

Composition Comprising mRNA Encoding Influenza Virus HA Protein and Use Thereof

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

The present application claims the benefit of priority to Chinese Patent Applications No. 202311244673.7, filed September 22, 2023 and No. 202411236824.9, filed September 4, 2024, which are incorporated by reference in their entirety herein.

Technical Field

The present invention relates to the technical field of biomedicine and vaccines, and in particular, to a composition comprising an mRNA encoding an influenza virus HA protein and use thereof.

Background

Seasonal influenza is a major public health problem of global concern and can cause serious illness and death in high-risk populations. The seasonal influenza virus H1N1 is an influenza A virus that has been spreading between people since the Spanish flu pandemic in 1918. It alternates with two other seasonal influenza viruses, H3N2 and influenza B, and continues to endanger human health.

Vaccines are an effective means for preventing and controlling the spread of viruses. Because seasonal influenza viruses are highly variable and uncertain, the viruses contained in corresponding vaccines must be updated regularly to optimize the immune effect of the vaccines. Compared with traditional vaccines, mRNA vaccines have a shorter development cycle and can be quickly updated and iterated for personalized treatment. In addition, combined immunization strategy provides a new approach to prevent seasonal influenza infection, reduce the number of vaccine injections, and reduce adverse immune reactions.

Summary

The present invention provides an mRNA vaccine targeting different seasonal influenza viruses simultaneously, wherein immunity is generated against a plurality of influenza viruses when the vaccine is inoculated once, and the immunity against the plurality of influenza viruses is further enhanced after the vaccine is inoculated twice. The quadrivalent combined mRNA vaccine of the invention can simultaneously provide multiple protections against various currently prevalent human influenza viruses (influenza A virus H1N1 subtype, influenza A virus H3N2 subtype, influenza B virus

Victoria lineage, and influenza B virus Yamagata lineage).

Influenza virus particles are spherical and consist of an outer membrane and a nucleocapsid. The outer surface of the outer membrane has two glycoprotein protrusions: haemagglutinin (HA) and neuraminidase (NA). When an influenza virus enters a host cell, the function of the HA protein is to connect with sialic acid on the cell surface, so that the virus adheres to the host surface and enters the host body through endocytosis. Under the influence of a low pH, it finally realizes the transition from a pre-fusion state to a post-fusion state, causing the fusion of the virus and the host cell membrane. After propagation of the new virus is completed, the NA protein is responsible for releasing the new virus from the host cell, making it easier for the virus to adhere to the host cell membrane.

Seasonal influenza viruses are divided into three types, i. e. influenza A, influenza B, and influenza C, wherein HA and NA of influenza A are easily mutated, resulting in immunogenic changes, and causing global pandemic multiple times. Therefore, the influenza A viruses are divided into multiple subtypes. Currently, HA of influenza A viruses can be divided into 18 subtypes, with the serial number being H1-H18, and NA of influenza A viruses can be divided into 11 subtypes, with the serial number being N1-N11. There is no subtype distinction between HA and NA of influenza B, and currently they mainly include Yamagata or Victoria strains. Influenza C viruses rarely cause epidemics.

In a first aspect, the invention provides an immunogenic composition comprising an mRNA encoding a hemagglutinin protein (HA protein) of one or more influenza virus strains.

In some embodiments, the HA protein of the one or more influenza virus strains is selected from influenza A virus HA proteins, influenza B virus HA proteins, or combinations thereof. In a preferred embodiment, the HA protein of the one or more influenza strains comprises an influenza A virus HA protein and an influenza B virus HA protein.

In some embodiments, the HA protein of the one or more influenza virus strains is one or more selected from (1)-(6):

- (1) a HA protein of an A/Wisconsin/588/2019(H1N1)pdm09-like virus;
- (2) a HA protein of an A/Darwin/6/2021(H3N2)-like virus;
- (3) a HA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage);
- (4) a HA protein of a B/Phuket/3073/2013-like virus (B/Yamagata lineage);

(5) a HA protein of an A/Wisconsin/67/2022(H1N1)pdm09-like virus; or

(6) a HA protein of an A/Massachusetts/18/2022(H3N2)-like virus.

In a preferred embodiment, the HA protein of the one or more influenza strains comprises:

(1) a HA protein of an A/Wisconsin/588/2019(H1N1)pdm09-like virus;

(2) a HA protein of an A/Darwin/6/2021(H3N2)-like virus;

(3) a HA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage); and

(4) a HA protein of a B/Phuket/3073/2013-like virus (B/Yamagata lineage).

In a preferred embodiment, the HA protein of the one or more influenza strains comprises:

(1) a HA protein of an A/Wisconsin/588/2019(H1N1)pdm09-like virus;

(2) a HA protein of an A/Darwin/6/2021(H3N2)-like virus; and

(3) a HA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage).

In a preferred embodiment, the HA protein of the one or more influenza strains comprises:

(3) a HA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage);

(4) a HA protein of a B/Phuket/3073/2013-like virus (B/Yamagata lineage);

(5) a HA protein of an A/Wisconsin/67/2022(H1N1)pdm09-like virus; and

(6) a HA protein of an A/Massachusetts/18/2022(H3N2)-like virus.

In a preferred embodiment, the HA protein of the one or more influenza strains comprises:

3) a HA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage);

5) a HA protein of an A/Wisconsin/67/2022(H1N1)pdm09-like virus; and

6) a HA protein of an A/Massachusetts/18/2022(H3N2)-like virus.

In some embodiments, the immunogenic composition further comprises an mRNA encoding a neuraminidase-NA protein of one or more influenza virus strains. In some embodiments, the NA protein of the one or more influenza virus strains is selected from influenza A virus NA proteins, influenza B virus NA proteins, or combinations thereof. In a preferred embodiment, the NA protein of the one or more influenza virus strains comprises an influenza A virus NA protein and an influenza B virus NA protein. In some embodiments, the NA protein of the one or more influenza virus strains

is selected from a NA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage).

In some embodiments, the immunogenic composition comprises an HA and NA protein, wherein the HA protein of the one or more influenza virus strains comprises four HA proteins selected from 1)-4), or three HA proteins selected from 1)-3), or four HA proteins selected from 3)-6), or three HA proteins selected from 3), 5), and 6):

- 1) a HA protein of an A/Wisconsin/588/2019(H1N1)pdm09-like virus;
- 2) a HA protein of an A/Darwin/6/2021(H3N2)-like virus;
- 3) a HA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage);
- 4) a HA protein of a B/Phuket/3073/2013-like virus (B/Yamagata lineage);
- 5) a HA protein of an A/Wisconsin/67/2022(H1N1)pdm09-like virus; or
- 6) a HA protein of an A/Massachusetts/18/2022(H3N2)-like virus; and

the NA protein is selected from a NA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage).

In some embodiments, the HA protein of the one or more influenza virus strains is one or more selected from HA proteins having amino acid sequences as shown in SEQ ID NOs: 1-6; and preferably, the HA protein comprises four HA proteins having amino acid sequences as shown in SEQ ID NOs: 1-4, or three HA proteins having amino acid sequences as shown in SEQ ID NOs: 1-3, or four HA proteins having amino acid sequences as shown in SEQ ID NOs: 3-6, or three HA proteins having amino acid sequences as shown in SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 6.

In some preferred embodiments, the immunogenic composition further comprises an NA protein of an influenza virus strain, wherein the amino acid sequence of the NA protein of the influenza virus strain is as shown in SEQ ID NO: 7.

In some embodiments, each of the mRNAs comprises, from 5' to 3', a capping structure, a 5'-UTR, an open reading frame (ORF), a 3'-UTR, and a polyA tail.

