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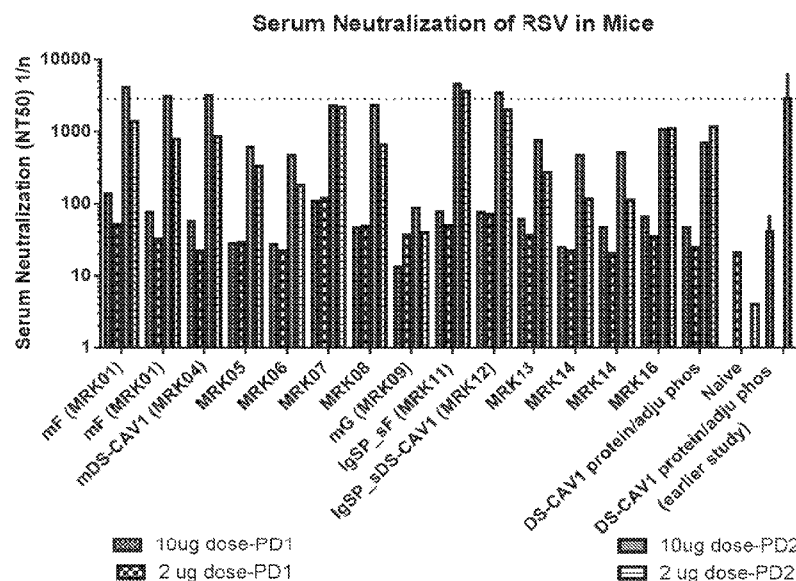
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(54) Title: RESPIRATORY SYNCYTIAL VIRUS VACCINE

Fig. 1



(57) Abstract: The disclosure relates to respiratory syncytial virus (RSV) ribonucleic acid (RNA) vaccines, as well as methods of using the vaccines and compositions comprising the vaccines.



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RESPIRATORY SYNCYTIAL VIRUS VACCINE

RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional
5 application number 62/471,801, filed March 15, 2017, which is incorporated by reference
herein in its entirety.

BACKGROUND

The human respiratory syncytial virus (RSV) is a negative-sense, single-stranded
10 RNA virus of the genus *Pneumovirinae* and of the family *Paramyxoviridae*. Symptoms in
adults typically resemble a sinus infection or the common cold, although the infection may be
asymptomatic. In older adults (*e.g.*, >60 years), RSV infection may progress to bronchiolitis
or pneumonia. Symptoms in children are often more severe, including bronchiolitis and
pneumonia. It is estimated that in the United States, most children are infected with RSV by
15 the age of three. The RSV virion consists of an internal nucleocapsid comprised of the viral
RNA bound to nucleoprotein (N), phosphoprotein (P), and large polymerase protein (L). The
nucleocapsid is surrounded by matrix protein (M) and is encapsulated by a lipid bilayer into
which the viral fusion (F) and attachment (G) proteins as well as the small hydrophobic
protein (SH) are incorporated. The viral genome also encodes two nonstructural proteins
20 (NS1 and NS2), which inhibit type I interferon activity as well as the M-2 protein.

Deoxyribonucleic acid (DNA) vaccination is one technique used to stimulate humoral
and cellular immune responses to foreign antigens, such as RSV antigens. The direct
injection of genetically engineered DNA (*e.g.*, naked plasmid DNA) into a living host results
in a small number of host cells directly producing an antigen, resulting in a protective
25 immunological response. With this technique, however, comes potential problems, including
the possibility of insertional mutagenesis, which could lead to the activation of oncogenes or
the inhibition of tumor suppressor genes.

SUMMARY

30 The RNA vaccines of the present disclosure may be used to induce a balanced
immune response against RSV, comprising both cellular and humoral immunity, without
risking the possibility of insertional mutagenesis, for example.

The RNA (*e.g.*, mRNA) vaccines may be utilized in various settings, depending on the prevalence of the infection, or the degree or level of unmet medical need. The RNA vaccines may be utilized to treat and/or prevent an infection by various genotypes, strains, and isolates of RSV. The RNA vaccines as provided herein have superior properties in that they produce much larger antibody titers and produce responses earlier than commercially-available anti-viral therapeutic treatments. While not wishing to be bound by theory, it is believed that the RNA vaccines of the present disclosure are better designed to produce the appropriate protein conformation upon translation, as the RNA vaccines co-opt natural cellular machinery. Unlike traditional vaccines, which are manufactured *ex vivo* and may trigger unwanted cellular responses, RNA vaccines as provided herein are presented to the cellular system in a more native fashion.

Some embodiments of the present disclosure provide respiratory syncytial virus (RSV) vaccines that include (i) at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding at least one RSV antigenic polypeptide (including immunogenic fragments thereof, *e.g.*, immunogenic fragments capable of raising an immune response to RSV), and (ii) a pharmaceutically acceptable carrier.

In some embodiments, the at least one RNA polynucleotide has at least one chemical modification.

In some embodiments, an antigenic polypeptide is glycoprotein G.

In some embodiments, an antigenic polypeptide is glycoprotein F.

In some embodiments, at least one antigenic polypeptide is glycoprotein F and at least one antigenic polypeptide is selected from G, M, N, P, L, SH, M2, NS1 and NS2.

In some embodiments, at least one antigenic polypeptide is glycoprotein F and at least two antigenic polypeptides are selected from G, M, N, P, L, SH, M2, NS1 and NS2.

In some embodiments, the RNA vaccines further comprise an adjuvant.

In some embodiments, at least one RNA polynucleotide is encoded by at least one nucleic acid sequence set forth as SEQ ID NO: 1, 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 242, 246, 257, 258, or 259, or homologs having at least 80% identity with a nucleic acid sequence set forth as SEQ ID NO: 1, 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 242, 246, 257, 258, or 259. In some embodiments, at least one RNA polynucleotide is encoded by at least one nucleic acid sequence set forth as SEQ ID NO: 1, 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 242, 246, 257, 258, or 259, or homologs having at least 90% (*e.g.* 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.8% or 99.9%) identity with a nucleic acid sequence set forth as SEQ ID NO: 1, 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 242, 246,

257, 258, or 259. In some embodiments, at least one RNA polynucleotide is encoded by at least one fragment of a nucleic acid sequence (*e.g.*, a fragment having at least one antigenic sequence or at least one epitope) set forth as SEQ ID NO: 1, 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 242, 246, 257, 258, or 259.

5 In some embodiments, at least one RNA polynucleotide comprises at least one nucleic acid sequence set forth as any of SEQ ID NO: 260-280, or homologs having at least 80% identity with a nucleic acid sequence set forth as any of SEQ ID NO: 260-280. In some embodiments, at least one RNA polynucleotide comprises at least one nucleic acid sequence set forth as any of SEQ ID NO: 260-280, or homologs having at least 90% (*e.g.* 90%, 91%, 10 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.8% or 99.9%) identity with a nucleic acid sequence set forth as any of SEQ ID NO: 260-280. In some embodiments, at least one RNA polynucleotide comprises at least one fragment of a nucleic acid sequence (*e.g.*, a fragment having at least one antigenic sequence or at least one epitope) set forth as any of SEQ ID NO: 260-280.

15 In some embodiments, the amino acid sequence of the RSV antigenic polypeptide is, or is a fragment of, or is a homolog having at least 80% (*e.g.*, 85%, 90%, 95%, 98%, 99%) identity to, the amino acid sequence set forth as SEQ ID NO: 3 or SEQ ID NO: 4.

In some embodiments, the amino acid sequence of the RSV antigenic polypeptide is, or is a fragment of, or is a homolog having at least 80% (*e.g.*, 85%, 90%, 95%, 98%, 99%) 20 identity to, the amino acid sequence set forth as SEQ ID NO: 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 243, or 245.

In some embodiments, at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic polypeptide having at least 90% identity to an amino acid sequence of the present disclosure and having membrane fusion activity. In some embodiments, at least one RNA 25 polynucleotide encodes an antigenic polypeptide having at least 95% identity to an amino acid sequence of the present disclosure and having membrane fusion activity. In some embodiments, at least one RNA polynucleotide encodes an antigenic polypeptide having at least 96% identity to an amino acid sequence of the present disclosure and having membrane fusion activity. In some embodiments, at least one RNA polynucleotide encodes an antigenic 30 polypeptide having at least 97% identity to an amino acid sequence of the present disclosure and having membrane fusion activity. In some embodiments, at least one RNA polynucleotide encodes an antigenic polypeptide having at least 98% identity to an amino acid sequence of the present disclosure and having membrane fusion activity. In some embodiments, at least one RNA polynucleotide encodes an antigenic polypeptide having at

least 99% identity to an amino acid sequence of the present disclosure and having membrane fusion activity. In some embodiments, at least one RNA polynucleotide encodes an antigenic polypeptide having 95-99% identity to an amino acid sequence of the present disclosure and having membrane fusion activity.

5 In some embodiments, at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic polypeptide having an amino acid sequence of the present disclosure and is codon optimized mRNA.

In some embodiments, at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic polypeptide having an amino acid sequence of the present disclosure and has less
10 than 80% identity to (corresponding) wild-type mRNA sequence. In some embodiments, at least one RNA polynucleotide encodes an antigenic polypeptide having an amino acid sequence of the present disclosure and has less than 75%, 85% or 95% identity to wild-type mRNA sequence. In some embodiments, at least one RNA polynucleotide encodes an antigenic polypeptide having an amino acid sequence of the present disclosure and has 30-
15 80%, 40-80%, 50-80%, 60-80%, 70-80%, 75-80% or 78-80% identity to wild-type mRNA sequence. In some embodiments, at least one RNA polynucleotide encodes an antigenic polypeptide having an amino acid sequence of the present disclosure and has 30-85%, 40-85%, 50-85%, 60-85%, 70-85%, 75-85%, or 80-85% identity to wild-type mRNA sequence. In some embodiments, at least one RNA polynucleotide encodes an antigenic polypeptide
20 having an amino acid sequence of the present disclosure and has 30-90%, 40-90%, 50-90%, 60-90%, 70-90%, 75-90%, 80-90%, or 85-90% identity to wild-type mRNA sequence.

In some embodiments, at least one RNA (*e.g.*, mRNA) polynucleotide is encoded by a nucleic acid (*e.g.*, DNA) having at least 90% identity to a nucleic acid sequence of the present disclosure. In some embodiments, at least one RNA polynucleotide is encoded by a
25 nucleic acid having at least 95% identity to a nucleic acid sequence of the present disclosure. In some embodiments, at least one RNA polynucleotide is encoded by a nucleic acid having at least 96% identity to a nucleic acid sequence of the present disclosure. In some embodiments, at least one RNA polynucleotide is encoded by a nucleic acid having at least 97% identity to a nucleic acid sequence of the present disclosure. In some embodiments, at
30 least one RNA polynucleotide is encoded by a nucleic acid having at least 98% identity to a nucleic acid sequence of the present disclosure. In some embodiments, at least one RNA polynucleotide is encoded by a nucleic acid having at least 99% identity to a nucleic acid sequence of the present disclosure. In some embodiments, at least one RNA polynucleotide

is encoded by a nucleic acid having 95-99% identity to a nucleic acid sequence of the present disclosure.

