

RESPIRATORY SYNCYTIAL VIRUS MRNA VACCINE, AND PREPARATION METHOD THEREFOR AND USE THEREOF

TECHNICAL FIELD

[0001] The present disclosure belongs to the technical field of nucleic acid vaccines, and particularly relates to a respiratory syncytial virus mRNA vaccine, and a preparation method therefor and use thereof.

BACKGROUND

[0002] Respiratory syncytial virus (RSV) is a negative-sense RNA virus of the genus *Pneumovirus* in the family Paramyxoviridae. In 1956, scientists isolated the RSV virus from chimpanzee respiratory specimens, which was called respiratory syncytial virus because it caused adjacent cells to fuse during cell culture and the cells to transform into a syncytium-like structure.

[0003] The RSV virus is an important pathogen causing lower respiratory tract infections in infants, the elderly, and patients with immune function deficiencies. RSV is airborne, enters the trachea and lungs by inhalation through the mouth and nose, and invades epithelial cells to cause airway trauma, such that the respiratory mucus is secreted from the surface of the airway to block the lumen, causing dyspnea. Common symptoms of an RSV infection include runny nose, fever, cough, and asthma, which can cause respiratory distress and even airway obstruction in serious cases, leading to respiratory failure and even death.

[0004] RSV is a common respiratory virus. It generally causes cold-like symptoms. The RSV infection mainly occurs in autumn, winter, and spring, and is one of the leading causes of severe respiratory diseases in infants under 5 years of age and the elderly over 65 years of age. For the vast majority of young people, RSV usually causes mild cold-like symptoms, but for infants, young children, and the elderly, the RSV infection is a potentially fatal disease.

[0005] The RSV infection has become a worldwide public health problem. RSV is the most common viral pathogen causing acute lower respiratory tract infections (ALRTIs) in children under 5 years of age worldwide, and is the leading cause of hospitalization for viral respiratory tract infections in infants and young children. Data show that the RSV infection accounts for 28% of all ALRTIs, with RSV hospital deaths in children accounting for 13% to 22% of deaths due to ALRTI.

[0006] The RSV infection is characterized by a widespread global epidemic, and the epidemic is affected by factors such as geographic location, temperature, and humidity. For example, the epidemic season of RSV starts from mid-October to mid-May of the following year in northern China, and occurs frequently in winter and spring in southern China, which is closely related to temperature. RSV has only one serotype, which is divided into subtypes A and B. These two subtypes are alternately epidemic in the northern part of the country.

[0007] Vaccines are the most economical and effective means of preventing RSV infection. WHO has listed the development of RSV vaccines as one of the urgent problems to be solved. However, since the development of RSV vaccines began in the 1960s, no effective vaccine certified by regulatory agencies has

been approved for marketing. Recently, major breakthroughs in RSV vaccines have been made one after another. On May 3, 2023, a respiratory syncytial virus (RSV) vaccine developed by GSK, Arexvy, received marketing approval from the FDA, making it the world's first RSV vaccine to be approved for marketing. The vaccine is mainly used for patients over 60 years of age to prevent lower respiratory tract (bronchus and lungs) diseases caused by the RSV infection. This was followed on June 1, 2023 by the US FDA's marketing approval of an RSV protein vaccine from Pfizer, Abrysvo™, for the prevention of acute respiratory tract diseases and lower respiratory tract diseases caused by RSV in adults over 60 years of age.

[0008] At present, the technical routes of RSV vaccines under development include attenuated live vaccines, subunit vaccines, recombinant vector vaccines, mRNA vaccines, etc. Traditional vaccines are usually based on inactivated or attenuated live virus vaccines, or subunit proteins derived from pathogens. Although these vaccines produce an effective immune response, they have a number of associated safety issues. For example, rather than having a protective effect on vaccinees, the early-developed inactivated RSV vaccines develop an enhanced respiratory disease (ERD), that is, not only do they fail to prevent the vaccinees from being infected with the RSV virus, but also lead to an aggravation of the condition of the vaccinees after they are infected with the RSV virus. In a clinical trial of an inactivated RSV vaccine, 16 out of the 20 infants in the trial group were severely ill and required hospitalization, with a hospitalization rate up to 80%, and two of them died.

[0009] mRNA vaccines function by introducing mRNA encoding a viral antigen into the body, and producing an antigen by expression in the body, which in turn stimulates the body to produce an immune response, and they have relatively high safety because mRNA exists only in the cytoplasm, is not integrated into a host genome, and can be naturally degraded in the body. mRNA vaccines themselves can be used as adjuvants to induce the body to produce natural immune effects. Moreover, mRNA vaccines can induce strong humoral immunity and cellular immunity, and generally have a stronger immune effect than traditional inactivated vaccines and subunit protein vaccines. mRNA vaccines do not need to be combined with other vaccine adjuvants, so that adverse reactions caused by other substances can be reduced. Compared with traditional vaccines, mRNA vaccines have a relatively simple production process flow and a short research and development period, and have the technical advantage of rapid response to the research and development of vaccines for new sudden infectious diseases. mRNA vaccines have been successfully made in the development of new coronavirus vaccines, and the safety and efficacy of mRNA vaccine technology have been demonstrated through large-scale population vaccination.

[0010] Neutralizing antibodies caused by the RSV infection are mainly directed against the F protein. RSV F protein is a type I fusion glycoprotein, which is relatively conserved among RSV types, and is an ideal target protein for vaccine antigens. The F protein transitions between two conformations: a metastable pre-fusion conformation (pre-F) and a stable post-fusion conformation (post-F). Although the antigenic epitopes targeted by the neutralizing antibodies are present in both conformations of the F protein, the characteristics of the natural immune response in humans to the RSV infection suggest that epitopes for most of the RSV neutralizing antibodies are located in the pre-fusion conformation of the F protein. Because of the instability of the pre-fusion conformation of the F protein, F protein subunit vaccines based

on inactivated RSV vaccines or extracted directly from RSV have failed.

[0011] Based on the above problems, in order to further reduce the risk of respiratory syncytial virus in immunocompromised populations (including children and the elderly), there is an urgent need to provide a novel mRNA vaccine for preventing RSV, which has good stability and can significantly improve the humoral immunity effect after vaccination.

SUMMARY

[0012] The present disclosure provides an mRNA vaccine encoding an RSV F protein, particularly a pre-fusion conformation pre-F of the F protein. The protein comprises 3 naturally occurring mutations of P102A, I379V, and M447V (SEQ ID NO: 210) based on a sequence of a wild-type protein of subtype RSV A2 set forth in SEQ ID NO: 1 (GenBank ID: 138251). The mutated sequence with these three amino acid residues is conserved in other RSV A virus strains, and based on this, candidate mutant antigen sequences are designed taking differences in the F protein sequences of the current mainstream virus strains into account.

[0013] RSV viruses include subtypes A and B, whose F proteins are approximately 90% identical in the amino acid sequence. For a sequence of an F0 precursor polypeptide of subtype A, for example, virus strain A2 (GenBank GI: 138251; Swiss Prot P03420), and a sequence of an F0 precursor polypeptide of subtype B, for example, virus strain 18537 (GenBank GI: 138250; Swiss Prot P13843), both are sequences of 574 amino acids, and signal peptide sequences of both have also been reported as amino acids 1 to 25 (GenBank and UniProt). In the two sequences, due to high homology of F protein sequences of both RSV virus type A and type B, the RSV pre-F mRNA and the vaccine prepared therefrom designed by the present disclosure are effective for preventing infections of RSV virus type A and type B.

[0014] As described herein, the three-dimensional structure of pre-F is used as a guide to engineer and construct a stabilized form of a pre-fusion F protein (“pre-F” antigen) and is used in animal models to produce an RSV neutralizing immune response that is significantly enhanced compared with that achieved by an immunogen of Abrysvo™ based on the RSV F protein and provides protection against RSV challenge in the animal models. The pre-F antigen can be used as a potential vaccine for RSV and as a diagnostic molecule.

[0015] In some embodiments, the recombinant RSV F protein comprises one or more amino acid substitutions that stabilize the protein in a pre-fusion conformation. For example, the substitutions stabilize a membrane distal portion of the F protein (comprising the N-terminal region of an F1 polypeptide) in a pre-fusion conformation. Which makes one or more antibodies specifically bind to pre-fusion conformation of RSV F protein or an antigenic site present in the pre-fusion conformation rather than in the post-fusion conformation of the RSV F protein, such as antigenic site Φ .

[0016] In some other embodiments, the recombinant RSV F protein may comprise one or more modifications (e.g., truncations and amino acid substitutions) to the C-terminus of the F1 polypeptide that, together with modifications stabilizing the membrane distal region of the F polypeptide, can increase the stabilization of the recombinant F protein in the pre-fusion conformation.

- [0017]** The present application relates to the following embodiments:
- [0018]** 1. An immunogenic composition, comprising a ribonucleic acid (RNA) of a respiratory syncytial virus (RSV), wherein the RNA encodes a wild-type RSV F protein or a variant thereof;
- [0019]** wherein a sequence of the wild-type RSV F protein is SEQ ID NO: 1.
- [0020]** 2. The composition according to embodiment 1, wherein the variant of the RSV F protein comprises one or more mutant forms selected from the following: a sequence truncation, a sequence deletion, and a site mutation.
- [0021]** 3. The composition according to embodiment 2, wherein the sequence truncation comprises a lack of a cytoplasmic domain, for example, the sequence truncation is to truncate amino acids at positions 550 to 574 of the C-terminus of the RSV F protein, and for example, the truncation is to retain amino acids at positions 1 to 549 of the RSV F protein relative to the wild-type F protein.
- [0022]** 4. The composition according to embodiment 2 or 3, wherein the sequence deletion is selected from one or more of the following: a deletion of amino acids at positions 104 to 136 and a deletion of amino acids at positions 104 to 144; optionally, the amino acids for the sequence deletion are replaced by a GSGS oligopeptide or a GS oligopeptide.
- [0023]** 5. The composition according to any one of embodiments 2 to 4, wherein the site mutation comprises one or more selected from the following: P102A, I379V, and M447V, for example, the site mutation comprises P102A and I379V; P102A and M447V; I379V and M447V; P102A, I379V, and M447V.
- [0024]** 6. The composition according to embodiment 5, wherein the site mutation further comprises one or more selected from the following: S46G, S215P, L373R, S213P, and N216P, for example, site mutations selected from: S46G, S215P, and L373R.
- [0025]** 7. The composition according to embodiment 5 or 6, wherein the site mutation further comprises one or more selected from the following:
- [0026]** (i) Q26C, N27C, T29C, E31C, F32C, Q34C, T36C, S41C, Y44C, Y53C, T54C, S55C, T100C, T103C, L138C, G139C, F140C, L141C, L142C, G145C, S146C, A147C, I148C, A149C, S150C, V152C, A153C, V154C, S155C, N183GC, S186C, L188C, S287C, I288C, S290C, I291C, I292C, V300C, L305C, Y306C, S319C, N325C, S330C, T337C, R339C, Q354C, T369C, M370C, N371C, S377C, N428C, Y458C, K461C, Q462C, K465C, L467C, Y468C, K470C, G471C;
- [0027]** (ii) T54H, T58L, S190I, S190F, V207L, T219I, V296I;
- [0028]** (iii) E92D, K465Q, D486S; and
- [0029]** (iv) V152I, C69Y, D73E, A74V, N88S.
- [0030]** 8. The composition according to embodiment 5 or 6, wherein the site mutation further comprises one or more combinations selected from the following:
- [0031]** (i) E31C, Q34C, T54C, S55C, T100C, I148C, A149C, V152C, V154C, S155C, L188C, I288C, S290C, R339C, S377C, Y458C, L467C, G471C;
- [0032]** (ii) S190F, V296I, V207L, T219I, S190I;
- [0033]** (iii) E92D, K465Q, D486S; and
- [0034]** (iv) V152I, C69Y, D73E, A74V, N88S.

[0035] 9. The composition according to embodiment 5 or 6, wherein the site mutation further comprises one or more combinations selected from the following:

[0036] (i) T54C, S55C, T100C, I148C, A149C, V152C, V154C, S155C, L188C, I288C, S290C, R339C, S377C, Y458C;

[0037] (ii) S190F, V296I, V207L, T219I, S190I;

[0038] (iii) E92D, K465Q, D486S; and

[0039] (iv) V152I, C69Y, D73E, A74V, N88S.

[0040] 10. The composition according to embodiment 5 or 6, wherein the site mutation further comprises one or more combinations selected from the following:

[0041] (i) T54C, I148C, A149C, V152C, S155C, I288C, S290C, Y458C;

[0042] (ii) S190F, V296I, V207L;

[0043] (iii) E92D, K465Q, D486S; and

[0044] (iv) V152I, C69Y, D73E, A74V, N88S.

[0045] 11. The composition according to any one of embodiments 1 to 10, wherein the variant of the RSV F protein comprises mutations P102A, I379V, and M447V, the variant of the RSV F protein is a truncated protein in which amino acids at positions 550 to 574 of SEQ ID NO: 1 are truncated, and amino acids at positions 104 to 144 are substituted by a GS linker, and the variant of the RSV F protein further comprises a combination of site mutations selected from any one of the following groups:

[0046]

Protein variant No.	Site mutation				
	Group 1	Group 2	Group 3	Group 4	Group 5
YK-RSV-061	A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	V152I
YK-RSV-003	A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	/
YK-RSV-062	A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	C69Y, D73E, A74V, N88S, V152I
YK-RSV-054	I148C, I288C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
YK-RSV-039	T54C, V152C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
YK-RSV-044	T100C, A149C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
YK-RSV-012	S55C, L188C	S190F, T219I	E92D, K465Q, D486S	S46G, S215P, L373R	/
YK-RSV-015	S55C, A149C, L188C, Y458C	S190I	E92D, K465Q, D486S	S46G, S215P, L373R	/
YK-RSV-001	A149C, S155C, S290C, Y458C	S190I	E92D, D486S, K465Q	S46G, S215P, L373R	/
YK-RSV-036	S55C, V154C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
YK-RSV-060	R339C, S377C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
YK-RSV-011	Q34C, G471C	S190F, T219I	E92D, K465Q, D486S	S46G, S215P, L373R	/

YK-RSV-030	E31C, L467C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
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[0047] 12. The composition according to any one of embodiments 1 to 10, wherein the variant of the RSV F protein comprises mutations P102A, I379V, and M447V, the variant of the RSV F protein is a truncated protein in which amino acids at positions 550 to 574 of SEQ ID NO: 1 are truncated, and amino acids at positions 104 to 144 are substituted by a GS linker, and the variant of the RSV F protein further comprises a combination of site mutations selected from any one of the following groups:

Protein variant No.	Site mutation				
	Group 1	Group 2	Group 3	Group 4	Group 5
YK-RSV-061	A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	V152I
YK-RSV-003	A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	/
YK-RSV-062	A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	C69Y, D73E, A74V, N88S, V152I
YK-RSV-054	I148C, I288C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
YK-RSV-039	T54C, V152C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
YK-RSV-044	T100C, A149C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
YK-RSV-012	S55C, L188C	S190F, T219I	E92D, K465Q, D486S	S46G, S215P, L373R	/
YK-RSV-015	S55C, A149C, L188C, Y458C	S190I	E92D, K465Q, D486S	S46G, S215P, L373R	/
YK-RSV-001	A149C, S155C, S290C, Y458C	S190I	E92D, D486S, K465Q	S46G, S215P, L373R	/
YK-RSV-036	S55C, V154C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
YK-RSV-060	R339C, S377C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/

[0048] 13. The composition according to any one of embodiments 1 to 10, wherein the variant of the RSV F protein comprises mutations P102A, I379V, and M447V, the variant of the RSV F protein is a truncated protein in which amino acids at positions 550 to 574 of SEQ ID NO: 1 are truncated, and amino acids at positions 104 to 144 are substituted by a GS linker, and the variant of the RSV F protein further comprises a combination of mutations selected from any one of the following groups:

Protein variant No.	Site mutation				
	Group 1	Group 2	Group 3	Group 4	Group 5
YK-RSV-061	A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	V152I
YK-RSV-003	A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	/
YK-RSV-062	A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	C69Y, D73E, A74V, N88S,

					V152I
YK-RSV-054	I148C, I288C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
YK-RSV-039	T54C, V152C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/

[0049] 14. The composition according to any one of embodiments 1 to 13, wherein the variant of the RSV F protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or 100% identity to a sequence of SEQ ID NO: 204 (YK-RSV-061), SEQ ID NO: 12 (YK-RSV-003), SEQ ID NO: 208 (YK-RSV-062), SEQ ID NO: 176 (YK-RSV-054), SEQ ID NO: 116 (YK-RSV-039), SEQ ID NO: 136 (YK-RSV-044), SEQ ID NO: 40 (YK-RSV-012), SEQ ID NO: 52 (YK-RSV-015), SEQ ID NO: 4 (YK-RSV-001), SEQ ID NO: 104 (YK-RSV-036), SEQ ID NO: 200 (YK-RSV-060), SEQ ID NO: 36 (YK-RSV-011), or SEQ ID NO: 80 (YK-RSV-030).

[0050] 15. The composition according to embodiment 14, wherein the variant of the RSV F protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or 100% identity to a sequence of SEQ ID NO: 204 (YK-RSV-061), SEQ ID NO: 12 (YK-RSV-003), SEQ ID NO: 208 (YK-RSV-062), SEQ ID NO: 176 (YK-RSV-054), SEQ ID NO: 116 (YK-RSV-039), SEQ ID NO: 136 (YK-RSV-044), SEQ ID NO: 40 (YK-RSV-012), SEQ ID NO: 52 (YK-RSV-015), SEQ ID NO: 4 (YK-RSV-001), SEQ ID NO: 104 (YK-RSV-036), or SEQ ID NO: 200 (YK-RSV-060).

[0051] 16. The composition according to embodiment 15, wherein the variant of the RSV F protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or 100% identity to a sequence of SEQ ID NO: 204 (YK-RSV-061), SEQ ID NO: 12 (YK-RSV-003), SEQ ID NO: 208 (YK-RSV-062), SEQ ID NO: 176 (YK-RSV-054), or SEQ ID NO: 116 (YK-RSV-039).

[0052] 17. The composition according to any one of embodiments 1 to 16, wherein an open reading frame (ORF) encoding the RSV F protein or the variant thereof comprises a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or 100% identity to SEQ ID NO: 203, SEQ ID NO: 11, SEQ ID NO: 207, SEQ ID NO: 175, SEQ ID NO: 115, SEQ ID NO: 135, SEQ ID NO: 39, SEQ ID NO: 51, SEQ ID NO: 3, SEQ ID NO: 103, SEQ ID NO: 199, SEQ ID NO: 35, or SEQ ID NO: 79.

[0053] 18. The composition according to embodiment 17, wherein the ORF comprises a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or 100% identity to SEQ ID NO: 203, SEQ ID NO: 11, SEQ ID NO: 207, SEQ ID NO: 175, SEQ ID NO: 115, SEQ ID NO: 135, SEQ ID NO: 39, SEQ ID NO: 51, SEQ ID NO: 3, SEQ ID NO: 103, or SEQ ID NO: 199.

[0054] 19. The composition according to embodiment 18, wherein the ORF comprises a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or 100% identity to SEQ ID NO: 203, SEQ ID NO: 11, SEQ ID NO: 207, SEQ ID NO: 175, or SEQ ID NO: 115.

[0055] 20. The composition according to any one of embodiments 1 to 19, wherein the RNA of the RSV further comprises a 5' untranslated region (UTR).

[0056] 21. The composition according to embodiment 20, wherein a sequence of the 5' UTR is: SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, or SEQ ID NO: 221.

[0057] 22. The composition according to embodiment 21, wherein the sequence of the 5' UTR is SEQ ID

NO: 217.

[0058] 23. The composition according to any one of embodiments 1 to 22, wherein the RNA of the RSV further comprises a 3' untranslated region (UTR).

[0059] 24. The composition according to embodiment 23, wherein a sequence of the 3' UTR is: SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224, or SEQ ID NO: 225.

[0060] 25. The composition according to embodiment 24, wherein the sequence of the 3' UTR is SEQ ID NO: 222.

[0061] 26. The composition according to any one of embodiments 1 to 25, wherein the RNA of the RSV further comprises a poly(A) tail.

[0062] 27. The composition according to embodiment 26, wherein the poly(A) tail has a length of 50 to 150 nucleotides.

[0063] 28. The composition according to any one of embodiments 1 to 27, wherein the RNA of the RSV further comprises a 5' cap structure.

[0064] 29. The composition according to embodiment 28, wherein the 5' cap structure is: 7mG(5')ppp(5')NlmpNp.

[0065] 30. The composition according to any one of embodiments 1 to 29, wherein a sequence of an open reading frame (ORF) encoding the RSV F protein or the variant thereof in the RSV RNA is codon-optimized.

[0066] 31. The composition according to embodiment 30, wherein the sequence of the ORF comprises at least one base modification.

[0067] 32. The composition according to embodiment 31, wherein the base modification comprises any one or more selected from the following: pseudouridine, N1-methylpseudouridine, N1-ethylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methylpseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-1-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methoxyuridine, and 2'-O-methyluridine.

[0068] 33. The composition according to embodiment 32, wherein the base modification comprises replacement of uracil by pseudouridine and/or N1-methylpseudouridine.

[0069] 34. The composition according to embodiment 31, wherein the base modification is 1 to 100% base modification, for example, 100% base modification.

[0070] 35. The composition according to any one of embodiments 1 to 34, wherein a sequence of the RNA of the RSV has at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or 100% identity to SEQ ID NO: 205, SEQ ID NO: 13, SEQ ID NO: 209, SEQ ID NO: 177, SEQ ID NO: 117, SEQ ID NO: 137, SEQ ID NO: 41, SEQ ID NO: 53, SEQ ID NO: 5, SEQ ID NO: 105, SEQ ID NO: 201, SEQ ID NO: 37, or SEQ ID NO: 81.

[0071] 36. The composition according to embodiment 35, wherein the sequence of the RNA of the RSV has at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or 100% identity to SEQ ID NO: 205, SEQ ID NO: 13, SEQ ID NO: 209, SEQ ID NO: 177, SEQ ID NO: 117, SEQ ID NO: 137, SEQ ID NO: 41, SEQ ID NO: 53, SEQ ID NO: 5, SEQ ID NO: 105, or SEQ ID NO: 201.

[0072] 37. The composition according to embodiment 36, wherein the sequence of the RNA of the RSV has at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or 100% identity to SEQ ID NO: 205, SEQ ID NO: 13, SEQ ID NO: 209, SEQ ID NO: 177, or SEQ ID NO: 117.

[0073] 38. The composition according to any one of embodiments 1 to 37, wherein the RNA of the RSV is mRNA.

[0074] 39. The composition according to any one of embodiments 1 to 38, wherein a sequence encoding the RSV F protein or the variant thereof has at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or 100% identity to SEQ ID NO: 202, SEQ ID NO: 10, SEQ ID NO: 206, SEQ ID NO: 174, SEQ ID NO: 114, SEQ ID NO: 134, SEQ ID NO: 38, SEQ ID NO: 50, SEQ ID NO: 2, SEQ ID NO: 102, SEQ ID NO: 198, SEQ ID NO: 34, or SEQ ID NO: 78.

[0075] 40. The composition according to embodiment 39, wherein the sequence encoding the RSV F protein or the variant thereof has at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or 100% identity to SEQ ID NO: 202, SEQ ID NO: 10, SEQ ID NO: 206, SEQ ID NO: 174, SEQ ID NO: 114, SEQ ID NO: 134, SEQ ID NO: 38, SEQ ID NO: 50, SEQ ID NO: 2, SEQ ID NO: 102, or SEQ ID NO: 198.

[0076] 41. The composition according to embodiment 40, wherein the sequence encoding the RSV F protein or the variant thereof has at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or 100% identity to SEQ ID NO: 202, SEQ ID NO: 10, SEQ ID NO: 206, SEQ ID NO: 174, or SEQ ID NO: 114.

[0077] 42. A method for preparing the composition according to any one of embodiments 1 to 41, comprising:

[0078] providing a template from which the RNA of the RSV can be produced by transcription;

[0079] performing transcription using the template under conditions suitable for the transcription for the RNA.

[0080] 43. The method according to embodiment 42, further comprising a purification step selected from the following: lithium chloride precipitation, affinity chromatography, buffer exchanged by ultrafiltration, cellulose chromatography.

[0081] 44. The composition according to any one of embodiments 1 to 41, wherein the composition is a vaccine and further comprises a pharmaceutically acceptable excipient.

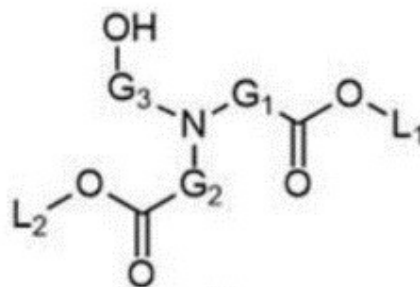
[0082] 45. The composition according to embodiment 44, wherein the pharmaceutically acceptable excipient comprises a lipid mixture, and the lipid mixture is, for example, a lipid nanoparticle (LNP).

[0083] 46. The composition according to embodiment 44 or 45, wherein the vaccine is an mRNA vaccine.

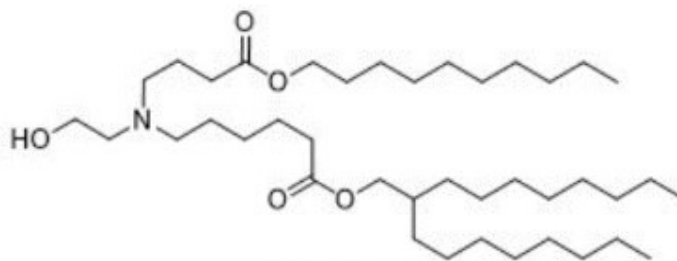
[0084] 47. The composition according to embodiment 45, wherein the lipid nanoparticle comprises, for example, a cationic lipid, a neutral lipid, a structural lipid, and a polymer-conjugated lipid.

[0085] 48. The composition according to embodiment 47, wherein the cationic lipid is a compound with a structure of formula I, or a N-oxide, a solvate, a pharmaceutically acceptable salt or a stereoisomer thereof, wherein G₁ is C₁₋₆ alkylene; G₂ is C₂₋₈ alkylene; G₃ is C₁₋₃ alkylene; L₁ is C₆₋₁₅ linear alkyl; L₂ is C₁₂₋₂₅ branched alkyl;

[0086] for example, YK-009 with a structure of formula I-I:



formula I

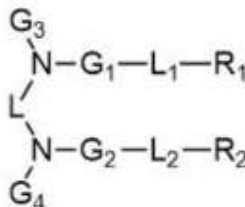


YK-009

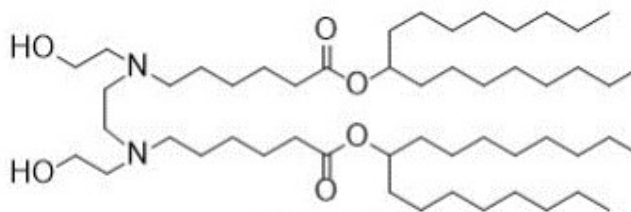
formula I-I

[0087] 49. The composition according to embodiment 47, wherein the cationic lipid is a compound with a structure of formula II, or a N-oxide, a solvate, a pharmaceutically acceptable salt or a stereoisomer thereof, wherein G_1 is C_{2-8} alkylene; G_2 is C_{2-8} alkylene; L_1 is $-C(O)O-$ or $-OC(O)-$; L_2 is $-C(O)O-$ or $-OC(O)-$; R_1 is C_{6-25} linear or branched alkyl; R_2 is C_{6-25} linear or branched alkyl; G_3 is $HO(CH_2)_2-$ or $HO(CH_2)_3-$; G_4 is $HO(CH_2)_2-$ or $HO(CH_2)_3-$; L is $(CH_2)_2-$ or $-(CH_2)_3-$ or $-(CH_2)_4-$;

[0088] for example, YK-401 with a structure of formula II-I or YK-402 with a structure of formula II-II:

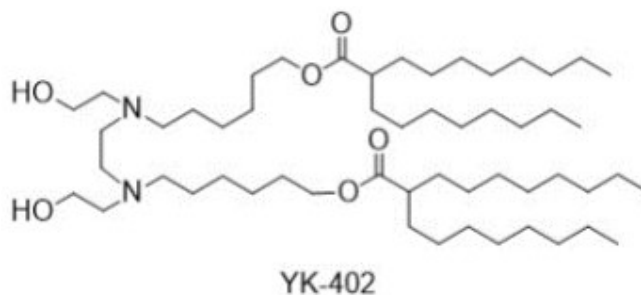


formula II



YK-401

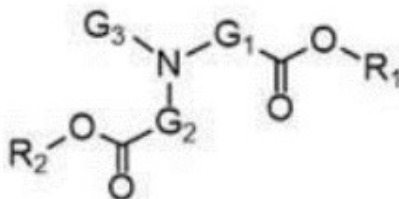
formula II-I



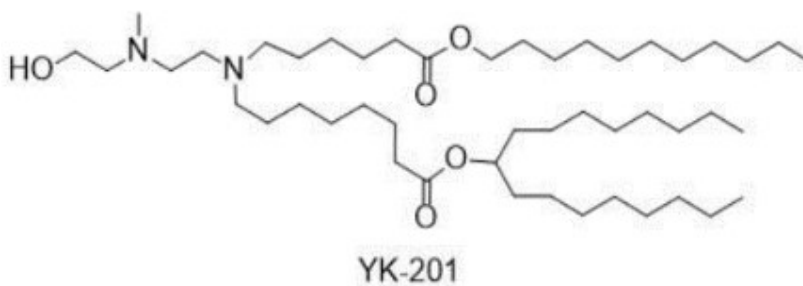
II-II

[0089] 50. The composition according to embodiment 47, wherein the cationic lipid is a compound with structure of formula III, or a N-oxide, a solvate, a pharmaceutically acceptable salt or a stereoisomer thereof, wherein G_1 is C_{1-6} alkylene; G_2 is C_{2-8} alkylene; R_1 is C_{6-20} linear or branched alkyl; R_2 is C_{12-25} branched alkyl; G_3 is: $HO(CH_2)_2N(CH_3)(CH_2)_2-$, $HO(CH_2)_2N(CH_2CH_3)(CH_2)_2-$, $(HO(CH_2)_2)_2N(CH_2)_2-$, $CH_3O(CH_2)_2N(CH_3)(CH_2)_2-$, $(CH_3)_2N(CH_2)_3SC(O)O(CH_2)_2-$, $(CH_3)_2N(CH_2)_3SC(O)-$, $CH_3NH(CH_2)_2N(CH_3)(CH_2)_2-$, or $CH_3CH_2NH(CH_2)_2-$;