In some embodiments, the capping structure can have m⁷G5'ppp5'(2'-OMe)NpG, wherein m⁷G is N7-methylguanosine; p is phosphate; ppp is triphosphate; 2'-OMe is 2'-methoxy modification; N is any nucleoside such as adenine nucleoside (A), guanine nucleotide (G), cytosine nucleoside (C) and uracil nucleoside (U), or other naturally occurring nucleoside or modified nucleoside.

In some embodiments, the 5'-UTR can comprise a 5'-UTR of a gene selected from the group consisting of a β -globin (HBB) gene, a heat shock protein 70 (Hsp70) gene, an axoneme dynein heavy chain 2 (DNAH2) gene, a 17 β -hydroxysteroid dehydrogenase 4 (HSD17B4) gene, or a homolog, fragment, or variant thereof. For example, the variant sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to a wild-type 5'-UTR sequence of a corresponding gene. In some embodiments, the 5'-UTR comprises a 5'-UTR derived from the 17 β -hydroxysteroid dehydrogenase 4 (HSD17B4) gene or a homolog, fragment, or variant thereof. In some embodiments, the 5'-UTR comprises a sequence as shown in SEQ ID NO: 15.

In some embodiments, the 3'-UTR may comprise a 3'-UTR of a gene selected from the group consisting of an albumin (ALB) gene, an α -globin gene, a β -globin (HBB) gene, a tyrosine hydroxylase gene, a heat shock protein 70 (Hsp70) gene, a lipoxygenase gene, and a collagen α gene, or a homolog, fragment, or variant thereof. For example, the variant sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to a wild-type 3'-UTR sequence of a corresponding gene. In some embodiments, the 3'-UTR comprises a 3'-UTR derived from an albumin (ALB) gene or a homolog, fragment, or variant thereof. Preferably, the 3'-UTR comprises a sequence as shown in SEQ ID NO: 16.

In some embodiments, the poly A tail may have 100-200 nucleotides in length, e. g., about 100 nucleotides, about 110 nucleotides, about 120 nucleotides, about 130 nucleotides, about 140 nucleotides, about 150 nucleotides, about 160 nucleotides, about 170 nucleotides, about 180 nucleotides, about 190 nucleotides, or about 200 nucleotides. In some embodiments, the polyA tail may have 100-150 nucleotides in length. In some embodiments, the poly A tail may have about 120 nucleotides in length.

In some embodiments, the mRNA encoding the HA protein of the one or more influenza virus strains is one or more selected from mRNAs having sequences as shown in SEQ ID NOs: 8-13, and preferably, comprises four mRNAs having sequences as shown in SEQ ID NOs: 8-11, or three mRNAs having sequences as shown in SEQ ID NOs: 8-10, or four mRNAs having sequences as shown in SEQ ID NOs: 10-13, or three mRNAs having sequences as shown in SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 13.

In some embodiments, the immunogenic composition comprises an mRNA encoding an NA protein of an influenza virus strain, and the sequence of the mRNA is SEQ ID NO: 14.

In some embodiments, one or more nucleotides in the mRNA may be modified. For example,

one or more nucleotides (e. g., all nucleotides) in the mRNA can each be replaced independently by a naturally occurring nucleotide analog or an artificially synthesized nucleotide analog.

In some embodiments, the naturally occurring nucleotide analog is selected from pseudouridine, 2-thiouridine, 5-methyluridine, 5-methylcytidine, and N6-methyladenosine. In some embodiments, the artificially synthesized nucleotide analog is selected from N-methylpseudouridine and 5-ethynyluridine.

In some embodiments, one or more uridine triphosphates in the mRNA are each independently replaced with pseudouridine triphosphate, 2-thiouridine triphosphate, 5-methyluridine triphosphate, N1-methylpseudouridine triphosphate, or 5-ethynyluridine triphosphate, and/or one or more cytidine triphosphates are replaced with 5-methylcytidine triphosphates, and/or one or more adenosine triphosphates are replaced with N6-methyladenosine triphosphate.

In some embodiments, one or more uridine triphosphates in the mRNA are each independently replaced with pseudouridine triphosphate, 1-methyl-pseudouridine triphosphate, or 5-ethynyluridine triphosphate, and/or one or more cytidine triphosphates are replaced with 5-methylcytidine triphosphate.

In some embodiments, the mRNA is encapsulated in a carrier material. In some embodiments, the carrier material may be selected from protamine, lipid nanoparticles (LNP), polymeric materials, and inorganic nanoparticles. In a preferred embodiment, the carrier material is an LNP. In some embodiments, the LNP may comprise one or more of ionized lipids, pegylated lipids, cholesterol and derivatives, and phospholipids. For example, the LNP may comprise any one or a combination of any two, any three, or all four of ionized lipids, pegylated lipids, cholesterol and derivatives, and phospholipids. In a preferred embodiment, the LNP comprises 8-(3-hydroxypropyl)(9,12-dienyloctadecyl-1)-amino-caprylic heptadecan-9-ol ester, distearoyl phosphatidylcholine (DSPC), 1,2-dimyristoyl-rac-glycerol-3-methoxy polyethylene glycol 2000 (DMG-PEG2000), and cholesterol.

In some embodiments, the molar concentration of each of the mRNAs in the composition is no more than 3, 2.5, 2, or 1.5 times the molar concentration of any other mRNA, or the molar concentration of each of the mRNAs in the composition is substantially the same. Preferably, the molar concentration of each of the mRNAs is substantially the same.

In some embodiments, the immunogenic composition comprises mRNAs encoding four HA proteins selected from 1)-4), or three HA proteins selected from 1)-3), or four HA proteins selected from 3)-6), or three HA proteins selected from 3), 5), and 6):

- 1) a HA protein of an A/Wisconsin/588/2019(H1N1)pdm09-like virus;

- 2) a HA protein of an A/Darwin/6/2021(H3N2)-like virus;
- 3) a HA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage);
- 4) a HA protein of a B/Phuket/3073/2013-like virus (B/Yamagata lineage);
- 5) a HA protein of an A/Wisconsin/67/2022(H1N1)pdm09-like virus; or
- 6) a HA protein of an A/Massachusetts/18/2022(H3N2)-like virus; and

preferably, when the immunogenic composition comprises four mRNAs, the molar ratio of the mRNAs is 1-3:1-3:1-3:1-3, e.g., 1-2.5:1-2.5:1-2.5:1-2.5, 1-2:1-2:1-2:1-2, 1-1.5:1-1.5:1-1.5:1-1.5, and preferably 1:1:1:1.

In some embodiments, the immunogenic composition comprises mRNAs encoding four HA proteins selected from 1)-4), or three HA proteins selected from 1)-3), or four HA proteins selected from 3)-6), or three HA proteins selected from 3), 5), and 6), and an mRNA encoding an NA protein selected from 7):

- 1) a HA protein of an A/Wisconsin/588/2019(H1N1)pdm09-like virus;
- 2) a HA protein of an A/Darwin/6/2021(H3N2)-like virus;
- 3) a HA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage);
- 4) a HA protein of a B/Phuket/3073/2013-like virus (B/Yamagata lineage);
- 5) a HA protein of an A/Wisconsin/67/2022(H1N1)pdm09-like virus;
- 6) a HA protein of an A/Massachusetts/18/2022(H3N2)-like virus; or
- 7) a NA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage); and

preferably, when the immunogenic composition comprises four mRNAs, the molar ratio of the mRNAs is 1-3:1-3:1-3:1-3, e.g., 1-2.5:1-2.5:1-2.5:1-2.5, 1-2:1-2:1-2:1-2, 1-1.5:1-1.5:1-1.5:1-1.5, and preferably 1:1:1:1.

In some embodiments, the composition may be a vaccine composition, and optionally, the vaccine composition further comprises one or more adjuvants.