In some embodiments, at least one mRNA polynucleotide is encoded by a nucleic acid having a sequence of the present disclosure and has less than 80% identity to wild-type mRNA sequence. In some embodiments, at least one mRNA polynucleotide is encoded by a nucleic acid having a sequence of the present disclosure and has less than 75%, 85% or 95% identity to a wild-type mRNA sequence. In some embodiments, at least one mRNA polynucleotide is encoded by a nucleic acid having a sequence of the present disclosure and has less than 30-80%, 40-80%, 50-80%, 60-80%, 70-80%, 75-80% or 78-80% identity to wild-type mRNA sequence. In some embodiments, at least one mRNA polynucleotide is encoded by a nucleic acid having a sequence of the present disclosure and has less than 30-85%, 40-85%, 50-85%, 60-85%, 70-85%, 75-85% or 80-85% identity to wild-type mRNA sequence. In some embodiments, at least one mRNA polynucleotide is encoded by a nucleic acid having a sequence of the present disclosure and has less than 30-90%, 40-90%, 50-90%, 60-90%, 70-90%, 75-90%, 80-90%, or 85-90% identity to wild-type mRNA sequence.

In some embodiments, at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic polypeptide having an amino acid sequence of the present disclosure and having at least 80% identity to wild-type mRNA sequence, but does not include wild-type mRNA sequence.

In some embodiments, the RSV vaccine includes at least one RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding at least one RSV antigenic polypeptide, said RNA polynucleotide having at least one chemical modification.

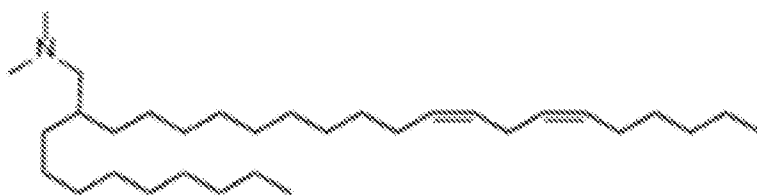
In some embodiments, the RSV vaccine includes at least one RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding at least one RSV antigenic polypeptide, said RNA polynucleotide having at least one chemical modification and at least one 5' terminal cap, wherein the RSV vaccine is formulated within a lipid nanoparticle.

In some embodiments, a 5' terminal cap is 7mG(5')ppp(5')NlmpNp.

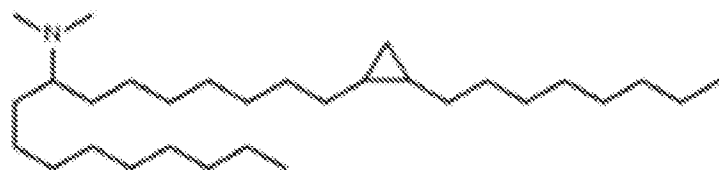
In some embodiments, at least one chemical modification is selected from the group consisting of pseudouridine, N1-methylpseudouridine, N1-ethylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 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-methyl uridine.

In some embodiments, a lipid nanoparticle comprises a cationic lipid, a PEG-modified lipid, a sterol and a non-cationic lipid. In some embodiments, a cationic lipid is an ionizable cationic lipid and the non-cationic lipid is a neutral lipid, and the sterol is a cholesterol. In some embodiments, a cationic lipid is selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine.

In some embodiments, the cationic lipid is

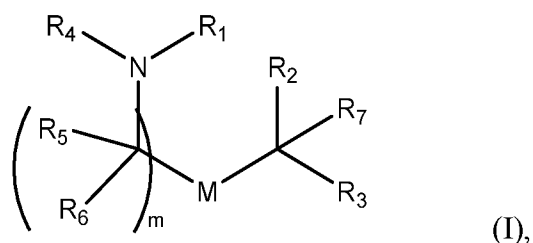


In some embodiments, the cationic lipid is



In some embodiments, at least one cationic lipid selected from compounds of Formula

(I):



or a salt or isomer thereof, wherein:

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, $-R^*YR''$, $-YR''$, and $-R''M'R'$;

R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, $-R^*YR''$, $-YR''$, and $-R^*OR''$, or R_2 and R_3 , together with the atom to which they are attached, form a heterocycle or carbocycle;

R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$,

-CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a carbocycle, heterocycle, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -N(R)₂, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(R)N(R)₂C(O)OR, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl,

-R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13.

In some embodiments, a subset of compounds of Formula (I) includes those in which when R₄ is -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, or -CQ(R)₂, then (i) Q is not -N(R)₂ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

In some embodiments, a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

- R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, $-CHQR$, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, $-OR$, $-O(CH_2)_nN(R)_2$, $-C(O)OR$, $-OC(O)R$, $-CX_3$, $-CX_2H$, $-CXH_2$, $-CN$, $-C(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-CRN(R)_2C(O)OR$, $-N(R)R_8$, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)C(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, $-N(OR)C(O)R$, $-N(OR)S(O)_2R$, $-N(OR)C(O)OR$, $-N(OR)C(O)N(R)_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, $-C(=NR_9)N(R)_2$, $-C(=NR_9)R$, $-C(O)N(R)OR$, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms selected from N, O, and S which is substituted with one or more substituents selected from oxo ($=O$), OH, amino, mono- or di-alkylamino, and C_{1-3} alkyl, and each n is independently selected from 1, 2, 3, 4, and 5;
- each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
- each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
- M and M' are independently selected from $-C(O)O-$, $-OC(O)-$, $-C(O)N(R')-$, $-N(R')C(O)-$, $-C(O)-$, $-C(S)-$, $-C(S)S-$, $-SC(S)-$, $-CH(OH)-$, $-P(O)(OR')O-$, $-S(O)_2-$, $-S-S-$, an aryl group, and a heteroaryl group;
- R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
- R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle;
- R_9 is selected from the group consisting of H, CN, NO_2 , C_{1-6} alkyl, $-OR$, $-S(O)_2R$, $-S(O)_2N(R)_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle;
- each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
- each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, $-R^*YR''$, $-YR''$, and H;
- each R'' is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
- each R^* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;
- each Y is independently a C_{3-6} carbocycle;
- each X is independently selected from the group consisting of F, Cl, Br, and I; and
- m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
- or salts or isomers thereof.

In some embodiments, a subset of compounds of Formula (I) includes those in which R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, $-R^*YR''$, $-YR''$, and $-R''M'R'$;

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR,

- 5 -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, 10 -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a 5- to 14-membered heterocycle and (i) R₄ is -(CH₂)_nQ in which n is 1 or 2, or (ii) R₄ is -(CH₂)_nCHQR in which n is 1, or (iii) R₄ is -CHQR, and -CQ(R)₂, then Q is either a 5- to 14-membered heteroaryl or 8- to 14-membered heterocycloalkyl;

- each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, 20 and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

- 25 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

- 30 each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

In some embodiments, a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

In some embodiments, a subset of compounds of Formula (I) includes those in which R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and

-R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₂₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

- 5 R₄ is -(CH₂)_nQ or -(CH₂)_nCHQR, where Q is -N(R)₂, and n is selected from 3, 4, and 5;
 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group,

10 and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

- 15 each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;
 each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₁₋₁₂ alkenyl;
 each Y is independently a C₃₋₆ carbocycle;
 each X is independently selected from the group consisting of F, Cl, Br, and I; and
 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

20 or salts or isomers thereof.

In some embodiments, a subset of compounds of Formula (I) includes those in which R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of C₁₋₁₄ alkyl, C₂₋₁₄

- 25 alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, and -CQ(R)₂, where Q is -N(R)₂, and n is selected from 1, 2, 3, 4, and 5;

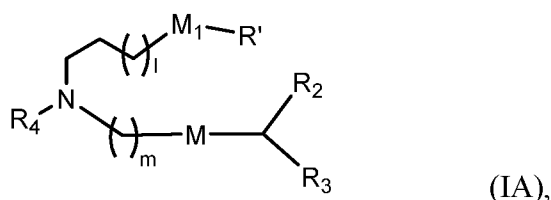
each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

- 30 each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;
 each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;
 each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₁₋₁₂ alkenyl;
 each Y is independently a C₃₋₆ carbocycle;
 each X is independently selected from the group consisting of F, Cl, Br, and I; and
 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
 or salts or isomers thereof.

In some embodiments, a subset of compounds of Formula (I) includes those of Formula (IA):



or a salt or isomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6, 7, 8, and 9; M₁ is a bond or M'; R₄ is unsubstituted C₁₋₃ alkyl, or -(CH₂)_nQ, in which Q is OH, -NHC(S)N(R)₂, -NHC(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)R₈, -NHC(=NR₉)N(R)₂, -NHC(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, heteroaryl or heterocycloalkyl; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, -S-S-, an aryl group, and a heteroaryl group; and R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, and C₂₋₁₄ alkenyl.

Some embodiments of the present disclosure provide a respiratory syncytial virus (RSV) vaccine that includes at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding at least one RSV antigenic polypeptide, wherein at least 80% of the uracil in the open reading frame have a chemical modification, optionally wherein the RSV vaccine is formulated in a lipid nanoparticle.

In some embodiments, 100% of the uracil in the open reading frame have a chemical modification. In some embodiments, a chemical modification is in the 5-position of the uracil. In some embodiments, a chemical modification is a N1-methyl pseudouridine. In some embodiments, a chemical modification is a N1-methyl pseudouridine in the 5-position of the uracil. In some embodiments, 100% of the uracil in the open reading frame are modified to include N1-methyl pseudouridine.

Some embodiments of the present disclosure provide methods of inducing an antigen specific immune response in a subject, comprising administering to the subject a RSV RNA (*e.g.*, mRNA) vaccine in an amount effective to produce an antigen specific immune response.

5 In some embodiments, an antigen specific immune response comprises a T cell response or a B cell response or both.

In some embodiments, a method of producing an antigen specific immune response involves a single administration of the RSV RNA (*e.g.*, mRNA) vaccine. In some embodiments, a method further includes administering to the subject a booster dose of the
10 RSV RNA (*e.g.*, mRNA) vaccine. A booster vaccine according to this invention may comprise any RSV RNA (*e.g.*, mRNA) vaccine disclosed herein and may be the same as the RSV RNA vaccine initially administered. In some embodiments, the same RSV RNA vaccine is administered annually for every RSV season.

In some embodiments, a RSV RNA (*e.g.*, mRNA) vaccine is administered to the
15 subject by intradermal, intranasal, or intramuscular injection. In some embodiments, a RSV RNA vaccine is administered to the subject by intramuscular injection.

Also provided herein are RSV RNA (*e.g.*, mRNA) vaccines for use in a method of inducing an antigen specific immune response in a subject, the method comprising administering the RSV vaccine to the subject in an amount effective to produce an antigen
20 specific immune response.

Further provided herein are uses of RSV RNA (*e.g.*, mRNA) vaccines in the manufacture of a medicament for use in a method of inducing an antigen specific immune response in a subject, the method comprising administering the RSV vaccine to the subject in an amount effective to produce an antigen specific immune response.