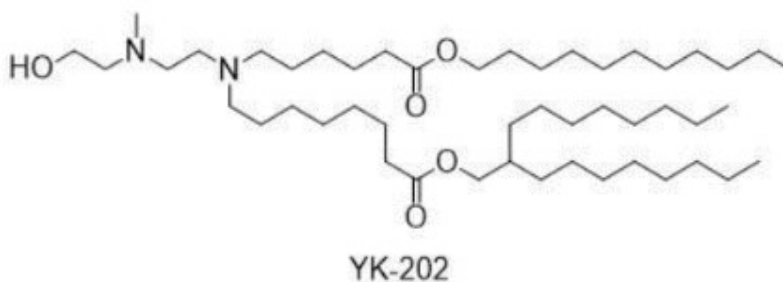
[0090] for example, YK-201 with a structure of formula III-I or YK-202 with a structure of formula III-II:



formula III



formula III-I

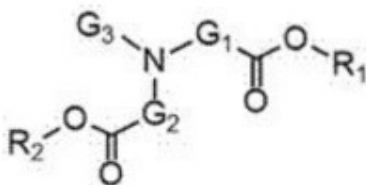


formula III-II

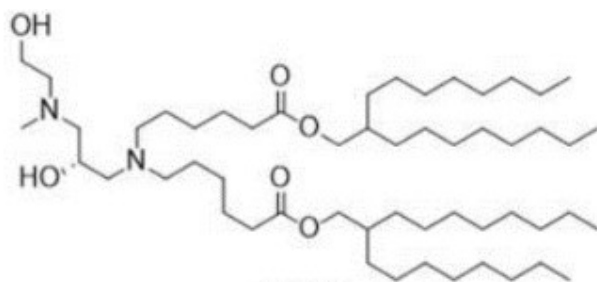
[0091] 51. The composition according to embodiment 47, wherein the cationic lipid is a compound with

a structure of formula IV, or a N-oxide, a solvate, a pharmaceutically acceptable salt or a stereoisomer thereof, wherein G₁ is C₁₋₈ alkylene; G₂ is C₂₋₈ alkylene; R₁ is C₆₋₂₅ linear or branched alkyl; R₂ is C₁₂₋₂₅ linear or branched alkyl; G₃ is HO(CH₂)₂N(R₃)CH₂CH(OH)CH₂-, wherein R₃ is -CH₃ or -CH₂CH₃ or -CH₂CH₂OH;

[0092] for example, YK-305 with a structure of formula IV-I or YK-310 with a structure of formula IV-II:

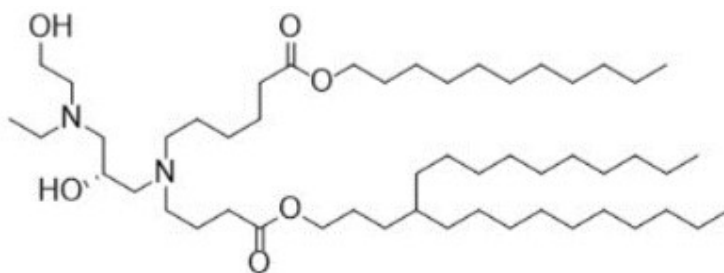


formula IV



YK-305

formula IV-I

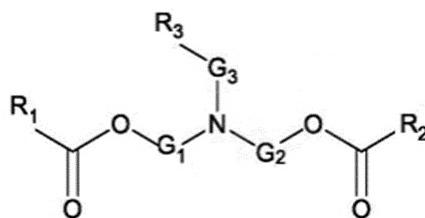


YK-310

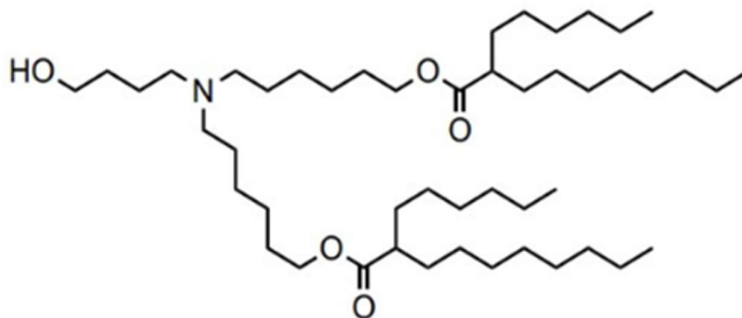
formula IV-II

[0093] 52. The composition according to embodiment 47, wherein the cationic lipid is a compound with a structure of formula V, or a N-oxide, a solvate, a pharmaceutically acceptable salt or a stereoisomer thereof, wherein G₁ and G₂ are each independently unsubstituted C₆-C₁₀ alkylene; G₃ is unsubstituted C₁-C₁₂ alkylene; R₁ and R₂ are each independently C₆-C₂₄ alkyl or C₆-C₂₄ alkenyl; R₃ is OR₅, N, -C(=O)OR₄, -OC(=O)R₄, or -NR₅C(=O)R₄; R₄ is C₁-C₁₂ hydrocarbyl; and R₅ is H or C₁-C₆ alkyl;

[0094] for example, ALC0315 with a structure of formula V-I:



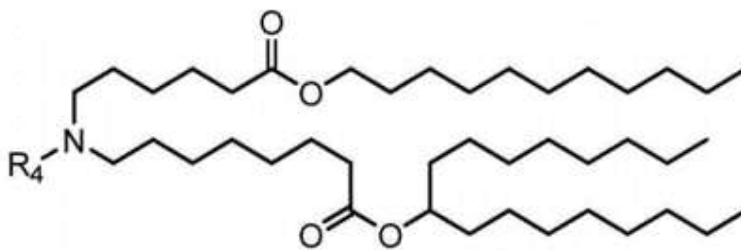
formula V



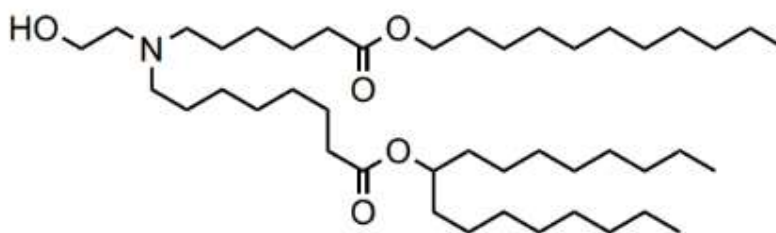
formula V-I

[0095] 53. The composition according to embodiment 47, wherein the cationic lipid is a compound with a structure of formula VI, or a N-oxide, a solvate, a pharmaceutically acceptable salt or a stereoisomer thereof, wherein R_4 is selected from $-(CH_2)_nQ$ and $-(CH_2)_nCHQR$; Q is selected from the group consisting of: $-OR$, $-OH$, $-O(CH_2)_nN(R)_2$, $-OC(O)R$, $-CX_3$, $-CN$, $-N(R)C(O)R$, $-N(H)C(O)R$, $-N(R)S(O)_2R$, $-N(H)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(H)C(O)N(R)_2$, $-N(H)C(O)N(H)(R)$, $-N(R)C(S)N(R)_2$, $-N(H)C(S)N(R)_2$, $-N(H)C(S)N(H)(R)$, $-N(R)S(O)_2R_8$, and a heterocyclic ring; n is 1, 2, or 3; wherein R is hydrogen, C_{1-3} alkyl, C_{2-3} alkenyl, or $(CH_2)_qOR^*$, wherein q is 1, 2, or 3, R^* is C_{1-12} alkyl or C_{2-12} alkenyl; X is fluoro, chloro, bromo, or iodo; R_8 is C_{3-6} cycloalkyl or a heterocyclic ring;

[0096] for example, SM102 with a structure of formula VI-I

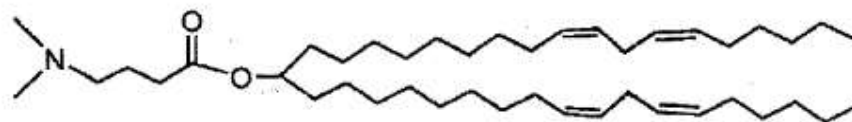


formula VI



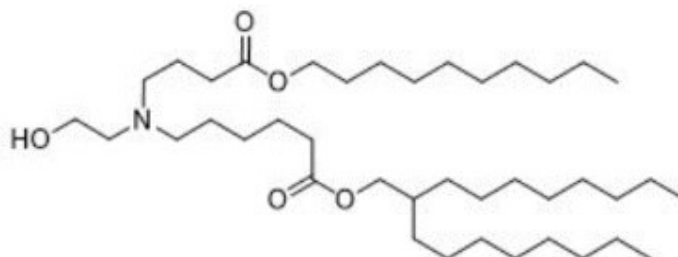
formula VI-I

[0097] 54. The composition according to embodiment 47, wherein the cationic lipid is a compound with a structure of formula VII, or a N-oxide, a solvate, a pharmaceutically acceptable salt, or a stereoisomer thereof:

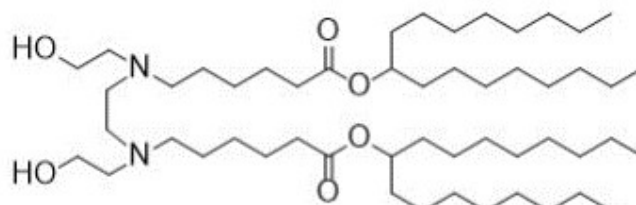


formula VII

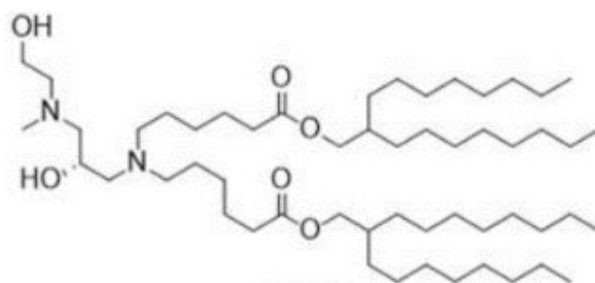
[0098] 55. The composition according to the preceding embodiment 47, wherein the cationic lipid comprises the following compounds: YK-009, YK-401, YK-305, ALC0315, SM102, DLIN-MC3



YK-009



YK-401



YK-305



[0104] 61. The composition according to any one of embodiments 47 to 60, wherein the neutral lipid is selected from one or more of the following: 1,2- dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC),

1,2-diundecanoyl-sn-glycero-phosphorylcholine (DUPC),
 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC),
 1,2-di-O-octadecadienyl-sn-glycero-3-phosphocholine (18:0 Diether PC),
 1-oleoyl-2-cholesterolhemisuccinyl-sn-glycero-3-phosphocholine (OChemPC),
 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine,
 1,2-diarachidonyl-sn-glycero-3-phosphorylcholine, 1,2-didocosaheptaenoyl-sn-glycero-3-phosphocholine,
 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE),
 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME16:0 PE),
 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine,
 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine,
 1,2-diarachidonyl-sn-glycero-3-phosphoethanolamine,
 1,2-didocosaheptaenoyl-sn-glycero-3-phosphoethanolamine,
 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), dipalmitoyl phosphatidylglycerol (DPPG), palmitoyl oleoyl phosphatidylethanolamine (POPE), distearoyl-phosphatidyl-ethanolamine (DSPE), dipalmitoyl phosphatidylethanolamine (DPPE), dimyristoyl phosphatidylethanolamine (DMPE), 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), 1-stearoyl-2-oleoyl-phosphatidylcholine (SOPC), sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyl oleoyl phosphatidylcholine, lysophosphatidylcholine, and lysophosphatidylethanolamine (LPE).

[0105] 62. The composition according to any one of embodiments 47 to 61, wherein the neutral lipid is DOPE and/or DSPC.

[0106] 63. The composition according to any one of embodiments 47 to 62, wherein the structural lipid is selected from one or more of the following: cholesterol, nonsterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, α -tocopherol and corticosteroid.

[0107] 64. The composition according to embodiment 63, wherein the structural lipid is cholesterol.

[0108] 65. The composition according to any one of embodiments 47 to 64, wherein the polymer-conjugated lipid is selected from one or more of the following: PEG-modified phosphatidylethanolamine, PEG-modified phosphatidic acid, PEG-modified ceramide, PEG-modified dialkylamine, PEG-modified diacylglycerol, PEG-modified dialkylglycerol.

[0109] 66. The composition according to the preceding embodiment 65, wherein the polymer-conjugated lipid is selected from one or more of the following: distearoyl phosphatidylethanolamine polyethylene glycol 2000 (DSPE-PEG2000), dimyristoylglycerol-3-methoxy polyethylene glycol 2000 (DMG-PEG2000), and methoxypoly(ethylene glycol) ditetradecylacetamide (ALC-0159).

[0110] 67. The composition according to any one of embodiments 44 to 66, wherein an effective dose of the RSV RNA is 25 to 200 μ g; preferably, 50 to 100 μ g.

[0111] 68. The composition according to any one of embodiments 44 to 67, wherein the vaccine is in a form of an injection.

[0112] 69. A method for preparing the composition according to any one of embodiments 44 to 68,

comprising: mixing the RNA of the RSV with the pharmaceutically acceptable excipient, for example, to encapsulate at least a portion of the RNA within a lipid nanoparticle.

[0113] 70. A use of the composition according to any one of embodiments 1 to 41 and 44 to 68 in the preparation of a vaccine for inducing a protective immune response to RSV in a subject, particularly a humoral immune response, for example, including the production of neutralizing antibodies, for example, over 40 years of age or under 10 years of age, for example, over 50 years of age, over 55 years of age, over 60 years of age, over 65 years of age, over 70 years of age, over 75 years of age, or over 80 years of age, or under 8 years of age, under 7 years of age, under 6 years of age, under 5 years of age, under 4 years of age, under 3 years of age, or under 2 years of age.

[0114] 71. A use of the composition according to any one of embodiments 1 to 41 and 44 to 68 in the preparation of a vaccine for preventing an RSV infection.

[0115] **The beneficial effect of the present disclosure lies in at least one of the following:**

[0116] The present application obtains at least 11 ribonucleic acids encoding a variant of an RSV F protein based on a wild-type RSV F protein through a particular variant form (including a sequence truncation, a site mutation, and/or a sequence deletion), and thus provides a novel mRNA vaccine for preventing RSV, which has high stability and can significantly improve the humoral immunity effect after inoculation. The effect includes at least one of the following:

[0117] increasing a binding rate of a pre-fusion epitope Φ on an RSV F mutant to a corresponding antibody;

[0118] improving the stability of a pre-fusion conformation of an RSV F mutant after treatment under different temperature, osmotic pressure, and pH conditions;

[0119] improving the binding quantity and binding capacity of an antibody to a specific antigenic epitope (epitope Φ , epitope Quaternary, and/or epitope V) for a pre-fusion conformation of an RSV F mutant;

[0120] increasing the titer of a neutralizing antibody produced after the immunization with an RSV F protein;

[0121] significantly reducing inflammatory responses and viral load in the body (including the lungs and turbinate);

[0122] being able to be simultaneously applied to a plurality of RSV subtypes.

[0123] Specifically, the results and technical effects of the present disclosure are as follows:

[0124] (1) Through a Western Blot test, the results show that the following 50 RSV F mutants all have significant protein expression: YK-RSV-001, YK-RSV-002, YK-RSV-003, YK-RSV-004, YK-RSV-005, YK-RSV-008, YK-RSV-009, YK-RSV-010, YK-RSV-011, YK-RSV-012, YK-RSV-013, YK-RSV-014, YK-RSV-015, YK-RSV-016, YK-RSV-017, YK-RSV-018, YK-RSV-019, YK-RSV-022, YK-RSV-029, YK-RSV-030, YK-RSV-031, YK-RSV-032, YK-RSV-033, YK-RSV-034, YK-RSV-035, YK-RSV-036, YK-RSV-037, YK-RSV-038, YK-RSV-039, YK-RSV-040, YK-RSV-041, YK-RSV-042, YK-RSV-043, YK-RSV-044, YK-RSV-045, YK-RSV-046, YK-RSV-048, YK-RSV-050, YK-RSV-051, YK-RSV-052, YK-RSV-053, YK-RSV-054, YK-RSV-055, YK-RSV-056, YK-RSV-057, YK-RSV-058, YK-RSV-059, YK-RSV-060, YK-RSV-061, and YK-RSV-062.

[0125] (2) By detecting the binding rates of epitopes on RSV F mutants to corresponding antibodies, the following 42 RSV F mutants (including YK-RSV-061, YK-RSV-003, YK-RSV-062, YK-RSV-054, YK-RSV-039, YK-RSV-044, YK-RSV-012, YK-RSV-015, YK-RSV-001, YK-RSV-036, YK-RSV-060, YK-RSV-005, YK-RSV-010, YK-RSV-017, YK-RSV-030, YK-RSV-038, YK-RSV-043, YK-RSV-055, YK-RSV-056, YK-RSV-057, YK-RSV-048, YK-RSV-052, YK-RSV-013, YK-RSV-002, YK-RSV-009, YK-RSV-050, YK-RSV-032, YK-RSV-019, YK-RSV-040, YK-RSV-046, YK-RSV-008, YK-RSV-059, YK-RSV-034, YK-RSV-004, YK-RSV-058, YK-RSV-016, YK-RSV-029, YK-RSV-035, YK-RSV-037, YK-RSV-011, YK-RSV-045, YK-RSV-051) are selected to have binding rates of an epitope Φ and an epitope II thereon to the corresponding antibodies of about 80% or more, all with good binding rates.

[0126] (3) By performing a stability test after stress treatment under different temperature, osmolarity, and pH conditions, the following 20 mutants (including YK-RSV-061, YK-RSV-003, YK-RSV-062, YK-RSV-054, YK-RSV-039, YK-RSV-044, YK-RSV-012, YK-RSV-015, YK-RSV-001, YK-RSV-036, YK-RSV-060, YK-RSV-009, YK-RSV-010, YK-RSV-011, YK-RSV-030, YK-RSV-043, YK-RSV-048, YK-RSV-050, YK-RSV-055, YK-RSV-057) are all selected to have a stress resistance parameter of above 0.44, up to 0.68, after storage at 4 °C for one week; a stress resistance parameter of above 0.64 after incubation at 50 °C for 60 min; a stress resistance parameter of above 0.61 after treatment under a high osmotic pressure condition for 60 min; a stress resistance parameter of above 0.70 after treatment under a low osmotic pressure condition for 60 min; a stress resistance parameter of above 0.56 after treatment under conditions of pH = 3.5 and pH = 10 for 60 min. The stress resistance parameters after treatment under the above different conditions are all superior to or comparable to those of the controls.

[0127] (4) By detecting binding levels of different epitopes to corresponding antibodies by ELISA, the following 15 mutants (including YK-RSV-061, YK-RSV-003, YK-RSV-062, YK-RSV-054, YK-RSV-039, YK-RSV-044, YK-RSV-012, YK-RSV-015, YK-RSV-001, YK-RSV-036, YK-RSV-060, YK-RSV-011, YK-RSV-030, YK-RSV-048, YK-RSV-050) are selected to have an epitope Φ , an epitope Quaternary, and an epitope V thereon that are all able to well bind to the corresponding antibodies, and have a relatively large number of binding epitopes and relatively strong binding capacity.

[0128] For example, the binding levels of the epitope Φ on YK-RSV-061, YK-RSV-003, and YK-RSV-062 to the RSV pre-F antibody (3C12) are 1.83 to 2.00 times that on the control PC-RSV-064; the binding levels of the epitope Quaternary to the RSV pre-F antibody (7H11) are 2.01-2.12 times that on the control PC-RSV-064.

[0129] The binding level of the epitope Φ on YK-RSV-054 to the RSV pre-F antibody (3C12) is 2.52 times that on the control PC-RSV-064; the binding level of the epitope Quaternary to the RSV pre-F antibody (7H11) is 1.82 times that on the control PC-RSV-064; the binding level of the epitope V to the RSV F antibody (7E11) is 3.30 times that on the control PC-RSV-064; the binding level of the epitope II to the RSV F antibody (11A9) is 1.79 times that on the control PC-RSV-064; the binding level of the epitope III to the RSV F antibody (4B9) is 2.27 times that on the control PC-RSV-064.

[0130] (5) The affinity of the target protein for different antibodies is analyzed by a BLI method. The detection results show that: the epitope Φ and the epitope Quaternary on 13 mutants (including YK-RSV-

061, YK-RSV-003, YK-RSV-062, YK-RSV-054, YK-RSV-039, YK-RSV-044, YK-RSV-012, YK-RSV-015, YK-RSV-001, YK-RSV-036, YK-RSV-060, YK-RSV-011, YK-RSV-030) bind with the strongest affinity to the corresponding antibodies.

[0131] The affinity constant K_D values for the binding of the epitope Φ on the mutants to the RSV pre-F antibody (3C12) are $3.26E-12$ M to $7.79E-11$ M, which are significantly lower than the K_D value (K_D of about $E-10$ M, with a difference of about 1 to 2 orders of magnitude) of the controls, indicating that the affinity of the antigens for the antibody is stronger.

[0132] The affinity constant K_D values for the binding of the epitope Quaternary on the mutants to the RSV pre-F antibody (7H11) are $3.05E-12$ M to $6.13E-10$ M, and except that the K_D values for YK-RSV-044, YK-RSV-060, and YK-RSV-030 are comparable to those of the controls, the K_D values for the other mutants are significantly lower than those of the controls (with a difference of 1 to 2 orders of magnitude), indicating that the affinity of the antigens for the antibody is stronger.

[0133] (6)

[0134] Mouse: binding antibody

[0135] The IgG titers specific for the pre-fusion F and post-fusion F proteins in the serum of mice on day 35 after the immunization are detected by an ELISA method. The detection results show that 11 test samples (including YK-RSV-061, YK-RSV-003, YK-RSV-062, YK-RSV-054, YK-RSV-039, YK-RSV-044, YK-RSV-012, YK-RSV-015, YK-RSV-001, YK-RSV-036, YK-RSV-060) are selected to have pre-F IgG antibody titers of 2.52 to 5.31 times that of the Abrysvo™ positive control, and have post-F IgG antibody titers of 1.13 to 5.21 times that of the Abrysvo™ positive control.

[0136] In all of the test samples being detected, the pre-F/post-F ratios are all higher than 1, indicating that the IgG titers produced by the pre-fusion F proteins are higher than those of IgG produced by the post-fusion F proteins.

[0137] Mouse: neutralizing antibody

[0138] **1) RSV type A virus:** the neutralizing antibody titers in the serum of mice on day 35 after the immunization are detected by a plaque reduction neutralization-based microneutralization test method, and 11 test samples (including YK-RSV-061, YK-RSV-003, YK-RSV-062, YK-RSV-054, YK-RSV-039, YK-RSV-044, YK-RSV-012, YK-RSV-015, YK-RSV-001, YK-RSV-036, YK-RSV-060) are selected to have neutralizing antibody titer IC_{50} values of 3.72 to 12.67 times that of the Abrysvo™ positive control, and have IC_{90} values of 2.34 to 14.41 times that of the Abrysvo™ positive control, which are all superior to those of the positive control.

[0139] **2) RSV type B virus:** 11 test samples (including YK-RSV-061, YK-RSV-003, YK-RSV-062, YK-RSV-054, YK-RSV-039, YK-RSV-044, YK-RSV-012, YK-RSV-015, YK-RSV-001, YK-RSV-036, YK-RSV-060) have neutralizing antibody titer IC_{50} values of 2.85 to 10.63 times that of the Abrysvo™ positive control, and have IC_{90} values of 2.88 to 14.23 times that of the Abrysvo™ positive control, with neutralizing antibody titers all significantly higher than that of the positive control.

[0140] (7)

[0141] Cotton rat: binding antibody

[0142] The IgG titers specific for the pre-fusion F and post-fusion F proteins in the serum of cotton rats on days 42 and 48 after the immunization are detected by an ELISA method. For 11 test samples (including YK-RSV-061, YK-RSV-003, YK-RSV-062, YK-RSV-054, YK-RSV-039, YK-RSV-044, YK-RSV-012, YK-RSV-015, YK-RSV-001, YK-RSV-036, YK-RSV-060):

[0143] The pre-F IgG antibody titers on day 42 are 3.05 to 13.10 times that of the Abrysvo™ positive control, and the post-F IgG antibody titers are 2.36 to 6.38 times that of the Abrysvo™ positive control, all of which are significantly higher than that of the positive control. The pre-F/post-F ratios of all the test samples range from 1.39 to 3.95, indicating that the IgG antibody titers produced by the pre-fusion F proteins are significantly higher than those produced by the post-fusion F proteins.

[0144] The pre-F IgG antibody titers on day 48 are 4.44 to 12.35 times that of the Abrysvo™ positive control, and the post-F IgG antibody titers are 1.51 to 6.83 times that of the Abrysvo™ positive control, all of which are significantly higher than those of the positive control. The pre-F/post-F ratios of all the test samples range from 1.78 to 4.29, indicating that the IgG antibody titers produced by the pre-fusion F proteins are still significantly higher than those produced by the post-fusion F proteins on day 48 after the immunization.

[0145] Cotton rat: neutralizing antibody

[0146] The neutralizing antibody titers produced by the RSV A2 virus in the serum of cotton rats are detected. The detection results show that, for 11 test samples (including YK-RSV-061, YK-RSV-003, YK-RSV-062, YK-RSV-054, YK-RSV-039, YK-RSV-044, YK-RSV-012, YK-RSV-015, YK-RSV-001, YK-RSV-036, YK-RSV-060):

[0147] 1) On day 42 after the immunization, for the RSV A2 virus, the neutralizing antibody titer IC₅₀ values are 2.10 to 14.30 times that of the Abrysvo™ positive control, and IC₉₀ values are 2.26 to 22.33 times that of the Abrysvo™ positive control, all of which are significantly higher than those of the positive control.

[0148] The neutralizing antibody titer IC₅₀ values produced by the RSV B virus are 2.16 to 14.63 times that of the Abrysvo™ positive control, and IC₉₀ values are 2.49 to 17.26 times that of the Abrysvo™ positive control, all of which are significantly higher than those of the positive control.

[0149] 2) On day 48 after the immunization, the neutralizing antibody IC₅₀ values produced by the RSV A2 virus are 1.70 to 7.57 times that of the Abrysvo™ positive control, and IC₉₀ values are 1.46 to 11.56 times that of the Abrysvo™ positive control, all of which are higher than those of the positive control.

[0150] (8) The results for pathological sections of the lungs of cotton rats show that, for 11 test samples (including YK-RSV-061, YK-RSV-003, YK-RSV-062, YK-RSV-054, YK-RSV-039, YK-RSV-044, YK-RSV-012, YK-RSV-015, YK-RSV-001, YK-RSV-036, YK-RSV-060): The peribronchiolar infiltration scores are 0.33 to 0.83, indicating mild inflammatory infiltration. All the test samples have interstitium infiltration scores superior to that of the positive control Abrysvo™, and the scores are 0.67 to 1.17, indicating mild inflammatory infiltration. All the test samples have low overall inflammatory response scores, and the total pathological scores are 1.00 to 2.00, all of which are lower than that of the control LNP. The scores of two indicators of perivascular infiltration and alveolar infiltration of the control and the test samples are all 0, indicating that there are no visible lesions in the tissue.

[0151] (9) The results for viral loads in the lungs and turbinates of cotton rats show that: the viral loads after immunization in the lungs of 11 test samples (including YK-RSV-061, YK-RSV-003, YK-RSV-062, YK-RSV-054, YK-RSV-039, YK-RSV-044, YK-RSV-012, YK-RSV-015, YK-RSV-001, YK-RSV-036, YK-RSV-060) are 1.61-2.08, which are lower than that of the positive control Abrysvo™; the viral loads in the turbinates are 0.62 to 1.51, which are only 0.19 to 0.45 times that of the positive control Abrysvo™, with significantly reduced viral loads, indicating that the test samples have a better antiviral effect.

BRIEF DESCRIPTION OF THE DRAWING

[0152] FIG. 1: antigen expression of YK-RSV-001-YK-RSV-062;

[0153] FIGs. 2(A) and 2(B) show RSV A2 pre-F binding antibody titers in the serum of mice on day 35, respectively;

[0154] FIGs. 3(A) and 3(B) show RSV A2 post-F binding antibody titers in the serum of mice on day 35, respectively;

[0155] FIGs. 4(A) and 4(B) show RSV A2 neutralizing antibody titer IC₅₀ in the serum of mice on day 35, respectively;

[0156] FIGs. 5(A) and 5(B) show RSV A2 neutralizing antibody titer IC₉₀ in the serum of mice on day 35, respectively;

[0157] FIGs. 6(A) and 6(B) show RSV B neutralizing antibody titer IC₅₀ in the serum of mice on day 35, respectively;

[0158] FIGs. 7(A) and 7(B) show RSV B neutralizing antibody titer IC₉₀ in the serum of mice on day 35, respectively;

[0159] FIGs. 8(A) and 8(B) show RSV A2 pre-F binding antibody titers in the serum of cotton rats on day 42, respectively;

[0160] FIGs. 9(A) and 9(B) show RSV A2 post-F binding antibody titers in the serum of cotton rats on day 42, respectively;

[0161] FIGs. 10(A) and 10(B) show RSV A2 pre-F binding antibody titers in the serum of cotton rats on day 48, respectively;

[0162] FIGs. 11(A) and 11(B) show RSV A2 post-F binding antibody titers in the serum of cotton rats on day 48, respectively;

[0163] FIGs. 12(A) and 12(B) show RSV A2 neutralizing antibody titer IC₅₀ in the serum of cotton rats on day 42, respectively;

[0164] FIGs. 13(A) and 13(B) show RSV A2 neutralizing antibody titer IC₉₀ in the serum of cotton rats on day 42, respectively;

[0165] FIGs. 14(A) and 14(B) show RSV B neutralizing antibody titer IC₅₀ in the serum of cotton rats on day 42, respectively;

[0166] FIGs. 15(A) and 15(B) show RSV B neutralizing antibody titer IC₉₀ in the serum of cotton rats on day 42, respectively;

[0167] FIGs. 16(A) and 16(B) show RSV A2 neutralizing antibody titer IC₅₀ in the serum of cotton rats

on day 48, respectively;

[0168] FIGs. 17(A) and 17(B) show RSV A2 neutralizing antibody titer IC₉₀ in the serum of cotton rats on day 48, respectively;

[0169] FIG. 18 shows the results for pathological sections of the lungs of cotton rats after treatment with the test samples of the present disclosure.

DETAILED DESCRIPTION

[0170] The present disclosure is further illustrated by the following examples. It should be understood that the embodiments of the present disclosure are given for illustration only and not for limitation, and the simple modifications of the present disclosure in the light of the technical solutions of the present disclosure are within the scope of the present disclosure.