As used herein, the term "vaccine composition" refers to a biological agent that induces or improves immunity to a particular disease. A vaccine composition is used to stimulate the immune system of an individual to induce the formation and/or proliferation of immune cells that specifically recognize a compound contained in the vaccine. At least a portion of the immune cells remain viable

for a period of time that can extend to 10, 20, or 30 years after vaccination. If an individual's immune system encounters a pathogen from which a compound capable of eliciting an immune response is derived within the aforementioned period, then the immune cells generated by vaccination are reactivated, and the immune response against the pathogen is enhanced compared to the immune response of an individual who has not been primed with the vaccine and is encountering the immunogenic compound of the pathogen for the first time.

In this context, "vaccinated", "immunized", "immunization" or "vaccination" refers to the administration of a vaccine to a subject with the aim of preventing the subject from developing one or more symptoms of a disease. In principle, vaccination comprises a prime vaccination and optionally one or more boost vaccinations. Prime vaccination or prime immunization is defined as a prime administration schedule for administration of a composition or unit dose disclosed herein to establish a protective immune response. Boost vaccination or boost immunization refers to an administration or administration schedule following a prime vaccination, for example at least 1 week, at least 2 weeks, at least 1 month, at least 6 months, at least 1 year, or even 5 or 10 years after the last administration of the prime vaccination schedule. Boost administration attempts to enhance or recreate the immune response produced by the prime vaccination.

The immune response to the composition or vaccine composition of the present invention is the formation of a humoral and/or cellular immune response in a subject to the antigenic protein present in the composition. For the purposes of the present invention, the "humoral immune response" refers to an immune response mediated by an antibody molecule, including a secretory (IgA) or IgG molecule, and the "cellular immune response" refers to an immune response mediated by T-lymphocytes and/or other white blood cells. An important aspect of cellular immunity involves antigen-specific responses of cell-lysed T cells ("CTLs"). CTLs are specific for peptide antigens that are presented in combination with proteins encoded by the major histocompatibility complex (MHC) and expressed on the cell surface. CTLs help induce and promote the destruction of intracellular microorganisms, or the lysis of cells infected by such microorganisms. Another aspect of cellular immunity involves antigen-specific responses of helper T cells. Helper T cells act to help stimulate the function and focus the activity of nonspecific effector cells against cells that display peptide antigens bound to MHC molecules on their surface. The cellular immune response also involves the production of cytokines, chemokines and other such molecules generated by activated T cells and/or other leukocytes, including those derived from CD4⁺ and CD8⁺ T cell-derived cells.

Therefore, the immune response can be one that stimulates production or activation of CTLs and/or helper T cells, and can also stimulate production of chemokines and/or cytokines. The composition or vaccine composition of the invention can also induce an antibody-mediated immune

response. Therefore, the immune response may include one or more of the following effects: antibody (e.g., IgA or IgG) production by B cells; and/or inhibitors, cytotoxicity or helper T cells and/or T cell activation specific for the protein present in the vaccine. These responses can be used to neutralize infectivity, and/or mediate antibody-complement or antibody dependent cell cytotoxicity (ADCC) to provide protection to the immunized individual. Such responses can be determined using standard immunoassays and neutralization assays known in the art.

As used herein, the term "adjuvant" refers to an agent that increases, stimulates, activates, enhances, or modulates the immune response to the active ingredient in the composition at the cellular or humoral level, for example, an immunological adjuvant stimulates the immune system to produce a response to an actual antigen but does not itself has an immune effect. Examples of such adjuvant include, but are not limited to, inorganic adjuvants (e.g., inorganic metal salts such as aluminum phosphate or aluminum hydroxide), organic adjuvants (e.g., saponin or squalene), oil-based adjuvants (such as Freund's complete and incomplete adjuvants), cytokines (such as IL-1 β , IL-2, IL-7, IL-12, IL-18, GM-CSF, and INF- γ), particulate adjuvants (e.g., immunostimulatory complexes (ISCOMS), liposomes, or biodegradable microspheres), viral particles, bacterial adjuvants (e.g., monophosphoryl lipid A or muropeptide), synthetic adjuvants (e.g., non-ionic block co-polymers, muropeptide analogues or synthetic lipid A), or synthetic polynucleotide adjuvants (e.g., poly-arginine or poly-lysine). Preferably, the adjuvant is selected from aluminum adjuvants (e.g., aluminum hydroxide, aluminum phosphate, aluminum sulfate, or alum), MF59, AS03, virosomes (e.g., hepatitis virus virosomes and influenza virus virosomes), AS04, heat reversible oil-in-water emulsions, ISA51, Freund's adjuvants, IL-12, CpG motifs, mannose, or any combinations thereof.

In a preferred embodiment, the adjuvant is selected from aluminum adjuvants (e.g. aluminum hydroxide, aluminum phosphate, aluminum sulfate, or alum), MF59, AS03, AS04, heat-reversible oil-in-water emulsions, ISA51, Freund's adjuvants, IL-12, CpG motifs, and mannose.

In some embodiments, the composition of the present invention further comprises one or more pharmaceutically acceptable carriers, excipients, or diluents.

As used herein, the term "pharmaceutically acceptable" refers to a carrier, excipient, or diluent that is suitable, within the scope of sound medical judgment, for use in contact with the tissues of humans and animals without excessive toxicity, irritation, allergic response, or other problems or complications, and commensurate with a reasonable benefit/risk ratio.

Exemplary carriers for use in the composition of the invention include saline, buffered saline, dextrose, and water. Exemplary excipients for use in the composition of the present invention include fillers, binders, disintegrants, coatings, sorbents, anti-adhesion agents, glidants, preservatives,

antioxidants, flavors, colorants, sweeteners, solvents, co-solvents, buffers, chelating agents, viscosity enhancer, surfactants, diluents, wetting agents, carriers, emulsifiers, stabilizers, and tension regulators. It is known to a person skilled in the art to select a suitable excipient to prepare the composition of the present invention. In general, the selection of a suitable excipient depends, inter alia, on the active agent used, the disease to be treated, and the desired dosage form of the composition.

Depending on the active agent employed, such as the mRNA, the composition of the invention can be prepared in various forms, such as solid, liquid, gaseous, or lyophilized form, in particular in the form of ointments, creams, transdermal patches, gels, powders, tablets, solutions, aerosols, granules, pellets, suspensions, emulsions, capsules, syrups, liquids, elixirs, extracts, tinctures, or fluid extracts, or in a form that is particularly suitable for the desired method of administration. The processes known to the present invention for producing medicaments are shown in Remington's Pharmaceutical Sciences (Ed. Maack Publishing Co. , Easton, Pa., 2012), 22nd Edition, and may include, for example, conventional mixing, dissolving, granulating, sugar-coating, levigating, emulsifying, encapsulating, embedding, or lyophilizing processes.

In some embodiments, the composition further comprises one or more additional therapeutic agents. For example, the therapeutic agent may be selected from additional antigenic proteins or polypeptides, antibodies, hormones or hormone mimetics, and small molecule drugs.

In a second aspect, the invention provides a method for preventing and/or treating a disease or condition associated with influenza virus infection in a subject, comprising administering to the subject an effective amount of the immunogenic composition of the first aspect of the invention.

As used herein, the term "preventing" or "treating" refers to a reduction in the risk of obtaining or developing a disease or condition, i.e. preventing the development of at least one of clinical symptoms of a disease in a subject susceptible to the disease or not exposed to the pathogen prior to the onset of the disease. For example, treatment may include: (i) preventing a disease, disorder, and/or condition in a patient who may be predisposed to the disease, disorder, and/or condition but has not yet been diagnosed as having the disease, disorder, and/or condition; (ii) inhibiting the disease, disorder, and/or condition, i.e. arresting its development; or (iii) relieving the disease, disorder, and/or condition, i.e. causing regression of the disease, disorder, and/or condition.