25 Some aspects of the present disclosure provide RSV RNA (*e.g.*, mRNA) vaccines formulated in an effective amount to produce an antigen specific immune response in a subject.

Other aspects of the present disclosure provide methods of inducing an antigen specific immune response in a subject, the method comprising administering to a subject the
30 RSV RNA (*e.g.*, mRNA) vaccine described herein in an effective amount to produce an antigen specific immune response in a subject.

In some embodiments, an anti-RSV antigenic polypeptide antibody titer produced in the subject is increased by at least 1 log relative to a control (*e.g.*, a control vaccine). In some

embodiments, the anti-RSV antigenic polypeptide antibody titer produced in the subject is increased by 1-3 log relative to a control (*e.g.*, a control vaccine).

In some embodiments, the anti-RSV antigenic polypeptide antibody titer produced in the subject is increased at least 2 times relative to a control (*e.g.*, a control vaccine). In some
5 embodiments, the anti-RSV antigenic polypeptide antibody titer produced in the subject is increased at least 5 times relative to a control (*e.g.*, a control vaccine). In some embodiments, the anti-RSV antigenic polypeptide antibody titer produced in the subject is increased at least 10 times relative to a control (*e.g.*, a control vaccine). In some embodiments, the anti-RSV antigenic polypeptide antibody titer produced in the subject is increased 2-10 times relative to
10 a control (*e.g.*, a control vaccine).

In some embodiments, the control is an anti-RSV antigenic polypeptide antibody titer produced in a subject who has not been administered RSV vaccine. In some embodiments, the control is an anti-RSV antigenic polypeptide antibody titer produced in a subject who has been administered a live attenuated or inactivated RSV vaccine. In some embodiments, the
15 control is an anti-RSV antigenic polypeptide antibody titer produced in a subject who has been administered a recombinant or purified RSV protein vaccine. In some embodiments, the control is an anti-RSV antigenic polypeptide antibody titer produced in a subject who has been administered an RSV virus-like particle (VLP) vaccine.

In some embodiments, the effective amount is a dose equivalent to at least a 2-fold
20 reduction in the standard of care dose of a recombinant RSV protein vaccine, wherein an anti-RSV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-RSV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified RSV protein vaccine, a live attenuated or inactivated RSV vaccine, or a RSV VLP vaccine.

In some embodiments, the effective amount is a dose equivalent to at least a 4-fold
25 reduction in the standard of care dose of a recombinant RSV protein vaccine, wherein an anti-RSV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-RSV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified RSV protein vaccine, a live attenuated or
30 inactivated RSV vaccine, or a RSV VLP vaccine.

In some embodiments, the effective amount is a dose equivalent to at least a 10-fold reduction in the standard of care dose of a recombinant RSV protein vaccine, wherein an anti-RSV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-RSV antigenic polypeptide antibody titer produced in a control subject administered the standard

of care dose of a recombinant or purified RSV protein vaccine, a live attenuated or inactivated RSV vaccine, or a RSV VLP vaccine.

In some embodiments, the effective amount is a dose equivalent to at least a 100-fold reduction in the standard of care dose of a recombinant RSV protein vaccine, wherein an anti-RSV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-RSV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified RSV protein vaccine, a live attenuated or inactivated RSV vaccine, or a RSV VLP vaccine.

In some embodiments, the effective amount is a dose equivalent to at least a 1000-fold reduction in the standard of care dose of a recombinant RSV protein vaccine, wherein an anti-RSV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-RSV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified RSV protein vaccine, a live attenuated or inactivated RSV vaccine, or a RSV VLP vaccine.

In some embodiments, the effective amount is a dose equivalent to a 2-fold to 1000-fold reduction in the standard of care dose of a recombinant RSV protein vaccine, wherein an anti-RSV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-RSV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified RSV protein vaccine, a live attenuated or inactivated RSV vaccine, or a RSV VLP vaccine.

In some embodiments, the effective amount is a total dose of 25 μ g to 1000 μ g, or 50 μ g to 1000 μ g, or 25 to 200 μ g. In some embodiments, the effective amount is a total dose of 50 μ g, 100 μ g, 200 μ g, 400 μ g, 800 μ g, or 1000 μ g. In some embodiments, the effective amount is a dose of 25 μ g administered to the subject a total of two times. In some embodiments, the effective amount is a dose of 50 μ g administered to the subject a total of two times. In some embodiments, the effective amount is a dose of 100 μ g administered to the subject a total of two times. In some embodiments, the effective amount is a dose of 200 μ g administered to the subject a total of two times. In some embodiments, the effective amount is a dose of 400 μ g administered to the subject a total of two times. In some embodiments, the effective amount is a dose of 500 μ g administered to the subject a total of two times.

In some embodiments, the effective amount administered to a subject is a total dose (of RSV RNA, *e.g.*, mRNA, vaccine) of 50 μ g to 1000 μ g.

In some embodiments, the efficacy (or effectiveness) of the RSV RNA (*e.g.*, mRNA) vaccine against RSV is greater than 60%.

Vaccine efficacy may be assessed using standard analyses (*see, e.g.*, Weinberg *et al.*, *J Infect Dis.* 2010 Jun 1;201(11):1607-10). For example, vaccine efficacy may be measured by double-blind, randomized, clinical controlled trials. Vaccine efficacy may be expressed as a proportionate reduction in disease attack rate (AR) between the unvaccinated (ARU) and vaccinated (ARV) study cohorts and can be calculated from the relative risk (RR) of disease among the vaccinated group with use of the following formulas:

Efficacy = (ARU – ARV)/ARU x 100; and

Efficacy = (1-RR) x 100.

Likewise, vaccine effectiveness may be assessed using standard analyses (*see, e.g.*, Weinberg *et al.*, *J Infect Dis.* 2010 Jun 1;201(11):1607-10). Vaccine effectiveness is an assessment of how a vaccine (which may have already proven to have high vaccine efficacy) reduces disease in a population. This measure can assess the net balance of benefits and adverse effects of a vaccination program, not just the vaccine itself, under natural field conditions rather than in a controlled clinical trial. Vaccine effectiveness is proportional to vaccine efficacy (potency) but is also affected by how well target groups in the population are immunized, as well as by other non-vaccine-related factors that influence the ‘real-world’ outcomes of hospitalizations, ambulatory visits, or costs. For example, a retrospective case control analysis may be used, in which the rates of vaccination among a set of infected cases and appropriate controls are compared. Vaccine effectiveness may be expressed as a rate difference, with use of the odds ratio (OR) for developing infection despite vaccination:

Effectiveness = (1 – OR) x 100.

In some embodiments, the efficacy (or effectiveness) of the RSV RNA (*e.g.*, mRNA) vaccine against RSV is greater than 65%. In some embodiments, the efficacy (or effectiveness) of the vaccine against RSV is greater than 70%. In some embodiments, the efficacy (or effectiveness) of the vaccine against RSV is greater than 75%. In some embodiments, the efficacy (or effectiveness) of the vaccine against RSV is greater than 80%. In some embodiments, the efficacy (or effectiveness) of the vaccine against RSV is greater than 85%. In some embodiments, the efficacy (or effectiveness) of the vaccine against RSV is greater than 90%.

In some embodiments, the vaccine immunizes the subject against RSV up to 1 year (*e.g.* for a single RSV season). In some embodiments, the vaccine immunizes the subject against RSV for up to 2 years. In some embodiments, the vaccine immunizes the subject

against RSV for more than 2 years. In some embodiments, the vaccine immunizes the subject against RSV for more than 3 years. In some embodiments, the vaccine immunizes the subject against RSV for more than 4 years. In some embodiments, the vaccine immunizes the subject against RSV for 5-10 years.

5 In some embodiments, the subject administered an RSV RNA (*e.g.*, mRNA) vaccine is about 5 years old or younger, is between the ages of about 1 year and about 5 years (*e.g.*, about 1, 2, 3, 4, 5 or 6 years), is between the ages of about 6 months and about 1 year (*e.g.*, about 6, 7, 8, 9, 10, 11 or 12 months), is about 6 months or younger, or is about 12 months or younger (*e.g.*, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 months or 1 month). In some embodiments, the
10 subject was born full term (*e.g.*, about 37-42 weeks). In some embodiments, the subject was born prematurely at about 36 weeks of gestation or earlier (*e.g.*, about 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26 or 25 weeks), the subject was born prematurely at about 32 weeks of gestation or earlier, or the subject was born prematurely between about 32 weeks and about 36 weeks of gestation.

15 In some embodiments, the subject is pregnant (*e.g.*, in the first, second or third trimester) when administered an RSV RNA (*e.g.*, mRNA) vaccine. RSV causes infections of the lower respiratory tract, mainly in infants and young children. One-third of RSV related deaths occur in the first year of life, with 99 percent of these deaths occurring in low-resource countries. It's so widespread in the United States that nearly all children become infected
20 with the virus before their second birthdays. Thus, the present disclosure provides RSV vaccines for maternal immunization to improve mother-to-child transmission of protection against RSV.

In some embodiments, the subject has a chronic pulmonary disease (*e.g.*, chronic obstructive pulmonary disease (COPD) or asthma). Two forms of COPD include chronic
25 bronchitis, which involves a long-term cough with mucus, and emphysema, which involves damage to the lungs over time. Thus, a subject administered a RSV RNA (*e.g.*, mRNA) vaccine may have chronic bronchitis or emphysema.

In some embodiments, the subject has been exposed to RSV, is infected with (has) RSV, or is at risk of infection by RSV.

30 In some embodiments, the subject is immunocompromised (has an impaired immune system, *e.g.*, has an immune disorder or autoimmune disorder).

In some embodiments, the subject is an elderly subject about 60 years old, about 70 years old, or older (*e.g.*, about 60, 65, 70, 75, 80, 85 or 90 years old).

In some embodiments, the subject is a young adult between the ages of about 20 years and about 50 years (*e.g.*, about 20, 25, 30, 35, 40, 45 or 50 years old).

Some aspects of the present disclosure provide Respiratory Syncytial Virus (RSV) RNA (*e.g.*, mRNA) vaccines containing a signal peptide linked to a RSV antigenic

polypeptide. Thus, in some embodiments, the RSV RNA (*e.g.*, mRNA) vaccines contain at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding a signal peptide linked to a RSV antigenic peptide. Also provided herein are nucleic acids encoding the RSV RNA (*e.g.*, mRNA) vaccines disclosed herein.

In some embodiments, the RSV antigenic peptide is RSV attachment protein (G). In some embodiments, the RSV antigenic peptide is RSV Fusion (F) glycoprotein. In some embodiments, the RSV antigenic peptide is nucleoprotein (N). In some embodiments, the RSV antigenic peptide is phosphoprotein (P). In some embodiments, the RSV antigenic peptide is large polymerase protein (L). In some embodiments, the RSV antigenic peptide is matrix protein (M). In some embodiments, the RSV antigenic peptide is small hydrophobic protein (SH). In some embodiments, the RSV antigenic peptide is nonstructural protein1(NS1). In some embodiments, the RSV antigenic peptide is nonstructural protein 2 (NS2).