[0171] As used herein, amino acid residues are abbreviated as follows: alanine (Ala; A), asparagine (Asn; N), aspartic acid (Asp; D), arginine (Arg; R), cysteine (Cys; C), glutamic acid (Glu; E), glutamine (Gln; Q), glycine (Gly; G), histidine (His; H), isoleucine (Ile; I), leucine (Leu; L), lysine (Lys; K), methionine (Met; M), phenylalanine (Phe; F), proline (Pro; P), serine (Ser; S), threonine (Thr; T), tryptophan (Trp; W), tyrosine (Tyr; Y), and valine (Val; V).

[0172] The following terms used herein have the meanings indicated below.

[0173] Vaccine

[0174] Vaccine refers to a substance comprising immunogen capable of eliciting a prophylactic or therapeutic immune response in an individual, which may be in the form of a pharmaceutical composition. Generally, vaccines elicit antigen-specific immune responses against antigens of pathogens, e.g., viral pathogens.

Nucleic acid

[0175] Nucleic acid is a polymer comprising nucleotides (nucleotide monomers). Thus, the nucleic acid is also referred to as polynucleotides. The nucleic acid may be or may comprise, for example, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), threose nucleic acid (TNA), glycol nucleic acid (GNA), peptide nucleic acid (PNA), locked nucleic acid (LNA, including LNA having a β -D-ribose configuration, α -LNA having an α -L-ribose configuration (diastereomer of LNA), 2'-amino-LNA having 2'-amino functionalization, and 2'-amino- α -LNA having 2'-amino functionalization), ethylene nucleic acid (ENA), cyclohexenyl nucleic acid (CeNA), and/or chimeras thereof and/or combinations thereof.

[0176] Messenger RNA (mRNA) is any RNA that encodes (at least one) protein (a naturally occurring, non-naturally occurring, or modified polymer of amino acids) and that can be translated *in vitro*, *in vivo*, *in situ*, or *ex vivo* to produce the encoded protein. Those skilled in the art will understand that unless otherwise stated, nucleic acid sequences set forth herein may be enumerated with "T" in a representative DNA sequence, but in the case where the sequence represents RNA (e.g., mRNA), "T" will be replaced by "U". Thus, for any DNA disclosed and identified herein by a particular sequence identification number, a corresponding RNA (e.g., mRNA) sequence complementary to the DNA is also disclosed, wherein each "T" of the DNA sequence is replaced by "U".

[0177] An open reading frame (ORF) is a contiguous stretch of DNA or RNA, beginning with an initiation codon (e.g., methionine (ATG or AUG)) and ending with a termination codon (e.g., TAA, TAG or TGA, or UAA, UAG or UGA). The ORF generally encodes a protein. It should be understood that the sequence disclosed herein may also comprise additional elements, e.g., 5' and 3' UTRs, but unlike ORF, these elements are not necessarily present in the RNA polynucleotide of the disclosure.

[0178] The composition of the present disclosure comprises RNA having an open reading frame (ORF) encoding a respiratory syncytial virus antigen. In some embodiments, the RNA is messenger RNA (mRNA). In some embodiments, the RNA (e.g., mRNA) further comprises a 5' UTR, a 3' UTR, a poly(A) tail, and/or a 5' cap analog (or 5' cap structure).

[0179] It should also be understood that the mRNA vaccine of the present disclosure may comprise any 5' untranslated region (UTR) and/or any 3' UTR. Exemplary UTR sequences are provided in the sequence listing; however, other UTR sequences may be used or substituted by any UTR sequence described herein. UTRs may also be omitted from the RNA polynucleotide provided herein.

[0180] Antigen

[0181] An antigen is a protein that is capable of inducing an immune response (e.g., causing an immune system to produce an antibody against an antigen). Herein, the use of the term “antigen” encompasses immunogenic proteins and immunogenic fragments (which induce (or are capable of inducing) an immune response to (at least one) respiratory syncytial virus, e.g., RSV A2 (138251)), unless otherwise stated. It should be understood that the term “protein” encompasses peptides and the term “antigen” encompasses fragments of antigens.

[0182] Exemplary sequences of a respiratory syncytial virus antigen and RNA encoding the respiratory syncytial virus antigen of the composition of the present disclosure are provided in the sequence listing.

[0183] Epitope

[0184] Epitope, also referred to as an antigenic determinant, refers to a particular region of an antigen molecule that can be recognized by a specific antibody, where one or more epitopes may be present on an antigenic molecule. The antigenic epitope can be recognized by a B-cell receptor (BCR), triggering subsequent antibody synthesis and secretion and mediating a humoral immune response. The pre-fusion pre-F conformation has six major antigenic sites/epitopes (including epitopes Φ , I, II, III, IV, and V), and by crystal structure analysis of the RSV F protein, it is found that the AM14 antibody specifically recognizes an epitope Quaternary spanning two protomers, including a region that undergoes extensive conformational changes in the transition of pre-fusion to post-fusion F proteins. These epitopes are highly antigenic, but relatively unstable. The post-fusion post-F conformation has only four antigenic sites/epitopes (including epitopes I, II, III, and IV), which are relatively stable but less antigenic. Compared with the epitopes I, II, III, and IV that are present in both pre-fusion and post-fusion conformations, the epitopes Φ and V are present only in the pre-F conformation, and studies have demonstrated that they are sites sensitive to neutralizing antibodies and can induce higher neutralizing antibody responses. The antibodies in the present disclosure recognize epitopes as follows:

[0185] (1) The RSV pre-F antibody (3C12) recognizing the epitope Φ specifically binds only to the F

protein in the pre-fusion conformation.

[0186] (2) The RSV pre-F antibody (7H11) recognizes the epitope Quaternary (spanning both epitopes IV and V) and specifically binds to the F protein in the pre-fusion conformation.

[0187] (3) The RSV F antibody (11A9) recognizing the epitope II can recognize both pre-fusion and post-fusion conformations of the F protein.

[0188] (4) The RSV F antibody (4B9) recognizing the epitope III preferentially recognizes the pre-fusion conformation while having weaker binding activity for the post-fusion conformation.

[0189] (5) The RSV pre-F antibody (7E11) recognizes the epitope V and specifically binds to the F protein in the pre-fusion conformation.

[0190] Studies have shown that whether to be able to bind specifically to the epitope Φ of the pre-fusion antigen and the ability to bind are closely related to the effect of the novel vaccine in preventing the RSV infection. The epitopes Φ and II account for 35% and < 10% of the neutralizing activity, respectively, and the neutralizing activity of RSV in human serum is mainly derived from the pre-fusion specific antibodies. Therefore, the use of pre-fusion antigens retaining the epitope Φ will help to induce or enhance neutralizing activity by vaccination.

[0191] The antigenic epitopes of the pre-fusion and post-fusion conformations of the RSV F protein are changed, with the epitope Φ retained only in the pre-fusion conformation and the epitope II retained in both the pre-fusion and post-fusion conformations. An antibody specific for the antigenic epitope Φ differs from neutralizing antibodies for other known epitopes on the RSV F protein in that it specifically recognizes only the pre-F structure. Studies have shown that whether to be able to bind specifically to the epitope Φ of the pre-fusion antigen and the ability to bind are closely related to the effect of the novel vaccine in preventing the RSV infection, and therefore, they are used as primary indicators for investigation. The epitope Φ is located at the top end of the pre-F trimer structure, has a small steric hindrance, and is beneficial to antibody binding. Thus, the higher the ability of the antibody to bind to the epitope Φ , the better the prophylactic effect on RSV.

[0192] Epitope binding assays are performed using monoclonal antibodies (mAbs), and the results show that the ability to bind to the epitope Φ -specific antibodies (e.g., number, binding rate, binding capacity, degree of binding, etc.) is related to the neutralizing activity, while the degree of binding to the epitope II is poorly related to the neutralizing activity. Moreover, the neutralizing activity of RSV in human serum is mainly derived from pre-F specific antibodies, and therefore, the neutralizing activity is improved by vaccination using the epitope Φ in the pre-fusion conformation.

[0193] Pre-fusion conformation of RSV F protein

[0194] The pre-fusion conformation of the RSV F protein is a structural conformation adopted by an RSV F protein prior to triggering a fusogenic event that results in the transition of RSV F to a post-fusion conformation and enables RSV F to become a mature RSV F protein in the secretory system after processing. In some embodiments, whether the antigen encoded by the mutant nucleic acid is stable in the pre-fusion conformation can be determined by binding to a particular epitope. The stable pre-fusion RSV F mutants of the present disclosure are in the pre-fusion conformation, i.e., they comprise (display) at least one epitope

which is specific for the F protein in the pre-fusion conformation. Epitopes specific for the F protein in the pre-fusion conformation are epitopes that are not present in the post-fusion conformation. The pre-fusion conformation of the RSV F protein may contain the same epitopes as those on the RSV F proteins expressed on natural RSV virions, and thus may provide advantages for eliciting protective neutralizing antibodies.

[0195] In some embodiments, stable pre-fusion RSV F mutants can specifically bind to the RSV pre-F antibody (3C12) recognizing the epitope Φ and to the RSV pre-F antibody (7H11) recognizing the epitope Quaternary.

[0196] Post-fusion conformation of RSV F protein

[0197] The post-fusion conformation of the RSV F protein is a structural conformation adopted by an RSV F protein that is not in the pre-fusion conformation, in which the N-terminus and C-terminus of the RSV F protein are proximal in a stable helix-helix. The post-fusion conformation of the RSV F protein has been described at the atomic level (see, e.g., McLellan et al., *J. Virol.*, 85, 7788, 2011; Swanson et al., *Proc. Natl. Acad. Sci. U.S.A.*, 108, 9619, 2011; and structural coordinates deposited under PDB accession No. 3RRR; each of which is incorporated herein by reference). In the post-fusion conformation, the RSV F protein cannot be bound by certain antibodies, e.g., antibodies recognizing the epitope Φ .

[0198] Variant

[0199] In some embodiments, the composition of the present disclosure comprises RNA encoding a respiratory syncytial virus antigen variant. An antigen variant or other polypeptide variant refers to a molecule whose amino acid sequence differs from that of the wild-type, natural, or reference sequence. An antigen/polypeptide variant may have substitutions, deletions, and/or insertions at certain positions within the amino acid sequence compared with the natural or reference sequence. Generally, the variant has at least 50% identity to the wild-type, natural, or reference sequence. In some embodiments, the variant has at least 80% or at least 90% identity, e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% or 100% identity compared with the wild-type, native, or reference sequence. Herein, “mutant” and “variant” are used interchangeably.

[0200] Mutation

[0201] The term “mutation” refers to a change in the amino acid sequence compared with the wild-type protein sequence. In the present disclosure, the antigen mutant has one or more base mutations compared with the wild-type RSV F protein. After mutation, a truncation, a deletion of an amino acid residue, or a substitution of an amino acid residue occurs in the amino acid sequence of the protein or polypeptide compared with an amino acid sequence of a reference protein or polypeptide. Herein, an amino acid substitution at a particular position in a protein sequence is indicated using the annotation “(amino acid residue in a wild-type protein) (amino acid position) (amino acid residue in an engineered protein)”. For example, Y44C refers to the substitution of a tyrosine (Y) residue at position 44 of an amino acid sequence of a reference protein with a cysteine (C) residue (in a variant of the reference protein). In the case of variations in amino acid residues at the same position between different wild-type sequences, the amino acid

code preceding the position number may be omitted from the annotation, such as “44C”.

[0202] Sequence truncation

[0203] Sequence truncation refers to a truncation of a contiguous portion of an amino acid sequence at the C terminus based on the wild-type RSV F protein. For example, in the present disclosure, the sequence truncation is to truncate amino acids at positions 550 to 574 of the C-terminus of the RSV F protein.

[0204] Sequence deletion

[0205] Sequence deletion refers to a deletion of a part of bases at amino acid sites based on the wild-type RSV F protein. For example, in the present disclosure, the sequence deletion is selected from one or more of the following: amino acid deletions at positions 104 to 136 and amino acid deletions at positions 104 to 144.

[0206] Site mutation

[0207] Site mutation refers to an alteration in one or more amino acid sites in the wild-type RSV F protein to stabilize the RSV F protein in the pre-fusion conformation. For example, in the present disclosure, the site mutation is selected from the following: P102A, I379V and M447V, S46G, S215P, L373R, S213P and N216P, Q26C, N27C, T29C, E31C, F32C, Q34C, T36C, S41C, Y44C, Y53C, T54C, S55C, T100C, T103C, L138C, G139C, F140C, L141C, L142C, G145C, S146C, A147C, I148C, A149C, S150C, V152C, A153C, V154C, S155C, N183GC, S186C, L188C, S287C, I288C, S290C, I291C, I292C, V300C, L305C, Y306C, S319C, N325C, S330C, T337C, R339C, Q354C, T369C, M370C, N371C, S377C, N428C, Y458C, K461C, Q462C, K465C, L467C, Y468C, K470C, G471C; T54H, T58L, S190I, S190F, V207L, T219I, V296I; E92D, K465Q, D486S; V152I, C69Y, D73E, A74V, N88S. In the above optional site mutations, one of the site mutations may occur at the amino acid at the same position, for example, the above mutations include T54C or T54H, which means that T at position 54 may be mutated to C or H, but not both.

[0208] Lack of cytoplasmic domain

[0209] The cytoplasmic domain consists of residues 550 to 574 of a polypeptide chain of a mature RSV F protein. For example, in the present disclosure, the lack of a cytoplasmic domain means that an amino acid sequence of 550 to 574 at the C-terminus of an antigen mutant is truncated.

[0210] “One or more”/“selected from”

[0211] Herein, “one or more” means one, two or more selected from the listed objects or elements. Similarly, “selected from” means that one, two or more are selected from the list of listed objects or elements, including any combination of the listed objects or elements, such as a combination of any two of the listed objects or elements, a combination of any three of the listed objects or elements, ..., a combination of any multiple of the listed objects or elements, including a combination of all the objects. In this sense, “more” may include two or three or more.

[0212] Variant antigens/polypeptides encoded by the nucleic acid of the present disclosure may contain amino acid changes that confer any one of a variety of desirable properties, e.g., improving their stability or enhancing immunogenicity in animals. Variant antigens/polypeptides may be prepared using conventional mutagenesis techniques and assayed as appropriate to determine whether they have the desired properties. Assays to determine expression levels and immunogenicity are well known in the art, and examples of such

assays are set forth in the Example section.

[0213] In some embodiments, the composition comprises an RNA or RNA ORF comprising a nucleotide sequence of any one of the sequences provided herein (see sequence listing), or a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% or 100% identity to the nucleotide sequence of any one of the sequences provided herein.

[0214] The term “identity” refers to a relationship between the sequences of two or more polypeptides (e.g., antigens) or polynucleotides (nucleic acids), as determined by comparing the sequences. The identity also refers to the degree of sequence correlation between or among sequences, as determined by the number of matches between two or more strings of amino acid residues or nucleic acid residues. An identity measure refers to the percentage of identity matches between the smaller of two or more sequences having gap alignments (if any) that are processed by a particular mathematical model or computer program (e.g., an “algorithm”). The identity of the relevant antigen or nucleic acid can be readily calculated by known methods. “Percent identity (%)”, when applied to a polypeptide or polynucleotide sequence, is defined as the percentage of residues in a candidate amino acid or nucleic acid sequence that are identical to residues (amino acid residues or nucleic acid residues) in an amino acid sequence or nucleic acid sequence of the second sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent identity. Methods and computer programs for alignment are well known in the art. It should be understood that the identity depends on the calculation of percent identity, but the value of identity may differ due to gaps and penalties introduced in the calculation. Generally, a variant of a particular polynucleotide or polypeptide (e.g., antigen) has at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% but less than 100% sequence identity to a particular reference polynucleotide or polypeptide, as determined by sequence alignment programs and parameters described herein and known to those of skill in the art. Such tools for alignment include those of the BLAST kit (Stephen F. Altschul et al. (1997), “Gapped BLAST and PSI-BLAST: a new generation of protein database search programs”, *Nucleic Acids Res.* 25: 3389-3402). Another common local alignment technique is based on the Smith-Waterman algorithm (Smith, T.F. and Waterman, M.S. (1981) “Identification of common molecular subsequences.” *J. Mol. Biol.* 147: 195-197). A general global alignment technique based on dynamic programming is the Needleman-Wunsch algorithm (Needleman, S. B. and Wunsch, C. D.(1970) “A general method applicable to the search for similarities in the amino acid sequences of two proteins.” *J. Mol. Biol.* 48: 443-453). Recently, a fast optimal global sequence alignment algorithm (FOGSAA) has been developed, which is said to produce global alignments of nucleotide and protein sequences more quickly than other optimal global alignment methods, including the Needleman-Wunsch algorithm.

[0215] Thus, polynucleotides encoding a peptide or a polypeptide containing substitutions, insertions and/or additions, deletions, and covalent modifications relative to a reference sequence, particularly the polypeptide (e.g., antigen) sequence disclosed herein, are included within the scope of the present disclosure. In some embodiments, sequences of (or encoding) a signal sequence, a termination sequence, a

transmembrane domain, a linker, etc., may be replaced by alternative sequences that perform the same or similar functions. In some embodiments, the stability may be improved, for example, by introducing larger amino acids into the cavity in the protein core. In other embodiments, the stability may be improved by replacing the embedded hydrogen bonding network by hydrophobic residue. Such sequences can be readily identified by those skilled in the art.

[0216] The RSV F mutant provided by the present disclosure comprises an F1 polypeptide and an F2 polypeptide, wherein both the F1 polypeptide and the F2 polypeptide comprise a plurality of introduced amino acid mutations relative to an amino acid sequence of a corresponding natural F protein. The introduction of such amino acid mutations into the RSV F mutant confers beneficial properties to the mutant, such as enhanced immunogenicity, improved stability, or formation of a particular desired physical form or conformation of the mutant, or improved stability.

[0217] Mutant (variant) structure of RSV F protein

[0218] The F glycoprotein of human RSV is first translated from mRNA into a single polypeptide precursor (F0) of 574 amino acids that contains a signal peptide sequence at the N-terminus. After translation, the signal peptide is removed by a signal peptidase in the endoplasmic reticulum. F0 is cleaved at two sites (between amino acid residues 109/110 and 136/137) by a cellular protease (particularly furin) in the trans-Golgi, a short glycosylated intervening sequence (also referred to as the pep27 region; amino acid residues 110 to 136), and two domains or subunits called F1 (C-terminal portion; amino acids 137 to 574) and F2 (N-terminal portion; amino acids 26 to 109). F2 is linked to F1, and the F1-F2 heterodimer assembles into a homotrimer in the virion.

[0219] Signal peptide

[0220] “Signal peptide” is a short amino acid sequence (e.g., about 18 to 25 amino acids in length) that is generally required for transmembrane translocation over the secretory pathway to control the entry of most proteins into the secretory pathway in eukaryotes and prokaryotes. The signal peptide is often, but not universally, located at the N-terminus of the polypeptide and is often cleaved off by a signal peptidase after the protein has crossed the membrane.

[0221] To enhance production, the F2 domain may be preceded by a secretion signal peptide, such as a natural F protein signal peptide or a heterologous signal peptide, for enhancing the production and secretion in a host cell in which the recombinant pre-F antigen is to be expressed.

[0222] F1 polypeptide

[0223] The term “F1 polypeptide” (F1) refers to a polypeptide chain of a mature RSV F protein. Natural F1 consists of approximate residues 137 to 574 of the RSV F0 precursor. As used herein, the term encompasses both the natural F1 polypeptide and the F1 polypeptide derived from the natural sequence that comprises modifications (e.g., amino acid substitutions, insertions, or deletions), e.g., modifications designed to stabilize the F mutant or enhance the immunogenicity of the F mutant.

[0224] F1 comprises a hydrophobic fusion peptide (FP) and a heptad repeat region A (HRA) at the N-terminus of 137 to 216 and comprises a heptad repeat region B (HRB), a transmembrane (TM, amino acid residues 530 to 550), and a cytoplasmic region (amino acid residues 551 to 574) at the C-terminus. In the

structure of the RSV F protein mutant, it is not strictly necessary to include the entire F1 domain. Typically, at least a subsequence (or fragment) of the F1 domain is selected and designed to maintain a stable conformation that includes immunodominant epitopes of the F protein. F1 contains many subsequences of epitopes recognized by neutralizing antibodies, for example, amino acid residues 262 to 275 are a subsequence of an epitope recognized by palivizumab; amino acid residues 423 to 436 are a subsequence of an epitope recognized by the ch101F monoclonal antibody of Centocor; amino acid residues 328 to 355 comprise a T cell epitope. Thus, the polypeptide of the F1 domain comprises at least about amino acids 262 to 436 of the polypeptide of the RSV F protein.

[0225] Linker

[0226] One or two furin recognition sites may be eliminated by deleting or substituting one or more amino acids of the furin recognition sites. By virtue of this design, the fusion peptide is not cleaved from F2, which prevents release from the globular head of the pre-fusion conformer and accessibility to adjacent membranes. It is predicted that the interaction between the fusion peptide and the membrane interface is a major problem of the instability of the pre-fusion state. In the fusion process, the interaction between the fusion peptide and the target membrane results in the exposure of the fusion peptide from the globular head structure, which enhances instability of the pre-fusion state and allows folding into a post-fusion conformer. This conformational change realizes the membrane fusion process. It is predicted that removal of one or both of the two furin cleavage sites prevents accessibility of the membrane to the N-terminal portion of the fusion peptide, which stabilizes the pre-fusion state. Optionally, an intervening pep27 peptide may also be removed or replaced by, for example, a linker peptide. Additionally, or optionally, a non-furin cleavage site (e.g., a metalloprotease site at positions 112 to 113) proximal to the fusion peptide may be removed or replaced.

[0227] In the present application, a linker peptide is used to replace the intervening pep27 peptide. The analysis of a structural model of the RSV F protein in the pre to fusion state suggests that pep27 produces a large unconstrained loop between F1 and F2, which does not contribute to the stabilization of the pre-fusion state and is removed after cleavage of the natural protein by furin. Commonly used protein linkers include flexible linkers, rigid linkers, and cleavable linkers. The linker used in the present application is a flexible linker.

[0228] The linker used in the present application can be used in this context without disrupting the conformation of the pre-F antigen.

[0229] F2 polypeptide

[0230] The term “F2 polypeptide” (F2) refers to a polypeptide chain of a mature RSV F protein. Natural F2 includes approximate residues 26 to 109 of the RSV F0 precursor. As used herein, the term encompasses both the natural F2 polypeptide and the F2 polypeptide derived from the natural sequence that comprises modifications (e.g., amino acid substitutions, insertions, or deletions), e.g., modifications designed to stabilize the F mutant or enhance the immunogenicity of the F mutant.

[0231] Those skilled in the art will recognize that it is not strictly necessary to include the entire F2 domain. Typically, consideration of conformation is important in selecting a subsequence (or a fragment)

of the F2 domain. As such, the F2 domain generally comprises a portion of the F2 domain that facilitates assembly and stability of the polypeptide.

[0232] Stabilization element

[0233] A naturally occurring eukaryotic mRNA molecule may contain stabilization elements, including, but not limited to, an untranslated region (UTR) at its 5' end (5' UTR) and/or at its 3' end (3' UTR), in addition to other structural features such as a 5'-cap structure or a 3'-poly(A) tail. Both the 5' UTR and the 3' UTR are elements that are generally transcribed from genomic DNA and are pre-mature mRNAs. Characteristic structural features of mature mRNA (e.g., the 5'-cap and the 3'-poly(A) tail) are generally added to transcribed (pre-mature) mRNA during mRNA processing.

[0234] In some embodiments, the composition comprises an RNA polynucleotide having an open reading frame encoding at least one antigenic polypeptide having at least one modification, and at least one 5' cap, and is formulated within a lipid nanoparticle. 5'-capping of a polynucleotide can be accomplished simultaneously during the *in vitro* transcription reaction according to the manufacturer's protocol using the following chemical RNA cap analogs to produce a 5'-guanosine cap structure.

[0235] 3'-poly(A) tail, i.e., poly(A) tail, is generally a stretch of adenine nucleotides added to the 3' end of transcribed mRNA, which is located in a region downstream, e.g., directly downstream (i.e., 3'), of the 3' UTR containing multiple consecutive adenosines monophosphates. In some cases, it may comprise about 10 to 400 adenine nucleotides. In some embodiments, the length of the 3'-poly(A) tail may be an essential element for the stability of individual mRNAs. In the relevant biological environment (e.g., in a cell, *in vivo*), the poly(A) tail serves to protect the mRNA from enzymatic degradation, e.g., in the cytoplasm, and to aid in transcription termination, and/or the export of mRNA from the nucleus and translation.

[0236] Untranslated region (UTR)

[0237] The mRNA of the present disclosure may comprise one or more regions or portions that serve or function as untranslated regions. When the mRNA is designed to encode at least one target antigen, the nucleic acid may comprise one or more of these untranslated regions (UTRs). The wild-type untranslated region of the nucleic acid is transcribed, but not translated. In the mRNA, 5' UTR starts at the transcription start site and continues to the initiation codon, but does not include the initiation codon; while the 3' UTR starts immediately after the termination codon and continues to the transcription termination signal. There is increasing evidence for a regulatory role played by UTRs in the stability and translation of nucleic acid molecules. Regulatory features of the UTRs can be incorporated into the polynucleotide of the present disclosure to particularly enhance the stability of the molecule. Particular features may also be incorporated to ensure controlled down-regulation of transcripts in the event that they are misdirected to undesired organ sites.

[0238] 5' UTR refers to a region of the mRNA that does not encode a polypeptide and is immediately upstream (5') of the initiation codon (the first codon of a mRNA transcript translated by a ribosome). 5' UTR does not encode the protein (is non-coding). Natural 5' UTRs have a feature of playing a role in translation initiation. They bear imprints such as the Kozak sequence, which is generally known to be involved in the process of initiating translation of many genes at the ribosome. The Kozak sequence has a

consensus CCR(A/G)CCAUGG, where R is a purine (adenine or guanine) three bases upstream of the initiation codon (AUG), and the initiation codon (AUG) is followed by another 'G'. 5' UTR is also known to form a secondary structure involved in the binding of elongation factors.

[0239] 3' UTR refers to a region of the mRNA that does not encode a polypeptide and is directly downstream (3') of the termination codon (a codon of a mRNA transcript that signals translation termination). 3' UTR does not encode the protein (is non-coding). Natural or wild-type 3' UTRs are known to have segments of adenosine and uridine embedded therein.

[0240] Those of ordinary skill in the art will understand that heterologous or synthetic 5' UTRs may be used with any desired 3' UTR sequence. For example, a heterologous 5' UTR may be used with a synthetic 3' UTR or a heterologous 3' UTR.

[0241] *In vitro* transcription of RNA

[0242] cDNA encoding the polynucleotide described herein can be transcribed using an *in vitro* transcription (IVT) system. *In vitro* transcription of RNA is known in the art and is described in international publication WO/2014/152027, which is incorporated herein by reference in its entirety.

[0243] In some embodiments, the RNA transcript is produced in an *in vitro* transcription reaction using a linearized DNA template to produce the RNA transcript.

[0244] In some embodiments, the *in vitro* transcription template encodes a 5' untranslated region (UTR), contains an open reading frame, and encodes a 3' UTR and a poly(A) tail. The particular nucleic acid sequence composition and length of the *in vitro* transcription template will depend on the mRNA encoded by the template.

[0245] The *in vitro* transcription system generally comprises a transcription buffer, nucleotide triphosphates (NTPs), an RNase inhibitor, and a polymerase.

[0246] The NTPs may be manufactured internally or from a supplier. The NTPs may be selected from, but are not limited to, those described herein, including natural and non-natural (modified) NTPs.

[0247] In some embodiments, the RNA transcript is capped by enzymatic capping. In some embodiments, the RNA comprises a 5' cap.

[0248] Lipid nanoparticle (LNP)

[0249] In some embodiments, the RNA (e.g., mRNA) of the present disclosure is formulated in a lipid nanoparticle (LNP). The lipid nanoparticle generally comprises an ionizable cationic lipid, a non-cationic lipid, sterol and PEG lipid components, and a target nucleic acid. The lipid nanoparticle of the present disclosure can be produced using components, compositions, and methods generally known in the art.

[0250] RSV F protein mutant stability

[0251] In some embodiments, the mutant antigen encoded by the nucleic acid of the present disclosure has superior properties, such as enhanced stability of the pre-fusion conformation, compared with the wild-type. In this example, assay methods used to determine the stability of the pre-fusion conformation have been specifically described.

[0252] In some embodiments, the stability of an antigen encoded by a mutant nucleic acid can be measured by determining the thermostability and the low temperature stability. "Thermostability", in this

example, means that after being subjected to an increased temperature (i.e., 50 °C) for 60 min, the mutant still exhibits the binding to at least one pre-fusion specific epitope, e.g., the epitope Φ as determined in the present application. “Low temperature stability”, in this example, means that after one week of low temperature storage at 4 °C, the mutant still displays the binding to at least one pre-fusion specific epitope, e.g., the epitope Φ as determined in the present application. Some pre-fusion RSV F mutants according to the present disclosure have increased stability when stored at a low temperature or when exposed to heat compared with RSV F polypeptides that do not have one or more of the mutations described above.

[0253] In some embodiments, the stability of an antigen encoded by a mutant nucleic acid can be measured by determining the stability under conditions of low and high osmotic pressure.

[0254] “High osmotic pressure stability”, in this example, means that after incubation for 60 min under a low osmotic pressure condition of 10 mM NaCl, the mutant still displays the binding to at least one pre-fusion specific epitope, e.g., the epitope Φ as determined in the present application. Some pre-fusion RSV F mutants according to the present disclosure have increased stability under a low osmotic pressure condition compared with RSV F polypeptides that do not have one or more of the mutations described above.

[0255] “High osmotic pressure stability”, in this example, means that after incubation for 60 min under a high osmotic pressure condition of 3.0 M MgCl₂, the mutant still displays the binding to at least one pre-fusion specific epitope, e.g., the epitope Φ as determined in the present application. Some pre-fusion RSV F mutants according to the present disclosure also have increased stability under a high osmotic pressure condition compared with RSV F polypeptides that do not have one or more of the mutations described above.

[0256] In some embodiments, the stability of an antigen encoded by a mutant nucleic acid can be measured by determining the stability under acid and base conditions.

[0257] “Acid and base stability”, in this example, means that after the RSV F mutant is incubated at pH 3.5 and pH 10 for 60 min at room temperature and subsequently neutralized to pH 7.4, the mutant still exhibits the binding to at least one pre-fusion specific epitope, e.g., the epitope Φ as determined in the present application. Some pre-fusion RSV F mutants according to the present disclosure have increased stability under acid and base conditions compared with RSV F polypeptides that do not have one or more of the mutations described above.