As used herein, the term "effective amount" means the amount of a compound that, when administered to a subject for treating or preventing a disease, is sufficient to effect such treatment or prevention. The "effective amount" may vary depending on the compound, the disease and the severity thereof, and the age, weight, etc. of a subject to be treated. The "therapeutically effective

amount" refers to an amount effective for therapeutic treatment. The "prophylactically effective amount" refers to an amount effective for prophylactic treatment.

As used herein, the term "administration" refers to the physical introduction of an agent into a subject by using any of a variety of methods and delivery systems known to a person skilled in the art. Exemplary routes of administration include intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other parenteral routes of administration, for example by injection or infusion.

As used herein, the terms "subject", "individual", and "patient" are well known in the art and are used interchangeably herein to refer to any subject in need of treatment, particularly a mammalian subject. Examples include, but are not limited to, humans and other primates, including non-human primates such as chimpanzees and other apes and monkey species. The terms "individual", "subject", and "patient" per se do not denote a particular age, gender, race, etc.

In embodiments of the method of the invention, the disease or condition is a disease or condition caused by infection with one or more of influenza A viruses and influenza B viruses. In some embodiments, the disease or condition is a disease or condition caused by one or more of: influenza A virus H1N1 subtype, influenza A virus H3N2 subtype, influenza B virus Victoria lineage, and influenza B virus Yamagata lineage. In a preferred embodiment, the disease or condition is a disease or condition caused by one or more of: A/Wisconsin/588/2019(H1N1)pdm09-like viruses, A/Darwin/6/2021(H3N2)-like viruses, B/Austria/1359417/2021-like viruses (B/Victoria lineage), B/Phuket/3073/2013-like viruses (B/Yamagata lineage), A/Victoria/2570/2019(H1N1) viruses, A/Cambodia/E0826360/2021(H3N2) viruses, A/California/04/2009(H1N1) viruses, and B/Florida/4/2006 viruses.

In some embodiments, the method comprises administering the immunogenic composition of the first aspect of the invention to the subject by using a prime-boost vaccination regimen. The "prime-boost vaccination regimen" refers to repeated administration of the immunogenic composition or the vaccine composition of the invention against a particular virus or group of viruses. In one embodiment, the prime-boost vaccination regimen involves at least two administrations of the immunogenic composition or vaccine composition for a particular virus or group of viruses. The first administration of the vaccine is referred to as "prime immunization", and any subsequent administration of the same vaccine, or a vaccine against the same virus or group of viruses as the first vaccine, is referred to as "boost immunization". Thus, in a further embodiment of the invention, the prime-boost vaccination regimen involves one administration of a vaccine for inducing an immune response and at least one subsequent administration for boosting the immune response. It will be appreciated that the invention also encompasses 2, 3, 4, or even 5 administrations for

boosting the immune response.

The time period between the prime immunization and boost immunization is optionally 1 week, 2 weeks, 4 weeks, 6 weeks, or 8 weeks. More specifically, it is 4 weeks or 8 weeks. If more than one boost is carried out, the subsequent boosts are applied 1 week, 2 weeks, 4 weeks, 6 weeks, or 8 weeks after the prior boost. For example, the time interval between any two boosts is 4 weeks or 8 weeks.

In certain embodiments, the immunogenic composition of the invention is administered via intranasal, intramuscular, subcutaneous, intradermal, intragastric, oral, or topical routes.

"Intranasal administration" is the application of the carrier of the invention to the mucosa of all respiratory passages including the lungs. More particularly, the composition is applied to the nasal mucosa. In one embodiment, intranasal administration is achieved by instillation, spraying, or aerosol. In another embodiment, the administration does not involve perforating the mucosa by mechanical means, such as a needle.

The term "intramuscular administration" refers to the injection of the carrier into any muscle of an individual. Exemplary intramuscular injections include administration in deltoid, vastus lateralis, ventrogluteal, and dorsogluteal regions.

The term "subcutaneous administration" refers to the injection of the carrier into the skin.

The term "intradermal administration" refers to the injection of the carrier into the dermis between layers of skin.

The term "oral administration" refers to the administration of the carrier to the gastric system via the mouth.

"Topical application" refers to the administration of the carrier to any part of the skin without penetrating the skin by a needle or comparable device. The carrier may also be topically applied to the mucosa of the mouth, nose, genital zone, and rectum.

In some embodiments, the immune response is elicited by an intranasal administration and the immune response is boosted by at least one intramuscular administration; the immune response is elicited by an intranasal administration and the immune response is boosted by at least one subcutaneous administration; the immune response is elicited by an intranasal administration and the immune response is boosted by at least one intradermal administration; the immune response is elicited by an intranasal administration and the immune response is boosted by at least one intragastric administration; the immune response is elicited by an intranasal administration and the

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administration; the immune response is elicited by an intragastric administration and the immune response is boosted by at least one intramuscular administration; the immune response is elicited by an intragastric administration and the immune response is boosted by at least one subcutaneous administration; the immune response is elicited by an intragastric administration and the immune response is boosted by at least one intradermal administration; the immune response is elicited by an intragastric administration and the immune response is boosted by at least one intragastric administration; the immune response is elicited by an intragastric administration and the immune response is boosted by at least one oral administration; the immune response is elicited by an intragastric administration and the immune response is boosted by at least one topical administration; the immune response is elicited by an intragastric administration and the immune response is boosted by at least one intranasal administration; the immune response is elicited by oral administration and the immune response is boosted by at least one intramuscular administration; the immune response is elicited by oral administration and the immune response is boosted by at least one subcutaneous administration; the immune response is elicited by oral administration and the immune response is boosted by at least one intradermal administration; the immune response is elicited by an oral administration and the immune response is boosted by at least one intragastric administration; the immune response is elicited by an oral administration and the immune response is boosted by at least one oral administration; the immune response is elicited by an oral administration and the immune response is boosted by at least one topical administration; the immune response is elicited by an oral administration and the immune response is boosted by at least one intranasal administration; the immune response is elicited by a topical administration and the immune response is boosted by at least one intramuscular administration; the immune response is elicited by a topical administration and the immune response is boosted by at least one subcutaneous administration; the immune response is elicited by a topical administration and the immune response is boosted by at least one intradermal administration; the immune response is elicited by a topical administration and the immune response is boosted by at least one intragastric administration; the immune response is elicited by a topical administration and the immune response is boosted by at least one oral administration; the immune response is elicited by a topical administration and the immune response is boosted by at least one topical administration; the immune response is elicited by a topical administration and the immune response is boosted by at least one intranasal administration. In one embodiment, the immune response is elicited by an intranasal administration and the immune response is boosted by at least one intramuscular administration. In yet another embodiment, the immune response is elicited by an intranasal administration and the immune response is boosted by at least one intranasal administration. In yet another embodiment, the immune response is elicited by an intramuscular administration and the

immune response is boosted by at least one intramuscular administration.

In a third aspect, the invention provides use of the immunogenic composition of the first aspect of the invention in the preparation of a medicament for preventing and/or treating a disease or condition associated with influenza virus infection in a subject.

In a fourth aspect, the invention provides the immunogenic composition of the first aspect of the invention, which is used for preventing and/or treating a disease or condition associated with influenza virus infection in a subject.

