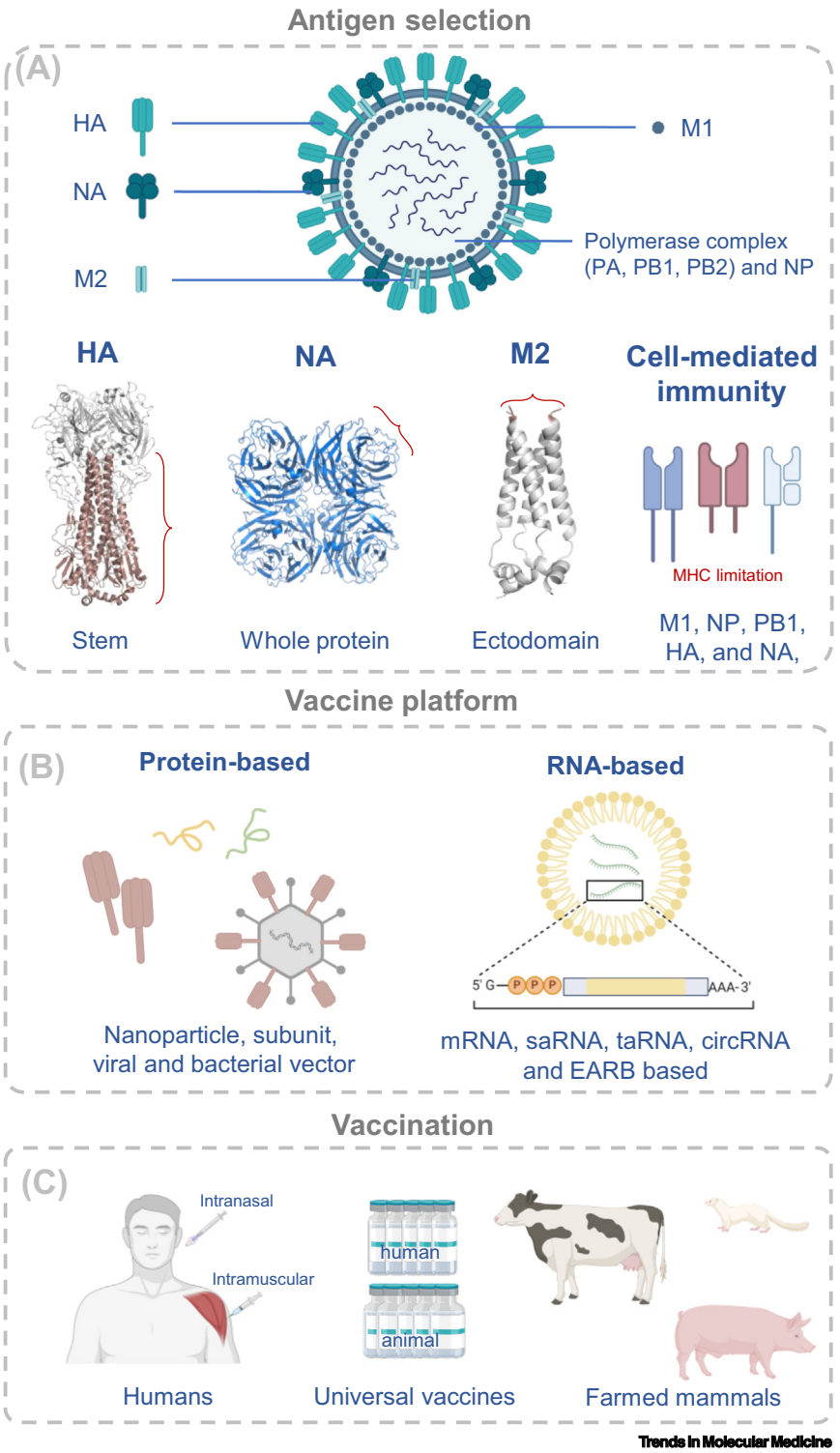

Figure 1. Antigen selection, vaccine platforms, and application of universal avian influenza virus (AIV) vaccines. (A) Viral structure and antigen selection: identification of epitopes in nucleoprotein (NP), polymerase

(Figure legend continued at the bottom of the next page.)

AIV in humans, developing nasal spray vaccines to target mucosal immunity should also be explored.

Several universal AIV vaccine candidates have entered preclinical and clinical trials and have shown substantial efficacy in animal models and humans (Table 1). When a universal AIV vaccine becomes available, questions regarding its usage and stockpiling should be considered. Farm animals should be prioritized to reduce the risk of antigenic shifts through reassortment, because mink and pigs are known to be 'mixing vessels', generating reassortant viruses from co-infection with more than one IAV (Figure 1C). Likewise, vaccination of 'high-risk' personnel, such as poultry farm workers and veterinarians, is important. A stockpile of universal vaccines can not only be used for immediate control in an outbreak, but also provides additional time for the development of more specific vaccines.

Concluding remarks

Prevention and mitigation of emerging AIVs will have a significant positive impact on public health and the economy. The combination of improved antigen design, state-of-the-art biotechnological methods, and novel vaccine platforms described here is expected to overcome the rapid evolution and diversity of AIVs. However, many challenges remain, such as duration of immune protection, logistics of production, distribution, and administration, particularly for low-income countries, and regulatory hurdles. For example, poultry are not routinely vaccinated against AIV in the USA. These challenges require ongoing collaborations and discussion. Nonetheless, universal AIV vaccines should be a key component of robust global animal and public health preparedness plans.

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Declaration of interests

The authors declare no competing interests.

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basic protein 1 (PB1), and matrix protein (M1), and in conserved hemagglutinin (HA) stem, neuraminidase (NA), and the extracellular domain of M2 of different AIV strains. PA: polymerase acidic protein. (B) Novel vaccine platforms: mRNA, self-amplifying RNA (saRNA), trans-amplifying RNA (taRNA), circular (circ)RNA, endogenous antigen–RNA bacterial (EARB) technology, nanoparticle, subunit, peptide, recombinant virus and bacterial vector-based approaches. (C) Stockpiling and vaccination strategies targeting humans and farmed mammals, such as cattle, mink, and pigs. All images adapted, with permission, from BioRender (biorender.com). Abbreviation: PA, polymerase acidic protein.