[0258] Bio-layer interferometry

[0259] Bio-Layer interferometry (referred to as BLI) is a label-free real-time monitoring optical detection technique, mainly used for comprehensive quantitative analysis of the interaction between biomolecules and protein concentration determination. BLI can monitor the entire intermolecular binding process in real time and calculate important data such as affinity constant (K_D), association rate constant (K_a), dissociation rate constant (K_D), etc., between molecules.

[0260] In some embodiments, the affinity of the RSV F protein mutants for antibodies recognizing different epitope is determined by BLI. In this example, the affinity for the RSV pre-F antibody (3C12) and the RSV pre-F antibody (7H11) was mainly detected.

[0261] Immunogenicity of vaccine

[0262] The term “immunogenicity” refers to the ability of a substance to cause, elicit, stimulate, or induce

an immune response against a particular antigen in an animal in the presence or absence of an adjuvant. In the sense of the present disclosure, an immunogen is a translation product of the provided artificial nucleic acid, preferably RNA, which comprises at least one coding sequence encoding at least one antigenic peptide or protein derived from the RSV as defined herein.

[0263] The term “neutralizing antibody” refers to an antibody that can prevent a pathogen from binding to a receptor on the surface of a host cell during infection by the pathogen, such that the pathogen cannot adhere to and invade the target cell for replication and reproduction. The effect of the vaccine is generally reflected visually by the level of “neutralizing antibody”.

[0264] In some embodiments, the ability of an immune composition (e.g., an RNA vaccine) to be effective is measured in a murine model. For example, the immune composition can be administered to a murine model and the induction of neutralizing antibody titers in the murine model is determined. In some embodiments, the antigen-specific immune response is measured as the geometric mean titer (GMT) of neutralizing antibody titers in the serum of mice or cotton rats. Geometric mean titer (GMT) is the mean antibody titer for a group of subjects calculated by multiplying all values and taking the n-th root of the number, where n is the number of subjects with available data.

[0265] Virus challenge studies can also be used to evaluate the efficacy of the vaccine of the present disclosure. For example, the immune composition may be administered to a murine model, the murine model is challenged with a virus, and the survival and/or immune response (e.g., neutralizing antibody response) of the murine model is determined.

[0266] “Heterocyclic ring” and “heterocyclyl” are used interchangeably herein and both refer to non-aromatic heterocyclyl in which one or more ring-forming atoms are heteroatoms such as oxygen, nitrogen, sulfur, etc., including monocyclic, fused, bridged, and spiro rings. Preferably, it has a 5- to 7-membered monocyclic ring or a 7- to 10-membered bicyclic or tricyclic ring, which may contain 1, 2, or 3 atoms selected from nitrogen, oxygen, and/or sulfur. Examples of “heterocyclyl” include, but are not limited to, morpholinyl, oxetanyl, thiomorpholinyl, tetrahydropyranyl, morpholinyl, piperidinyl, 2-oxo-piperidinyl, pyrrolidinyl, 2-oxo-pyrrolidinyl, piperazin-2-one, and piperazinyl. The heterocyclyl may be substituted or unsubstituted.

[0267] 1.1 Example 1: Design of RSV F antigen mutants

[0268] The present disclosure mainly referred to an RSV A2 (138251) protein sequence, on the basis of which P102A, I379V, and M447V mutations (52 antigen sequences of candidate mutants (Nos. 1 to 52) and 6 control sequences (Nos. 53 to 58) all contained the above 3 mutations, with other mutation forms shown in the table below) were performed.

[0269] 52 antigen sequences of candidate mutants (Nos. 1 to 52) and 6 control sequences (Nos. 53 to 58), 1 wild sequence (No. 59), and 1 positive control (No. 60) were designed in total using various mutation combinations, with specific mutation combinations of the mutants shown in the table below.

[0270] The positive control is derived from the marketed vaccine of Pfizer, Abrysvo™ (NDC 0069-0344-05), and different from the mRNA vaccines designed by the present disclosure, it is a protein vaccine.

[0271] Design thought of control sequences: on the basis of the mutants designed by the present

application, different control sequences were designed to investigate the effect of different site mutations. For example,

[0272] (1) To investigate the influence of mutations in groups 2 and 3 of site mutations, PC-RSV-063 was designed based on a YK-RSV-003 mutation combination.

[0273] (2) To investigate the influence of mutations in group 1 of site mutations, PC-RSV-067 and PC-RSV-068 were designed by changing mutation types in group 1 based on a YK-RSV-061 mutation combination.

[0274] (3) To investigate the influence of mutations in groups 2 and 3 of site mutations, PC-RSV-064, PC-RSV-065, and PC-RSV-066 were designed based on a YK-RSV-061 mutation combination.

[0275] 1. By referring to “in design cycle 4, RSV F variant: sc9-10 DS-Cav1 A149C Y458C S46G K465Q S215P E92D; Mutations: A149C, S155C, S190F, V207L, S290C, L373R, Y458C, S46G, K465Q, S215P, E92D; F1-F2 Linker residues: 103 to 145; Linker sequence: GS; End residue: 513” in “M Gordon Joyce et al., Iterative structure-based improvement of a fusion-glycoprotein vaccine against RSV, *Nature Structural & Molecular Biology*, volume 23, 811–820 (2016), doi:10.1038/nsmb.3267”, a mutant PC-RSV-063 was designed.

[0276] 2. By referring to “SEQ ID NO: 6” in the patent application publication “WO2023/166079 A1”, a mutant PC-RSV-064 was designed.

Table 1 Amino acid mutation sites of RSV F proteins

No.	Antigen No.	Sequence truncation	Sequence deletion	Site mutation				
				Group 1	Group 2	Group 3	Group 4	Group 5
1	YK-RSV-001	550 to 574 aa were truncated based on the wild-type RSV F protein, that is, 1 to 549 aa were retained	104 to 144 aa were deleted and replaced by a GS linker	A149C, S155C, S290C, Y458C	S190I	E92D, D486S, K465Q	S46G, S215P, L373R	/
2	YK-RSV-002			A149C, S155C, S290C, Y458C	T54H, S190F	E92D, K465Q, D486S	S46G, S215P, L373R	/
3	YK-RSV-003			A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	/
4	YK-RSV-004			A149C, S155C, S290C, Y458C	S190I	E92D, K465Q, D486S	S46G, S213P, L373R	/
5	YK-RSV-005			A149C, S155C, S290C, Y458C	S190I	E92D, K465Q, D486S	S46G, N216P, L373R	/
6	YK-RSV-008			S55C, L188C,	S190I	E92D, K465Q, D486S	S46G, S215P, L373R	/
7	YK-RSV-009			Q34C, G471C	T58L, S190F	E92D, K465Q, D486S	S46G, S215P, L373R	/
8	YK-RSV-010			S55C, L188C	T58L, S190F	E92D, K465Q, D486S	S46G, S215P, L373R	/
9	YK-RSV-011			Q34C, G471C	S190F, T219I	E92D, K465Q, D486S	S46G, S215P, L373R	/
10	YK-RSV-012			S55C, L188C	S190F, T219I	E92D, K465Q, D486S	S46G, S215P, L373R	/
11	YK-RSV-013			T103C, I148C, Y458C	S190F	E92D, K465Q, D486S	S46G, S215P, L373R	/
12	YK-RSV-014			Q34C, A149C, Y458C, G471C	S190I	E92D, K465Q, D486S	S46G, S215P, L373R	/
13	YK-RSV-015			S55C, A149C, L188C, Y458C	S190I	E92D, K465Q, D486S	S46G, S215P, L373R	/
14	YK-RSV-016			Q34C, A149C, Y458C, G471C	T58L, S190F	E92D, K465Q, D486S	S46G, S215P, L373R	/
15	YK-RSV-017			S55C, A149C, L188C,	T58L, S190F	E92D, K465Q,	S46G, S215P, L373R	/

				Y458C		D486S		
16	YK-RSV-018			Q34C, A149C, Y458C, G471C	S190F, T219I	E92D, K465Q, D486S	S46G, S215P, L373R	/
17	YK-RSV-019			S55C, A149C, L188C, Y458C	S190F, T219I	E92D, K465Q, D486S	S46G, S215P, L373R	/
18	YK-RSV-022			T103C, I148C, N183GC, N428C	S190F	E92D, K465Q, D486S	S46G, S215P, L373R	/
19	YK-RSV-029			T29C, K465C	S190F, V207L	E92D	S46G, S215P, L373R	/
20	YK-RSV-030			E31C, L467C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
21	YK-RSV-031			F32C, Y468C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
22	YK-RSV-032			Q34C, K470C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
23	YK-RSV-033			T36C, G471C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
24	YK-RSV-034			Q26C, K461C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
25	YK-RSV-035			N27C, Q462C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
26	YK-RSV-036			S55C, V154C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
27	YK-RSV-037			Y53C, A153C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
28	YK-RSV-038			S55C, S186C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
29	YK-RSV-039			T54C, V152C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
30	YK-RSV-040			T54C, A153C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
31	YK-RSV-041			S41C, S319C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
32	YK-RSV-042			Y44C, S319C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
33	YK-RSV-043			T100C, S150C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
34	YK-RSV-044			T100C, A149C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
35	YK-RSV-045			T100C, V300C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
36	YK-RSV-046		104-136 aa, amino acids deleted from the sequence were replaced by a GSGS linker	L138C, T337C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
37	YK-RSV-047			G139C, Q354C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
38	YK-RSV-048			L142C, N371C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
39	YK-RSV-049			G145C, M370C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
40	YK-RSV-050			L141C, N371C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
41	YK-RSV-051			F140C, T369C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
42	YK-RSV-052		104-144 aa,	S146C, L305C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/

43	YK-RSV-053		amino acids deleted from the sequence were replaced by a GS linker	A149C, Y306C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
44	YK-RSV-054			I148C, I288C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
45	YK-RSV-055			A147C, I292C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
46	YK-RSV-056			A147C, S287C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
47	YK-RSV-057			A147C, I291C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
48	YK-RSV-058			S330C, G471C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
49	YK-RSV-059			N325C, G471C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
50	YK-RSV-060			R339C, S377C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
51	YK-RSV-061			A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	V152I
52	YK-RSV-062			A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	C69Y, D73E, A74V, N88S, V152I
53	PC-RSV-063			A149C, S155C, S290C, Y458C	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
54	PC-RSV-064			A149C, S155C, S290C, Y458C	S190F	E92D, K465Q	S46G, S215P, L373R	V152I
55	PC-RSV-065			A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q	S46G, S215P, L373R	V152I
56	PC-RSV-066			A149C, S155C, S290C, Y458C	S190F	E92D, K465Q, D486S	S46G, S215P, L373R	V152I
57	PC-RSV-067			T103C, I148C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	V152I
58	PC-RSV-068			S55C, L188C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	V152I
59	RSV A2 (138251)-wt	Full length (1 to 574 aa)	/	/	/	/	/	/
60	Abrysvo™	Retention (1 to 513 aa)	/	T103C, I148C	S190I	D486S	/	/

[0277] 1.2 Example 2: Preparation of RSV F antigen mRNA vaccines

[0278] The antigen sequences, after being designed, were each synthesized on a vector pVaX1 with optimized 5'-UTR, 3'-UTR, and polyA tail. All plasmids were synthesized by Azenta Life Sciences. The synthesized plasmid was subjected to linearized enzymatic cleavage, an mRNA stock solution was prepared by *in vitro* transcription, and LNP encapsulation was performed to obtain an mRNA formulation sample.

[0279] mRNA transcribed with pVaX1 as a basic backbone was used for subsequent analysis of the binding of antigenic epitopes by Western Blot or flow cytometry, detection of the binding of antigenic epitopes by ELISA, and detection of affinity of antigens for antibodies by BLI. An HIS-tag and a T4 fibrin folder were added to the vector backbone for detecting the stability of a trimer formed by secreted proteins.

[0280] The specific preparation process of an mRNA sample was as follows.

[0281] 1.2.1 Preparation of mRNA stock solution

[0282] 1) Experimental materials

[0283] NEB® Golden Gate assembly kit (BsaI-HFv2) (NEB, Cat. No.: R3733);

[0284] DNA product purification kit (purchased from Tiangen Biotech (Beijing) Co., Ltd. (referred to as Tiangen), Cat. No.: DP205);

[0285] ATP (purchased from Hongene Biotech Corporation (referred to as Hongene), Cat. No.: R1-051);

[0286] CTP (Hongene, Cat. No.: R3-056);

[0287] GTP (Hongene, Cat. No.: R2-057);

[0288] N1-Me-pUTP (Hongene, Cat. No.: R5-064);

[0289] Cap1-GAG m7G(5')ppp(5')(2'OMeA)pG (Hongene, Cat. No.: ON-134);

[0290] T7 RNA polymerase (Hongene, Cat. No.: ON-004);

[0291] RNase inhibitor (Hongene, Cat. No.: ON-039);

[0292] Pyrophosphatase (Hongene, Cat. No.: ON-025);

[0293] 10× IVT reaction buffer (Hongene, Cat. No.: ON-062);

[0294] DNase I (Hongene, Cat. No.: ON-109).

[0295] 2) Experimental procedures

[0296] (1) Linearized enzymatic cleavage for plasmid

[0297] Plasmid DNA was linearized using a BsaI-HFv2 endonuclease, and the enzymatic cleavage system comprised plasmid DNA inserted with a target gene, a 10× rCutSmart buffer, BsaI-HFv2, and RNase-free ddH₂O. The temperature of the enzymatic cleavage reaction was 37 °C and the time for the enzymatic cleavage reaction was 15 to 30 min. The enzymatic cleavage system is shown in the table below.

Table 2. Enzymatic cleavage reaction system

Component	50 μL of reaction system
DNA	1 μg
10× rCutSmart buffer	5 μl(1 ×)
BsaI-HFv2	1 μl
RNase-free ddH ₂ O	Making up to 50 μL

[0298] After the enzymatic cleavage reaction was completed, the linearized product was extracted using a DNA product purification kit, the concentration of the product was determined using an ultra-micro UV

spectrophotometer (Denovix), and the length and state of a linearized plasmid template was detected by agarose gel.

[0299] (2) Co-transcriptional capping

[0300] mRNA was synthesized by transcription with T7 RNA polymerase using the linearized plasmid prepared in the above step as a template and NTP solutions (NTPs) and a cap1 cap analog as starting materials. The above cap1 cap analog was Cap1-GAG, having a structure of m7G(5')ppp(5')(2'-OMeA)pG, and with a molecular formula of C₃₂H₄₃N₁₅O₂₄P₄.

[0301] The specific reaction system is shown in the table below. The formulated reaction system was placed in a constant temperature incubator at 37 °C for shaking culture for 3 h.

Table 3. Transcription reaction system

Reaction component	Final concentration/volume of addition	
T7 transcription buffer solution (10×)	T7 transcription buffer: 1/10 total volume	
NTPs	ATP	10 mM
	CTP	10 mM
	GTP	10 mM
	N1-Me-Pseudo UTP	10 mM
Cap1	10 mM	
Linearized plasmid	50 µg/ml	
RNase inhibitors	1 KU/ml	
Inorganic pyrophosphatase	Adding 10 to 40 U per 1 mg of linearized plasmid	
T7 RNA polymerase	Adding 150 to 300 KU per 1 mg of linearized plasmid	
RNase-free ddH ₂ O	Making up to a target volume of 20 µL	

[0302] 1 µL of DNase I was added to the above 20 µL of co-transcriptional capping reaction system after the reaction was completed, and the mixture was uniformly mixed and then placed at 37 °C for digestion for 30 min to obtain a co-transcriptional capped product.

[0303] (3) Purification by LiCl precipitation method

[0304] The co-transcriptional capped product obtained above was purified by a lithium chloride precipitation method as follows:

[0305] Adding LiCl: LiCl was added to the co-transcriptional capped product to make a final concentration of 2.8 M, and low temperature precipitation was performed for 2 h.

[0306] Precipitation: The mixture was centrifuged at a high speed of 12,000 rpm for 15 min, and the precipitate was retained.

[0307] Washing: The precipitate was washed with 75% ethanol 2 times, and dissolved with RNase-free ddH₂O to obtain an mRNA stock solution.

[0308] Detection: the purified transcript was determined for concentration by ultra-micro UV spectrophotometer (Denovix) and integrity by capillary electrophoresis.

[0309] **1.2.2 Preparation of mRNA-LNP (mRNA vaccine)**

[0310] A cationic lipid, DSPC, cholesterol, and DMG-PEG2000 were separately weighed accurately and dissolved with anhydrous ethanol, and a excipient mixed solution was formulated according to a molar ratio (controlling the molar ratio of the cationic lipid, the neutral lipid, the structural lipid, and the polymer-

conjugated lipid to be 49:10:39.5:1.5) as an organic phase for later use.

[0311] mRNA was dissolved with a citric acid buffer and diluted to formulate a solution as an aqueous phase for later use. The organic phase and the aqueous phase were separately extracted with a BD™ syringe. An LNP intermediate solution was prepared in a microfluidic device with the organic phase placed in the left pump, and the aqueous phase placed in the right pump.

[0312] The above LNP intermediate solution was diluted with a PBS solution and subjected to concentration by ultrafiltration on an ultrafiltration device. The solution, after being concentrated to a certain volume, was diluted with a PBS solution and concentrated on the ultrafiltration device, and the ultrafiltration was stopped when the final concentration volume was less than 5 mL.

[0313] 1.3 Example 3: Detection of expression of RSV F antigen mutants by Western Blot

[0314] To verify whether the designed antigen mRNAs can be expressed in the cells, the mRNAs were transfected into the cells, and the expression of the antigen mRNAs in the cells was detected using Western Blot.

[0315] 1) Experimental materials

[0316] 293T cells (purchased from ATCC cell bank);

[0317] primary antibody: HRSV-F (purchased from AbMax Biotechnology Co., Ltd., hereinafter referred to as AbMax, Cat. No.: 11049-R302);

[0318] secondary antibody: goat anti-mouse IgG (H + L) secondary antibody, HRP (purchased from Shanghai Beyotime Biotechnology Co., Ltd., hereinafter referred to as Beyotime, Cat. No.: A0286);

[0319] experimental group: a protein sample mixed solution obtained by lysis treatment after cells were transfected by a series of mRNA vaccines prepared in Example 2;

[0320] internal reference protein: glyceraldehyde-3-phosphate dehydrogenase GAPDH;

[0321] 2) Experimental procedures

[0322] Cell culture and transfection: 293T cells were seeded at 1.3×10^5 cells/well in a 24-well plate. The next day, mRNA-LNP samples (containing 300 ng of mRNA) were added directly to the cells, and the mixture was uniformly mixed.

[0323] Preparation of protein samples: (1) the cells were taken out from the incubator after 16 h of transfection, pipetted repeatedly using a pipettor to allow the cells to be completely separated from the bottom of the dish, then transferred into a 1.5 mL centrifuge tube, and centrifuged at 1500 rpm for 3 min at 25 °C. (2) The supernatant was discarded, and the cells were resuspended in 1 mL of PBS by pipetting and centrifuged at 1500 rpm for 3 min. (3) The previous step was repeated 2 times. (4) 50 µL of RIPA lysis buffer (0.5 µL of 100× protein inhibitor was added to the RIPA lysis buffer before use) was added to each tube of cells, and the mixture was incubated on ice for 30 min, during which the samples were vortexed for 30 s every 5 min. (5) The centrifuge was pre-cooled, and after the cells were fully lysed, the samples were centrifuged at 12,000 rpm for 10 min at 4 °C, and the supernatant was transferred into a new 1.5 mL centrifuge tube by pipetting to obtain test protein samples.

[0324] Development and analysis: the concentrations of the protein samples were detected using a BCA quantification kit. The samples were pipetted for spotting, 4 µL of marker was spotted, and 200 V

electrophoresis was performed for 30 min. The membrane transfer was performed using a dry membrane transfer apparatus. The gel was taken out, a membrane transfer “sandwich” was made and covered with a negative plate, and the membrane transfer was performed at 21 V, 23 V, and 25 V for 1 min, 4 min, and 2 min, respectively; then blocking was performed at room temperature for 1 h. Primary antibody incubation: incubation was performed at room temperature for 1 h. Membrane washing: with a 1× TBST buffer solution as a washing buffer, washing was performed for 5 min/time for 3 times. Secondary antibody incubation: incubation was performed at room temperature for 1 h. Membrane washing: washing was performed using a 1× TBST buffer solution for 5 min/time for 3 times. Color development: 1 mL of developing solution and 1 mL of fixing solution were mixed at 1:1, and the mixture was added dropwise to the membrane for color development and photographing.

[0325] 1.4 Example 4: Detection of binding of epitopes Φ and II of RSV F antigen mutants by flow cytometry

[0326] To investigate whether the RSV F antigen mutants have pre-fusion epitopes, an RSV pre-F antibody (3C12) (specifically binding to the RSV pre-F epitope Φ) and an RSV F antibody (11A9) (binding to pre-F and post-F epitopes II) were detected by flow cytometry in this example, and RSV F protein mutants in the pre-F conformation were expected to bind to the antibodies tested.

[0327] 1) Experimental materials

[0328] 293T cells (purchased from ATCC cell bank); RSV pre-F antibody (3C12), epitope Φ (Novoprotein, Cat. No.: DA101); RSV F antibody (11A9), epitope II (Novoprotein, Cat. No.: DA091);

[0329] experimental groups: mRNA vaccines corresponding to the expressed antigens in Example 3;

[0330] 2) Experimental procedures

[0331] (1) Cell transfection: 293T cells were seeded at 1.3×10^5 cells/well in a 24-well plate. The next day, mRNA-LNP samples (containing 300 ng of mRNA) were added directly to the cells, and the mixture was uniformly mixed.

[0332] (2) Cell collection: The cells were taken out of the incubator after 16 h of transfection, the medium was pipetted off, 500 μL of 0.1% BSA solution was pipetted to wash the medium remaining in the well plate, taking care not to resuspend the cells, and the 0.1% BSA solution was pipetted off. 500 μL of 0.1% BSA solution was added to each well, and the cells were pipetted and transferred to a 1.5 mL centrifuge tube.

[0333] (3) Centrifugation: The cells were harvested by centrifugation at 400 g for 5 min at 4 °C in the dark, and the supernatant was discarded.

[0334] (4) Straining: 50.6 μL of RSV fluorescent antibody diluent was added to each of the control sample and the test samples to resuspend the cells, and the samples were incubated at 4 °C for 2 h in the dark.

[0335] (5) Cleaning: After the incubation was completed, 500 μL of 0.1% BSA solution was added to each tube, the cells were harvested by centrifugation at 400 g for 5 min at 4 °C in the dark, and the supernatant was discarded. This operation was repeated three times.

[0336] (6) Resuspension of cells: 200 μL of 0.1% BSA solution was added to each sample, and the cells were carefully pipetted to prepare a cell suspension.

[0337] (7) Detection on flow cytometer: The binding kinetics of RSV F mutants to antibodies were

measured using flow cytometry, with 1×10^4 cells detected in each measurement.

[0338] 1.5 Example 5: Detection of stability of RSV F antigen mutants by ELISA

[0339] To investigate the stability of the RSV F antigen mutants under different pH, temperature, osmotic pressure and other conditions, the mutants were subjected to stress tests under different treatment conditions in this example.

[0340] 1) Experimental materials

[0341] 293T cells (purchased from ATCC cell bank);

[0342] Primary antibody: RSV pre-F antibody (3C12), epitope Φ (purchased from Suzhou Novoprotein Scientific Co., Ltd. (hereinafter referred to as Novoprotein), Cat. No.: DA101);

[0343] Standard: RSV pre-F trimer protein (Novoprotein, Cat. No.: DRA230);

[0344] Secondary antibody: peroxidase AffiniPure goat anti-human IgG (H + L) (purchased from Yeasen Biotechnology (Shanghai) Co., Ltd. (hereinafter referred to as Yeasen), Cat. No.: N/A);

[0345] Experimental group: mRNA vaccines after screening in Example 4;

[0346] 2) Experimental procedures:

[0347] (1) Cell transfection and collection: The transfection procedure was the same as in Example 3, and the cell supernatant was collected.

[0348] (2) Different pH conditions: The RSV F protein solution was adjusted to pH 3.5 and pH 10 with 10% NaOH and a 1 M HCl solution, respectively, and incubated at room temperature for 60 min, followed by neutralization to pH 7.4.

[0349] (3) High-temperature treatment: The RSV F protein solution was incubated at 50 °C for 60 min.

[0350] (4) Low-temperature treatment: The RSV F protein solution was stored at low temperature of 4 °C for one week.

[0351] (5) Low osmotic pressure treatment: The cell supernatant containing 110 mM NaCl was diluted to 10 mM NaCl with a 2.5 mM Tris buffer (pH 7.5) and incubated at room temperature for 60 min.

[0352] (6) High osmotic pressure treatment: The cell supernatant was adjusted to 3.0 M MgCl₂ with 4.5 M MgCl₂ and incubated at room temperature for 60 min.

[0353] (7) Detection of antigen expression by ELISA:

[0354] Coating: A 96-well microplate was coated with a working solution and incubated at 2 to 8 °C for 16 to 20 h.

[0355] Plate washing: Phosphate buffered saline PBST was added to each well, and the plate was washed 5 times using a plate washer and patted dry on absorbent paper.

[0356] Blocking: After a blocking solution was added, the cells were placed on a constant temperature microplate shaker and incubated at 400 rpm for 1.5 h at 37 °C.

[0357] Plate washing: PBST was added to each well, and the plate was washed 5 times using a plate washer and patted dry on absorbent paper.

[0358] Primary antibody incubation: A primary antibody dilution was added to each well, and the plate was sealed with a sealing film, and then incubated at 400 rpm for 1.5 h at 37 °C.

[0359] Plate washing: PBST was added to each well, and the plate was washed 5 times using a plate

washer and patted dry on absorbent paper.

[0360] Secondary antibody incubation: A secondary antibody dilution was added to each well, and the plate was sealed with a sealing film, and then incubated at 400 rpm for 45 min at 37 °C.

[0361] Plate washing: PBST was added to each well, and the plate was washed 5 times using a plate washer and patted dry on absorbent paper.

[0362] Color development: A chromogenic solution was added, and the plate was sealed with a sealing film, and then placed on a constant temperature microplate shaker and incubated at 400 rpm for 25 min at 37 °C.

[0363] Reaction stopping: After the color development was completed, a stop buffer was added at 50 µL/well to the microplate to stop the reaction.

[0364] Plate reading: Absorbance values (OD values) were read within 5 min using a multimode microplate reader, with dual wavelengths of 450 nm and 630 nm set.

[0365] 1.6 Example 6: Detection of binding of RSV F antigen mutants to different epitopes by ELISA

[0366] To investigate the binding of the RSV F antigen mutants to antibodies recognizing different epitopes, the binding amount was determined by an ELISA method in this example.

[0367] 1) Experimental materials

[0368] 293T cells (purchased from ATCC cell bank);

[0369] Primary antibody: RSV pre-F antibody (3C12), epitope Φ (Novoprotein, Cat. No.: DA101);

RSV pre-F antibody (7H11), epitope Quaternary (Novoprotein, Cat. No.: DA109);

[0370] RSV F antibody (11A9), epitope II (Novoprotein, Cat. No.: DA091);

[0371] RSV F antibody (4B9), epitope III (Novoprotein, Cat. No.: DA110);

[0372] RSV pre-F antibody (7E11), epitope V (Cat. No.: DA119);

[0373] Standard: RSV-pre-F trimer protein (Novoprotein, Cat. No.: DRA230);

[0374] Secondary antibody: peroxidase AffiniPure goat anti-human IgG (H + L) (Yeasten, Cat. No.: N/A);

[0375] Experimental group: mRNA vaccines after screening in Example 5;

[0376] 2) Experimental procedures

[0377] Cell culture and transfection, and protein sample preparation: the experimental procedures were the same as in Example 3.

[0378] Detection of antigen expression by ELISA: the experimental procedures were the same as in Example 5.

[0379] 1.7 Example 7: Detection of affinity of RSV F antigen mutants for different antibodies by BLI

[0380] To investigate the affinity of the RSV F antigen mutants for antibodies specifically recognizing epitopes in a pre-fusion conformation, the affinity was determined by a bio-layer interferometry (BLI) method in this example.

[0381] 1) Experimental materials

[0382] 293T cells (purchased from ATCC cell bank);

- [0383] RSV pre-F antibody (3C12), epitope Φ (Novoprotein, Cat. No.: DA101);
- [0384] RSV pre-F antibody (7H11), epitope Quaternary (Novoprotein, Cat. No.: DA109);
- [0385] Experimental group: mRNA vaccines after screening in Example 6;
- [0386] 2) Experimental procedures:
- [0387] Protein sample preparation: the experimental procedure was the same as in Example 3.
- [0388] The antibodies were each diluted to 10 $\mu\text{g/mL}$ with a $1\times$ PBST solution.
- [0389] Antibodies RSV pre-F antibody (3C12) and RSV pre-F antibody (7H11) were each captured with a ProA probe in a $1\times$ PBST solution, with a capture level limited to 4.0 nm; then, they were sequentially reacted with the diluted target protein, and the solid phase conjugates were subjected to dissociation analysis in a $1\times$ PBST buffer after complete reaction.
- [0390] The detection was performed using a protein interaction analyzer, and the results were analyzed using Data Analysis12.0 software to obtain the binding rate, dissociation rate, and affinity constants.
- [0391] **1.8 Example 8: Study of immunogenicity of RSV F antigen mutants in mice**
- [0392] To investigate antibody titers of the RSV F antigens produced in mice, the mice were subjected to *in vivo* immunization in this example.
- [0393] **1.8.1 Immunization of Balb/c mice**
- [0394] 1) Experimental materials
- [0395] Animals: Balb/c mice, female, 6 to 8 weeks;
- [0396] negative control: LNP sample;
- [0397] positive control: AbrysvoTM;
- [0398] test samples: YK-RSV-001, YK-RSV-011, YK-RSV-030, YK-RSV-036, YK-RSV-044, YK-RSV-060, YK-RSV-062, YK-RSV-061, YK-RSV-003, YK-RSV-012, YK-RSV-015, YK-RSV-039, and YK-RSV-054;
- [0399] 2) Immunization regimen for mice

Table 4. Immunization regimen for mice

Group	Number of animals	Immunization					Blood sampling time point	Time of sacrifice
		Test vaccine	Administration dose ($\mu\text{g}/\text{animal}$)	Administration volume ($\mu\text{L}/\text{animal}$)	Route	Frequency		
1	6	LNP	0	100	Intramuscular injection	Days 0 and 21	Day 35	Day 49
2	6	Abrysvo TM	1	100				
3	6	YK-RSV-001	5	100				
4	6	YK-RSV-011	5	100				
5	6	YK-RSV-030	5	100				

6	6	YK-RSV-036	5	100				
7	6	YK-RSV-044	5	100				
8	6	YK-RSV-060	5	100				
9	6	YK-RSV-062	5	100				
10	6	YK-RSV-061	5	100				
11	8	YK-RSV-003	5	100				
12	8	YK-RSV-012	5	100				
13	8	YK-RSV-015	5	100				
14	8	YK-RSV-039	5	100				
15	8	YK-RSV-054	5	100				

Note: The administration volume is calculated according to the actual concentration of the administration dose

[0400] 3) Sample collection and pretreatment

[0401] 0.2 mL of blood was taken from the orbital venous plexus of each animal, and the serum was separated (centrifugation at 4000 rpm/min for 10 min at 4 °C) and stored in a refrigerator at 4 °C for further treatment.