In some embodiments, the signal peptide is a IgE signal peptide. In some embodiments, the signal peptide is an IgE HC (Ig heavy chain epsilon-1) signal peptide. In some embodiments, the signal peptide has the sequence MDWTWILFLVAAATRVHS (SEQ ID NO: 281). In some embodiments, the signal peptide is an IgGκ signal peptide. In some embodiments, the signal peptide has the sequence METPAQLLFLLLLWLPDTTG (SEQ ID NO: 282). In some embodiments, the signal peptide is encoded by sequence TGGAGACTCCCGCTCAGCTGCTGTTTTTGCTCCTCCTATGGCTGCCGGATACCACC GGC (SEQ ID NO: 287) or AUGGAGACUCCCGCUCAGCUGCUGUUUUUGCUCCU CCUAUGGCUGCCGGAUACCACCGGC (SEQ ID NO: 288). In some embodiments, the signal peptide is selected from: a Japanese encephalitis PRM signal sequence (MLGSNSGQRVVFTILLLLVAPAYS; SEQ ID NO: 283), VSVg protein signal sequence (MKCLLYLAFLFIGVNCA; SEQ ID NO: 284) and Japanese encephalitis JEV signal sequence (MWLVSLAIVTACAGA; SEQ ID NO: 285). In some embodiments, the signal peptide is MELLILKANAITTILTAVTFC (SEQ ID NO: 289).

Also provided herein are respiratory syncytial virus (RSV) vaccines, comprising at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding membrane-bound RSV F protein, membrane-bound DS-Cav1 (stabilized prefusion RSV F

protein), or a combination of membrane-bound RSV F protein and membrane-bound DS-Cav1, and a pharmaceutically acceptable carrier.

In some embodiments, a RNA polynucleotide comprises the sequence of SEQ ID NO: 5 and/or the sequence of SEQ ID NO: 7.

5 In some embodiments, an effective amount of an RSV RNA (*e.g.*, mRNA) vaccine (*e.g.*, a single dose of the RSV vaccine) results in a 2 fold to 200 fold (*e.g.*, about 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or 200 fold) increase in serum neutralizing antibodies against RSV, relative to a control (*e.g.*, a control vaccine). In some embodiments, a single dose of the RSV RNA (*e.g.*, mRNA)
10 vaccine results in an about 5 fold, 50 fold, or 150 fold increase in serum neutralizing antibodies against RSV, relative to a control (*e.g.*, a control vaccine). In some embodiments, a single dose of the RSV RNA (*e.g.*, mRNA) vaccine results in an about 2 fold to 10 fold, or an about 40 to 60 fold increase in serum neutralizing antibodies against RSV, relative to a control (*e.g.*, a control vaccine).

15 In some embodiments, the serum neutralizing antibodies are against RSV A and/or RSV B.

In some embodiments, the RSV vaccine is formulated in a MC3 lipid nanoparticle (*see, e.g.*, U.S. Publication No. 2013/0245107 A1 and International Publication No. WO 2010/054401).

20 Also provided herein are methods of inducing an antigen specific immune response in a subject, the method comprising administering to a subject the RSV RNA (*e.g.*, mRNA) vaccine comprising at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding membrane-bound RSV F protein, membrane-bound DS-Cav1 (stabilized prefusion RSV F protein), or a combination of membrane-bound RSV F protein
25 and membrane-bound DS-Cav1, and a pharmaceutically acceptable carrier, in an effective amount to produce an antigen specific immune response in a subject.

In some embodiments, the methods further comprise administering a booster dose of the RSV RNA (*e.g.*, mRNA) vaccine. In some embodiments, the methods further comprise administering a second booster dose of the RSV vaccine.

30 In some embodiments, efficacy of RNA vaccines RNA (*e.g.*, mRNA) can be significantly enhanced when combined with a flagellin adjuvant, in particular, when one or more antigen-encoding mRNAs is combined with an mRNA encoding flagellin.

RNA (*e.g.*, mRNA) vaccines combined with the flagellin adjuvant (*e.g.*, mRNA-encoded flagellin adjuvant) have superior properties in that they may produce much larger

antibody titers and produce responses earlier than commercially available vaccine formulations. While not wishing to be bound by theory, it is believed that the RNA vaccines, for example, as mRNA polynucleotides, are better designed to produce the appropriate protein conformation upon translation, for both the antigen and the adjuvant, as the RNA (e.g., mRNA) vaccines co-opt natural cellular machinery. Unlike traditional vaccines, which are manufactured *ex vivo* and may trigger unwanted cellular responses, RNA (e.g., mRNA) vaccines are presented to the cellular system in a more native fashion.

Some embodiments of the present disclosure provide RNA (e.g., mRNA) vaccines that include at least one RNA (e.g., mRNA) polynucleotide having an open reading frame encoding at least one antigenic polypeptide (including immunogenic fragments thereof, e.g., immunogenic fragments capable of raising an immune response to RSV) and at least one RNA (e.g., mRNA polynucleotide) having an open reading frame encoding a flagellin adjuvant.

In some embodiments, at least one flagellin polypeptide (e.g., encoded flagellin polypeptide) is a flagellin protein. In some embodiments, at least one flagellin polypeptide (e.g., encoded flagellin polypeptide) is an immunogenic flagellin fragment. In some embodiments, at least one flagellin polypeptide and at least one antigenic polypeptide are encoded by a single RNA (e.g., mRNA) polynucleotide. In other embodiments, at least one flagellin polypeptide and at least one antigenic polypeptide are each encoded by a different RNA polynucleotide.

In some embodiments at least one flagellin polypeptide has at least 80%, at least 85%, at least 90%, or at least 95% identity to a flagellin polypeptide having a sequence of SEQ ID NO: 173-175.

In some embodiments the nucleic acid vaccines described herein are chemically modified. In other embodiments the nucleic acid vaccines are unmodified.

Yet other aspects provide compositions for and methods of vaccinating a subject comprising administering to the subject a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a first respiratory virus antigenic polypeptide, wherein the RNA polynucleotide does not include a stabilization element, and wherein an adjuvant is not co-formulated or co-administered with the vaccine.

In other aspects the invention is a composition for or method of vaccinating a subject comprising administering to the subject a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide wherein a dosage of between 10 µg/kg and 400 µg/kg of the nucleic acid vaccine is administered to

the subject. In some embodiments the dosage of the RNA polynucleotide is 1-5 µg, 5-10 µg, 10-15 µg, 15-20 µg, 10-25 µg, 20-25 µg, 20-50 µg, 30-50 µg, 40-50 µg, 40-60 µg, 60-80 µg, 60-100 µg, 50-100 µg, 80-120 µg, 40-120 µg, 40-150 µg, 50-150 µg, 50-200 µg, 80-200 µg, 100-200 µg, 120-250 µg, 150-250 µg, 180-280 µg, 200-300 µg, 50-300 µg, 80-300 µg, 100-300 µg, 40-300 µg, 50-350 µg, 100-350 µg, 200-350 µg, 300-350 µg, 320-400 µg, 40-380 µg, 40-100 µg, 100-400 µg, 200-400 µg, or 300-400 µg per dose. In some embodiments, the nucleic acid vaccine is administered to the subject by intradermal or intramuscular injection. In some embodiments, the nucleic acid vaccine is administered to the subject on day zero. In some embodiments, a second dose of the nucleic acid vaccine is administered to the subject on day twenty one.

In some embodiments, a dosage of 25 micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some embodiments, a dosage of 100 micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some embodiments, a dosage of 50 micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some embodiments, a dosage of 75 micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some embodiments, a dosage of 150 micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some embodiments, a dosage of 400 micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some embodiments, a dosage of 200 micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some embodiments, the RNA polynucleotide accumulates at a 100 fold higher level in the local lymph node in comparison with the distal lymph node. In other embodiments the nucleic acid vaccine is chemically modified and in other embodiments the nucleic acid vaccine is not chemically modified.

Aspects of the invention provide a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide, wherein the RNA polynucleotide does not include a stabilization element, and a pharmaceutically acceptable carrier or excipient, wherein an adjuvant is not included in the vaccine. In some embodiments, the stabilization element is a histone stem-loop. In some embodiments, the stabilization element is a nucleic acid sequence having increased GC content relative to wild type sequence.

Aspects of the invention provide nucleic acid vaccines comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide, wherein

the RNA polynucleotide is present in the formulation for in vivo administration to a host, which confers an antibody titer superior to the criterion for seroprotection for the first antigen for an acceptable percentage of human subjects. In some embodiments, the antibody titer produced by the mRNA vaccines of the invention is a neutralizing antibody titer. In some
5 embodiments the neutralizing antibody titer is greater than a protein vaccine. In other
embodiments the neutralizing antibody titer produced by the mRNA vaccines of the invention is greater than an adjuvanted protein vaccine. In yet other embodiments the neutralizing
antibody titer produced by the mRNA vaccines of the invention is 1,000- 10,000, 1,200-
10,000, 1,400- 10,000, 1,500- 10,000, 1,000- 5,000, 1,000- 4,000, 1,800- 10,000, 2000-
10 10,000, 2,000- 5,000, 2,000- 3,000, 2,000- 4,000, 3,000- 5,000, 3,000- 4,000, or 2,000- 2,500.
A neutralization titer is typically expressed as the highest serum dilution required to
achieve a 50% reduction in the number of plaques.

Also provided are nucleic acid vaccines comprising one or more RNA
polynucleotides having an open reading frame encoding a first antigenic polypeptide, wherein
15 the RNA polynucleotide is present in a formulation for in vivo administration to a host for
eliciting a longer lasting high antibody titer than an antibody titer elicited by an mRNA
vaccine having a stabilizing element or formulated with an adjuvant and encoding the first
antigenic polypeptide. In some embodiments, the RNA polynucleotide is formulated to
produce a neutralizing antibodies within one week of a single administration. In some
20 embodiments, the adjuvant is selected from a cationic peptide and an immunostimulatory
nucleic acid. In some embodiments, the cationic peptide is protamine.

Aspects provide nucleic acid vaccines comprising one or more RNA polynucleotides
having an open reading frame comprising at least one chemical modification or optionally no
nucleotide modification, the open reading frame encoding a first antigenic polypeptide,
25 wherein the RNA polynucleotide is present in the formulation for in vivo administration to a
host such that the level of antigen expression in the host significantly exceeds a level of
antigen expression produced by an mRNA vaccine having a stabilizing element or formulated
with an adjuvant and encoding the first antigenic polypeptide.

Other aspects provide nucleic acid vaccines comprising one or more RNA
30 polynucleotides having an open reading frame comprising at least one chemical modification
or optionally no nucleotide modification, the open reading frame encoding a first antigenic
polypeptide, wherein the vaccine has at least 10 fold less RNA polynucleotide than is
required for an unmodified mRNA vaccine to produce an equivalent antibody titer. In some
embodiments, the RNA polynucleotide is present in a dosage of 25-100 micrograms.

Aspects of the invention also provide a unit of use vaccine, comprising between 10ug and 400 µg of one or more RNA polynucleotides having an open reading frame comprising at least one chemical modification or optionally no nucleotide modification, the open reading frame encoding a first antigenic polypeptide, and a pharmaceutically acceptable carrier or excipient, formulated for delivery to a human subject. In some embodiments, the vaccine further comprises a cationic lipid nanoparticle.