In embodiments of the use of the invention, the disease or condition is a disease or condition caused by infection with one or more of influenza A viruses and influenza B viruses. In some embodiments, the disease or condition is a disease or condition caused by one or more of influenza A virus H1N1 subtype, influenza A virus H3N2 subtype, influenza B virus Victoria lineage, and influenza B virus Yamagata lineage. In a preferred embodiment, the disease or condition is a disease or condition caused by one or more of A/Wisconsin/588/2019(H1N1) pdm09-like virus, A/Darwin/6/2021(H3N2)-like virus, B/Austria/1359417/2021-like virus (B/Victoria lineage), B/Phuket/3073/2013-like virus (B/Yamagata lineage), A/Victoria/2570/2019(H1N1) virus, A/Cambodia/E0826360/2021(H3N2) virus, A/California/04/2009(H1N1) virus, and B/Florida/4/2006 virus.

In a fifth aspect, the invention provides a method for preventing and/or treating a disease or a condition associated with influenza virus infection, comprising: administering the immunogenic composition to a subject suffering from the disease or the condition associated with influenza virus infection.

In embodiments of the method for preventing and/or treating a disease or a condition associated with influenza virus infection, the disease or the condition is a disease or a condition associated with influenza A virus and/or influenza B virus infection.

In a sixth aspect, the invention provides the immunogenic composition of the first aspect of the invention used as a medicine for preventing and/or treating a disease or a condition associated with influenza virus infection.

In embodiments of the use as a medicine for preventing and/or treating a disease or a condition associated with influenza virus infection, the disease or the condition is a disease or a condition associated with influenza A virus and/or influenza B virus infection.

Brief Description of the Drawings

The accompanying drawings, which form a part of the present invention, are used to provide a further understanding of the present invention. The schematic embodiments of the present invention and the description thereof are used to explain the present invention, and do not form improper limits to the present invention. In the drawings:

Fig. 1 shows a graph of the results of a Western blot of the HA proteins expressed by RBMRNA-01, RBMRNA-02, RBMRNA-03, and RBMRNA-04 of the invention.

Fig. 2 shows a graph of the results of IgG antibody levels in mouse serum against HA proteins after vaccination with one dose of quadrivalent vaccine.

Fig. 3 shows a graph of the results of IgG antibody levels in mouse serum against HA proteins after vaccination with two doses of quadrivalent vaccine.

Fig. 4 shows a graph of the results of hemagglutination inhibition (HI) antibody levels in mouse serum after vaccination with two doses of quadrivalent vaccine.

Fig. 5 shows a graph of the results of cytokine secretion by mouse spleen lymphocytes stimulated by different HA proteins induced by the quadrivalent vaccine of the present invention.

Detailed Description of the Embodiments

It should be noted that the embodiments of the present invention and the features in the embodiments can be combined with each other without conflict. Hereinafter, the present invention will be described in detail with reference to examples.

Example 1 Preparation of mRNA

A DNA coding sequence was designed based on the sequence of an A/Wisconsin/588/2019(H1N1) pdm09-like virus HA protein. After adding 5'-UTR, 3'-UTR, and polyA sequences to the DNA coding sequence, the sequence was inserted into a pT7TS plasmid by homologous recombination to form a recombinant vector pT7TS-2.0, and the final recombinant plasmid was named RBMRNA-01 plasmid. The elements contained in the RBMRNA-01 plasmid included a start sequence (SEQ ID NO: 17), a T7 promoter sequence (SEQ ID NO: 18), a 5'-UTR sequence (SEQ ID NO: 15), a 3'-UTR sequence (SEQ ID NO: 16), a 3'-terminal polyadenylic acid (poly A) sequence, an ampicillin resistance gene promoter, and a kanamycin sulfate resistance gene. These non-coding structures are used to regulate the stability, translation efficiency, and immunogenicity of an mRNA transcribed from the RBMRNA-01 plasmid.

The coding region and polyA region of the RBMRNA-01 plasmid were sequenced, and the

inserted target gene sequencing was completely consistent with the reference sequence. The RBMRNA-01 plasmid was transcribed in vitro to obtain an mRNA (named RBMRNA-01), and the mRNA was translated to obtain a protein (named RBMRNA-01 protein, the sequence was as shown in SEQ ID NO: 1).

With reference to the described method, plasmids for HA proteins or NA proteins of another influenza viruses were designed and obtained, the plasmids were transcribed in vitro to obtain corresponding mRNAs, and the mRNAs were translated to obtain corresponding proteins.

Table 1. Plasmids designed from different influenza strains and coding mRNAs and proteins thereof

Viral protein name	Plasmid and corresponding DNA coding sequence	mRNA name	Protein name
A HA protein of an A/Wisconsin/588/2019(H1N1)pdm09-like virus	RBMRNA-01 plasmid	RBMRNA-01 (SEQ ID NO: 8)	RBMRNA-01 protein (SEQ ID NO: 1)
A HA protein of an A/Darwin/6/2021(H3N2)-like virus	RBMRNA-02 plasmid	RBMRNA-02 (SEQ ID NO: 9)	RBMRNA-02 protein (SEQ ID NO: 2)
A HA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage)	RBMRNA-03 plasmid	RBMRNA-03 (SEQ ID NO: 10)	RBMRNA-03 protein (SEQ ID NO: 3)
A HA protein of a B/Phuket/3073/2013-like virus (B/Yamagata lineage)	RBMRNA-04 plasmid	RBMRNA-04 (SEQ ID NO: 11)	RBMRNA-04 protein (SEQ ID NO: 4)
A NA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage)	RBMRNA-05 plasmid	RBMRNA-05 (SEQ ID NO: 14)	RBMRNA-05 protein (SEQ ID NO: 7)

Example 2 Expression validation of mRNA

293T cells were transfected with 5µg of RBMRNA-01, RBMRNA-02, RBMRNA-03, and RBMRNA-04 obtained in Example 1 with reference to the instructions of Lipofectamine Messenger MAX (ThermoFisher Scientific), and cells not transfected with the mRNA were used as a negative

control (NC). 24 hours after transfection, the expression of the HA proteins was examined by Western Blot. Primary antibodies used included: Influenza A H1N1 (A/California/04/2009) Hemagglutinin/HA antibody (Sino Biological, Cat#11055-T62), Influenza A H3N2 Hemagglutinin/HA antibody (Sino Biological, Cat#11056-MM03), Influenza B virus (B/Florida/4/2006) Hemagglutinin/HA antibody (Sino Biological, Cat#11053-T62), and Influenza B Hemagglutinin/HA antibody (Sino Biological, Cat#11053-MM06), labelled with goat anti-rabbit-HRP secondary antibody or goat anti-mouse-HRP secondary antibody. Results are shown in Figure 1. The four antigen proteins can be expressed significantly in cells.

Example 3 Assessment of IgG antibody titers and HI antibody titers induced by quadrivalent mRNA vaccine

mRNA vaccines, referred to as RBMRNA-01 vaccine, RBMRNA-02 vaccine, RBMRNA-03 vaccine, and RBMRNA-04 vaccine, respectively, were prepared using the mRNAs: RBMRNA-01, RBMRNA-02, RBMRNA-03, and RBMRNA-04 of Example 1.

The mRNA was encapsulated using lipid nanoparticles that comprised the following components: 8-(3-hydroxypropyl) (9,12-dienyl-octadecyl-1)-amino-caprylic heptadecan-9-ol ester, distearoyl phosphatidylcholine (DSPC), 1,2-dimyristoyl-rac-glycerol-3-methoxypolyethylene glycol 2000 (DMG-PEG2000), and cholesterol. The preparation method comprised dissolving the described components in an ethanol solution, mixing the lipid ethanol solution with an mRNA aqueous solution via a microfluidic mixer to obtain lipid nanoparticles, and performing dialysis, ultrafiltration and micro-membrane filtration on the mixture to obtain an LNP vaccine formulation of the mRNA. A specific vaccine preparation method is disclosed in patent application CN 112480217 A.