[0402] 4) Health detection

[0403] Once daily, including but not limited to death, illness, respiration, secretions, faeces, and food and water consumption.

[0404] 5) Humane endpoint

[0405] Any mice that lost more than 20% of their body weight during the experiment (with body weight on day 0 as baseline weight before infection, and body weight on the day of inoculation as baseline weight after infection) or/and exhibited symptoms of dying, would be euthanized and scored as dead animals in the results, as specified by the IACUC protocol.

[0406] **1.8.2 Detection of neutralizing antibody titers of RSVs in serum of mice**

[0407] 1) Objective

[0408] The neutralizing antibody titers of respiratory syncytial viruses RSV A2 and RSV B18357 in the serum were detected by a spot reduction-based micro-neutralization test method.

[0409] 2) Key reagents

[0410] Primary antibody: research grade palivizumab (purchased from Wuhan Chemstan Biotechnology Co., Ltd., hereinafter referred to as Wuhan Chemstan, Cat. No.: CSD00024);

[0411] secondary antibody: rabbit anti-human IgG H & L (HRP) (Abcam, Cat. No.: ab6759);

[0412] 3) Calculation formula for data:

[0413] (1) Sample inhibition rate: calculated by Excel (single-well calculation), sample inhibition rate = $(1 - (\text{number of sample spots}/\text{number of virus control spots})) \times 100\%$.

[0414] (2) The sample dilution factor as an X axis and the inhibition rate as a Y axis were input into GraphPad software, and IC₅₀ value and the linear correlation coefficient R² of the sample were calculated by four-parameter fitting, wherein the IC₅₀ value was the neutralizing antibody titer of the sample. The titer was retained as an integer and the linear correlation coefficient R² was retained to the second decimal place.

[0415] (3) Mean value: $\frac{\sum_{i=1}^n X_i}{n}$, where X_i represents the ith measurement, and n represents the number of measurements; calculated by Excel, and retained to the second decimal place. Note: If the calculation result is the titer value, it is retained as an integer.

[0416] 4) Experimental procedures

[0417] After the test serum sample was subjected to gradient dilution, a certain amount of RSV virus was added. After a neutralization reaction, the mixture was added to a cell plate for culture for 22 to 26 h, and finally, the neutralizing antibody titer level of the serum sample was calculated according to the formation of spots. The operation process was as follows:

[0418] Cell preparation and plating: HEp-2 cells were purchased from Yuchi (Shanghai) Biotechnology Co., Ltd. The cells were thawed and cultured before the experiment, and the cells used in the detection were 3 to 20 generations after the thawing. The cells were digested in advance and seeded into a 96-well plate at 2.5×10^5 cells/mL and 0.1 mL/well, and the plate was placed in an incubator at 37 °C with 5% CO₂ for culture for 17 to 23 h. When no abnormality was observed in the cells, the serum sample was taken and diluted in a gradient.

[0419] Control setting: Controls were set in a 96-well plate (neutralization plate), and the dilutions were added to cell controls (CCs) and virus controls (VCs), with duplicate wells set.

[0420] Virus addition: viruses diluted with the diluent were taken and added to the sample wells and the VC wells of the 96-well plate, the 96-well plate was gently patted to uniformly mix the samples and the viruses, and then placed in an incubator at 37 °C with 5% CO₂ for incubation for neutralization reactions.

[0421] Cell preparation, infection, and culture: a 96-well plate seeded with the cells in advance was taken, the original culture medium was discarded by pipetting, a mixed solution from the neutralization plate was pipetted and added to the 96-well plate placed with the cells, which was then placed in an incubator at 37 °C with 5% CO₂ for culture.

[0422] Fixing and staining: after the culture, the original culture medium was discarded by pipetting, and a certain volume of fixative was added to each well. After fixation, the fixative was discarded, and the plate was repeatedly washed with 1× PBS 2 times. After a blocking buffer was added for blocking, the blocking buffer was discarded, and the plate was repeatedly washed with 1× PBS 2 times; after a primary antibody working solution was added for incubation, the antibody was discarded, and the plate was repeatedly washed with 1× PBS 2 times; after an HRP secondary antibody working solution was added for

incubation, the antibody was discarded, and the plate was repeatedly washed with 1× PBS 2 times; a chromogenic solution was added, and the plate was incubated at room temperature for 5 to 20 min; the chromogenic solution was discarded, the plate was patted dry, and spots were counted by an enzyme-linked immunospot reader.

[0423] Calculation for results: neutralizing antibody titers: the inhibition rates of gradients of the sample were calculated, the dilution factor-inhibition rate of the sample was input into GraphPad software for fitting, and the IC₅₀ and IC₉₀ values of the sample were calculated, wherein the IC₅₀ and IC₉₀ values were the neutralizing antibody titers of the sample.

[0424] 1.8.3 Detection of binding antibody titers of RSVs in serum of mice

[0425] 1) Objective

[0426] Serum samples in an immunogenicity test of RSV mRNA vaccines were detected by a qualified self-constructed indirect ELISA method, with detection indexes including RSV pre-F protein- and post-F protein-specific antibody titers, which provide data support for analysis of immunogenicity and protection against challenge of vaccines.

[0427] 2) Key reagents

[0428] Coating working solution: RSV-pre-F-11 protein (Vazyme, Cat. No.: RM2187); RSV-post-F-11 protein (Vazyme, Cat. No.: RM2188);

[0429] positive quality control: monoclonal anti-RSV-pre-F0 specific antibody* (Acro, Cat. No.: RS0-Y132); monoclonal anti-RSV-post-F0 specific antibody* (Acro, Cat. No.: RSV-Y180);

[0430] secondary antibody: rabbit anti-mouse IgG HL (HRP) (Abcam, Ab6728);

[0431] 3) Calculation formula for data

[0432] Mean: Mean = Average (OD value X1:OD value Xn);

[0433] signal-to-noise ratio (SNR): SNR = mean OD value of sample/mean OD value of NC;

[0434] coefficient of variation (CV%): CV% = STDEV (OD value X1:OD value Xn)/mean OD value × 100;

[0435] calculation of binding antibody titers: the titer values in each analysis batch were calculated using the FORECAST (x, known_y's, known_x's) function, where x represents the Cut off value, known_y's represents two dilution factors across the Cut off value, and known_x's represents two OD values across the Cut off value, and the corresponding dilution factor obtained was the titer value for each sample.

[0436] 4) Experimental procedures

[0437] The procedures were the same as in the ELISA method in Example 5. In the primary antibody incubation step, the diluted test serum sample was added, and after being sealed with a sealing film, the plate was placed on a constant temperature microplate shaker and incubated at 400 rpm for 1.5 h at 37 °C.

[0438] 1.9 Example 9: Study of immunogenicity of RSV F antigen mutants in cotton rats

[0439] To investigate antibody titers of the RSV F antigens in cotton rats and the immunoprotective effect after challenge, cotton rats were subjected to *in vivo* immunization in this example.

[0440] 1.9.1 Immunization of cotton rats

[0441] 1) Experimental materials

- [0442] Animals: cotton rats, female, 6-8 weeks;
- [0443] negative control: LNP sample;
- [0444] positive control: Abrysvo™;
- [0445] test samples: YK-RSV-001, YK-RSV-061, YK-RSV-062, YK-RSV-003, YK-RSV-012, YK-RSV-015, YK-RSV-036, YK-RSV-039, YK-RSV-044, YK-RSV-054, and YK-RSV-060;
- [0446] 2) Immunization regimen for cotton rats

Table 5. Immunization regimen for cotton rats

Group	Number of animals	Immunization				Blood sampling time point	Time of sacrifice
		Test vaccine	Administration dose (µg/animal)	Administration volume (µL/animal)	Route	Frequency	
1	6	LNP	0	100	Intramuscular injection	Days 0 and 28	Days 42 and 48
2	6	Abrysvo™	50	208			
3	6	YK-RSV-001	5	100			
4	6	YK-RSV-061	5	100			
5	6	YK-RSV-062	5	100			
6	6	YK-RSV-003	5	100			
7	6	YK-RSV-012	5	100			
8	6	YK-RSV-015	5	100			
9	6	YK-RSV-036	5	100			
10	6	YK-RSV-039	5	100			
11	6	YK-RSV-044	5	100			
12	6	YK-RSV-054	5	100			
13	6	YK-RSV-060	5	100			

Note: The administration volume is calculated according to the actual concentration of the administration dose. The injection dose of Abrysvo™ includes 25 µg of each of type A and type B.

- [0447] 3) Tissue sample collection
- [0448] The lungs and turbinates were aseptically taken on Day 54.
- [0449] 4) Information of challenge

[0450] On day 49, the challenge was performed by nasal drip, the virus strain was RSV A2 virus, and the challenge volume was 50 μ L (5×10^5 PFU).

[0451] 1.9.2 Determination of binding antibody and neutralizing antibody titers of RSVs in serum of cotton rats

[0452] The detection methods for the binding antibody and neutralizing antibody titers were the same as in Examples 4.8.2 and 4.8.3.

[0453] 1.9.3 Detection of respiratory syncytial virus (RSV) titers in tissue samples of cotton rats by a plaque assay

[0454] 1) Objective

[0455] The respiratory syncytial virus (RSV) titers in the left lung and turbinate tissue samples of cotton rats were determined by a plaque assay, which was completed for all biological samples.

[0456] 2) Key reagents

[0457] Primary antibody: research grade palivizumab (Wuhan Chemstan, Cat. No.: CSD00024);

[0458] secondary antibody: rabbit anti-human IgG H & L (HRP) (Abcam, Cat. No.: ab6759);

[0459] quality control virus RSV A2;

[0460] (3) Experimental procedures

[0461] After the test samples were subjected to gradient dilution, the cells were infected. After the infection solution was removed, a covering layer solution was added, and the cells were continued to be cultured until plaques were formed. The results for the virus titers in the tissue samples were finally calculated according to the appearance of plaques or lesions. The operation process was as follows:

[0462] Cell preparation and plating: HEp-2 cells were purchased from Yuchi (Shanghai) Biotechnology Co., Ltd. The cells were thawed and cultured before the experiment, and the cells used in the detection were 3 to 20 generations after the thawing. The cells were digested in advance and seeded into a 24-well plate at 2×10^5 cells/mL and 1 mL/well, and the plate was placed in an incubator at 37 °C with 5% CO₂ for culture for 17 to 23 h.

[0463] Treatment of tissue samples: after the left lung and the turbinate tissue samples were obtained, a virus preservation solution was added, the tissues were ground, and the samples were centrifuged to obtain supernatants, which were subjected to gradient dilution.

[0464] Cell treatment: a cell plate seeded in advance was taken from an incubator at 37 °C with carbon dioxide, the culture supernatant was discarded by pipetting, then the plate was repeatedly washed with 1× PBS 2 times, and the residual liquid was discarded by pipetting.

[0465] Virus infection and adsorption: the supernatant of the diluted tissue homogenate was taken and added to corresponding wells of the cell plate, and the 24-well plate was placed in an incubator at 37 °C with 5% CO₂ to enable the viruses to be fully adsorbed on the cells.

[0466] Addition of covering layer: after the adsorption was completed, the virus solution was discarded by pipetting, a covering layer was added to each well, the cell plate was placed in an incubator at 37 °C with 5% CO₂ for culture, and the formation of lesions and plaques were observed daily.

[0467] Fixing: after typical plaque appeared in the virus wells, the covering layer was discarded by

pipetting, and 4% paraformaldehyde was added to each well for fixing.

[0468] Straining: after the cell plate was fixed, the fixative was discarded, and the cell plate was repeatedly washed with 1× PBS 3 times and blocked with a blocking buffer; the blocking buffer was discarded, the plate was repeatedly washed with 1× PBS 3 times, and a primary antibody working solution was added for incubation; the antibody was discarded, the plate was repeatedly washed with 1× PBS 3 times, and an HRP secondary antibody working solution was added for incubation; the antibody was discarded, the plate was washed with 1× PBS 3 times, a chromogenic solution was added, and the plate was incubated at room temperature for 10 to 20 min; the chromogenic solution was discarded, and spots were observed and counted by a film camera.

[0469] Calculation for results:

[0470] Virus titer [PFU/mL] = (mean plaque number × sample dilution factor)/inoculum size (mL);

[0471] Tissue virus titer [PFU/g]^{1*} = [(mean plaque number × tissue sample dilution factor)/inoculum size (mL) × volume of tissue grinding fluid (mL)]/tissue weight (g).

[0472] The tissue virus Lg titer [Lg PFU/g] is the logarithm of the tissue virus titer [PFU/g] at a base of 10.

[0473] 1*: plaques of all dilutions which can be accurately counted are selected for statistical calculation, and the arithmetic mean is used during the calculation.

[0474] 1.9.4 Detection of lung tissue inflammation indexes by HE staining

[0475] 1) Objective

[0476] The lung tissue inflammation indexes were detected by HE staining.

[0477] 2) Key reagents

[0478] Xylene solution (Shanghai Zhanyun Chemical Co., Ltd., Cat. No. 1330-20-7); hematoxylin solution (Mayer) (Baso, Cat. No.: BASO-BA-4021); eosin solution (alcohol soluble) (Baso, Cat. No.: BASO-BA-4022);

[0479] 3) Experimental procedures

[0480] Lung tissues were perfused, fixed in 4% paraformaldehyde for more than 24 h, dehydrated, and sliced at the maximum parenchyma area with a section thickness of 4 μm. A staining rack loaded with the slices was placed in an oven at 60 to 65 °C for baking the slices for 1 h. After paraffin was melted to be in a transparent liquid state, the staining rack was placed in a room temperature environment for 10 min for cooling. After the cooling was completed, HE staining was performed, and finally the slices were sealed.

[0481] 4) Results

[0482] Manual semi-quantitative scoring was performed with the scoring criteria shown in the table below. Data were analyzed by Mean + SEM and analyzed by one-way ANOVA.

Table 6. Semi-quantitative histopathological scoring criteria

Histological characterization	Score	Criteria
Alveolar region Inflammatory infiltrations of macrophages, lymphocytes, neutrophils, etc., are observed in the alveolar tissue	0	No visible lesion
	1	Mild
	2	Moderate
	3	Severe

	4	Serious
Perivascular region Inflammatory infiltrations of macrophages, lymphocytes, neutrophils, etc., are observed in the perivascular region	0	No visible lesion
	1	Mild
	2	Moderate
	3	Severe
	4	Serious
Peribronchiolar region Inflammatory infiltrations of macrophages, lymphocytes, neutrophils, etc., are observed in the peribronchiolar region	0	No visible lesion
	1	Mild
	2	Moderate
	3	Severe
	4	Serious
Interstitium Inflammatory infiltrations of macrophages, lymphocytes, neutrophils, etc., are observed in the interstitium	0	No visible lesion
	1	Mild
	2	Moderate
	3	Severe
	4	Serious

[0483] 2 Analysis of results

[0484] 2.1 Results for expression of RSV F antigen mutants

[0485] YK-RSV-001, YK-RSV-002, YK-RSV-003, YK-RSV-004, YK-RSV-005, YK-RSV-008, YK-RSV-009, YK-RSV-010, YK-RSV-011, YK-RSV-012, YK-RSV-013, YK-RSV-014, YK-RSV-015, YK-RSV-016, YK-RSV-017, YK-RSV-018, YK-RSV-019, YK-RSV-022, YK-RSV-029, YK-RSV-030, YK-RSV-031, YK-RSV-032, YK-RSV-033, YK-RSV-034, YK-RSV-035, YK-RSV-036, YK-RSV-037, YK-RSV-038, YK-RSV-039, YK-RSV-040, YK-RSV-041, YK-RSV-042, YK-RSV-043, YK-RSV-044, YK-RSV-045, YK-RSV-046, YK-RSV-048, YK-RSV-050, YK-RSV-051, YK-RSV-052, YK-RSV-053, YK-RSV-054, YK-RSV-055, YK-RSV-056, YK-RSV-057, YK-RSV-058, YK-RSV-059, YK-RSV-060, YK-RSV-061, and YK-RSV-062 mutants all showed significant protein expression at a size of about 75 kDa, which was consistent with the theoretical size of the antigen, and could be used as effective candidate antigens for next immunogenicity screening and evaluation. Thus, the above 50 mutants would be further determined for antigenic epitope binding by flow cytometry for further screening.

[0486] 2.2 Results for determination of epitope binding of RSV F antigen mutants

[0487] Among major neutralizing active antigenic epitopes of the RSV F protein, the epitope Φ is located only in the pre-fusion conformation, and the epitope II is retained in both the pre-fusion and post-fusion conformations.

[0488] The ability of the antibodies to specifically bind to the epitope Φ correlates with neutralizing activity, and the low binding rates to the epitope Φ affect neutralizing activity, resulting in poor neutralizing activity.

[0489] The RSV pre-F antibody (3C12) used in this example specifically binds to the RSV pre-F epitope Φ , and the RSV F antibody (11A9) binds to the pre-F and post-F epitopes II. Whether the pre-fusion conformations of the RSV F mutants are stable can be further determined by detecting the binding of the epitope Φ and the epitope II on the mutants to corresponding epitopes of antibodies 3C12 and 11A9, respectively, by flow cytometry. Data for epitope binding are shown in the table below.

Table 7. Results for epitope binding of RSV F mutants – general table

No.	RSV pre-F antibody (3C12)	RSV F antibody (11A9)	$\Phi/\Pi(\%)$
	Epitope Φ (%)	Epitope Π (%)	
YK-RSV-001	91.7	97.6	94.0
YK-RSV-002	88.0	93.5	94.1
YK-RSV-003	94.5	96.9	97.5
YK-RSV-004	82.1	90.1	91.1
YK-RSV-005	91.6	95.2	96.2
YK-RSV-008	83.9	92.5	90.7
YK-RSV-009	87.4	93.1	93.9
YK-RSV-010	90.3	92.8	97.3
YK-RSV-011	78.9	86.6	91.1
YK-RSV-012	93.3	97.2	96.0
YK-RSV-013	89.3	94.3	94.7
YK-RSV-014	0.0	0.1	0.0
YK-RSV-015	94.9	98.5	96.3
YK-RSV-016	79.8	83.4	95.7
YK-RSV-017	91.3	94.6	96.5
YK-RSV-018	68.4	76.3	89.6
YK-RSV-019	84.5	90.4	93.5
YK-RSV-022	0.2	2.1	9.5
YK-RSV-029	79.6	86.7	91.8
YK-RSV-030	91.0	96.8	94.0
YK-RSV-031	75.5	83.4	90.5
YK-RSV-032	86.1	94.8	90.8
YK-RSV-033	72.0	82.4	87.4
YK-RSV-034	82.5	91.0	90.7
YK-RSV-035	79.6	87.1	91.4
YK-RSV-036	93.3	96.7	96.5
YK-RSV-037	79.0	86.9	90.9
YK-RSV-038	90.7	95.7	94.8
YK-RSV-039	93.0	97.1	95.8
YK-RSV-040	84.3	92.0	91.6
YK-RSV-041	73.7	81.5	90.4
YK-RSV-042	49.8	61.1	81.5
YK-RSV-043	90.7	95.2	95.3
YK-RSV-044	92.9	96.9	95.9
YK-RSV-045	78.3	86.0	91.0
YK-RSV-046	84.0	89.8	93.5
YK-RSV-048	89.6	95.1	94.2
YK-RSV-050	86.5	93.0	93.0
YK-RSV-051	76.8	87.1	88.2
YK-RSV-052	89.4	93.7	95.4
YK-RSV-053	40.0	67.0	59.7
YK-RSV-054	94.4	97.5	96.8
YK-RSV-055	93.3	96.2	97.0
YK-RSV-056	94.6	97.1	97.4
YK-RSV-057	94.4	97.2	97.1
YK-RSV-058	81.2	88.7	91.5
YK-RSV-059	83.1	89.8	92.5

YK-RSV-060	91.6	95.2	96.2
YK-RSV-061	93.8	95.7	98.0
YK-RSV-062	92.5	94.5	97.9
PC-RSV-064	88.0	91.2	96.5
PC-RSV-065	84.0	92.6	90.7
PC-RSV-066	85.1	90.8	93.7
PC-RSV-067	84.9	89.8	94.5
PC-RSV-068	87.7	90.5	96.9
RSV A2 (138251)-wt	1.0	23.1	4.3

[0490] There was a total of 20 mutants with a binding rate of the epitope Φ on the RSV F mutant to the antibody 3C12 of greater than 90%. The binding rates of the epitope Φ on these RSV F mutants to the antibody 3C12 were all superior to those of controls PC-RSV-064, PC-RSV-065, PC-RSV-066, PC-RSV-067, PC-RSV-068, and wild-type RSV A2 (138251)-wt.

[0491] 1) The binding rates of the epitope Φ on the following 11 RSV F mutants to the antibody 3C12 were higher than 90%, ranging from 91.6% to 94.9%, which was significantly superior to those of controls

[0492] The mutants within this range are shown in the table below, including YK-RSV-061, YK-RSV-003, YK-RSV-062, YK-RSV-054, YK-RSV-039, YK-RSV-044, YK-RSV-012, YK-RSV-015, YK-RSV-001, YK-RSV-036, and YK-RSV-060.

Table 8. Results for epitope binding of RSV F mutants – 1

No.	RSV pre-F antibody (3C12)	RSV F antibody (11A9)	Φ /II (%)
	Epitope Φ (%)	Epitope II (%)	
YK-RSV-061	93.8	95.7	98.0
YK-RSV-003	94.5	96.9	97.5
YK-RSV-062	92.5	94.5	97.9
YK-RSV-054	94.4	97.5	96.8
YK-RSV-039	93.0	97.1	95.8
YK-RSV-044	92.9	96.9	95.9
YK-RSV-012	93.3	97.2	96.0
YK-RSV-015	94.9	98.5	96.3
YK-RSV-001	91.7	97.6	94.0
YK-RSV-036	93.3	96.7	96.5
YK-RSV-060	91.6	95.2	96.2
PC-RSV-064	88.0	91.2	96.5
PC-RSV-065	84.0	92.6	90.7
PC-RSV-066	85.1	90.8	93.7
PC-RSV-067	84.9	89.8	94.5
PC-RSV-068	87.7	90.5	96.9
RSV A2 (138251)-wt	1.0	23.1	4.3

[0493] 2) The binding rates of the epitope Φ on the following 9 RSV F mutants to the antibody 3C12

were also all higher than 90%, which was significantly superior to those of controls

[0494] The binding rates of the epitope Φ on 9 mutants to the antibody 3C12 in the table below were 90.3% to 94.6%. The mutants within this range are shown in the table below.

Table 9. Results for epitope binding of RSV F mutants – 2

No.	RSV pre-F antibody (3C12)	RSV F antibody (11A9)	Φ /II(%)
	Epitope Φ (%)	Epitope II (%)	
YK-RSV-056	94.6	97.1	97.4
YK-RSV-057	94.4	97.2	97.1
YK-RSV-055	93.3	96.2	97.0
YK-RSV-005	91.6	95.2	96.2
YK-RSV-017	91.3	94.6	96.5
YK-RSV-030	91.0	96.8	94.0
YK-RSV-038	90.7	95.7	94.8
YK-RSV-043	90.7	95.2	95.3
YK-RSV-010	90.3	92.8	97.3
PC-RSV-064	88.0	91.2	96.5
PC-RSV-065	84.0	92.6	90.7
PC-RSV-066	85.1	90.8	93.7
PC-RSV-067	84.9	89.8	94.5
PC-RSV-068	87.7	90.5	96.9
RSV A2 (138251)-wt	1.0	23.1	4.3

[0495] 3) The binding rates of the epitope Φ on the following 15 RSV F mutants to the antibody 3C12 were 80%-90%, which was superior to or comparable to those of controls

[0496] The mutants within this range are shown in the table below. The binding rates of the epitope Φ on these mutants to the antibody 3C12 were 81.2% to 89.6%, the binding rates of the epitope II on these mutants to the antibody 11A9 were 88.7% to 95.1%, and the ratio of Φ /II was 90.7% to 95.4%.

Table 10. Results for epitope binding of RSV F mutants – 3

No.	RSV pre-F antibody (3C12)	RSV F antibody (11A9)	Φ /II(%)
	Epitope Φ (%)	Epitope II (%)	
YK-RSV-048	89.6	95.1	94.2
YK-RSV-052	89.4	93.7	95.4
YK-RSV-013	89.3	94.3	94.7
YK-RSV-002	88.0	93.5	94.1
YK-RSV-009	87.4	93.1	93.9
YK-RSV-050	86.5	93.0	93.0
YK-RSV-032	86.1	94.8	90.8
YK-RSV-019	84.5	90.4	93.5
YK-RSV-040	84.3	92.0	91.6
YK-RSV-046	84.0	89.8	93.5
YK-RSV-008	83.9	92.5	90.7
YK-RSV-059	83.1	89.8	92.5
YK-RSV-034	82.5	91.0	90.7

YK-RSV-004	82.1	90.1	91.1
YK-RSV-058	81.2	88.7	91.5
PC-RSV-064	88.0	91.2	96.5
PC-RSV-065	84.0	92.6	90.7
PC-RSV-066	85.1	90.8	93.7
PC-RSV-067	84.9	89.8	94.5
PC-RSV-068	87.7	90.5	96.9
RSV A2 (138251)-wt	1.0	23.1	4.3

[0497] There were a total of 15 mutants with a binding rate of the epitope Φ on the RSV F mutant to the antibody 3C12 of less than 80%.

[0498] 4) The binding rates of the epitope Φ on 7 RSV F mutants to the antibody 3C12 were less than 80%

[0499] The binding rates of the epitope Φ on the following 7 mutants to the antibody 3C12 were 76.8% to 79.8%, the binding rates of the epitope II to antibody 11A9 were 83.4% to 87.1%, and the ratio of Φ /II was 88.2% to 95.7%. The mutants within this range are shown in the table below.

Table 11. Results for epitope binding of RSV F mutants – 4

No.	RSV pre-F antibody (3C12)	RSV F antibody (11A9)	Φ /II(%)
	Epitope Φ (%)	Epitope II (%)	
YK-RSV-016	79.8	83.4	95.7
YK-RSV-029	79.6	86.7	91.8
YK-RSV-035	79.6	87.1	91.4
YK-RSV-037	79.0	86.9	90.9
YK-RSV-011	78.9	86.6	91.1
YK-RSV-045	78.3	86.0	91.0
YK-RSV-051	76.8	87.1	88.2
PC-RSV-064	88.0	91.2	96.5
PC-RSV-065	84.0	92.6	90.7
PC-RSV-066	85.1	90.8	93.7
PC-RSV-067	84.9	89.8	94.5
PC-RSV-068	87.7	90.5	96.9
RSV A2 (138251)-wt	1.0	23.1	4.3

[0500] In summary, the binding rates of the epitope Φ on the 42 mutants selected above (see Tables 8, 9, 10, and 11) to the corresponding antibodies were relatively high.

[0501] 2.3 Results for stability screening of RSV F antigen mutants

[0502] The RSV F pre-fusion conformation is in a metastable state and can spontaneously rearrange to a highly stable post-fusion conformation. The trigger for the transition from the pre-fusion conformation to the post-fusion conformation during cellular entry is not clear. However, when the F protein is extracted from the membrane with surfactants such as Triton X-100, Triton X-114, NP-40, Brij-35, Brij-58, Tween 20,

Tween 80, octylglucoside, octylthioglucoside, SDS, CHAPS, and CHAPSO, or expressed as an extracellular domain, the F glycoprotein is readily transferred to the post-fusion conformation after physical or chemical stress or storage (J. S. McLellan et al., 2013; J. O. Ngwuta et al., 2015). RSV F in the pre-fusion conformation helps the vaccine to induce or enhance neutralizing activity, and the physical stability directly affects the protein conformation. Therefore, antigens with superior stability were selected through different treatment conditions in this example.

[0503] In this example, stability screening of the mutants through different treatment conditions was defined as a stress test, including storage at 4 °C for one week; incubation at 50 °C for 60 min; treatment at room temperature under low osmotic pressure and high osmotic pressure conditions for 60 min; treatment at room temperature under acid and base conditions for 60 min. The binding of the mutants to the RSV pre-F antibody (3C12) recognizing the epitope Φ was detected by ELISA. The stress resistance parameter in this example was calculated as follows:

[0504] Stress resistance parameter = the binding amount of stressed sample to antibody ÷ the binding amount of unstressed sample to antibody;

[0505] More stable mutants were expected to have relatively high stress resistance. The results for stability screening were as follows:

[0506] 1) The mutants all retained the binding capacity to the antibody under different stress tests.

[0507] From the results for the ELISA, it could be seen that the mutants still retained affinity for the RSV pre-F antibody (3C12) after different stress tests, as the RSV pre-F antibody (3C12) specifically bound to the epitope Φ of the pre-fusion conformation of the RSV F protein, indicating that the mutants could still be stabilized in the pre-fusion conformation after different stress tests.

[0508] 2) Some mutants were still very stable after stress tests.

[0509] Through an inter-group analysis of the stability results of all mutants, it was found that the mutants with a stress resistance parameter of less than 0.4 after storage for one week at 4 °C, a stress resistance parameter of less than 0.5 after incubation at 50 °C for 60 min, a stress resistance parameter of less than 0.5 after high osmotic pressure treatment for 60 min, and a stress resistance parameter of less than 0.5 after low osmotic pressure treatment for 60 min showed relatively significant differences among groups, and thus are used as criteria for antigen screening.