Aspects of the invention provide methods of creating, maintaining or restoring antigenic memory to a respiratory virus strain in an individual or population of individuals comprising administering to said individual or population an antigenic memory booster nucleic acid vaccine comprising (a) at least one RNA polynucleotide, said polynucleotide comprising at least one chemical modification or optionally no nucleotide modification and two or more codon-optimized open reading frames, said open reading frames encoding a set of reference antigenic polypeptides, and (b) optionally a pharmaceutically acceptable carrier or excipient. In some embodiments, the vaccine is administered to the individual via a route selected from the group consisting of intramuscular administration, intradermal administration and subcutaneous administration. In some embodiments, the administering step comprises contacting a muscle tissue of the subject with a device suitable for injection of the composition. In some embodiments, the administering step comprises contacting a muscle tissue of the subject with a device suitable for injection of the composition in combination with electroporation.

Aspects of the invention provide methods of vaccinating a subject comprising administering to the subject a single dosage of between 25 µg/kg and 400 µg/kg of a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide in an effective amount to vaccinate the subject.

Other aspects provide nucleic acid vaccines comprising one or more RNA polynucleotides having an open reading frame comprising at least one chemical modification, the open reading frame encoding a first antigenic polypeptide, wherein the vaccine has at least 10 fold less RNA polynucleotide than is required for an unmodified mRNA vaccine to produce an equivalent antibody titer. In some embodiments, the RNA polynucleotide is present in a dosage of 25-100 micrograms.

Other aspects provide nucleic acid vaccines comprising an LNP formulated RNA polynucleotide having an open reading frame comprising no nucleotide modifications (unmodified), the open reading frame encoding a first antigenic polypeptide, wherein the vaccine has at least 10 fold less RNA polynucleotide than is required for an unmodified

mRNA vaccine not formulated in a LNP to produce an equivalent antibody titer. In some embodiments, the RNA polynucleotide is present in a dosage of 25-100 micrograms.

The data presented in the Examples demonstrate significant enhanced immune responses using the formulations of the invention. Both chemically modified and unmodified RNA vaccines are useful in the invention. Surprisingly, in contrast to prior art reports that it was preferable to use chemically unmodified mRNA formulated in a carrier for the production of vaccines, it is described herein that chemically modified mRNA-LNP vaccines required a much lower effective mRNA dose than unmodified mRNA, i.e., tenfold less than unmodified mRNA when formulated in carriers other than LNP. Both the chemically modified and unmodified RNA vaccines of the invention produce better immune responses than mRNA vaccines formulated in a different lipid carrier.

In other aspects the invention encompasses a method of treating an elderly subject age 60 years or older comprising administering to the subject a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a respiratory virus antigenic polypeptide in an effective amount to vaccinate the subject.

In other aspects the invention encompasses a method of treating a young subject age 17 years or younger comprising administering to the subject a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a respiratory virus antigenic polypeptide in an effective amount to vaccinate the subject.

In other aspects the invention encompasses a method of treating an adult subject comprising administering to the subject a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a respiratory virus antigenic polypeptide in an effective amount to vaccinate the subject.

In some aspects the invention is a method of vaccinating a subject with a combination vaccine including at least two nucleic acid sequences encoding respiratory antigens wherein the dosage for the vaccine is a combined therapeutic dosage wherein the dosage of each individual nucleic acid encoding an antigen is a sub therapeutic dosage. In some embodiments, the combined dosage is 25 micrograms of the RNA polynucleotide in the nucleic acid vaccine administered to the subject. In some embodiments, the combined dosage is 100 micrograms of the RNA polynucleotide in the nucleic acid vaccine administered to the subject. In some embodiments the combined dosage is 50 micrograms of the RNA polynucleotide in the nucleic acid vaccine administered to the subject. In some embodiments, the combined dosage is 75 micrograms of the RNA polynucleotide in the nucleic acid vaccine administered to the subject. In some embodiments, the combined

dosage is 150 micrograms of the RNA polynucleotide in the nucleic acid vaccine administered to the subject. In some embodiments, the combined dosage is 400 micrograms of the RNA polynucleotide in the nucleic acid vaccine administered to the subject. In some embodiments, the sub therapeutic dosage of each individual nucleic acid encoding an antigen is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 micrograms. In other embodiments the nucleic acid vaccine is chemically modified and in other embodiments the nucleic acid vaccine is not chemically modified.

In some embodiments, the RNA polynucleotide is one of SEQ ID NO: 1, 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 242, 246, 257, 258, or 259 and includes at least one chemical modification. In other embodiments, the RNA polynucleotide is one of SEQ ID NO: 1, 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 242, 246, 257, 258, or 259 and does not include any nucleotide modifications, or is unmodified. In yet other embodiments, the at least one RNA polynucleotide encodes an antigenic protein of any of SEQ ID NO: 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 243, or 245 and includes at least one chemical modification. In other embodiments, the RNA polynucleotide encodes an antigenic protein of any of SEQ ID NO: 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 243, or 245 and does not include any nucleotide modifications, or is unmodified.

The details of various embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and the drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, features and advantages will be apparent from the following description of particular embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of various embodiments of the invention.

Fig. 1 shows data from an immunogenicity study in mice, designed to evaluate the immune response to RSV vaccine antigens delivered using various mRNA vaccines formulated with MC3 LNP in comparison to protein antigens. The data demonstrated strong neutralizing antibody titers.

Fig. 2 shows that that RNA/LNP vaccines gave much higher cellular immune responses than the protein antigen.

Figs. 3A-3C show data from an intracellular cytokine staining assay to test immunogenicity in mice, demonstrating that RSV-F mRNA/NLP vaccines and RSV-G mRNA/LNP vaccines, but not DS-CAV1 protein antigens, elicit robust Th1 biased CD4+ immune responses in mice.

5 Figs. 4A-4C show data from an intracellular cytokine staining assay to test immunogenicity in mice, demonstrating that RSV-F mRNA/NLP vaccines and RSV-G mRNA/LNP vaccines, but not DS-CAV1 protein antigens, elicit robust Th1 biased CD8+ immune responses in mice.

10 Fig. 5 shows data from an immunogenicity study in mice, demonstrating strong neutralizing antibody titers equivalent to those achieved with a protein antigen adjuvanted with ADJU-PHOS[®].

15 Figs. 6A-6C show data from an intracellular cytokine staining assay to test immunogenicity in mice, demonstrating that RSV-F mRNA/LNP vaccines and RSV-G mRNA/LNP vaccines, but not DS-CAV1 protein antigens, elicit robust Th1 biased CD4+ immune responses in mice.

Figs. 7A-7C show data from an intracellular cytokine staining assay to test immunogenicity in mice, confirming that RSV-F mRNA/LNP vaccines, but not RSV-G mRNA/LNP vaccines or DS-CAV1 protein antigens, elicit robust TH1 biased CD8+ immune responses in mice.

20 Fig. 8 shows data from an assay, demonstrating that no virus was recovered from lungs of any of mice immunized with RSV mRNA vaccines formulated with MC3 LNP, and only one animal at the lower dose of DS-CAV1 protein /ADJU-PHOS[®] vaccine had any virus detectable in the nose.

25 Fig. 9 shows data from an immunogenicity study in cotton rats, demonstrating strong neutralizing antibody titers in animals immunized with various RSV mRNA vaccines formulated with MC3 LNP.

Fig. 10 shows data from a cotton rat competition ELISA, characterizing the antigenic Ø and antigenic site II response to various RSV mRNA vaccines.

30 Fig. 11 shows data from a cotton rat challenge assay, demonstrating protective effects of RSV mRNA vaccines formulated with MC3 LNP.

Fig. 12 shows a graph representative of serum neutralizing antibody titers (NT50 individual and GMT with 95% confidence intervals) to RSV A induced in African Green Monkeys by RSV mRNA vaccines and control formulations.

Figs. 13A-13B show graphs representative of serum antibody competition ELISA titers (IT50 individual and GMT with 95% confidence intervals) against palivizumab (site II) (Fig. 13A) and D25 (site Ø) (Fig. 13B) measured at week 10 (2 weeks PD3).

Figs. 14A-14B show graphs representative of mean lung viremia detected post challenge (Fig. 13A) and mean nasal viremia detected post challenge (Fig. 13B) in African Green Monkeys with 95% confidence intervals.

Fig. 15 shows a graph representative of serum neutralizing antibody titers (NT50 individual and GMT with 95% confidence intervals) to RSV A induced in RSV-experienced African Green Monkeys by various RSV mRNA vaccine and control formulations at 2 weeks post vaccination.

Fig. 16 shows a graph representative of serum neutralizing antibody titers (GMT with 95% confidence intervals) to RSV A induced in RSV-experienced African Green Monkeys by various RSV mRNA vaccine and control formulations.

Figs. 17A-17B show graphs representative of serum antibody competition ELISA titers (IT50 individual and GMT with 95% confidence intervals) against palivizumab (site II) (Fig. 17A) and D25 (site Ø) (Fig. 17B) measured at baseline and 4 weeks post immunization.

Figs. 18A-18B show graphs representative of RSV F-specific CD4⁺ (Fig. 18A) and CD8⁺ (Fig. 18B) T cell responses induced in RSV experienced African Green Monkeys by various vaccine and control formulations.

Fig. 19 shows a graph representative of serum neutralizing antibody titers (NT50 individual and GMT with 95% confidence intervals) to RSV A and RSV B induced in cotton rats at weeks 4 (4 weeks post dose 1 against RSV A (circle) and RSV B (square)) and 8 (4 weeks post dose 2 against RSV A (triangle pointing up) and RSV B (triangle pointing down)) by various vaccine and control formulations.

Fig. 20 shows a graph representative of mean lung (circles) and nose (squares) viral copies with 95% confidence intervals measured in cotton rats post challenge with RSV B 18357.

DETAILED DESCRIPTION

Embodiments of the present disclosure provide RNA (*e.g.*, mRNA) vaccines that include a (at least one) polynucleotide encoding a respiratory syncytial virus (RSV) antigen. RSV is a negative-sense, single-stranded RNA virus of the genus *Pneumovirinae*. The virus is present in at least two antigenic subgroups, known as Group A and Group B, primarily resulting from differences in the surface G glycoproteins. Two RSV surface glycoproteins –

G and F – mediate attachment with and attachment to cells of the respiratory epithelium. F surface glycoproteins mediate coalescence of neighboring cells. This results in the formation of syncytial cells. RSV is the most common cause of bronchiolitis. Most infected adults develop mild cold-like symptoms such as congestion, low-grade fever, and wheezing. Infants and small children may suffer more severe symptoms such as bronchiolitis and pneumonia. The disease may be transmitted among humans via contact with respiratory secretions.