The described four vaccines were mixed in an mRNA molar ratio of 1:1 to prepare a quadrivalent mRNA vaccine (the quadrivalent vaccine) for BALB/c mouse immunoassay. Mice for immunization were BALB/c mice of SPF grade health, 6-8 weeks old, and females, and were randomly divided into a solvent control (PBS) group and an immunization group according to the body weight of the mice in the experiment, with 5 mice/group. The immunization group was administered the quadrivalent vaccine by intramuscular injection at an injection dose of 20 µg/mouse, and solvent control group was administered an equal volume of PBS, and immunized twice on day 0 (D0) and day 21 (D21), respectively, for prime and boost immunizations. Serum was collected at 14 days after the prime immunization and boost immunization, the IgG antibody titer against influenza virus HA protein in the serum was detected by indirect ELISA, and hemagglutination inhibition (HI) assay was used to detect hemagglutination inhibition antibody titer in the serum.

Antigenic proteins involved in indirect ELISA assay are: Influenza A H1N1

(A/Wisconsin/588/2019)/(A/Victoria/2570/2019) HA protein (Sino Biological, Cat#40787-V08H1), Influenza A H3N2 (A/Darwin/6/2021) HA protein (Sino Biological, Cat#40868-V08H), Influenza B (B/PHUKET/3073/2013) HA protein (Sino Biological, Cat# 40498-V08B), and Influenza B (B/Austria/1359417/2021) HA protein (Sino Biological, Cat#40862-V08H).

The specific method for the hemagglutination inhibition assay was as follows:

(1) Preparation of a 4HAU hemagglutinin working solution: the HA titer of the used influenza virus solution was detected by means of a hemagglutination experiment, and the influenza virus solution was diluted with PBS to a titer of 4 hemagglutination units.

(2) Treatment of non-specific hemagglutination inhibition activity in mouse serum by means of a receptor-destroying enzyme (RDE): 1 volume of mouse serum was incubated in a 37 °C water bath for 16 h after mixing with 3 volumes of RDE, followed by incubation at 56 °C for 1 h to inactivate the activity of RDE.

(3) HI test: 25 μ L of PBS was added into the well 2 to well 9 of a V-shaped 96-well hemagglutination plate, 25 μ L of RDE-treated serum was added into the first well and second well of each row, and the serum to be tested was diluted 2-fold serially from well 2 to well 9. 25 μ L was aspirated and the dilution was complete. 25 μ L of PBS was added to well 10 as a virus control well, and 50 μ L of PBS was added to well 11 as a red blood cell control well. 25 μ L of 4HAU antigen was added to each of wells 1-10 and mixed thoroughly by shaking. Leave at room temperature for at least 20 minutes. 25 μ L of 1% chicken red blood cell suspension was added to each well and mixed thoroughly. Leave at room temperature for 20-30 minutes, and then determine the hemagglutination results.

(4) Result determination: the highest serum dilution factor that completely inhibited 4 HAU antigens was determined as the HI antibody titer of the serum.

IgG antibody titers against the HA protein in mouse serum after vaccination with one dose and two doses of the quadrivalent vaccine are shown in Fig. 2 and 3, respectively. The results show that IgG antibody levels in mouse serum against HA of influenza A virus H1N1 and H3N2 and Yamagata lineage influenza B virus and Victoria lineage influenza B virus HA are significantly elevated after vaccination with one dose of the quadrivalent vaccine compared to the solvent control group mice, with an average titer of 4 Log₁₀ or more (Fig. 2). After two doses of the quadrivalent vaccine are inoculated, the serum HA IgG antibody level of mice is higher than that of the first vaccination, and the average titer reaches 6 Log₁₀ or more (fig. 3).

The HI antibody titer in the mouse serum after vaccination with two doses of the quadrivalent

vaccine is shown in Fig. 4. The results show that HI titer against the A/Victoria/2570/2019 (H1N1) strain and the B/Austria/1359417/2021 (B-Victoria) strain reaches 2304 and 1408 respectively after vaccination with 2 doses of the quadrivalent vaccine, and HI antibodies against multiple H3N2 subtypes and multiple influenza B strains are produced, including A/Cambodia/E0826360/2021 (H3N2), A/Darwin/6/2021 (H3N2), A/Hong Kong/2671/2019 (H3N2) 45040, B/Washington/02/2019 (B/Victoria), B/Phuket/3073/2013 (B/Yamagata), etc. It can be determined from Fig. 4 that even with regard to A/Hong Kong/2671/2019 (H3N2) 45040 and B/Washington/02/2019 (B/Victoria), the HI titer also reaches 1:80-1:160. However, it is recognized in the art that a protection effect of 50% can be produced when the HI titer reaches 1:40. The above results show that the quadrivalent vaccine can induce a strong humoral immune response, and can stimulate a broad immune response against antigenically diverse influenza A and B viruses.

Example 4 Quadrivalent mRNA vaccine-induced Th1-biased cellular immune response

BALB/c mouse immunization assays were performed using the quadrivalent vaccine prepared in Example 3. Mice for immunization were BALB/c mice of SPF grade health, 6-8 weeks old, and females, and were randomly divided into a solvent control (PBS) group and an immunization group according to the body weight of the mice in the experiment, with 3 per group. The immunization group was administered the quadrivalent vaccine by intramuscular injection at an injection dose of 20 µg/mouse, and the solvent control group was administered an equal volume of PBS, followed by two immunizations at day 0 and day 21, respectively, for prime and boost immunizations. 14 days after the boost, the mouse spleen was taken to isolate splenic lymphocytes, and the levels of Th1-type cytokine INF-γ and Th2-type cytokine IL4 were detected by ELISPOT assay. The specific operations of detecting INF-γ and IL-4 were performed according to the instruction of the Dayou (达优) Mouse IFN-γ/IL-4 ELISPOT kit. No stimulant was added to the negative control wells, 10 µL of a positive stimulant (see the instruction of the kit) was added to the positive control wells, and 1 µg of Influenza A H1N1 (A/Wisconsin/588/2019)/(A/Victoria/2570/2019) HA protein (Sino Biological, Cat#40787-V08H1), Influenza A H3N2 (A/Darwin/6/2021) HA protein (Sino Biological, Cat#40868-V08H), Influenza B (B/PHUKET/3073/2013) HA protein (Sino Biological, Cat# 40498-V08B), and Influenza B (B/Austria/1359417/2021) HA protein (Sino Biological, Cat#40862-V08H) was respectively added to each experimental well for stimulating lymphocytes. The results are shown in Fig. 5.

The results show that, after boost immunization, stimulating splenic lymphocytes again with HA protein antigens can generate specific cellular immune responses against four antigens, wherein the cellular response against the influenza A virus H3N2 HA protein is the strongest. In addition, the frequency of T lymphocytes secreting the Th1 type cytokine INF-γ is significantly higher than the

frequency of T lymphocytes secreting the Th2 type cytokine IL-4, indicating that the immune response induced by the quadrivalent vaccine is a Th1- biased immune response.

Example 5 Evaluation of protective effects of quadrivalent and pentavalent vaccines against influenza virus attack

RBMRNA-01, RBMRNA-02, RBMRNA-03, RBMRNA-04, and RBMRNA-05 in Example 1 were respectively used to prepare mRNA vaccines, referred to as RBMRNA-01 vaccine, RBMRNA-02 vaccine, RBMRNA-03 vaccine, RBMRNA-04, RBMRNA-05 vaccine, respectively.