Table 12. Results for stability screening of RSV F mutants – general table

No.	Determination of physical stability, RSV pre-F antibody (3C12)					
	Temperature (°C)		Osmotic pressure (mM)		pH	
	4	50	3000	10	3.5	10
YK-RSV-001	0.67±0.10	0.70 ± 0.06	0.86±0.22	0.97±0.26	0.75±0.03	1.00±0.10
YK-RSV-002	0.61±0.11	0.46±0.06	0.44±0.13	0.84±0.14	0.65±0.08	0.97±0.14
YK-RSV-003	0.65±0.01	0.81 ± 0.03	0.83±0.06	0.89±0.17	1.04±0.32	1.02±0.17
YK-RSV-004	0.72±0.06	0.48 ± 0.05	0.46±0.13	0.91±0.23	0.97±0.21	1.00±0.27
YK-RSV-005	0.55±0.09	0.44±0.06	0.47±0.08	0.57±0.00	0.64±0.20	0.88±0.14
YK-RSV-008	0.65±0.06	0.45± 0.08	0.33±0.02	0.81±0.09	0.76±0.21	0.99±0.19
YK-RSV-009	0.71±0.11	0.84 ± 0.04	0.69±0.15	0.78±0.15	0.60±0.14	0.91±0.04
YK-RSV-010	0.86±0.08	1.04 ± 0.09	0.84±0.11	0.97±0.07	0.82±0.05	0.93±0.05
YK-RSV-011	0.85±0.08	0.76 ± 0.01	0.80±0.11	0.97±0.23	0.63±0.18	0.86±0.01

YK-RSV-012	0.69±0.11	1.22 ± 0.24	0.73±0.08	0.76±0.17	0.67±0.07	1.22±0.00
YK-RSV-013	0.69±0.11	0.91 ± 0.23	0.43±0.04	0.59±0.08	0.84±0.19	0.92±0.04
YK-RSV-015	0.57±0.08	0.70 ± 0.06	0.72±0.03	0.73±0.18	0.80±0.12	0.87±0.12
YK-RSV-016	0.28±0.05	0.80 ± 0.01	0.53±0.07	0.77±0.08	0.91±0.06	0.98±0.14
YK-RSV-017	0.35±0.07	0.60 ± 0.04	0.46±0.06	0.59±0.03	0.76±0.11	0.93±0.06
YK-RSV-019	0.37±0.08	0.58±0.05	0.63±0.04	0.81±0.04	0.77±0.05	0.88±0.09
YK-RSV-029	0.32±0.06	0.57±0.08	0.40±0.01	0.61±0.05	0.63±0.03	0.82±0.01
YK-RSV-030	0.44±0.08	1.05±0.00	0.83±0.06	0.89±0.15	0.89±0.06	1.00±0.11
YK-RSV-032	0.34±0.08	0.78±0.02	0.37±0.02	0.66±0.14	1.01±0.35	0.98±0.06
YK-RSV-034	0.37±0.07	0.68±0.07	0.34±0.15	0.84±0.14	0.81±0.09	0.91±0.04
YK-RSV-035	0.31±0.07	0.39±0.02	0.21±0.06	0.39±0.05	0.57±0.03	0.82±0.01
YK-RSV-036	0.69±0.08	0.75±0.08	0.72±0.02	0.80±0.06	0.98±0.03	1.16±0.23
YK-RSV-037	0.27±0.07	0.50±0.06	0.21±0.03	0.54±0.11	0.68±0.10	1.24±0.11
YK-RSV-038	0.32±0.08	0.70±0.10	0.55±0.13	0.74±0.08	0.88±0.17	0.82±0.26
YK-RSV-039	0.62±0.08	0.82±0.21	0.80±0.05	0.92±0.06	0.74±0.04	0.73±0.07
YK-RSV-040	0.38±0.08	0.91±0.14	0.61±0.09	0.75±0.13	0.93±0.09	1.00±0.23
YK-RSV-043	0.70±0.13	0.66±0.06	0.72±0.09	0.84±0.18	0.90±0.13	0.75±0.28
YK-RSV-044	0.56±0.08	0.75±0.13	0.73±0.00	0.86±0.20	0.87±0.00	0.97±0.04
YK-RSV-045	0.29±0.02	0.41±0.03	0.46±0.05	0.57±0.09	0.47±0.09	0.97±0.07
YK-RSV-046	0.24±0.07	0.63±0.03	0.41±0.02	0.46±0.14	0.83±0.04	1.03±0.03
YK-RSV-048	0.46±0.08	0.70±0.07	0.61±0.17	0.75±0.00	0.61±0.07	1.13±0.19
YK-RSV-050	0.46±0.07	0.69±0.15	0.66±0.15	0.87±0.19	1.23±0.27	1.16±0.37
YK-RSV-051	0.33±0.05	0.60±0.08	0.44±0.00	0.51±0.13	0.69±0.14	1.00±0.17
YK-RSV-052	0.42±0.10	0.69±0.09	0.30±0.06	0.88±0.21	0.80±0.04	1.22±0.14
YK-RSV-054	0.71±0.08	0.75±0.18	0.83±0.20	0.96±0.23	0.91±0.13	0.97±0.04
YK-RSV-055	0.48±0.11	0.78±0.19	0.71±0.23	0.72±0.20	0.69±0.08	0.79±0.26
YK-RSV-056	0.69±0.10	0.91±0.09	0.44±0.08	0.82±0.07	0.64±0.12	0.72±0.14
YK-RSV-057	0.61±0.10	0.64±0.17	0.64±0.15	0.70±0.07	0.56±0.08	0.56±0.09
YK-RSV-058	0.36±0.07	0.73±0.09	0.50±0.05	0.60±0.04	0.72±0.03	0.81±0.05
YK-RSV-059	0.52±0.10	0.74±0.07	0.44±0.03	0.49±0.02	0.73±0.03	0.82±0.09
YK-RSV-060	0.56±0.08	0.82±0.13	0.61±0.07	0.80±0.13	0.73±0.11	0.76±0.24
YK-RSV-061	0.68±0.08	0.87±0.05	0.85±0.24	0.88±0.17	1.02±0.24	0.98±0.28
YK-RSV-062	0.65±0.04	0.84 ± 0.04	0.87±0.16	0.89±0.05	0.97±0.04	0.95±0.09
PC-RSV-063	0.45±0.08	0.66±0.11	0.52±0.04	0.71±0.05	0.73±0.02	0.91±0.02
PC-RSV-064	0.34±0.04	0.62±0.05	0.57±0.05	0.74±0.05	0.65±0.06	0.80±0.08
PC-RSV-065	0.45±0.07	0.71±0.10	0.62±0.06	0.76±0.12	0.72±0.05	0.81±0.11
PC-RSV-066	0.49±0.09	0.46±0.07	0.67±0.11	0.70±0.03	0.68±0.10	0.77±0.11
PC-RSV-067	0.58±0.08	0.68±0.08	0.42±0.07	0.68±0.12	0.70±0.07	0.83±0.08
PC-RSV-068	0.55±0.06	0.73±0.12	0.47±0.09	0.69±0.19	0.63±0.09	0.86±0.10

[0510] The following mutants had a stress resistance parameter of above 0.44, up to 0.86, after storage at 4 °C for one week; a stress resistance parameter of above 0.64 after incubation at 50 °C for 60 min; a stress resistance parameter of above 0.61 after treatment under a high osmotic pressure condition for 60 min; a stress resistance parameter of above 0.70 after treatment under a low osmotic pressure condition for 60 min; stress resistance parameters both above 0.56 after treatment under conditions of pH = 3.5 and pH = 10 for 60 min. The stress resistance parameters after treatment under different conditions were all superior to or comparable to those of the controls.

Table 13. Results for stability screening of RSV F mutants – 1

No.	Determination of physical stability, RSV pre-F antibody (3C12)
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	Temperature (°C)		Osmotic pressure (mM)		pH	
	4	50	3000	10	3.5	10
YK-RSV-061	0.68±0.08	0.87±0.05	0.85±0.24	0.88±0.17	1.02±0.24	0.98±0.28
YK-RSV-003	0.65±0.01	0.81 ± 0.03	0.83±0.06	0.89±0.17	1.04±0.32	1.02±0.17
YK-RSV-062	0.65±0.04	0.84 ±0.04	0.87±0.16	0.89±0.05	0.97±0.04	0.95±0.09
YK-RSV-054	0.71±0.08	0.75±0.18	0.83±0.20	0.96±0.23	0.91±0.13	0.97±0.04
YK-RSV-039	0.62±0.08	0.82±0.21	0.80±0.05	0.92±0.06	0.74±0.04	0.73±0.07
YK-RSV-044	0.56±0.08	0.75±0.13	0.73±0.00	0.86±0.20	0.87±0.00	0.97±0.04
YK-RSV-012	0.69±0.11	1.22 ± 0.24	0.73±0.08	0.76±0.17	0.67±0.07	1.22±0.00
YK-RSV-015	0.57±0.08	0.70 ± 0.06	0.72±0.03	0.73±0.18	0.80±0.12	0.87±0.12
YK-RSV-001	0.67±0.10	0.70 ± 0.06	0.86±0.22	0.97±0.26	0.75±0.03	1.00±0.10
YK-RSV-036	0.69±0.08	0.75±0.08	0.72±0.02	0.80±0.06	0.98±0.03	1.16±0.23
YK-RSV-060	0.56±0.08	0.82±0.13	0.61±0.07	0.80±0.13	0.73±0.11	0.76±0.24
PC-RSV-063	0.45±0.08	0.66±0.11	0.52±0.04	0.71±0.05	0.73±0.02	0.91±0.02
PC-RSV-064	0.34±0.04	0.62±0.05	0.57±0.05	0.74±0.05	0.65±0.06	0.80±0.08
PC-RSV-065	0.45±0.07	0.71±0.10	0.62±0.06	0.76±0.12	0.72±0.05	0.81±0.11
PC-RSV-066	0.49±0.09	0.46±0.07	0.67±0.11	0.70±0.03	0.68±0.10	0.77±0.11
PC-RSV-067	0.58±0.08	0.68±0.08	0.42±0.07	0.68±0.12	0.70±0.07	0.83±0.08
PC-RSV-068	0.55±0.06	0.73±0.12	0.47±0.09	0.69±0.19	0.63±0.09	0.86±0.10

Table 14. Results for stability screening of RSV F mutants – 2

No.	Determination of physical stability, RSV pre-F antibody (3C12)					
	Temperature (°C)		Osmotic pressure (mM)		pH	
	4	50	3000	10	3.5	10
YK-RSV-009	0.71±0.11	0.84 ± 0.04	0.69±0.15	0.78±0.15	0.60±0.14	0.91±0.04
YK-RSV-010	0.86±0.08	1.04 ± 0.09	0.84±0.11	0.97±0.07	0.82±0.05	0.93±0.05
YK-RSV-011	0.85±0.08	0.76 ± 0.01	0.80±0.11	0.97±0.23	0.63±0.18	0.86±0.01
YK-RSV-030	0.44±0.08	1.05±0.00	0.83±0.06	0.89±0.15	0.89±0.06	1.00±0.11
YK-RSV-043	0.70±0.13	0.66±0.06	0.72±0.09	0.84±0.18	0.90±0.13	0.75±0.28
YK-RSV-048	0.46±0.08	0.70±0.07	0.61±0.17	0.75±0.00	0.61±0.07	1.13±0.19
YK-RSV-050	0.46±0.07	0.69±0.15	0.66±0.15	0.87±0.19	1.23±0.27	1.16±0.37
YK-RSV-055	0.48±0.11	0.78±0.19	0.71±0.23	0.72±0.20	0.69±0.08	0.79±0.26
YK-RSV-057	0.61±0.10	0.64±0.17	0.64±0.15	0.70±0.07	0.56±0.08	0.56±0.09
PC-RSV-063	0.45±0.08	0.66±0.11	0.52±0.04	0.71±0.05	0.73±0.02	0.91±0.02
PC-RSV-064	0.34±0.04	0.62±0.05	0.57±0.05	0.74±0.05	0.65±0.06	0.80±0.08
PC-RSV-065	0.45±0.07	0.71±0.10	0.62±0.06	0.76±0.12	0.72±0.05	0.81±0.11
PC-RSV-066	0.49±0.09	0.46±0.07	0.67±0.11	0.70±0.03	0.68±0.10	0.77±0.11
PC-RSV-067	0.58±0.08	0.68±0.08	0.42±0.07	0.68±0.12	0.70±0.07	0.83±0.08
PC-RSV-068	0.55±0.06	0.73±0.12	0.47±0.09	0.69±0.19	0.63±0.09	0.86±0.10

[0511] 3) Summary:

[0512] (1) From the results for physical stability screening, it could be seen that the stability was significantly improved under certain stress tests as compared to that of the controls, and 20 mutants (including YK-RSV-061, YK-RSV-003, YK-RSV-062, YK-RSV-054, YK-RSV-039, YK-RSV-044, YK-RSV-012, YK-RSV-015, YK-RSV-001, YK-RSV-036, YK-RSV-060, YK-RSV-009, YK-RSV-010, YK-RSV-011, YK-RSV-030, YK-RSV-043, YK-RSV-048, YK-RSV-050, YK-RSV-055, and YK-RSV-057) had superior stability under each stress test as compared with the other mutant groups, showing stronger overall

stability. The binding capacity of the mutants to different epitope antibodies was further detected by ELISA as follows.

[0513] 2.4 ELISA results of the binding of RSV F antigen mutants to antibodies recognizing different epitopes

[0514] Among the epitopes Φ , Quaternary, V, II, and III to which the present application relates, antibodies recognizing the epitopes Φ , Quaternary, and V specifically bound only to the pre-fusion conformation compared with the epitopes II and III that are present in both the pre-fusion and post-fusion conformations. Studies have demonstrated that specific epitopes present only in the pre-fusion conformation are sites to which neutralizing antibodies are sensitive and can induce a higher neutralizing antibody response.

[0515] In the present application, the binding of different mutant antigens to antibodies recognizing different epitopes was detected by ELISA, and the strength of the binding of the antigens to the antibodies was measured by the binding amount. (1) The RSV pre-F antibody (3C12) recognizing the epitope Φ binds only to the F protein in the pre-fusion conformation; (2) the RSV pre-F antibody (7H11) recognizes the epitope Quaternary (spanning both epitopes IV and V) and specifically binds to the F protein in the pre-fusion conformation; (3) the RSV F antibody (11A9) recognizing the epitope II can recognize both the pre-fusion and post-fusion conformations of the F protein; (4) the RSV F antibody (4B9) recognizing the epitope III preferentially recognizes the pre-fusion conformation and has relatively weak binding activity for the post-fusion conformation; (5) the RSV pre-F antibody (7E11) recognizing the epitope V binds only to the F protein in the pre-fusion conformation. The results for the ELISA were as follows.

Table 15. ELISA results of RSV F mutants-general table

No.	ELISA results (ng/mL)									
	RSV pre-F antibody (3C12)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV pre-F antibody (7H11)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV F antibody (7E11)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV F antibody (11A9)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV F antibody (4B9)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody
	Epitope Φ		Epitope Quaternary		Epitope V		Epitope II		Epitope III	
YK-RSV-001	5136	1.86	7401	2.05	431	1.34	1593	0.76	2133	0.74
YK-RSV-003	5541	2.00	7249	2.01	452	1.41	1994	0.95	3171	1.11
YK-RSV-009	521	0.19	29	0.01	43	0.13	203	0.10	358	0.12
YK-RSV-010	838	0.30	39	0.01	139	0.43	336	0.16	534	0.19
YK-RSV-011	8036	2.90	6549	1.81	866	2.70	4388	2.09	7072	2.47
YK-RSV-012	6709	2.42	4919	1.36	459	1.43	3219	1.53	4689	1.63
YK-RSV-015	5932	2.14	8943	2.48	420	1.31	1886	0.90	2933	1.02
YK-RSV-030	5376	1.94	2485	0.69	441	1.37	1822	0.87	3688	1.29
YK-RSV-036	7027	2.54	7769	2.15	1069	3.33	3384	1.61	7424	2.59
YK-RSV-039	9467	3.42	5583	1.55	584	1.82	4097	1.95	7659	2.67
YK-RSV-043	9445	3.41	922	0.26	223	0.69	4610	2.20	6529	2.28
YK-RSV-044	6909	2.50	2031	0.56	442	1.38	4039	1.92	7078	2.47
YK-RSV-048	3306	1.19	2873	0.80	217	0.68	2255	1.07	4655	1.62
YK-RSV-050	3068	1.11	3722	1.03	923	2.88	2474	1.18	3494	1.22
YK-RSV-054	6979	2.52	6589	1.82	1060	3.30	3754	1.79	6502	2.27
YK-RSV-055	8589	3.10	4317	1.20	91	0.28	3856	1.84	7298	2.54
YK-RSV-057	5210	1.88	6346	1.76	129	0.40	2823	1.34	4779	1.67
YK-RSV-060	5993	2.17	2203	0.61	409	1.27	2657	1.27	5613	1.96

YK-RSV-061	5261	1.90	7661	2.12	595	1.85	2505	1.19	3683	1.28
YK-RSV-062	5059	1.83	7350	2.03	487	1.52	2063	0.98	3260	1.14
PC-RSV-063	2727	0.99	3557	0.98	461	1.44	2053	0.98	2993	1.04
PC-RSV-064	2768	1.00	3612	1.00	321	1.00	2100	1.00	2868	1.00
PC-RSV-065	3378	1.22	3705	1.03	436	1.36	1864	0.89	3102	1.08
PC-RSV-066	2169	0.78	3213	0.89	374	1.17	1682	0.80	2058	0.72
PC-RSV-067	2828	1.02	2869	0.79	298	0.93	2509	1.19	3608	1.26
PC-RSV-068	3168	1.14	3667	1.02	312	0.97	3191	1.52	4321	1.51

[0516] 1) The epitopes Φ , Quaternary, and V on the following 11 mutants were all able to well bind to corresponding antibodies with relatively strong binding capacity, and the binding capacity of the epitopes II and/or III to the corresponding antibodies was superior to or comparable to that on the controls.

[0517] For example, the binding levels of the epitope Φ on YK-RSV-061, YK-RSV-003, and YK-RSV-062 to the RSV pre-F antibody (3C12) were 1.83 to 2.00 times that on the control PC-RSV-064; the binding levels of the epitope Quaternary to the RSV pre-F antibody (7H11) were 2.01 to 2.12 times that on the control PC-RSV-064.

[0518] The binding level of the epitope Φ on YK-RSV-054 to the RSV pre-F antibody (3C12) was 2.52 times that on the control PC-RSV-064; the binding level of the epitope Quaternary to the RSV pre-F antibody (7H11) was 1.82 times that on the control PC-RSV-064; the binding level of the epitope V to the RSV F antibody (7E11) was 3.30 times that on the control PC-RSV-064; the binding level of the epitope II to the RSV F antibody (11A9) was 1.79 times that on the control PC-RSV-064; the binding level of the epitope III to the RSV F antibody (4B9) was 2.27 times that on the control PC-RSV-064.

Table 16. ELISA results of RSV F mutants – 1

No.	ELISA results (ng/mL)									
	RSV pre-F antibody (3C12)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV pre-F antibody (7H11)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV F antibody (7E11)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV F antibody (11A9)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV F antibody (4B9)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody
	Epitope Φ		Epitope Quaternary		Epitope V		Epitope II		Epitope III	
YK-RSV-061	5261	1.90	7661	2.12	595	1.85	2505	1.19	3683	1.28
YK-RSV-003	5541	2.00	7249	2.01	452	1.41	1994	0.95	3171	1.11
YK-RSV-062	5059	1.83	7350	2.03	487	1.52	2063	0.98	3260	1.14
YK-RSV-054	6979	2.52	6589	1.82	1060	3.30	3754	1.79	6502	2.27
YK-RSV-039	9467	3.42	5583	1.55	584	1.82	4097	1.95	7659	2.67
YK-RSV-044	6909	2.50	2031	0.56	442	1.38	4039	1.92	7078	2.47
YK-RSV-012	6709	2.42	4919	1.36	459	1.43	3219	1.53	4689	1.63
YK-RSV-015	5932	2.14	8943	2.48	420	1.31	1886	0.90	2933	1.02
YK-RSV-001	5136	1.86	7401	2.05	431	1.34	1593	0.76	2133	0.74
YK-RSV-036	7027	2.54	7769	2.15	1069	3.33	3384	1.61	7424	2.59
YK-RSV-060	5993	2.17	2203	0.61	409	1.27	2657	1.27	5613	1.96
PC-RSV-063	2727	0.99	3557	0.98	461	1.44	2053	0.98	2993	1.04
PC-RSV-064	2768	1.00	3612	1.00	321	1.00	2100	1.00	2868	1.00
PC-RSV-065	3378	1.22	3705	1.03	436	1.36	1864	0.89	3102	1.08
PC-RSV-066	2169	0.78	3213	0.89	374	1.17	1682	0.80	2058	0.72
PC-RSV-067	2828	1.02	2869	0.79	298	0.93	2509	1.19	3608	1.26
PC-RSV-068	3168	1.14	3667	1.02	312	0.97	3191	1.52	4321	1.51

[0519] 2) The epitopes Φ , Quaternary, and V on the following 4 mutants were also able to well bind to corresponding antibodies with relatively strong binding capacity.

[0520] For example, the binding level of the epitope Φ on YK-RSV-011 to the RSV pre-F antibody (3C12) was 2.90 times that on the control PC-RSV-064; the binding level of the epitope Quaternary to the RSV pre-F antibody (7H11) was 1.81 times that on the control PC-RSV-064; the binding level of the epitope V to the RSV F antibody (7E11) was 2.70 times that on the control PC-RSV-064; the binding level of the epitope II to the RSV F antibody (11A9) was 2.09 times that on the control PC-RSV-064; the binding level of the epitope III to the RSV F antibody (4B9) was 2.47 times that on the control PC-RSV-064.

Table 17. Results for ELISA results of RSV F mutants – 2

No.	ELISA results (ng/mL)									
	RSV pre-F antibody (3C12)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV pre-F antibody (7H11)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV F antibody (7E11)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV F antibody (11A9)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV F antibody (4B9)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody
	Epitope Φ		Epitope Quaternary		Epitope V		Epitope II		Epitope III	
YK-RSV-011	8036	2.90	6549	1.81	866	2.70	4388	2.09	7072	2.47
YK-RSV-030	5376	1.94	2485	0.69	441	1.37	1822	0.87	3688	1.29
YK-RSV-048	3306	1.19	2873	0.80	217	0.68	2255	1.07	4655	1.62
YK-RSV-050	3068	1.11	3722	1.03	923	2.88	2474	1.18	3494	1.22
PC-RSV-063	2727	0.99	3557	0.98	461	1.44	2053	0.98	2993	1.04
PC-RSV-064	2768	1.00	3612	1.00	321	1.00	2100	1.00	2868	1.00
PC-RSV-065	3378	1.22	3705	1.03	436	1.36	1864	0.89	3102	1.08
PC-RSV-066	2169	0.78	3213	0.89	374	1.17	1682	0.80	2058	0.72
PC-RSV-067	2828	1.02	2869	0.79	298	0.93	2509	1.19	3608	1.26
PC-RSV-068	3168	1.14	3667	1.02	312	0.97	3191	1.52	4321	1.51

[0521] In summary, the above contents showed the detection of the binding levels of 5 different epitopes on the mutants, and the mutants with better binding level (see Tables 16 and 17) were further screened for candidate antigens.

[0522] 2.5 Results for detection of affinity of the RSV F antigen mutants for antibodies recognizing different epitopes

[0523] Bio-Layer interferometry (referred to as BLI) is a label-free real-time monitoring optical detection technique, mainly used for comprehensive quantitative analysis of the interaction between biomolecules and protein concentration determination. The BLI can monitor the binding process between molecules in real time and calculate important data such as affinity constant (K_D), association rate constant (K_{on} or k_a), dissociation rate constant (K_{off} , K_{dis} , or k_d), etc., between molecules.

[0524] $K_D = K_{off} \div K_{on}$;

[0525] K_D : the affinity constant, representing the strength of the binding of two molecules, wherein the greater the K_D , the weaker the binding, and otherwise, the stronger the binding;

[0526] K_{on} : the association rate constant;

[0527] K_{dis} (k_{off}): the dissociation rate constant;

[0528] K_{on} and K_{off} mainly represent the mode of molecular interaction, whether it is fast binding and fast dissociation, slow binding and slow dissociation, or the like.

[0529] In the present application, the affinity of target proteins for 2 antibodies was analyzed by a BLI method, wherein the RSV pre-F antibody (3C12) recognizes the epitope Φ , and the RSV pre-F antibody (7H11) recognizes the epitope Quaternary. The detection results were as follows:

Table 18. Results for affinity of different epitopes on RSV F mutants for corresponding antibodies – general table

No.	K _D value (M)	
	RSV pre-F antibody (3C12)	RSV pre-F antibody (7H11)
	Epitope Φ	Epitope Quaternary
YK-RSV-039	3.26E-12	2.83E-11
YK-RSV-036	2.10E-11	8.09E-12
YK-RSV-044	4.66E-11	4.05E-10
YK-RSV-001	4.73E-11	9.38E-12
YK-RSV-054	5.16E-11	1.25E-11
YK-RSV-060	5.18E-11	6.13E-10
YK-RSV-061	6.21E-11	3.05E-12
YK-RSV-062	6.26E-11	3.62E-12
YK-RSV-003	6.35E-11	3.47E-12
YK-RSV-012	6.87E-11	3.82E-11
YK-RSV-011	6.93E-11	3.27E-11
YK-RSV-015	7.41E-11	5.09E-12
YK-RSV-030	7.79E-11	2.88E-10
YK-RSV-048	1.83E-10	2.27E-10
YK-RSV-050	5.52E-10	1.15E-10
PC-RSV-063	1.36E-10	1.07E-10
PC-RSV-064	1.24E-10	1.56E-10
PC-RSV-065	1.62E-10	1.22E-10
PC-RSV-066	2.63E-10	1.33E-10
PC-RSV-067	5.29E-10	3.06E-10
PC-RSV-068	6.61E-10	2.25E-10

[0530] 1) The following mutants had the strongest binding affinity for the antibodies and were significantly superior to the controls

[0531] The affinity constant K_D values for the binding of the epitope Φ on the following mutants to the RSV pre-F antibody (3C12) were 3.26E-12 M to 7.79E-11 M, which were significantly lower than the K_D values (K_D of E-10 M, with a difference of 1 to 2 orders of magnitude) of the controls, indicating that the affinity of the antigens for the antibody was stronger.

[0532] The affinity constant K_D values for the binding of the epitope Quaternary on the mutants to the RSV pre-F antibody (7H11) were 3.05E-12 M to 6.13E-10 M, and except that the K_D values for YK-RSV-044, YK-RSV-060, and YK-RSV-030 were comparable to those of the controls, the K_D values for the other mutants were significantly lower than those of the controls (with a difference of 1 to 2 orders of magnitude), indicating that the affinity of the antigens for the antibody was stronger.

[0533] The following mutants will be subjected to animal experiments for further screening.

Table 19. Results for affinity of different epitopes on RSV F mutants for corresponding antibodies

No.	K _D value (M)
-----	--------------------------

	RSV pre-F antibody (3C12)	RSV pre-F antibody (7H11)
	Epitope Φ	Epitope Quaternary
YK-RSV-039	3.26E-12	2.83E-11
YK-RSV-036	2.10E-11	8.09E-12
YK-RSV-044	4.66E-11	4.05E-10
YK-RSV-001	4.73E-11	9.38E-12
YK-RSV-054	5.16E-11	1.25E-11
YK-RSV-060	5.18E-11	6.13E-10
YK-RSV-061	6.21E-11	3.05E-12
YK-RSV-062	6.26E-11	3.62E-12
YK-RSV-003	6.35E-11	3.47E-12
YK-RSV-012	6.87E-11	3.82E-11
YK-RSV-011	6.93E-11	3.27E-11
YK-RSV-015	7.41E-11	5.09E-12
YK-RSV-030	7.79E-11	2.88E-10
PC-RSV-063	1.36E-10	1.07E-10
PC-RSV-064	1.24E-10	1.56E-10
PC-RSV-065	1.62E-10	1.22E-10
PC-RSV-066	2.63E-10	1.33E-10
PC-RSV-067	5.29E-10	3.06E-10
PC-RSV-068	6.61E-10	2.25E-10

[0534] 2.6 Results for immunogenicity of RSV F antigen mutants in Balb/c mice

[0535] Mice are small in size, fast in growth, low in feeding cost, and applicable to many commercially available detection reagents. For the above reasons, mice are currently the most widely used in animal models of RSV infection. Prince reported mice as animal models of hRSV infection for the first time in 1979. Balb/c mice are used more frequently, and after infection by transluminal drip, the RSV titer is 10^5 to 10^7 PFU, and significant inflammatory changes in the lower respiratory tract and a significant increase in part of cytokines and CC/CXC chemokines such as TNF- α , IL-6, and IFN- γ in tracheal perfusate can be observed in animals, and the infiltration of peribronchiolar and perivascular mononuclear cells (lymphocytes and macrophages) or interstitial pneumonia can be observed by histopathology. Mice infected with different RSV subtypes (such as A/Long, A2, and clinical isolates) have different symptoms. Mice infected with RSV show an increased respiratory rate with airway obstruction and airway hyperresponsiveness, and the airway hyperreactivity lasts for a long time, which is very similar to the RSV infection in children. Moreover, mice are one of the most common model animals for pharmacological and toxicological studies, with a clear genetic background, a large number of strains, and the ability to meet particular experimental needs through gene-targeted transfection or knockout techniques. Therefore, mice were used as animal models for detection in the following experiments.

[0536] 2.6.1 Results for detection of binding antibody titers

[0537] The IgG titers specific for the pre-fusion and post-fusion F proteins in the serum of mice on day 35 after the immunization were detected by an ELISA method. The detection results are shown in the table below:

[0538] 1. The pre-F IgG antibody titers of the following 11 test samples were 2.52 to 5.31 times that of

the Abrysvo™ positive control, and the post-F IgG antibody titers were 1.13 to 5.21 times that of the Abrysvo™ positive control.

[0539] The pre-F IgG antibody titers of YK-RSV-061, YK-RSV-003, and YK-RSV-062 were 4.29 to 5.31 times that of the Abrysvo™ positive control, and the post-F IgG antibody titers were 4.32 to 5.21 times that of the Abrysvo™ positive control.

[0540] Among them, the pre-F IgG antibody titers of the YK-RSV-061 mutant were the highest, with the pre-F IgG antibody titer being 5.31 times that of the Abrysvo™ positive control, and the post-F IgG antibody titers being 5.21 times that of the Abrysvo™ positive control.

[0541] In all of the test samples being detected, the pre-F/post-F ratios were all higher than 1, indicating that the IgG titers produced by the pre-fusion F proteins of the test samples were higher than those produced by the post-fusion F proteins.