The genome of RSV encodes at least three surface glycoproteins, including F, G, and SH, four nucleocapsid proteins, including L, P, N, and M2, and one matrix protein, M. Glycoprotein F directs viral penetration by fusion between the virion and the host membrane. Glycoprotein G is a type II transmembrane glycoprotein and is the major attachment protein. SH is a short integral membrane protein. Matrix protein M is found in the inner layer of the lipid bilayer and assists virion formation. Nucleocapsid proteins L, P, N, and M2 modulate replication and transcription of the RSV genome. It is thought that glycoprotein G tethers and stabilizes the virus particle at the surface of bronchial epithelial cells, while glycoprotein F interacts with cellular glycosaminoglycans to mediate fusion and delivery of the RSV virion contents into the host cell (Krzyszaniak MA *et al. PLoS Pathog* 2013;9(4)).

RSV RNA (*e.g.*, mRNA) vaccines, as provided herein, may be used to induce a balanced immune response, comprising both cellular and humoral immunity, without many of the risks associated with DNA vaccination.

The entire contents of International Application No. PCT/US2015/027400, International Publication No. WO2015164674A, are incorporated herein by reference.

It has been discovered that the mRNA vaccines described herein are superior to current vaccines in several ways. First, the lipid nanoparticle (LNP) delivery is superior to other formulations including a protamine base approach described in the literature and no additional adjuvants are to be necessary. The use of LNPs enables the effective delivery of chemically modified or unmodified mRNA vaccines. Additionally it has been demonstrated herein that both modified and unmodified LNP formulated mRNA vaccines were superior to conventional vaccines by a significant degree. In some embodiments the mRNA vaccines of the invention are superior to conventional vaccines by a factor of at least 10 fold, 20 fold, 40 fold, 50 fold, 100 fold, 500 fold or 1,000 fold.

Although attempts have been made to produce functional RNA vaccines, including mRNA vaccines and self-replicating RNA vaccines, the therapeutic efficacy of these RNA vaccines have not yet been fully established. Quite surprisingly, the inventors have discovered, according to aspects of the invention a class of formulations for delivering

mRNA vaccines in vivo that results in significantly enhanced, and in many respects synergistic, immune responses including enhanced antigen generation and functional antibody production with neutralization capability. These results can be achieved even when significantly lower doses of the mRNA are administered in comparison with mRNA doses used in other classes of lipid based formulations. The formulations of the invention have demonstrated significant unexpected in vivo immune responses sufficient to establish the efficacy of functional mRNA vaccines as prophylactic and therapeutic agents. Additionally, self-replicating RNA vaccines rely on viral replication pathways to deliver enough RNA to a cell to produce an immunogenic response. The formulations of the invention do not require viral replication to produce enough protein to result in a strong immune response. Thus, the mRNA of the invention are not self-replicating RNA and do not include components necessary for viral replication.

The invention involves, in some aspects, the surprising finding that lipid nanoparticle (LNP) formulations significantly enhance the effectiveness of mRNA vaccines, including chemically modified and unmodified mRNA vaccines. The efficacy of mRNA vaccines formulated in LNP was examined in vivo using several distinct antigens. The results presented herein demonstrate the unexpected superior efficacy of the mRNA vaccines formulated in LNP over other commercially available vaccines.

In addition to providing an enhanced immune response, the formulations of the invention generate a more rapid immune response with fewer doses of antigen than other vaccines tested. The mRNA-LNP formulations of the invention also produce quantitatively and qualitatively better immune responses than vaccines formulated in a different carriers.

The data described herein demonstrate that the formulations of the invention produced significant unexpected improvements over existing antigen vaccines. Additionally, the mRNA-LNP formulations of the invention are superior to other vaccines even when the dose of mRNA is lower than other vaccines. Various mRNA vaccines formulated with MC3 LNP were compared in mice to protein antigen vaccination. The data demonstrated that in comparison to existing vaccines, the mRNA vaccines produced stronger neutralizing antibody titers, much higher cellular immune responses than the protein antigen, elicited robust Th1 biased CD4⁺ and CD8⁺ immune responses in mice and reduction in virus in the lungs. No virus was recovered from lungs of any of mice immunized with RSV mRNA vaccines formulated with MC3 LNP, in contrast to only one animal at the lower dose of protein/adjuvant vaccine formulation. Significant neutralizing antibody titers were also achieved in rats and monkeys.

The LNP used in the studies described herein has been used previously to deliver siRNA in various animal models as well as in humans. In view of the observations made in association with the siRNA delivery of LNP formulations, the fact that LNP is useful in vaccines is quite surprising. It has been observed that therapeutic delivery of siRNA formulated in LNP causes an undesirable inflammatory response associated with a transient IgM response, typically leading to a reduction in antigen production and a compromised immune response. In contrast to the findings observed with siRNA, the LNP-mRNA formulations of the invention are demonstrated herein to generate enhanced IgG levels, sufficient for prophylactic and therapeutic methods rather than transient IgM responses.

Antigens/Antigenic Polypeptides

At least two antigenic subgroups (A and B) of RSV are known to exist. This antigenic dimorphism is due primarily to difference in the surface G glycoproteins. Two surface glycoproteins, G and F, are present in the envelope and mediate attachment and fusion with cells of the respiratory epithelium. The F proteins also mediate coalescence of neighboring cells to form the characteristic syncytial cells for which the virus receives its name. The epidemiologic and biologic significance of the two antigenic variants of RSV is uncertain. Nonetheless, there is some evidence to suggest that Group A infections tend to be more severe.

The RSV genome is ~15,000 nucleotides in length and is composed of a single strand of RNA with negative polarity. It has 10 genes encoding 11 proteins—there are 2 open reading frames of M2. The genome is transcribed sequentially from NS1 to L with reduction in expression levels along its length.

NS1 and NS2 inhibit type I interferon activity. In some embodiments, a RSV vaccine comprises at least one RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding products of NS1, or NS2.

N encodes nucleocapsid protein that associates with the genomic RNA forming the nucleocapsid. In some embodiments, a RSV vaccine comprises at least one RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding nucleocapsid protein.

M encodes the Matrix protein required for viral assembly. In some embodiments, a RSV vaccine comprises at least one RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding Matrix protein.

SH, G and F form the viral coat. The G protein is a surface protein that is heavily glycosylated and functions as the attachment protein. The F protein is another important

surface protein that mediates fusion, allowing entry of the virus into the cell cytoplasm and also allowing the formation of syncytia. The F protein is homologous in both subtypes of RSV; antibodies directed at the F protein are neutralizing. In contrast, the G protein differs considerably between the two subtypes. In some embodiments, a RSV vaccine comprises at least one RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding SH, G or F protein, or a combination thereof.

Nucleolin at the cell surface is the receptor for the RSV fusion protein. Interference with the nucleolin-RSV fusion protein interaction has been shown to be therapeutic against RSV infection in cell cultures and animal models. In some embodiments, a RSV vaccine comprises at least one RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding nucleolin.

M2 is the second matrix protein also required for transcription and encodes M2-1 (elongation factor) and M2-2 (transcription regulation). M2 contains CD8 epitopes. In some embodiments, a RSV vaccine comprises at least one RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding the second matrix protein.

L encodes the RNA polymerase. In some embodiments, a RSV vaccine comprises at least one RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding the RNA polymerase (L).

The phosphoprotein P is a cofactor for the L protein. In some embodiments, a RSV vaccine comprises at least one RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding phosphoprotein P.

Some embodiments of the present disclosure provide RSV vaccines that include at least one RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding glycoprotein G.

Some embodiments of the present disclosure provide RSV vaccines that include at least one RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding glycoprotein F.

Some embodiments of the present invention disclose RSV vaccines that include at least one RNA (*e.g.* mRNA) polynucleotide having an open reading frame encoding a polypeptide in the post-fusion form. Further embodiments of the present invention disclose RSV vaccines that include at least one RNA (*e.g.* mRNA) polynucleotide having an open reading frame encoding a polypeptide in the pre-fusion form. In some embodiments, the polypeptides comprise glycoproteins in a prefusion conformation, for example, but not limited to, prefusion glycoprotein F or DS-CAV1. Without wishing to be bound by theory,

certain polypeptides, when in a prefusion conformation, may contain more epitopes for neutralizing antibodies relative to the post-fusion conformation of the same proteins. For example, prefusion glycoprotein F has a unique antigen site (“antigenic site 0”) at its membrane distal apex. Antigenic site 0 may, but not necessarily, comprise residues 62-69 and 196-209 of a RSV F protein sequence. In some instances, such as, but not limited to, prefusion glycoprotein F, prefusion polypeptides may exhibit many fold greater immune responses than those achieved with post-fusion polypeptides. Prefusion RSV glycoproteins and their methods of use are described in WO2014/160463, incorporated by reference herein its entirety.

In some embodiments, RSV vaccines include at least one RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding glycoprotein F or glycoprotein G obtained from RSV strain A2 (RSV A2). Other RSV strains are encompassed by the present disclosure, including subtype A strains and subtype B strains.

In some embodiments, a RSV vaccine has at least one RNA (*e.g.*, mRNA) having at least one modification, including but not limited to at least one chemical modification.

In some embodiments, a RSV antigenic polypeptide is longer than 25 amino acids and shorter than 50 amino acids. The term “antigenic polypeptide” includes immunogenic fragments thereof (*e.g.*, immunogenic fragments capable of raising an immune response to RSV). Thus, polypeptides include gene products, naturally occurring polypeptides, synthetic polypeptides, homologs, orthologs, paralogs, fragments and other equivalents, variants, and analogs of the foregoing. A polypeptide may be a single molecule or may be a multi-molecular complex such as a dimer, trimer or tetramer. Polypeptides may also comprise single chain or multichain polypeptides such as antibodies or insulin and may be associated or linked. Most commonly, disulfide linkages are found in multichain polypeptides. The term polypeptide may also apply to amino acid polymers in which at least one amino acid residue is an artificial chemical analogue of a corresponding naturally-occurring amino acid.

The term “polypeptide variant” refers to molecules which differ in their amino acid sequence from a native or reference sequence. The amino acid sequence variants may possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence, as compared to a native or reference sequence. Ordinarily, variants possess at least 50% identity to a native or reference sequence. In some embodiments, variants share at least 80%, or at least 90% identity with a native or reference sequence.

In some embodiments “variant mimics” are provided. As used herein, a “variant mimic” contains at least one amino acid that would mimic an activated sequence. For

example, glutamate may serve as a mimic for phospho-threonine and/or phospho-serine. Alternatively, variant mimics may result in deactivation or in an inactivated product containing the mimic. For example, phenylalanine may act as an inactivating substitution for tyrosine, or alanine may act as an inactivating substitution for serine.

5 “Orthologs” refers to genes in different species that evolved from a common ancestral gene by speciation. Normally, orthologs retain the same function in the course of evolution. Identification of orthologs is critical for reliable prediction of gene function in newly sequenced genomes.

10 “Analog” is meant to include polypeptide variants that differ by one or more amino acid alterations, for example, substitutions, additions or deletions of amino acid residues that still maintain one or more of the properties of the parent or starting polypeptide.

 “Paralogs” are genes (or proteins) related by duplication within a genome. Orthologs retain the same function in the course of evolution, whereas paralogs evolve new functions, even if these are related to the original one.