A quadrivalent vaccine was prepared by mixing RBMRNA-01, RBMRNA-02, RBMRNA-03, and RBMRNA-04 according to an mRNA molar ratio of 1:1; and a pentavalent vaccine was prepared by mixing RBMRNA-01, RBMRNA-02, RBMRNA-03, RBMRNA-04, and RBMRNA-05 according to an mRNA molar ratio of 1:1. Both the quadrivalent vaccine and the pentavalent vaccine were used in a BALB/c mouse immunoassay. The cross neutralization activity of the vaccines was evaluated by detecting neutralizing antibodies against different strains, and the lung tissue virus titer of mice was tested by TCID₅₀ assay, so as to evaluate the protective effects of the quadrivalent and pentavalent vaccines against influenza A and B virus attack.

BALB/c mice were selected for the experiment, and each virus attack protection experiment was provided with an attack control group, a quadrivalent vaccine group, and a pentavalent vaccine group, with 4 mice per group. The quadrivalent vaccine group and the pentavalent vaccine group were respectively administrated with 20 µg of the quadrivalent vaccine and the pentavalent vaccine by intramuscular injection per mouse, and the attack control group was administrated with an equal volume of saline. Immunization was performed twice, on day 0 (prime immunization) and day 21 (boost immunization). Blood was collected two weeks after the boost immunization, and the serum was isolated to detect neutralizing antibodies, and virus attack was performed after blood collection. For the influenza A virus attack protection experiment, the attack control group (4 mice per group), the quadrivalent vaccine group (4 mice per group), and the pentavalent vaccine group (4 mice per group) were subjected to nasal infection with A/California/04/2009 (H1N1) virus, respectively, with an infection dose of 2×10^4 TCID₅₀ per mice. For the influenza B virus attack experiment, the attack control group (4 mice per group), the quadrivalent vaccine group (4 mice per group), and the pentavalent vaccine group (4 mice per group) were subjected to nasal infection with B/Austria/1359417/2021 virus, respectively, with an infection dose of 6.5×10^5 TCID₅₀ per mice. All mice were dissected 4 days after attack infection, right lung tissue was sampled and tested for virus titer by TCID₅₀ experiment. The limit of detection was 10 TCID₅₀/mL.

The specific method for neutralizing antibody detection was as follows:

- (1) 100 μ L of MDCK cells were added to each well of a 96-well cell culture plate to achieve 1×10^4 cells per well. The culture plate was placed in a 5% CO₂ cell incubator at 37 °C and incubated horizontally. After the cells grew to a monolayer, a micro-neutralization test was performed.
- (2) The serum was inactivated in a 56 °C water bath for 30 min, and centrifuged at 6000 g for 3 min, and the supernatant was transferred to a 1.5 mL centrifuge tube.
- (3) The inactivated serum was diluted 2-fold with a virus maintenance solution.
- (4) The virus was diluted to a working concentration (200 TCID₅₀/100 μ L) with the virus maintenance solution, and was added to the diluted serum at a ratio of 1:1, and the mixture, after uniform mixing, was placed in a 37 °C incubator for 1 hour.
- (5) The cell culture medium was removed from the 96-well culture plate filled with a cell monolayer, and the 96-well culture plate was washed twice with PBS.
- (6) The mixed solution after the neutralization was sequentially added to the described monolayer cells, and the cells was incubated in a 5% CO₂ cell incubator at 37 °C for 5-7 days.
- (7) The number of wells comprising cell lesion was recorded by daily observation, and when the number of wells comprising cell lesion no longer changed, the final experiment results were obtained.
- (8) The highest serum dilution factor that can protect 50% of the cell wells from virus infection was determined as the neutralizing antibody titer of the serum.

As shown in Table 2, virus replication could be detected in the lung tissue of mice in the attack control group 4 days after infection with A/California/04/2009 (H1N1) or B/Austria/1359417/2021, while no virus was detected in the lung tissue of mice in the quadrivalent vaccine group and the pentavalent vaccine group. The results show that the quadrivalent vaccine and the pentavalent vaccine can effectively protect mice from infection by A/California/04/2009 (H1N1) and B/Florida/4/2006 viruses, and have prevention effects on influenza A and B viruses.

The results of neutralizing antibodies are shown in Table 3. Both the quadrivalent vaccine and the pentavalent vaccine can produce neutralizing antibodies against influenza A and B viruses. The pentavalent vaccine has stronger cross neutralization activity against different virus strains than the quadrivalent vaccine.

Table 2. Virus titer of right lung tissue (TCID₅₀/ml)

	A/California/04/2009 (H1N1) attack	B/Austria/1359417/2021 attack
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Animal number	Attack control group	Quadrivalent vaccine group	Pentavalent vaccine group	Attack control group	Quadrivalent vaccine group	Pentavalent vaccine group
1	500000	<10	<10	85063	<10	<10
2	232079	<10	<10	15811	<10	<10
3	340646	<10	<10	10772	<10	<10
4	158114	<10	<10	23208	<10	<10

Table 3. Neutralizing antibody titer of mixed serum (n=4)

Strain Vaccine	B/Austria/135 9417/2021 (B/Victoria lineage)	B/Phuket/307 3/2013 (Yamagata lineage)	B/Florida/78/2 015 (B/Victoria lineage)	A/Hong Kong/4801/20 14 (H3N2)	A/California/0 4/2009 (H1N1)
Quadrivalent vaccine	6451	1016	<40	1180	226
Pentavalent vaccine	7608	905	174	1607	320

The above description is only the preferred embodiments of the present invention, and is not intended to limit the present invention. For a person skilled in the art, the present invention may have various modifications and variations. Any modifications, equivalent replacements, improvements and the like made within the spirit and principle of the present invention shall fall within the scope of protection of the present invention.

Claims

1. An immunogenic composition comprising an mRNA encoding a hemagglutinin protein-HA protein of one or more influenza virus strains.
2. The immunogenic composition of claim 1, wherein the HA protein of the one or more influenza strains is selected from influenza A virus HA proteins and influenza B virus HA proteins.
3. The immunogenic composition of claim 2, wherein the HA protein of the one or more influenza strains is one or more selected from 1)-6):
 - 1) a HA protein of an A/Wisconsin/588/2019(H1N1)pdm09-like virus;
 - 2) a HA protein of an A/Darwin/6/2021(H3N2)-like virus;
 - 3) a HA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage);
 - 4) a HA protein of a B/Phuket/3073/2013-like virus (B/Yamagata lineage);
 - 5) a HA protein of an A/Wisconsin/67/2022(H1N1)pdm09-like virus; or
 - 6) a HA protein of an A/Massachusetts/18/2022(H3N2)-like virus.
4. The immunogenic composition of any one of claims 1-3, further comprising an mRNA encoding a neuraminidase-NA protein of one or more influenza virus strains.
5. The immunogenic composition of claim 4, wherein the NA protein of the one or more influenza virus strains is selected from influenza A virus NA proteins and influenza B virus NA proteins.
6. The immunogenic composition of claim 5, wherein the NA protein of the one or more influenza virus strains is selected from a NA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage).
7. The immunogenic composition of claim 4, wherein the HA protein of the one or more influenza virus strains comprises four HA proteins selected from 1)-4), or three HA proteins selected from 1)-3), or four HA proteins selected from 3)-6), or three HA proteins selected from 3), 5), and 6):
 - 1) a HA protein of an A/Wisconsin/588/2019(H1N1)pdm09-like virus;
 - 2) a HA protein of an A/Darwin/6/2021(H3N2)-like virus;
 - 3) a HA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage);
 - 4) a HA protein of a B/Phuket/3073/2013-like virus (B/Yamagata lineage);
 - 5) a HA protein of an A/Wisconsin/67/2022(H1N1)pdm09-like virus; or

6) a HA protein of an A/Massachusetts/18/2022(H3N2)-like virus.