Table 20. RSV F protein-specific IgG antibody titers in serum of mice (day 35) – 1

Test sample	pre-F IgG		post-F IgG		pre-F/ post-F
	Antibody GMT ($\times 10^4$)	Test sample GMT/Abrysvo™ GMT (times)	Antibody GMT ($\times 10^4$)	Test sample GMT/Abrysvo™ GMT (times)	
YK-RSV-061	669	5.31	542	5.21	1.23
YK-RSV-003	618	4.90	510	4.90	1.21
YK-RSV-062	541	4.29	449	4.32	1.20
YK-RSV-054	495	3.93	405	3.89	1.22
YK-RSV-039	493	3.91	386	3.71	1.28
YK-RSV-044	452	3.59	257	2.47	1.76
YK-RSV-012	462	3.67	310	2.98	1.49
YK-RSV-015	474	3.76	411	3.95	1.15
YK-RSV-001	449	3.56	332	3.19	1.35
YK-RSV-036	386	3.06	375	3.61	1.03
YK-RSV-060	318	2.52	118	1.13	2.69
LNP	0.01	0.00	0.01	0.00	1.00
Abrysvo™	126	1.00	104	1.00	1.21

[0542] 2. The pre-F IgG antibody titers of YK-RSV-011 and YK-RSV-030 were 2.38 to 2.50 times that of the Abrysvo™ positive control, and the post-F IgG antibody titers were 1.30 to 2.81 times that of the Abrysvo™ positive control.

Table 21. RSV F protein-specific IgG antibody titers in serum of mice (day 35) – 2

Test sample	pre-F IgG		post-F IgG		pre-F/post-F
	Antibody GMT ($\times 10^4$)	Test sample GMT/Abrysvo™ GMT (times)	Antibody GMT ($\times 10^4$)	Test sample GMT/Abrysvo™ GMT (times)	
YK-RSV-011	300	2.38	135	1.30	2.22
YK-RSV-030	315	2.50	292	2.81	1.08
LNP	0.01	0.00	0.01	0.00	1.00
Abrysvo™	126	1.00	104	1.00	1.21

[0543] The above test samples would be further evaluated for immunogenicity by determining the level of neutralizing antibodies produced.

[0544] 2.6.2 Results for detection of neutralizing antibody titers

[0545] The neutralizing antibody titers in the serum of mice on day 35 after the immunization were detected by a spot reduction-based micro-neutralization assay. The results are shown in the table below and in FIGs. 4 to 7 (each including FIGs. (A) and (B)):

[0546] 1. Results for RSV type A virus-neutralizing antibody titers

[0547] The neutralizing antibody titer IC₅₀ values of the following 11 test samples were 3.72 to 12.67 times that of the Abrysvo™ positive control, and IC₉₀ values were 2.34 to 14.41 times that of the Abrysvo™ positive control, all of which were higher than those of the positive control.

[0548] The neutralizing antibody titer IC₅₀ values of YK-RSV-061, YK-RSV-003, and YK-RSV-062 were 6.34 to 12.67 times that of the Abrysvo™ positive control, and IC₉₀ values were 9.56 to 14.41 times that of the Abrysvo™ positive control, indicating significantly higher neutralizing antibody titers.

[0549] Among them, YK-RSV-061 had the highest neutralizing antibody titers, with IC₅₀ being 12.67 times that of the Abrysvo™ positive control, and IC₉₀ being 14.41 times that of the positive control.

Table 22. Neutralizing antibody titers of RSV A2 in serum of mice (day 35)

Test sample	IC ₅₀		IC ₉₀	
	Titer	Test sample IC ₅₀ /Abrysvo™ IC ₅₀ (times)	Titer	Test sample IC ₉₀ /Abrysvo™ IC ₉₀ (times)
YK-RSV-061	51997	12.67	3472	14.41
YK-RSV-003	30330	7.39	2311	9.59
YK-RSV-062	26022	6.34	2305	9.56
YK-RSV-054	24748	6.03	1705	7.07
YK-RSV-039	20758	5.06	1586	6.58
YK-RSV-044	19263	4.69	1283	5.32
YK-RSV-012	19706	4.80	1362	5.65
YK-RSV-015	20496	5.00	1458	6.05
YK-RSV-001	18693	4.56	1097	4.55
YK-RSV-036	17880	4.36	722	3.00
YK-RSV-060	15272	3.72	563	2.34
LNP	40	0.01	40	0.17
Abrysvo™	4103	1.00	241	1.00

[0550] 2. Results for RSV type B virus-neutralizing antibody titers

[0551] The above 11 test samples obtained by screening were further detected for the levels of neutralizing antibodies produced for RSV B18357 virus. The detection results are shown in the table below:

[0552] The neutralizing antibody titer IC₅₀ values of the following 11 mutants were 2.85 to 10.63 times that of the Abrysvo™ positive control, and IC₉₀ values were 2.88 to 14.23 times that of the Abrysvo™ positive control, indicating that the neutralizing antibody titers were all significantly higher than those of the positive control.

[0553] The neutralizing antibody titer IC₅₀ values of YK-RSV-061, YK-RSV-003, and YK-RSV-062 were 5.68 to 10.63 times that of the Abrysvo™ positive control, and IC₉₀ values were 6.87-14.23 times that of the Abrysvo™ positive control, indicating very high neutralizing antibody titers.

[0554] Among them, YK-RSV-061 had the highest neutralizing antibody titers, with IC₅₀ being 10.63 times that of the Abrysvo™ positive control, and IC₉₀ being 14.23 times that of the positive control.

Table 23. Neutralizing antibody titers of RSV B18357 in serum of mice (day 35)

Test sample	IC ₅₀		IC ₉₀	
	Titer	Test sample IC ₅₀ /Abrysvo™ IC ₅₀ (times)	Titer	Test sample IC ₉₀ /Abrysvo™ IC ₉₀ (times)
YK-RSV-061	34877	10.63	3088	14.23
YK-RSV-003	23943	7.30	1712	7.89
YK-RSV-062	18615	5.68	1490	6.87
YK-RSV-054	16175	4.93	1243	5.73
YK-RSV-039	12814	3.91	976	4.50
YK-RSV-044	10303	3.14	877	4.04
YK-RSV-012	11641	3.55	895	4.12
YK-RSV-015	12359	3.77	970	4.47
YK-RSV-001	9661	2.95	829	3.82
YK-RSV-036	9420	2.87	713	3.29
YK-RSV-060	9337	2.85	625	2.88
LNP	40	0.01	40	0.18
Abrysvo™	3280	1.00	217	1.00

[0555] In summary, the above 11 test samples (including YK-RSV-061, YK-RSV-003, YK-RSV-062, YK-RSV-054, YK-RSV-039, YK-RSV-044, YK-RSV-012, YK-RSV-015, YK-RSV-001, YK-RSV-036, and YK-RSV-060) were all able to produce relatively high neutralizing antibody titers for different subtypes of RSV A2 and RSV B18357.

[0556] 2.7 Results for immunogenicity of RSV F antigen mutants in cotton rats

[0557] Dreizin reported cotton rats as animal models of hRSV infection for the first time in 1971. After the cotton rats are infected by hRSV, the virus is mainly replicated and amplified in the lungs and lower respiratory tract. Mild to moderate bronchiolitis or pneumonia, airway epithelial cell shedding, and pulmonary atelectasis can be observed by pathological examination, the symptoms of the upper respiratory tract infection are significant, and changes in the related cytokines are not significant. Cotton rats are standard animal models for the evaluation of the efficacy of vaccines, antiviral drugs, and monoclonal antibodies (such as palivizumab). Therefore, cotton rats were used as animal models for detection in the following experiments.

[0558] 2.7.1 Results for detection of binding antibody titers

[0559] The IgG titers specific for the pre-fusion and post-fusion F proteins in the serum of cotton rats on days 42 and 48 after the immunization were detected by an ELISA method (see FIGs. 8 to 11 (each including FIGs. (A) and (B))). The detection results of IgG titers on day 42 are shown in the table below:

[0560] (1) On day 42, the pre-F IgG antibody titers of the following 11 test samples were 3.05 to 13.10 times that of the Abrysvo™ positive control, and the post-F IgG antibody titers were 2.36 to 6.38 times that of the Abrysvo™ positive control, all of which were significantly higher than those of the positive control.

[0561] The pre-F IgG antibody titers of YK-RSV-061, YK-RSV-003, and YK-RSV-062 were 9.76 to

13.10 times that of the Abrysvo™ positive control, and the post-F IgG antibody titers were 4.66 to 6.33 times that of the Abrysvo™ positive control.

[0562] Among them, the IgG antibody titers of the test sample YK-RSV-061 were the highest, with the pre-F IgG antibody titer being 13.10 times that of the Abrysvo™ positive control, and the post-F IgG antibody titers being 6.33 times that of the Abrysvo™ positive control.

[0563] The pre-F/post-F ratios of all the test samples range from 1.39 to 3.95, indicating that the IgG antibody titers produced by the pre-fusion F proteins are significantly higher than those of the post-fusion F proteins.

Table 24. RSV F protein-specific IgG antibody titers in serum of cotton rats (day 42)

Test sample	pre-F IgG		post-F IgG		pre-F/post-F
	Antibody GMT ($\times 10^4$)	Test sample GMT/Abrysvo™ GMT (times)	Antibody GMT ($\times 10^4$)	Test sample GMT/Abrysvo™ GMT (times)	
YK-RSV-061	2017	13.10	570	6.33	3.54
YK-RSV-003	1681	10.92	523	5.81	3.21
YK-RSV-062	1503	9.76	419	4.66	3.59
YK-RSV-054	1037	6.73	376	4.18	2.76
YK-RSV-039	982	6.38	574	6.38	1.71
YK-RSV-044	921	5.98	277	3.08	3.32
YK-RSV-012	885	5.75	319	3.54	2.77
YK-RSV-015	837	5.44	212	2.36	3.95
YK-RSV-001	524	3.40	376	4.18	1.39
YK-RSV-036	495	3.21	291	3.23	1.70
YK-RSV-060	469	3.05	302	3.36	1.55
LNP	0.01	0.00	0.01	0.00	1.00
Abrysvo™	154	1.00	90	1.00	1.71

[0564] Meanwhile, the IgG titers in the serum of cotton rats on day 48 were detected. The detection results are shown in the table below:

[0565] (2) On day 48, the pre-F IgG antibody titers of the 11 test samples were 4.44 to 12.35 times that of the Abrysvo™ positive control, and the post-F IgG antibody titers were 1.51 to 6.83 times that of the Abrysvo™ positive control, all of which were significantly higher than those of the positive control.

[0566] The pre-F IgG antibody titers of YK-RSV-061, YK-RSV-003, and YK-RSV-062 were 9.02 to 12.35 times that of the Abrysvo™ positive control, and the post-F IgG antibody titers were 4.03 to 6.83 times that of the Abrysvo™ positive control.

[0567] Among them, the IgG antibody titers of the test sample YK-RSV-061 were the highest, with the pre-F IgG antibody titer being 12.35 times that of the Abrysvo™ positive control, and the post-F IgG antibody titers being 6.83 times that of the Abrysvo™ positive control.

[0568] The pre-F/post-F ratios of all the test samples range from 1.78 to 4.29, indicating that the IgG antibody titers produced by the pre-fusion F proteins are still significantly higher than those produced by the post-fusion F proteins on day 48 after the immunization.

Table 25. RSV F protein-specific IgG antibody titers in serum of cotton rats (day 48)

Test sample	pre-F IgG		post-F IgG		pre-F/post-F
	Antibody GMT ($\times 10^4$)	Test sample GMT/Abrysvo TM GMT (times)	Antibody GMT ($\times 10^4$)	Test sample GMT/Abrysvo TM GMT (times)	
YK-RSV-061	1013	12.35	444	6.83	2.28
YK-RSV-003	806	9.83	302	4.65	2.67
YK-RSV-062	740	9.02	262	4.03	2.82
YK-RSV-054	524	6.39	293	4.51	1.79
YK-RSV-039	511	6.23	287	4.42	1.78
YK-RSV-044	495	6.04	242	3.72	2.05
YK-RSV-012	471	5.74	154	2.37	3.06
YK-RSV-015	451	5.50	180	2.77	2.51
YK-RSV-001	430	5.24	154	2.37	2.79
YK-RSV-036	420	5.12	98	1.51	4.29
YK-RSV-060	364	4.44	131	2.02	2.78
LNP	0.01	0.00	0.01	0.00	1.00
Abrysvo TM	82	1.00	65	1.00	1.26

[0569] 2.7.2 Results for detection of neutralizing antibody titers

[0570] The neutralizing antibody titers produced for RSV A2 virus in the serum of cotton rats were detected on days 42 and 48 (see FIGs. 12 (A) and 12 (B), FIGs. 13 (A) and 13 (B), FIGs. 16 (A) and 16 (B), and FIGs. 17 (A) and 17 (B)). The detection results for the neutralizing antibody titers on day 42 are shown in the table below:

[0571] (1) On day 42 after the immunization, the neutralizing antibody titer IC₅₀ values of the following 11 test samples detected in the serum of cotton rats were 2.10 to 14.30 times that of the AbrysvoTM positive control, and IC₉₀ values were 2.26 to 22.33 times that of the AbrysvoTM positive control, all of which were significantly higher than those of the positive control.

[0572] The neutralizing antibody titer IC₅₀ values of YK-RSV-061, YK-RSV-003, and YK-RSV-062 were 8.84 to 14.30 times that of the AbrysvoTM positive control, and IC₉₀ values were 16.46 to 22.33 times that of the AbrysvoTM positive control.

[0573] Among them, IC₅₀ of YK-RSV-061 was 14.30 times that of the AbrysvoTM positive control, and IC₉₀ was 22.33 times that of the positive control, indicating the highest neutralizing antibody titers.

Table 26. Neutralizing antibody titers of RSV A2 in serum of cotton rats (day 42)

Test sample	IC ₅₀		IC ₉₀	
	Titer	Test sample IC ₅₀ /Abrysvo TM IC ₅₀ (times)	Titer	Test sample IC ₉₀ /Abrysvo TM IC ₉₀ (times)
YK-RSV-061	60321	14.30	5628	22.33
YK-RSV-003	46401	11.00	4852	19.25
YK-RSV-062	37265	8.84	4147	16.46
YK-RSV-054	24933	5.91	2238	8.88
YK-RSV-039	16421	3.89	1017	4.04
YK-RSV-044	14249	3.38	984	3.90
YK-RSV-012	10774	2.55	944	3.75

YK-RSV-015	9842	2.33	870	3.45
YK-RSV-001	9547	2.26	723	2.87
YK-RSV-036	9206	2.18	650	2.58
YK-RSV-060	8839	2.10	570	2.26
LNP	40	0.01	40	0.16
Abrysvo™	4217	1.00	252	1.00

[0574] (2) The neutralizing antibody titers produced for RSV B virus in the serum of cotton rats on day 42 were also detected. The results are shown in the table below and in FIGs. 14 (A) and 14 (B) and FIGs. 15 (A) and 15 (B):

[0575] The neutralizing antibody titer IC₅₀ values of the following 11 test samples were 2.16 to 14.63 times that of the Abrysvo™ positive control, and IC₉₀ values were 2.49 to 17.26 times that of the Abrysvo™ positive control, all of which were significantly higher than those of the positive control.

[0576] The neutralizing antibody titer IC₅₀ values of YK-RSV-061, YK-RSV-003, and YK-RSV-062 were 11.52 to 14.63 times that of the Abrysvo™ positive control, and IC₉₀ values were 13.44 to 17.26 times that of the Abrysvo™ positive control.

[0577] Among them, IC₅₀ of YK-RSV-061 was 14.63 times that of the Abrysvo™ positive control, and IC₉₀ was 17.26 times that of the positive control, indicating the highest neutralizing antibody titers.

Table 27. Neutralizing antibody titers of RSV B in serum of cotton rats (day 42)

Test sample	IC ₅₀		IC ₉₀	
	Titer	Test sample IC ₅₀ /Abrysvo™ IC ₅₀ (times)	Titer	Test sample IC ₉₀ /Abrysvo™ IC ₉₀ (times)
YK-RSV-061	94685	14.63	4384	17.26
YK-RSV-003	82331	12.72	3913	15.41
YK-RSV-062	74557	11.52	3414	13.44
YK-RSV-054	53282	8.23	2035	8.01
YK-RSV-039	38643	5.97	1289	5.07
YK-RSV-044	34308	5.30	1111	4.37
YK-RSV-012	31475	4.86	994	3.91
YK-RSV-015	25684	3.97	992	3.91
YK-RSV-001	21034	3.25	871	3.43
YK-RSV-036	16633	2.57	767	3.02
YK-RSV-060	13980	2.16	632	2.49
LNP	40	0.01	40	0.16
Abrysvo™	6472	1.00	254	1.00

[0578] (3) On day 48 after the immunization, the neutralizing antibody titers produced for RSV A2 virus in the serum of cotton rats were detected. The detection results are shown in the table below:

[0579] The neutralizing antibody titer IC₅₀ values of the following 11 test samples were 1.70 to 7.57 times that of the Abrysvo™ positive control, and IC₉₀ values were 1.46 to 11.56 times that of the Abrysvo™ positive control, all of which were higher than those of the positive control.

[0580] The neutralizing antibody titer IC₅₀ values of YK-RSV-061, YK-RSV-003, and YK-RSV-062 were 4.51 to 7.57 times that of the Abrysvo™ positive control, and IC₉₀ values were 8.75 to 11.56 times that of

the Abrysvo™ positive control.

[0581] Among them, IC₅₀ of YK-RSV-061 was 7.57 times that of the Abrysvo™ positive control, and IC₉₀ was 11.56 times that of the positive control, indicating the highest neutralizing antibody titers.

Table 28. Neutralizing antibody titers of RSV A2 in serum of cotton rats (day 48)

Test sample	IC ₅₀		IC ₉₀	
	Titer	Test sample IC ₅₀ /Abrysvo™ IC ₅₀ (times)	Titer	Test sample IC ₉₀ /Abrysvo™ IC ₉₀ (times)
YK-RSV-061	40016	7.57	4185	11.56
YK-RSV-003	24244	4.59	3377	9.33
YK-RSV-062	23814	4.51	3168	8.75
YK-RSV-054	15798	2.99	1612	4.45
YK-RSV-039	13376	2.53	1581	4.37
YK-RSV-044	12567	2.38	1418	3.92
YK-RSV-012	11331	2.14	913	2.52
YK-RSV-015	10210	1.93	759	2.10
YK-RSV-001	10436	1.98	735	2.03
YK-RSV-036	9399	1.78	655	1.81
YK-RSV-060	8995	1.70	529	1.46
LNP	40	0.01	40	0.11
Abrysvo™	5284	1.00	362	1.00

[0582] In summary, on days 42 and 48 after the immunization of cotton rats, the binding antibody and neutralizing antibody titer levels produced by all the test samples were significantly higher than those of the control Abrysvo™, and the humoral immunity effect was significantly improved. Among them, the test sample YK-RSV-061 had the best immune effect. All the test samples also had a good immune effect on different subtypes of virus, including RSV types A and B.

[0583] 2.7.3 Results for pathological sections of the lungs of cotton rats

[0584] Pathological sections of the lungs of cotton rats are shown in FIG. 18, and the scoring results are shown in the table below:

Table 29. Scoring results for pathological sections of the lungs of cotton rats

Test sample	Perivascular infiltration	Peribronchiolar infiltration	Alveolar infiltration	Interstitial infiltration	Total score
YK-RSV-061	0.00	0.33	0.00	0.67	1.00
YK-RSV-003	0.00	0.50	0.00	0.83	1.33
YK-RSV-062	0.00	0.50	0.00	1.00	1.50
YK-RSV-054	0.00	0.50	0.00	1.00	1.50
YK-RSV-039	0.00	0.67	0.00	1.00	1.67
YK-RSV-044	0.00	0.67	0.00	1.17	1.84
YK-RSV-012	0.00	0.67	0.00	1.00	1.67
YK-RSV-015	0.00	0.33	0.00	1.00	1.33
YK-RSV-001	0.00	0.83	0.00	1.00	1.83
YK-RSV-036	0.00	0.83	0.00	1.17	2.00
YK-RSV-060	0.00	0.67	0.00	0.83	1.50

LNP	0.00	2.33	0.00	1.50	3.83
Abrysvo™	0.00	0.67	0.00	1.33	2.00

[0585] From the above results, it could be seen that the peribronchiolar infiltration scores of the test samples were all 0.33 to 0.83, indicating mild inflammatory infiltration.

[0586] All the test samples had interstitial infiltration scores superior to those of the positive control Abrysvo™, and the scores were 0.67 to 1.17, indicating mild inflammatory infiltration.

[0587] All the test samples had low overall inflammatory response scores, and the total pathological scores were 1.00 to 2.00, all of which were lower than that of the control LNP. The scores of two indicators of perivascular infiltration and alveolar infiltration of the control and the test samples were all 0, indicating that there were no visible lesions in the tissue.

[0588] 2.7.4 Results for viral loads in the lungs and turbinates of cotton rats

[0589] After the cotton rats were sacrificed on day 54, the lung and turbinate tissues were aseptically taken for the determination of viral titers. The results are shown in the table below.

Table 30. Viral loads in the lungs and turbinates of cotton rats (day 54)

Test sample	Viral load in the lungs		Viral load in the turbinate	
	GMT	Test sample viral load/Abrysvo™ viral load (times)	GMT	Test sample viral load/Abrysvo™ viral load (times)
YK-RSV-061	1.61	0.75	0.62	0.19
YK-RSV-003	1.69	0.79	0.80	0.24
YK-RSV-062	1.85	0.86	0.94	0.28
YK-RSV-054	1.94	0.91	1.10	0.33
YK-RSV-039	1.82	0.85	1.08	0.32
YK-RSV-044	1.91	0.89	1.44	0.43
YK-RSV-012	1.84	0.86	1.40	0.42
YK-RSV-015	2.06	0.96	1.44	0.43
YK-RSV-001	1.85	0.86	1.27	0.38
YK-RSV-036	1.89	0.88	1.40	0.42
YK-RSV-060	2.08	0.97	1.51	0.45
LNP	4.52	2.11	4.12	1.23
Abrysvo™	2.14	1.00	3.35	1.00

[0590] The viral loads in the lungs of cotton rats were determined, and the results showed that the tissue viral load of the negative control LNP after the immunization was 4.52, which was significantly higher than those of the positive control Abrysvo™ and the other test samples. The tissue viral load of the control Abrysvo™ after the immunization was 2.14, and the tissue viral loads of the other test samples after the immunization were 1.61 to 2.08, which were lower than that of the positive control Abrysvo™.

[0591] The viral loads in the turbinates of cotton rats were determined, and the results showed that the tissue viral load of the negative control LNP after the immunization was 4.12, which was significantly higher than those of the other test samples. The tissue viral load of the control Abrysvo™ after the immunization was 3.35, the tissue viral load of the other test samples after the immunization was 0.62 to 1.51, which was only 0.19 to 0.45 times that of the control Abrysvo™, with a significant reduction in the viral load, indicating that the test samples had a better antiviral effect.

[0592] 2.8 Analysis of mutant differences

[0593] In summary, in the present disclosure, by designing 52 antigen sequences encoding the RSV F protein mutants, a total of at least 11 (YK-RSV-061, YK-RSV-003, YK-RSV-062, YK-RSV-054, YK-RSV-039, YK-RSV-044, YK-RSV-012, YK-RSV-015, YK-RSV-001, YK-RSV-036, and YK-RSV-060), particularly at least 5 (YK-RSV-061, YK-RSV-003, YK-RSV-062, YK-RSV-054, and YK-RSV-039) of these sequences were selected, which had superior effects to those of the positive control Abrysvo™ and the designed controls PC-RSV-063, PC-RSV-064, PC-RSV-065, PC-RSV-066, PC-RSV-067, and PC-RSV-068.

[0594] (1)

Table 31. Comparison of mutant differences

No.	Sequence truncation	Sequence deletion	Site mutation				
			Group 1	Group 2	Group 3	Group 4	Group 5
YK-RSV-003	550 to 574 aa were truncated	104 to 144 aa were deleted, with amino acids replaced by a GS linker	A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	/
YK-RSV-061	based on the wild-type RSV F protein, that is, (1		A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	V152I
YK-RSV-062	to 549 aa) were retained		A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	C69Y, D73E, A74V, N88S, V152I

Table 32. ELISA results of RSV F mutants

No.	ELISA results (ng/mL)									
	RSV pre-F antibody (3C12)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV pre-F antibody (7H11)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV F antibody (7E11)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV F antibody (11A9)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV F antibody (4B9)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody
	Epitope Φ		Epitope Quaternary		Epitope V		Epitope II		Epitope III	
YK-RSV-003	5541	2.00	7249	2.01	452	1.41	1994	0.95	3171	1.11
YK-RSV-061	5261	1.90	7661	2.12	595	1.85	2505	1.19	3683	1.28
YK-RSV-062	5059	1.83	7350	2.03	487	1.52	2063	0.98	3260	1.14
PC-RSV-064	2768	1.00	3612	1.00	321	1.00	2100	1.00	2868	1.00

Table 33. Neutralizing antibody titers of RSV A2 in serum of mice (day 35)

Test sample	IC ₅₀		IC ₉₀	
	Titer	Test sample IC ₅₀ /Abrysvo™ IC ₅₀ (times)	Titer	Test sample IC ₉₀ /Abrysvo™ IC ₉₀ (times)
YK-RSV-061	51997	12.67	3472	14.41
YK-RSV-003	30330	7.39	2311	9.59
YK-RSV-062	26022	6.34	2305	9.56
LNP	40	0.01	40	0.17
Abrysvo™	4103	1.00	241	1.00

Table 34. Neutralizing antibody titers of RSV B18357 in serum of mice (day 35)

Test sample	IC ₅₀		IC ₉₀	
	Titer	Test sample IC ₅₀ /Abrysvo™ IC ₅₀ (times)	Titer	Test sample IC ₉₀ /Abrysvo™ IC ₉₀ (times)
YK-RSV-061	34877	10.63	3088	14.23
YK-RSV-003	23943	7.30	1712	7.89
YK-RSV-062	18615	5.68	1490	6.87
LNP	40	0.01	40	0.18
Abrysvo™	3280	1.00	217	1.00

[0595] Compared with the YK-RSV-003 mutant, YK-RSV-061 had 1 additional site mutation V152I on its basis; YK-RSV-062 had 5 additional site mutations C69Y, D73E, A74V, N88S, and V152I on its basis;

[0596] epitopes on three mutants of YK-RSV-003, YK-RSV-061, and YK-RSV-062 all had good binding levels to the corresponding antibodies. For example, the binding levels of the epitope Quaternary on YK-RSV-061, YK-RSV-003, and YK-RSV-062 to the RSV pre-F antibody (7H11) were 2.01 to 2.12 times that on the control PC-RSV-064.

[0597] However, the neutralizing antibody titers IC₅₀ and IC₉₀ of YK-RSV-061 for RSV type A virus were 12.67 and 14.41 times those of the Abrysvo™ positive control, respectively, while the neutralizing antibody titers IC₅₀ and IC₉₀ of YK-RSV-003 were 7.39 and 9.59 times those of the Abrysvo™ positive control. Although the two differed by only 1 site mutation V152I, the neutralizing antibody titers of YK-RSV-061 were significantly increased, which was an unexpected result. The neutralizing antibody titers of YK-RSV-062 were comparable to those of YK-RSV-003. Similarly, the neutralizing antibody titers of YK-RSV-061 for RSV type B virus were also significantly superior.

[0598] (2)

Table 35. Comparison of mutant differences

No.	Sequence truncation	Sequence deletion	Site mutation				
			Group 1	Group 2	Group 3	Group 4	Group 5
YK-RSV-003	550 to 574 aa were truncated based on the wild-type RSV F protein, that is, (1 to 549 aa) were retained	104 to 144 aa, amino acids deleted from the sequence were replaced by a GS linker	A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	/
YK-RSV-061			A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	V152I
YK-RSV-062			A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	C69Y,D73E,A74V,N88S,V152I
PC-RSV-063			A149C, S155C, S290C Y458C,	S190F, V207L	E92D, K465Q	S46G, S215P, L373R	/
PC-RSV-064			A149C, S155C, S290C, Y458C	S190F	E92D, K465Q	S46G, S215P, L373R	V152I
PC-RSV-065			A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q	S46G, S215P, L373R	V152I
PC-RSV-066			A149C, S155C, S290C, Y458C	S190F	E92D, K465Q, D486S	S46G, S215P, L373R	V152I
PC-RSV-067			T103C, I148C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	V152I
PC-RSV-068			S55C, L188C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	V152I

Table 36. ELISA results n of RSV F mutants

No.	ELISA results (ng/mL)									
	RSV pre-F antibody (3C12)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV pre-F antibody (7H11)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV F antibody (7E11)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV F antibody (11A9)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody	RSV F antibody (4B9)	Binding amount to antibody/ binding amount of PC-RSV-064 to antibody
	Epitope Φ		Epitope Quaternary		Epitope V		Epitope II		Epitope III	
YK-RSV-061	5261	1.90	7661	2.12	595	1.85	2505	1.19	3683	1.28
YK-RSV-003	5541	2.00	7249	2.01	452	1.41	1994	0.95	3171	1.11
YK-RSV-062	5059	1.83	7350	2.03	487	1.52	2063	0.98	3260	1.14
PC-RSV-063	2727	0.99	3557	0.98	461	1.44	2053	0.98	2993	1.04
PC-RSV-064	2768	1.00	3612	1.00	321	1.00	2100	1.00	2868	1.00
PC-RSV-065	3378	1.22	3705	1.03	436	1.36	1864	0.89	3102	1.08
PC-RSV-066	2169	0.78	3213	0.89	374	1.17	1682	0.80	2058	0.72
PC-RSV-067	2828	1.02	2869	0.79	298	0.93	2509	1.19	3608	1.26
PC-RSV-068	3168	1.14	3667	1.02	312	0.97	3191	1.52	4321	1.51

Table 37. Results for affinity of RSV F mutants for different epitope antibodies

No.	K_D value (M)	
	RSV pre-F antibody (3C12)	RSV pre-F antibody (7H11)
	Epitope Φ	Epitope Quaternary
YK-RSV-061	6.21E-11	3.05E-12
YK-RSV-003	6.35E-11	3.47E-12
YK-RSV-062	6.26E-11	3.62E-12
PC-RSV-063	1.36E-10	1.07E-10
PC-RSV-064	1.24E-10	1.56E-10
PC-RSV-065	1.62E-10	1.22E-10
PC-RSV-066	2.63E-10	1.33E-10
PC-RSV-067	5.29E-10	3.06E-10
PC-RSV-068	6.61E-10	2.25E-10

[0599] Compared with the YK-RSV-003 mutant, the control PC-RSV-063 had 1 mutation type in group 2 of site mutations changed, from V296I to V207L; and a D486S site mutation in group 3 of site mutations deleted. However, the binding level of the epitope Quaternary on YK-RSV-003 to the RSV pre-F antibody (7H11) was 2.04 times that of the control PC-RSV-063, with a significant difference in binding levels.