15 The present disclosure provides several types of compositions that are polynucleotide or polypeptide based, including variants and derivatives. These include, for example, substitutional, insertional, deletion and covalent variants and derivatives. The term “derivative” is used synonymously with the term “variant,” but generally refers to a molecule that has been modified and/or changed in any way relative to a reference molecule or starting
20 molecule.

 As such, polynucleotides encoding peptides or polypeptides containing substitutions, insertions and/or additions, deletions and covalent modifications with respect to reference sequences, in particular the polypeptide sequences disclosed herein, are included within the scope of this disclosure. For example, sequence tags or amino acids, such as one or more
25 lysines, can be added to peptide sequences (*e.g.*, at the N-terminal or C-terminal ends). Sequence tags can be used for peptide detection, purification or localization. Lysines can be used to increase peptide solubility or to allow for biotinylation. Alternatively, amino acid residues located at the carboxy and amino terminal regions of the amino acid sequence of a peptide or protein may optionally be deleted providing for truncated sequences. Certain
30 amino acids (*e.g.*, C-terminal or N-terminal residues) may alternatively be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger sequence which is soluble, or linked to a solid support. In alternative embodiments, sequences for (or encoding) signal sequences, termination sequences, transmembrane domains, linkers, multimerization domains (such as, *e.g.*, foldon regions) and the like may be

substituted with alternative sequences that achieve the same or a similar function. Such sequences are readily identifiable to one of skill in the art. It should also be understood that some of the sequences provided herein contain sequence tags or terminal peptide sequences (*e.g.*, at the N-terminal or C-terminal ends) that may be deleted, for example, prior to use in the preparation of an RNA (*e.g.*, mRNA) vaccine.

“Substitutional variants” when referring to polypeptides are those that have at least one amino acid residue in a native or starting sequence removed and a different amino acid inserted in its place at the same position. Substitutions may be single, where only one amino acid in the molecule has been substituted, or they may be multiple, where two or more amino acids have been substituted in the same molecule.

As used herein the term “conservative amino acid substitution” refers to the substitution of an amino acid that is normally present in the sequence with a different amino acid of similar size, charge, or polarity. Examples of conservative substitutions include the substitution of a non-polar (hydrophobic) residue such as isoleucine, valine and leucine for another non-polar residue. Likewise, examples of conservative substitutions include the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, and between glycine and serine. Additionally, the substitution of a basic residue, such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another acidic residue are additional examples of conservative substitutions. Examples of non-conservative substitutions include the substitution of a non-polar (hydrophobic) amino acid residue such as isoleucine, valine, leucine, alanine, methionine for a polar (hydrophilic) residue such as cysteine, glutamine, glutamic acid or lysine and/or a polar residue for a non-polar residue.

“Features” when referring to polypeptide or polynucleotide are defined as distinct amino acid sequence-based or nucleotide-based components of a molecule respectively. Features of the polypeptides encoded by the polynucleotides include surface manifestations, local conformational shape, folds, loops, half-loops, domains, half-domains, sites, termini or any combination thereof.

As used herein when referring to polypeptides the term “domain” refers to a motif of a polypeptide having one or more identifiable structural or functional characteristics or properties (*e.g.*, binding capacity, serving as a site for protein-protein interactions).

As used herein, when referring to polypeptides the terms “site” as it pertains to amino acid based embodiments, is used synonymously with “amino acid residue” and “amino acid side chain.” As used herein, when referring to polynucleotides the terms “site” as it pertains

to nucleotide based embodiments, is used synonymously with “nucleotide.” A site represents a position within a peptide or polypeptide or polynucleotide that may be modified, manipulated, altered, derivatized or varied within the polypeptide or polynucleotide based molecules.

5 As used herein, the terms “termini” or “terminus,” when referring to polypeptides or polynucleotides, refers to an extremity of a polypeptide or polynucleotide respectively. Such extremity is not limited only to the first or final site of the polypeptide or polynucleotide but may include additional amino acids or nucleotides in the terminal regions. Polypeptide-based molecules may be characterized as having both an N-terminus (terminated by an amino acid with a free amino group (NH₂)) and a C-terminus (terminated by an amino acid with a free carboxyl group (COOH)). Proteins are in some cases made up of multiple polypeptide chains brought together by disulfide bonds or by non-covalent forces (multimers, oligomers). These proteins have multiple N-termini and C-termini. Alternatively, the termini of the polypeptides may be modified such that they begin or end, as the case may be, with a non-polypeptide based moiety such as an organic conjugate.

10 As recognized by those skilled in the art, protein fragments, functional protein domains, and homologous proteins are also considered to be within the scope of polypeptides of interest. For example, provided herein is any protein fragment (meaning a polypeptide sequence at least one amino acid residue shorter than a reference polypeptide sequence but otherwise identical) of a reference protein 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or greater than 100 amino acids in length. In another example, any protein that includes a stretch of 20, 30, 40, 50, or 100 amino acids that are 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% identical to any of the sequences described herein can be utilized in accordance with the present disclosure. In some embodiments, a polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, or more mutations, as shown in any of the sequences provided or referenced herein. In some 25 embodiments, a protein fragment is longer than 25 amino acids and shorter than 50 amino acids.

Polypeptide or polynucleotide molecules of the present disclosure may share a certain degree of sequence similarity or identity with the reference molecules (*e.g.*, reference polypeptides or reference polynucleotides), for example, with art-described molecules (*e.g.*, engineered or designed molecules or wild-type molecules). The term “identity,” as known in the art, refers to a relationship between the sequences of two or more polypeptides or polynucleotides, as determined by comparing the sequences. In the art, identity also means the degree of sequence relatedness between them as determined by the number of matches

between strings of two or more amino acid residues or nucleic acid residues. Identity measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (e.g., “algorithms”). Identity of related peptides can be readily calculated by known methods.

5 “% identity” as it applies to polypeptide or polynucleotide sequences is defined as the percentage of residues (amino acid residues or nucleic acid residues) in the candidate amino acid or nucleic acid sequence that are identical with the residues in the amino acid sequence or nucleic acid sequence of a second sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent identity. Methods and computer
10 programs for the alignment are well known in the art. It is understood that identity depends on a calculation of percent identity but may differ in value due to gaps and penalties introduced in the calculation. Generally, variants of a particular polynucleotide or polypeptide have at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% but less than 100% sequence identity to
15 that particular reference polynucleotide or polypeptide as determined by sequence alignment programs and parameters described herein and known to those skilled in the art. Such tools for alignment include those of the BLAST suite (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 popular local alignment technique is based on the Smith-
20 Waterman algorithm (Smith, T.F. & 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. & 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). More recently a Fast Optimal
25 Global Sequence Alignment Algorithm (FOGSAA) has been developed that purportedly produces global alignment of nucleotide and protein sequences faster than other optimal global alignment methods, including the Needleman–Wunsch algorithm. Other tools are described herein, specifically in the definition of “identity” below.

As used herein, the term “homology” refers to the overall relatedness between
30 polymeric molecules, e.g. between nucleic acid molecules (e.g. DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Polymeric molecules (e.g. nucleic acid molecules (e.g. DNA molecules and/or RNA molecules) and/or polypeptide molecules) that share a threshold level of similarity or identity determined by alignment of matching residues are termed homologous. Homology is a qualitative term that describes a relationship between

molecules and can be based upon the quantitative similarity or identity. Similarity or identity is a quantitative term that defines the degree of sequence match between two compared sequences. In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical or similar. The term “homologous” necessarily refers to a comparison between at least two sequences (polynucleotide or polypeptide sequences). Two polynucleotide sequences are considered homologous if the polypeptides they encode are at least 50%, 60%, 70%, 80%, 90%, 95%, or even 99% for at least one stretch of at least 20 amino acids. In some embodiments, homologous polynucleotide sequences are characterized by the ability to encode a stretch of at least 4–5 uniquely specified amino acids. For polynucleotide sequences less than 60 nucleotides in length, homology is determined by the ability to encode a stretch of at least 4–5 uniquely specified amino acids. Two protein sequences are considered homologous if the proteins are at least 50%, 60%, 70%, 80%, or 90% identical for at least one stretch of at least 20 amino acids.

Homology implies that the compared sequences diverged in evolution from a common origin. The term “homolog” refers to a first amino acid sequence or nucleic acid sequence (*e.g.*, gene (DNA or RNA) or protein sequence) that is related to a second amino acid sequence or nucleic acid sequence by descent from a common ancestral sequence. The term “homolog” may apply to the relationship between genes and/or proteins separated by the event of speciation or to the relationship between genes and/or proteins separated by the event of genetic duplication.

Nucleic Acids/Polynucleotides

RSV vaccines, as provided herein, comprise at least one (one or more) ribonucleic acid (RNA) polynucleotide having an open reading frame encoding at least one RSV antigenic polypeptide. The term “nucleic acid,” in its broadest sense, includes any compound and/or substance that comprises a polymer of nucleotides. These polymers are referred to as polynucleotides.

In some embodiments, at least one RNA polynucleotide is encoded by at least one nucleic acid sequence set forth as SEQ ID NO: 1, 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 242, 246, 257, 258, or 259, or homologs having at least 80% identity with a nucleic acid sequence set forth as SEQ ID NO: 1, 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 242, 246, 257, 258, or 259. In some embodiments, at least one RNA polynucleotide is encoded by at

least one nucleic acid sequence set forth as SEQ ID NO: 1, 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 242, 246, 257, 258, or 259, or homologs having at least 90% (*e.g.* 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.8% or 99.9%) identity with a nucleic acid sequence set forth as SEQ ID NO: 1, 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 242, 246, 257, 258, or 259. In some embodiments, at least one RNA polynucleotide is encoded by at least one fragment of a nucleic acid sequence (*e.g.*, a fragment having at least one antigenic sequence or at least one epitope) set forth as SEQ ID NO: 1, 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 242, 246, 257, 258, or 259. In some embodiments, the at least one RNA polynucleotide has at least one chemical modification. In some embodiments, the at least one RNA polynucleotide is an mRNA polynucleotide, wherein each uracil (100% of the uracils) of the mRNA polynucleotide is chemically modified. In some embodiments, the at least one RNA polynucleotide is an mRNA polynucleotide, wherein each uracil (100% of the uracils) of the mRNA polynucleotide is chemically modified to include a N1-methyl pseudouridine.

In some embodiments, the amino acid sequence of the RSV antigenic polypeptide is, or is a (antigenic) fragment of, or is a homolog having at least 80% (*e.g.*, 85%, 90%, 95%, 98%, 99%) identity to, the amino acid sequence set forth as SEQ ID NO: 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 243, or 245.

Nucleic acids (also referred to as polynucleotides) may be or may include, for example, ribonucleic acids (RNAs), deoxyribonucleic acids (DNAs), threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs), including LNA having a β -D-ribo configuration, α -LNA having an α -L-ribo configuration (a diastereomer of LNA), 2'-amino-LNA having a 2'-amino functionalization, and 2'-amino- α -LNA having a 2'-amino functionalization), ethylene nucleic acids (ENA), cyclohexenyl nucleic acids (CeNA) or chimeras or combinations thereof.