8. The immunogenic composition of claim 6, wherein the HA protein of the one or more influenza virus strains comprises four HA proteins selected from 1)-4), or three HA proteins selected from 1)-3), or four HA proteins selected from 3)-6), or three HA proteins selected from 3), 5), and 6):

1) a HA protein of an A/Wisconsin/588/2019(H1N1)pdm09-like virus;

2) a HA protein of an A/Darwin/6/2021(H3N2)-like virus;

3) a HA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage);

4) a HA protein of a B/Phuket/3073/2013-like virus (B/Yamagata lineage);

5) a HA protein of an A/Wisconsin/67/2022(H1N1)pdm09-like virus; or

6) a HA protein of an A/Massachusetts/18/2022(H3N2)-like virus; and

the NA protein is selected from a NA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage).

9. The immunogenic composition of claim 3 or 7 or 8, wherein the HA protein of the one or more influenza virus strains is one or more selected from HA proteins having amino acid sequences as shown in SEQ ID NOs:1-6;

and preferably, the HA protein comprises four HA proteins having amino acid sequences as shown in SEQ ID NOs: 1-4, or three HA proteins having amino acid sequences as shown in SEQ ID NOs: 1-3, or four HA proteins having amino acid sequences as shown in SEQ ID NOs: 3-6, or three HA proteins having amino acid sequences as shown in SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 6.

10. The immunogenic composition of any one of claim 6 or 8, wherein the amino acid sequence of the NA protein of the influenza strain is as shown in SEQ ID NO: 7.

11. The immunogenic composition of any one of claims 1-10, wherein each of the mRNA comprises, from 5' to 3', a capping structure, a 5'-UTR, an open reading frame, a 3'-UTR, and a polyA tail.

12. The immunogenic composition of claim 11, wherein the 5'-UTR comprises a 5'-UTR derived from a 17 β -hydroxysteroid dehydrogenase 4 (HSD17B4) gene or a homolog, fragment, or variant thereof, and preferably, the 5'-UTR comprises a sequence as shown in SEQ ID NO: 15;

wherein the 3'-UTR comprises a 3'-UTR derived from an albumin ALB gene or a homolog, fragment, or variant thereof, preferably, the 3'-UTR comprises a sequence as shown in SEQ ID

NO: 16; and/or

wherein the polyA tail is between 100 and 150 nucleotides in length.

13. The immunogenic composition of any one of claims 1-12, wherein the mRNA encoding the HA protein of the one or more influenza virus strains is one or more selected from mRNAs having sequences as shown in SEQ ID NOs: 8-13; and

preferably, the mRNA comprises four mRNAs having sequences as shown in SEQ ID NOs: 8-11, or three mRNAs having sequences as shown in SEQ ID NOs: 8-10, or four mRNAs having sequences as shown in SEQ ID NOs: 10-13, or three mRNAs having sequences as shown in SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 13.

14. The immunogenic composition of any one of claims 1-13, wherein a sequence of the mRNA encoding the NA protein of the influenza virus strain, is SEQ ID NO: 14.

15. The immunogenic composition of any one of claims 1-14, wherein one or more uridine triphosphates in the mRNA are each independently replaced with pseudouridine triphosphate, 2-thiouridine triphosphate, 5-methyluridine triphosphate, N1-methylpseudouridine triphosphate, or 5-ethynyluridine triphosphate, and/or one or more cytidine triphosphates are replaced with 5-methylcytidine triphosphates, and/or one or more adenosine triphosphates are replaced with N6-methyladenosine triphosphates; and

preferably, one or more uridine triphosphates in the mRNA are each independently replaced with pseudouridine triphosphate, 1-methyl-pseudouridine triphosphate, or 5-ethynyluridine triphosphate, and/or one or more cytidine triphosphates are replaced with 5-methylcytidine triphosphate.

16. The immunogenic composition of any one of claims 1-15, wherein the mRNA is encapsulated in a carrier material.

17. The immunogenic composition of claim 16, wherein the carrier material is selected from lipid nanoparticles-LNPs, protamine, polymeric materials, and inorganic nanoparticles.

18. The immunogenic composition of any one of claims 1-17, wherein the molar concentration of each of the mRNAs in the immunogenic composition is no more than three times the molar concentration of any other of the mRNAs; and

preferably, the molar concentration of each of the mRNAs is substantially the same.

19. The immunogenic composition of claim 18, wherein the immunogenic composition comprises

mRNAs encoding four HA proteins selected from 1)-4), or three HA proteins selected from 1)-3), or four HA proteins selected from 3)-6), or three HA proteins selected from 3), 5), and 6):

- 1) a HA protein of an A/Wisconsin/588/2019(H1N1)pdm09-like virus;
- 2) a HA protein of an A/Darwin/6/2021(H3N2)-like virus;
- 3) a HA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage);
- 4) a HA protein of a B/Phuket/3073/2013-like virus (B/Yamagata lineage);
- 5) a HA protein of an A/Wisconsin/67/2022(H1N1)pdm09-like virus; or
- 6) a HA protein of an A/Massachusetts/18/2022(H3N2)-like virus.

preferably, when the immunogenic composition comprises four mRNAs, the molar ratio of the mRNAs is 1-3:1-3:1-3:1-3, and preferably 1:1:1:1.

20. The immunogenic composition of claim 19, wherein the immunogenic composition comprises mRNAs encoding four HA proteins selected from 1)-4), or three HA proteins selected from 1)-3), or four HA proteins selected from 3)-6), or three HA proteins selected from 3), 5), and 6), and an mRNA encoding the NA protein selected from 7):

- 1) a HA protein of an A/Wisconsin/588/2019(H1N1)pdm09-like virus;
- 2) a HA protein of an A/Darwin/6/2021(H3N2)-like virus;
- 3) a HA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage);
- 4) a HA protein of a B/Phuket/3073/2013-like virus (B/Yamagata lineage);
- 5) a HA protein of an A/Wisconsin/67/2022(H1N1)pdm09-like virus; and
- 6) a HA protein of an A/Massachusetts/18/2022(H3N2)-like virus; or
- 7) a NA protein of a B/Austria/1359417/2021-like virus (B/Victoria lineage); and

preferably, when the immunogenic composition comprises four mRNAs, the molar ratio of the mRNAs is 1-3:1-3:1-3:1-3, and preferably 1:1:1:1.

21. The immunogenic composition of any one of claims 1-19, wherein the immunogenic composition further comprises an adjuvant.

22. The immunogenic composition of claim 21, wherein the adjuvant is any one or more selected from aluminum adjuvants, MF59, AS03, AS04, thermoreversible oil-in-water emulsions, ISA51, Freund's adjuvant, IL-12, CpG motifs, and mannose; and

preferably, the aluminum adjuvant is any one or more selected from aluminum hydroxide, aluminum phosphate, aluminum sulfate, or alum.

23. Use of the immunogenic composition of any one of claims 1-22 in the preparation of a vaccine for preventing and/or treating a disease or a condition associated with influenza virus infection.

24. The use of claim 23, wherein the disease or the condition is a disease or a condition associated with influenza A virus and/or influenza B virus infection.

25. A method for preventing and/or treating a disease or a condition associated with influenza virus infection, comprising:

administering the immunogenic composition of any one of claims 1-22 to a subject suffering from the disease or the condition associated with influenza virus infection.

26. The method of claim 25, wherein the disease or the condition is a disease or a condition associated with influenza A virus and/or influenza B virus infection.

27. The immunogenic composition of any one of claims 1-22 for use as a medicine for preventing and/or treating a disease or a condition associated with influenza virus infection.

28. The immunogenic composition of claim 27, wherein the disease or the condition is a disease or a condition associated with influenza A virus and/or influenza B virus infection.