[0600] Compared with the YK-RSV-061 mutant, the controls PC-RSV-065 and PC-RSV-066 each had 1 site mutation (D486S or V296I) reduced, the control PC-RSV-064 had 2 site mutations (D486S and V296I) reduced, and the controls PC-RSV-067 and PC-RSV-068 had the mutation type in group 1 of site mutations changed. The results showed that: the affinity constant K_D for the binding of the epitope Φ on the YK-RSV-061 mutant to the RSV pre-F antibody (3C12) was 6.21E-11 M; the affinity constant K_D for the binding of the epitope Quaternary to the RSV pre-F antibody (7H11) was 3.05E-12 M, and the K_D values for the other controls were all E-10 M, with a difference of 1 to 2 orders of magnitude, indicating that the affinity of the antigen YK-RSV-061 for the antibodies was higher than that of the other controls.

[0601] From the above comparison, it could be seen that the binding level and the binding force of the epitope on the YK-RSV-003 mutant to a corresponding antibody were both significantly higher than those

of the controls PC-RSV-063 to PC-RSV-068; meanwhile, in conjunction with the comparison results in section (1), the YK-RSV-061 mutant had a significantly superior effect to the YK-RSV-003 mutant, and the YK-RSV-062 had a comparable effect to the YK-RSV-003, so that YK-RSV-061, YK-RSV-003, and YK-RSV-062 were all superior to the controls PC-RSV-063 to PC-RSV-068.

[0602] The table below shows the complete sequence information and the serial numbers thereof in the present application. Taking the antigen sequence of YK-RSV-001 as an example, SEQ ID NOs: 3 and 4 are the DNA sequence and amino acid sequence of the open reading frame of the antigen YK-RSV-001, respectively; SEQ ID NOs: 2 and 5 are the DNA sequence and mRNA sequence of the antigen YK-RSV-001, respectively.

Table 38. Sequence numbers, names, and types thereof in the present application

SEQ ID NO:	Name	Sequence type
1	RSV A2 (138251)-wt	AA
2	YK-RSV-001-DNA	DNA
3	YK-RSV-001- ORF-NT	ORF-NT
4	YK-RSV-001- ORF-AA	ORF-AA
5	YK-RSV-001- mRNA	mRNA
6	YK-RSV-002- DNA	DNA
7	YK-RSV-002- ORF-NT	ORF-NT
8	YK-RSV-002- ORF-AA	ORF-AA
9	YK-RSV-002-mRNA	mRNA
10	YK-RSV-003-DNA	DNA
11	YK-RSV-003-ORF-NT	ORF-NT
12	YK-RSV-003-ORF-AA	ORF-AA
13	YK-RSV-003-mRNA	mRNA
14	YK-RSV-004-DNA	DNA
15	YK-RSV-004- ORF-NT	ORF-NT
16	YK-RSV-004- ORF-AA	ORF-AA
17	YK-RSV-004- mRNA	mRNA
18	YK-RSV-005-DNA	DNA
19	YK-RSV-005- ORF-NT	ORF-NT
20	YK-RSV-005- ORF-AA	ORF-AA
21	YK-RSV-005- mRNA	mRNA
22	YK-RSV-008-DNA	DNA
23	YK-RSV-008-ORF-NT	ORF-NT
24	YK-RSV-008-ORF-AA	ORF-AA
25	YK-RSV-008-mRNA	mRNA
26	YK-RSV-009-DNA	DNA

27	YK-RSV-009-ORF-NT	ORF-NT
28	YK-RSV-009-ORF-AA	ORF-AA
29	YK-RSV-009-mRNA	mRNA
30	YK-RSV-010-DNA	DNA
31	YK-RSV-010-ORF-NT	ORF-NT
32	YK-RSV-010-ORF-AA	ORF-AA
33	YK-RSV-010-mRNA	mRNA
34	YK-RSV-011-DNA	DNA
35	YK-RSV-011-ORF-NT	ORF-NT
36	YK-RSV-011-ORF-AA	ORF-AA
37	YK-RSV-011-mRNA	mRNA
38	YK-RSV-012-DNA	DNA
39	YK-RSV-012-ORF-NT	ORF-NT
40	YK-RSV-012-ORF-AA	ORF-AA
41	YK-RSV-012-mRNA	mRNA
42	YK-RSV-013-DNA	DNA
43	YK-RSV-013-ORF-NT	ORF-NT
44	YK-RSV-013-ORF-AA	ORF-AA
45	YK-RSV-013-mRNA	mRNA
46	YK-RSV-014-DNA	DNA
47	YK-RSV-014-ORF-NT	ORF-NT
48	YK-RSV-014-ORF-AA	ORF-AA
49	YK-RSV-014-mRNA	mRNA
50	YK-RSV-015-DNA	DNA
51	YK-RSV-015-ORF-NT	ORF-NT
52	YK-RSV-015-ORF-AA	ORF-AA
53	YK-RSV-015-mRNA	mRNA
54	YK-RSV-016-DNA	DNA
55	YK-RSV-016-ORF-NT	ORF-NT
56	YK-RSV-016-ORF-AA	ORF-AA
57	YK-RSV-016-mRNA	mRNA
58	YK-RSV-017-DNA	DNA
59	YK-RSV-017-ORF-NT	ORF-NT
60	YK-RSV-017-ORF-AA	ORF-AA
61	YK-RSV-017-mRNA	mRNA
62	YK-RSV-018-DNA	DNA
63	YK-RSV-018-ORF-NT	ORF-NT

64	YK-RSV-018-ORF-AA	ORF-AA
65	YK-RSV-018-mRNA	mRNA
66	YK-RSV-019-DNA	DNA
67	YK-RSV-019-ORF-NT	ORF-NT
68	YK-RSV-019-ORF-AA	ORF-AA
69	YK-RSV-019-mRNA	mRNA
70	YK-RSV-022-DNA	DNA
71	YK-RSV-022-ORF-NT	ORF-NT
72	YK-RSV-022-ORF-AA	ORF-AA
73	YK-RSV-022-mRNA	mRNA
74	YK-RSV-029-DNA	DNA
75	YK-RSV-029-ORF-NT	ORF-NT
76	YK-RSV-029-ORF-AA	ORF-AA
77	YK-RSV-029-mRNA	mRNA
78	YK-RSV-030-DNA	DNA
79	YK-RSV-030-ORF-NT	ORF-NT
80	YK-RSV-030-ORF-AA	ORF-AA
81	YK-RSV-030-mRNA	mRNA
82	YK-RSV-031-DNA	DNA
83	YK-RSV-031-ORF-NT	ORF-NT
84	YK-RSV-031-ORF-AA	ORF-AA
85	YK-RSV-031-mRNA	mRNA
86	YK-RSV-032-DNA	DNA
87	YK-RSV-032-ORF-NT	ORF-NT
88	YK-RSV-032-ORF-AA	ORF-AA
89	YK-RSV-032-mRNA	mRNA
90	YK-RSV-033-DNA	DNA
91	YK-RSV-033-ORF-NT	ORF-NT
92	YK-RSV-033-ORF-AA	ORF-AA
93	YK-RSV-033-mRNA	mRNA
94	YK-RSV-034-DNA	DNA
95	YK-RSV-034-ORF-NT	ORF-NT
96	YK-RSV-034-ORF-AA	ORF-AA
97	YK-RSV-034-mRNA	mRNA
98	YK-RSV-035-DNA	DNA
99	YK-RSV-035-ORF-NT	ORF-NT
100	YK-RSV-035-ORF-AA	ORF-AA

101	YK-RSV-035-mRNA	mRNA
102	YK-RSV-036-DNA	DNA
103	YK-RSV-036-ORF-NT	ORF-NT
104	YK-RSV-036-ORF-AA	ORF-AA
105	YK-RSV-036-mRNA	mRNA
106	YK-RSV-037-DNA	DNA
107	YK-RSV-037-ORF-NT	ORF-NT
108	YK-RSV-037-ORF-AA	ORF-AA
109	YK-RSV-037-mRNA	mRNA
110	YK-RSV-038-DNA	DNA
111	YK-RSV-038-ORF-NT	ORF-NT
112	YK-RSV-038-ORF-AA	ORF-AA
113	YK-RSV-038-mRNA	mRNA
114	YK-RSV-039-DNA	DNA
115	YK-RSV-039-ORF-NT	ORF-NT
116	YK-RSV-039-ORF-AA	ORF-AA
117	YK-RSV-039-mRNA	mRNA
118	YK-RSV-040-DNA	DNA
119	YK-RSV-040-ORF-NT	ORF-NT
120	YK-RSV-040-ORF-AA	ORF-AA
121	YK-RSV-040-mRNA	mRNA
122	YK-RSV-041-DNA	DNA
123	YK-RSV-041-ORF-NT	ORF-NT
124	YK-RSV-041-ORF-AA	ORF-AA
125	YK-RSV-041-mRNA	mRNA
126	YK-RSV-042-DNA	DNA
127	YK-RSV-042-ORF-NT	ORF-NT
128	YK-RSV-042-ORF-AA	ORF-AA
129	YK-RSV-042-mRNA	mRNA
130	YK-RSV-043-DNA	DNA
131	YK-RSV-043-ORF-NT	ORF-NT
132	YK-RSV-043-ORF-AA	ORF-AA
133	YK-RSV-043-mRNA	mRNA
134	YK-RSV-044-DNA	DNA
135	YK-RSV-044-ORF-NT	ORF-NT
136	YK-RSV-044-ORF-AA	ORF-AA
137	YK-RSV-044-mRNA	mRNA

138	YK-RSV-045-DNA	DNA
139	YK-RSV-045-ORF-NT	ORF-NT
140	YK-RSV-045-ORF-AA	ORF-AA
141	YK-RSV-045-mRNA	mRNA
142	YK-RSV-046-DNA	DNA
143	YK-RSV-046-ORF-NT	ORF-NT
144	YK-RSV-046-ORF-AA	ORF-AA
145	YK-RSV-046-mRNA	mRNA
146	YK-RSV-047-DNA	DNA
147	YK-RSV-047-ORF-NT	ORF-NT
148	YK-RSV-047-ORF-AA	ORF-AA
149	YK-RSV-047-mRNA	mRNA
150	YK-RSV-048-DNA	DNA
151	YK-RSV-048-ORF-NT	ORF-NT
152	YK-RSV-048-ORF-AA	ORF-AA
153	YK-RSV-048-mRNA	mRNA
154	YK-RSV-049-DNA	DNA
155	YK-RSV-049-ORF-NT	ORF-NT
156	YK-RSV-049-ORF-AA	ORF-AA
157	YK-RSV-049-mRNA	mRNA
158	YK-RSV-050-DNA	DNA
159	YK-RSV-050-ORF-NT	ORF-NT
160	YK-RSV-050-ORF-AA	ORF-AA
161	YK-RSV-050-mRNA	mRNA
162	YK-RSV-051-DNA	DNA
163	YK-RSV-051-ORF-NT	ORF-NT
164	YK-RSV-051-ORF-AA	ORF-AA
165	YK-RSV-051-mRNA	mRNA
166	YK-RSV-052-DNA	DNA
167	YK-RSV-052-ORF-NT	ORF-NT
168	YK-RSV-052-ORF-AA	ORF-AA
169	YK-RSV-052-mRNA	mRNA
170	YK-RSV-053-DNA	DNA
171	YK-RSV-053-ORF-NT	ORF-NT
172	YK-RSV-053-ORF-AA	ORF-AA
173	YK-RSV-053-mRNA	mRNA
174	YK-RSV-054-DNA	DNA

175	YK-RSV-054-ORF-NT	ORF-NT
176	YK-RSV-054-ORF-AA	ORF-AA
177	YK-RSV-054-mRNA	mRNA
178	YK-RSV-055-DNA	DNA
179	YK-RSV-055-ORF-NT	ORF-NT
180	YK-RSV-055-ORF-AA	ORF-AA
181	YK-RSV-055-mRNA	mRNA
182	YK-RSV-056-DNA	DNA
183	YK-RSV-056-ORF-NT	ORF-NT
184	YK-RSV-056-ORF-AA	ORF-AA
185	YK-RSV-056-mRNA	mRNA
186	YK-RSV-057-DNA	DNA
187	YK-RSV-057-ORF-NT	ORF-NT
188	YK-RSV-057-ORF-AA	ORF-AA
189	YK-RSV-057-mRNA	mRNA
190	YK-RSV-058-DNA	DNA
191	YK-RSV-058-ORF-NT	ORF-NT
192	YK-RSV-058-ORF-AA	ORF-AA
193	YK-RSV-058-mRNA	mRNA
194	YK-RSV-059-DNA	DNA
195	YK-RSV-059-ORF-NT	ORF-NT
196	YK-RSV-059-ORF-AA	ORF-AA
197	YK-RSV-059-mRNA	mRNA
198	YK-RSV-060-DNA	DNA
199	YK-RSV-060-ORF-NT	ORF-NT
200	YK-RSV-060-ORF-AA	ORF-AA
201	YK-RSV-060-mRNA	mRNA
202	YK-RSV-061-DNA	DNA
203	YK-RSV-061-ORF-NT	ORF-NT
204	YK-RSV-061-ORF-AA	ORF-AA
205	YK-RSV-061-mRNA	mRNA
206	YK-RSV-062-DNA	DNA
207	YK-RSV-062-ORF-NT	ORF-NT
208	YK-RSV-062-ORF-AA	ORF-AA
209	YK-RSV-062-mRNA	mRNA
210	RSV A2 (138251)-mt	AA
211	PC-RSV-063	AA

212	PC-RSV-064	AA
213	PC-RSV-065	AA
214	PC-RSV-066	AA
215	PC-RSV-067	AA
216	PC-RSV-068	AA
217	5' UTR	/
218	5' UTR	/
219	5' UTR	/
220	5' UTR	/
221	5' UTR	/
222	3' UTR	/
223	3' UTR	/
224	3' UTR	/
225	3' UTR	/

What is claimed is:

1. An immunogenic composition, comprising a ribonucleic acid (RNA) of a respiratory syncytial virus (RSV), wherein the RNA encodes a variant of a wild-type RSV F protein;

wherein a sequence of the wild-type RSV F protein is SEQ ID NO: 1;

the variant of the RSV F protein comprises mutations P102A, I379V, and M447V, the variant of the RSV F protein is a truncated protein in which amino acids at positions 550 to 574 of SEQ ID NO: 1 are truncated, and amino acids at positions 104 to 144 are substituted by a GS linker, and the variant of the RSV F protein further comprises a combination of site mutations selected from any one of the following groups of Group 1 to Group 5, therefore obtain the variant of the RSV F protein selected from YK-RSV-061, YK-RSV-003 and/or YK-RSV-062:

Protein variant No.	Site mutation				
	Group 1	Group 2	Group 3	Group 4	Group 5
YK-RSV-061	A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	V152I
YK-RSV-003	A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	/
YK-RSV-062	A149C, S155C, S290C, Y458C	S190F, V296I	E92D, K465Q, D486S	S46G, S215P, L373R	C69Y, D73E, A74V, N88S, V152I

2. The composition according to claim 1, wherein an amino acid sequence of the variant of the RSV F protein is: SEQ ID NO: 204, SEQ ID NO: 12 or SEQ ID NO: 208; or,

a nucleotide sequence of an open reading frame (ORF) encoding the variant of the RSV F protein is: SEQ ID NO: 203, SEQ ID NO: 11 or SEQ ID NO: 207; or,

a sequence of the RNA of the RSV is: SEQ ID NO: 205, SEQ ID NO: 13 or SEQ ID NO: 209; or,

a DNA sequence encoding the variant of the RSV F protein is: SEQ ID NO: 202, SEQ ID NO: 10 or SEQ ID NO: 206.

3. The composition according to claim 1, wherein the RNA of the RSV further comprises a 5' untranslated region (UTR).

4. The composition according to claim 3, wherein a sequence of the 5' untranslated region is: SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, or SEQ ID NO: 221.

5. The composition according to claim 1, wherein the RNA of the RSV further comprises a 3' untranslated region (UTR).

6. The composition according to claim 5, wherein a sequence of the 3' untranslated region is: SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224, or SEQ ID NO: 225.

7. The composition according to claim 1, wherein the RNA of the RSV further comprises a poly(A) tail.

8. The composition according to claim 1, wherein the RNA of the RSV further comprises a 5' cap structure.

9. The composition according to claim 8, wherein the 5' cap structure is: 7mG(5')ppp(5')NlmpNp.

10. The composition according to claim 1, wherein a sequence of an open reading frame (ORF) encoding the RSV F protein or the variant thereof in the RSV RNA is codon-optimized.

11. The composition according to claim 10, wherein the sequence of the ORF comprises at least one

base modification.

12. The composition according to claim 11, wherein the base modification comprises any one or more selected from the following: pseudouridine, N1-methylpseudouridine, N1-ethylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-1-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methoxyuridine, and 2'-O-methyluridine.

13. The composition according to claim 1, wherein the composition is a vaccine and further comprises a pharmaceutically acceptable excipient.

14. The composition according to claim 13, wherein the pharmaceutically acceptable excipient comprises a lipid mixture, and the lipid mixture is a lipid nanoparticle (LNP).

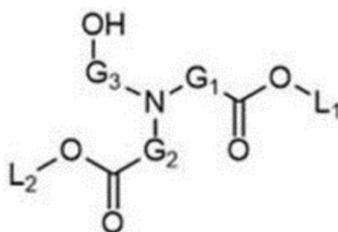
15. The composition according to claim 13, wherein the vaccine is an mRNA vaccine; or,

an effective dose of the RSV RNA is 25 to 200 µg; or,

an effective dose of the RSV RNA is 50 to 100 µg.

16. The composition according to claim 14, wherein the lipid nanoparticle comprises a cationic lipid, a neutral lipid, a structural lipid, and a polymer-conjugated lipid.

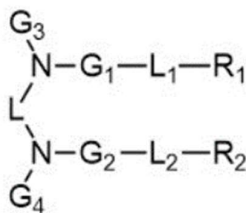
17. The composition according to claim 16, wherein the cationic lipid is a compound with a structure of formula I, or a N-oxide, a solvate, a pharmaceutically acceptable salt or a stereoisomer thereof, wherein G₁ is C₁₋₆ alkylene; G₂ is C₂₋₈ alkylene; G₃ is C₁₋₃ alkylene; L₁ is C₆₋₁₅ linear alkyl; L₂ is C₁₂₋₂₅ branched alkyl



formula I

; or,

the cationic lipid is a compound with a structure of formula II, or a N-oxide, a solvate, a pharmaceutically acceptable salt or a stereoisomer thereof, wherein G₁ is C₂₋₈ alkylene; G₂ is C₂₋₈ alkylene; L₁ is -C(O)O- or -OC(O)-; L₂ is -C(O)O- or -OC(O)-; R₁ is C₆₋₂₅ linear or branched alkyl; R₂ is C₆₋₂₅ linear or branched alkyl; G₃ is HO(CH₂)₂- or HO(CH₂)₃-; G₄ is HO(CH₂)₂- or HO(CH₂)₃-; L is (CH₂)₂- or -(CH₂)₃- or -(CH₂)₄-

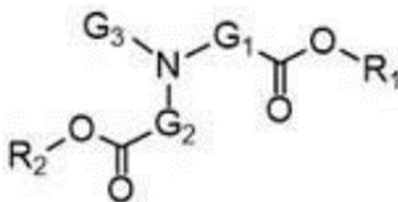


formula II

; or,

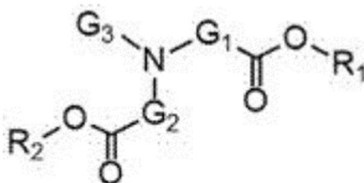
the cationic lipid is a compound with structure of formula III, or a N-oxide, a solvate, a pharmaceutically

acceptable salt or a stereoisomer thereof, wherein G_1 is C_{1-6} alkylene; G_2 is C_{2-8} alkylene; R_1 is C_{6-20} linear or branched alkyl; R_2 is C_{12-25} branched alkyl; G_3 is: $HO(CH_2)_2N(CH_3)(CH_2)_2-$, $HO(CH_2)_2N(CH_2CH_3)(CH_2)_2-$, $(HO(CH_2)_2)_2N(CH_2)_2-$, $CH_3O(CH_2)_2N(CH_3)(CH_2)_2-$, $(CH_3)_2N(CH_2)_3SC(O)O(CH_2)_2-$, $(CH_3)_2N(CH_2)_3SC(O)-$, $CH_3NH(CH_2)_2N(CH_3)(CH_2)_2-$, or $CH_3CH_2NH(CH_2)_2-$



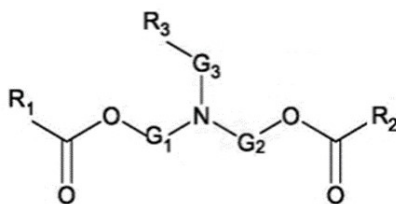
formula III ; or,

the cationic lipid is a compound with a structure of formula IV, or a N-oxide, a solvate, a pharmaceutically acceptable salt or a stereoisomer thereof, wherein G_1 is C_{1-8} alkylene; G_2 is C_{2-8} alkylene; R_1 is C_{6-25} linear or branched alkyl; R_2 is C_{12-25} linear or branched alkyl; G_3 is $HO(CH_2)_2N(R_3)CH_2CH(OH)CH_2-$, wherein R_3 is $-CH_3$ or $-CH_2CH_3$ or $-CH_2CH_2OH$



formula IV ; or,

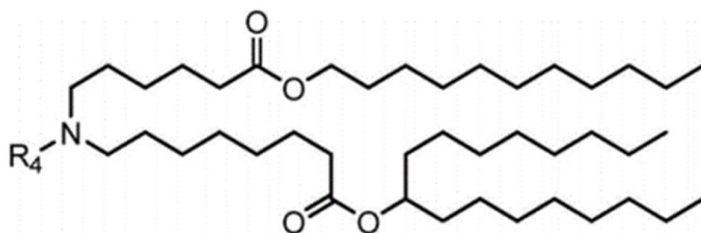
the cationic lipid is a compound with a structure of formula V, or a N-oxide, a solvate, a pharmaceutically acceptable salt or a stereoisomer thereof, wherein G_1 and G_2 are each independently unsubstituted C_6-C_{10} alkylene; G_3 is unsubstituted C_1-C_{12} alkylene; R_1 and R_2 are each independently C_6-C_{24} alkyl or C_6-C_{24} alkenyl; R_3 is OR_5 , N , $-C(=O)OR_4$, $-OC(=O)R_4$, or $-NR_5C(=O)R_4$; R_4 is C_1-C_{12} alkyl; and R_5 is H or C_1-C_6 alkyl



formula V ; or,

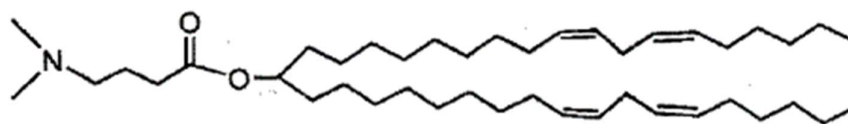
the cationic lipid is a compound with a structure of formula VI, or a N-oxide, a solvate, a pharmaceutically acceptable salt or a stereoisomer thereof, wherein R_4 is selected from $-(CH_2)_nQ$ and $-(CH_2)_nCHQR$; Q is selected from the group consisting of: $-OR$, $-OH$, $-O(CH_2)_nN(R)_2$, $-OC(O)R$, $-CX_3$, $-CN$, $-N(R)C(O)R$, $-N(H)C(O)R$, $-N(R)S(O)_2R$, $-N(H)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(H)C(O)N(R)_2$, $-$

$N(H)C(O)N(H)(R)$, $-N(R)C(S)N(R)_2$, $-N(H)C(S)N(R)_2$, $-N(H)C(S)N(H)(R)$, $-N(R)S(O)_2R_8$, and a heterocyclic ring; n is 1, 2, or 3; wherein R is hydrogen, C_{1-3} alkyl, C_{2-3} alkenyl, or $(CH_2)_qOR^*$, wherein q is 1, 2, or 3, R^* is C_{1-12} alkyl or C_{2-12} alkenyl; X is fluoro, chloro, bromo, or iodo; R_8 is C_{3-6} cycloalkyl or a heterocyclic ring



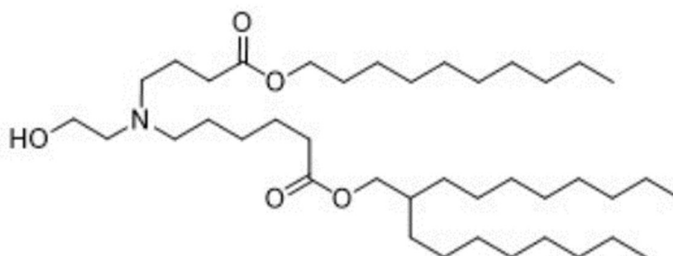
formula VI ; or,

the cationic lipid is a compound with a structure of formula VII, or a N-oxide, a solvate, a pharmaceutically acceptable salt, or a stereoisomer thereof:

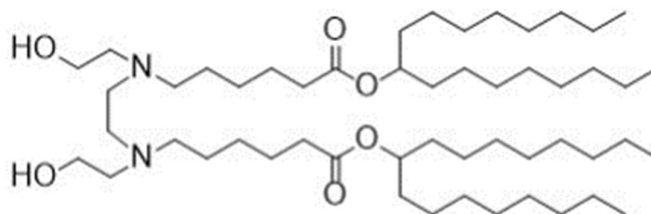


formula VII ; or,

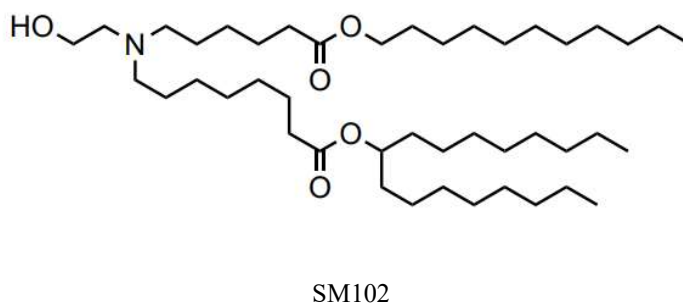
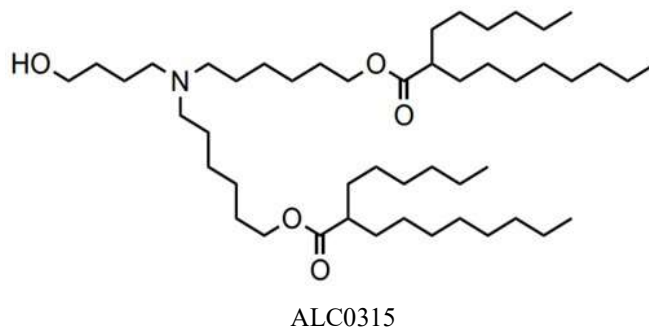
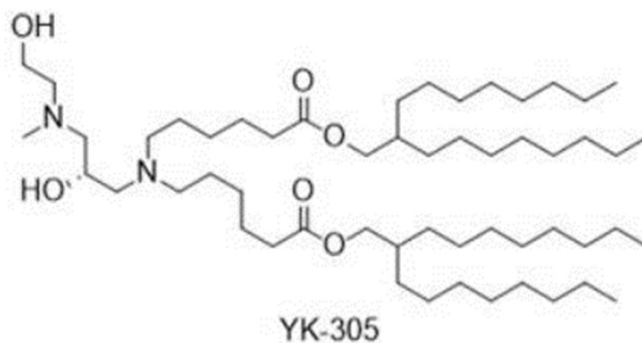
the cationic lipid is selected from the following compounds: YK-009, YK-401, YK-305, ALC0315, SM102, DLIN-MC3



YK-009



YK-401



18. The composition according to claim 16, wherein the cationic lipid and the neutral lipid are at a molar ratio of (1-10):1; or,

the cationic lipid and the structural lipid are at a molar ratio of (1-5):1.

19. The composition according to claim 16, wherein the cationic lipid, the neutral lipid, the structural lipid, and the polymer-conjugated lipid are at a molar ratio of (25-75):(5-25):(15-65):(0.5-10); or

the cationic lipid, the neutral lipid, the structural lipid, and the polymer-conjugated lipid are at a molar ratio of (35-49):(7.5-15):(35-55):(1-5).

20. The composition according to claim 16, wherein the neutral lipid is selected from one or more of phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, ceramide, sterol, and derivatives thereof; or

the neutral lipid is selected from one or more of the following: 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycero-phosphorylcholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecadienyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterolhemisuccinyl-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonyl-sn-glycero-3-phosphorylcholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-diphyanoyl-sn-glycero-3-phosphoethanolamine (ME 16:0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), dipalmitoyl phosphatidylglycerol (DPPG), palmitoyl oleoyl phosphatidylethanolamine (POPE), distearoyl-phosphatidyl-ethanolamine (DSPE), dipalmitoyl phosphatidylethanolamine (DPPE), dimyristoyl phosphatidylethanolamine (DMPE), 1-stearyl-2-oleoyl-stearoylethanolamine (SOPE), 1-stearyl-2-oleoyl-phosphatidylcholine (SOPC), sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyl oleoyl phosphatidylcholine, lysophosphatidylcholine, and lysophosphatidylethanolamine (LPE); or

the neutral lipid is DOPE and/or DSPC; or

the structural lipid is selected from one or more of the following: cholesterol, nonsterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, α -tocopherol and corticosteroid.

21. The composition according to claim 16, wherein the polymer-conjugated lipid is selected from one or more of the following: PEG-modified phosphatidylethanolamine, PEG-modified phosphatidic acid, PEG-modified ceramide, PEG-modified dialkylamine, PEG-modified diacylglycerol, PEG-modified dialkylglycerol.

22. The composition according to the preceding claim 21, wherein the polymer-conjugated lipid is selected from one or more of the following: distearoyl phosphatidylethanolamine polyethylene glycol 2000 (DSPE-PEG2000), dimyristoylglycerol-3-methoxy polyethylene glycol 2000 (DMG-PEG2000), and methoxypoly(ethylene glycol) ditetradecylacetamide (ALC-0159).

23. The composition according to any one of claims 13 to 22, wherein the vaccine is in a form of an injection.

24. A method for preparing the composition according to any one of claims 13 to 23, comprising: mixing the RNA of the RSV with the pharmaceutically acceptable excipient.

25. A use of the composition according to any one of claims 1 to 23 in the preparation of a vaccine for inducing a protective immune response to RSV in a subject.

26. A use of the composition according to any one of claims 1 to 23 in the preparation of a vaccine for

inducing a humoral immune response to RSV in a subject.

27. A use of the composition according to any one of claims 1 to 23 in the preparation of a vaccine for preventing an RSV infection.