In some embodiments, polynucleotides of the present disclosure function as messenger RNA (mRNA). "Messenger RNA" (mRNA) refers to any polynucleotide that encodes a (at least one) polypeptide (a naturally-occurring, non-naturally-occurring, or modified polymer of amino acids) and can be translated to produce the encoded polypeptide *in vitro*, *in vivo*, *in situ* or *ex vivo*. The skilled artisan will appreciate that, except where otherwise noted, polynucleotide sequences set forth in the instant application will recite "T"s in a representative DNA sequence but where the sequence represents RNA (*e.g.*, mRNA), the "T"s would be substituted for "U"s. Thus, any of the RNA polynucleotides encoded by a DNA identified by a particular sequence identification number may also comprise the

corresponding RNA (*e.g.*, mRNA) sequence encoded by the DNA, where each “T” of the DNA sequence is substituted with “U.”

It should be understood that the mRNA polynucleotides of the vaccines as provided herein are synthetic molecules, *i.e.*, they are not naturally-occurring molecules. That is, the mRNA polynucleotides of the present disclosure are isolated mRNA polynucleotides. As is known in the art, “isolated polynucleotides” refer to polynucleotides that are substantially physically separated from other cellular material (*e.g.*, separated from cells and/or systems that produce the polynucleotides) or from other material that hinders their use in the vaccines of the present disclosure. Isolated polynucleotides are substantially pure in that they have been substantially separated from the substances with which they may be associated in living or viral systems. Thus, mRNA polynucleotide vaccines are not associated with living or viral systems, such as cells or viruses. The mRNA polynucleotide vaccines do not include viral components (*e.g.*, viral capsids, viral enzymes, or other viral proteins, for example, those needed for viral-based replication), and the mRNA polynucleotide vaccines are not packaged within, encapsulated within, linked to, or otherwise associated with a virus or viral particle. In some embodiments, the mRNA vaccines comprise a lipid nanoparticle that consists of, or consists essentially of, one or more mRNA polynucleotides (*e.g.*, mRNA polynucleotides encoding one or more RSV antigen(s)).

The basic components of an mRNA molecule typically include at least one coding region, a 5' untranslated region (UTR), a 3' UTR, a 5' cap and a poly-A tail. Polynucleotides of the present disclosure may function as mRNA but can be distinguished from wild-type mRNA in their functional and/or structural design features, which serve to overcome existing problems of effective polypeptide expression using nucleic-acid based therapeutics. In some embodiments, the RNA is a messenger RNA (mRNA) having an open reading frame encoding at least one RSV antigen. In some embodiments, the RNA (*e.g.*, mRNA) further comprises a (at least one) 5' UTR, 3' UTR, a polyA tail and/or a 5' cap.

In some embodiments, a RNA polynucleotide (*e.g.*, mRNA) of a RSV vaccine encodes 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-10, 5-9, 5-8, 5-7, 5-6, 6-10, 6-9, 6-8, 6-7, 7-10, 7-9, 7-8, 8-10, 8-9 or 9-10 antigenic polypeptides. In some embodiments, a RNA polynucleotide (*e.g.*, mRNA) of a RSV RNA (*e.g.*, mRNA) vaccine encodes at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 antigenic polypeptides. In some embodiments, a RNA polynucleotide (*e.g.*, mRNA) of a RSV vaccine encodes at least 100 antigenic polypeptides, or at least 200 antigenic polypeptides. In some embodiments, a RNA polynucleotide (*e.g.*, mRNA) of a RSV vaccine

encodes 1-10, 5-15, 10-20, 15-25, 20-30, 25-35, 30-40, 35-45, 40-50, 1-50, 1-100, 2-50 or 2-100 antigenic polypeptides.

Polynucleotides (*e.g.*, mRNAs) of the present disclosure, in some embodiments, are codon optimized. Codon optimization methods are known in the art and may be used as provided herein. Codon optimization, in some embodiments, may be used to match codon frequencies in target and host organisms to ensure proper folding; bias GC content to increase mRNA stability or reduce secondary structures; minimize tandem repeat codons or base runs that may impair gene construction or expression; customize transcriptional and translational control regions; insert or remove protein trafficking sequences; remove/add post translation modification sites in encoded protein (*e.g.*, glycosylation sites); add, remove or shuffle protein domains; insert or delete restriction sites; modify ribosome binding sites and mRNA degradation sites; adjust translational rates to allow the various domains of the protein to fold properly; or reduce or eliminate problem secondary structures within the polynucleotide. Codon optimization tools, algorithms and services are known in the art – non-limiting examples include services from GeneArt (Life Technologies), DNA2.0 (Menlo Park CA) and/or proprietary methods. In some embodiments, the open reading frame (ORF) sequence is optimized using optimization algorithms.

In some embodiments, a codon optimized sequence shares less than 95% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (*e.g.*, an antigenic protein or polypeptide)). In some embodiments, a codon optimized sequence shares less than 90% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (*e.g.*, an antigenic protein or polypeptide)). In some embodiments, a codon optimized sequence shares less than 85% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (*e.g.*, an antigenic protein or polypeptide)). In some embodiments, a codon optimized sequence shares less than 80% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (*e.g.*, an antigenic protein or polypeptide)). In some embodiments, a codon optimized sequence shares less than 75% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (*e.g.*, an antigenic protein or polypeptide)).

In some embodiments, a codon optimized sequence shares between 65% and 85% (*e.g.*, between about 67% and about 85% or between about 67% and about 80%) sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (*e.g.*, an antigenic protein or polypeptide)). In some embodiments, a codon optimized sequence shares between 65% and 75% or about 80% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (*e.g.*, an antigenic protein or polypeptide)).

In some embodiments, the RSV vaccine includes at least one RNA polynucleotide having an open reading frame encoding at least one RSV antigenic polypeptide having at least one modification, at least one 5' terminal cap, and is formulated within a lipid nanoparticle. 5'-capping of polynucleotides may be completed concomitantly during the *in vitro*-transcription reaction using the following chemical RNA cap analogs to generate the 5'-guanosine cap structure according to manufacturer protocols: 3'-O-Me-m7G(5')ppp(5') G [the ARCA cap]; G(5')ppp(5')A; G(5')ppp(5')G; m7G(5')ppp(5')A; m7G(5')ppp(5')G (New England BioLabs, Ipswich, MA). 5'-capping of modified RNA may be completed post-transcriptionally using a Vaccinia Virus Capping Enzyme to generate the "Cap 0" structure: m7G(5')ppp(5')G (New England BioLabs, Ipswich, MA). Cap 1 structure may be generated using both Vaccinia Virus Capping Enzyme and a 2'-O methyl-transferase to generate: m7G(5')ppp(5')G-2'-O-methyl. Cap 2 structure may be generated from the Cap 1 structure followed by the 2'-O-methylation of the 5'-antepenultimate nucleotide using a 2'-O methyl-transferase. Cap 3 structure may be generated from the Cap 2 structure followed by the 2'-O-methylation of the 5'-preantepenultimate nucleotide using a 2'-O methyl-transferase. Enzymes may be derived from a recombinant source.

When transfected into mammalian cells, the modified mRNAs have a stability of between 12-18 hours, or greater than 18 hours, *e.g.*, 24, 36, 48, 60, 72, or greater than 72 hours.

In some embodiments a codon optimized RNA may be one in which the levels of G/C are enhanced. The G/C-content of nucleic acid molecules (*e.g.*, mRNA) may influence the stability of the RNA. RNA having an increased amount of guanine (G) and/or cytosine (C) residues may be functionally more stable than RNA containing a large amount of adenine (A) and thymine (T) or uracil (U) nucleotides. As an example, WO2002/098443 discloses a pharmaceutical composition containing an mRNA stabilized by sequence modifications in the translated region. Due to the degeneracy of the genetic code, the modifications work by

substituting existing codons for those that promote greater RNA stability without changing the resulting amino acid. The approach is limited to coding regions of the RNA.

Signal peptides

5 In some embodiments, antigenic polypeptides encoded by RSV polynucleotides comprise a signal peptide. Signal peptides, comprising the N-terminal 15-60 amino acids of proteins, are typically needed for the translocation across the membrane on the secretory pathway and thus universally control the entry of most proteins both in eukaryotes and prokaryotes to the secretory pathway. Signal peptides generally include of three regions: an
10 N-terminal region of differing length, which usually comprises positively charged amino acids; a hydrophobic region; and a short carboxy-terminal peptide region. In eukaryotes, the signal peptide of a nascent precursor protein (pre-protein) directs the ribosome to the rough endoplasmic reticulum (ER) membrane and initiates the transport of the growing peptide chain across it. The signal peptide is not responsible for the final destination of the mature
15 protein, however. Secretory proteins devoid of further address tags in their sequence are by default secreted to the external environment. Signal peptides are cleaved from precursor proteins by an endoplasmic reticulum (ER)-resident signal peptidase or they remain uncleaved and function as a membrane anchor. During recent years, a more advanced view of signal peptides has evolved, showing that the functions and immunodominance of certain
20 signal peptides are much more versatile than previously anticipated.

Signal peptides typically function to facilitate the targeting of newly synthesized protein to the endoplasmic reticulum (ER) for processing. ER processing produces a mature Envelope protein, wherein the signal peptide is cleaved, typically by a signal peptidase of the host cell. A signal peptide may also facilitate the targeting of the protein to the cell
25 membrane. RSV vaccines of the present disclosure may comprise, for example, RNA polynucleotides encoding an artificial signal peptide, wherein the signal peptide coding sequence is operably linked to and is in frame with the coding sequence of the RSV antigenic polypeptide. Thus, RSV vaccines of the present disclosure, in some embodiments, produce an antigenic polypeptide comprising a RSV antigenic polypeptide fused to a signal peptide.
30 In some embodiments, a signal peptide is fused to the N-terminus of the RSV antigenic polypeptide. In some embodiments, a signal peptide is fused to the C-terminus of the RSV antigenic polypeptide.

In some embodiments, the signal peptide fused to the RSV antigenic polypeptide is an artificial signal peptide. In some embodiments, an artificial signal peptide fused to the RSV

antigenic polypeptide encoded by the RSV RNA (*e.g.*, mRNA) vaccine is obtained from an immunoglobulin protein, *e.g.*, an IgE signal peptide or an IgG signal peptide. In some embodiments, a signal peptide fused to the RSV antigenic polypeptide encoded by a RSV RNA (*e.g.*, mRNA) vaccine is an Ig heavy chain epsilon-1 signal peptide (IgE HC SP) having the sequence of: MDWTWILFLVAAATRVHS (SEQ ID NO: 281). In some embodiments, a signal peptide fused to a RSV antigenic polypeptide encoded by the RSV RNA (*e.g.*, mRNA) vaccine is an IgGk chain V-III region HAH signal peptide (IgGk SP) having the sequence of METPAQLLFLLLLWLPDTTG (SEQ ID NO: 282). In some embodiments, the RSV antigenic polypeptide encoded by a RSV RNA (*e.g.*, mRNA) vaccine has an amino acid sequence set forth in one of SEQ ID NO: 1 to SEQ ID NO: 28 fused to a signal peptide of SEQ ID NO: 281 or SEQ ID NO: 282. The examples disclosed herein are not meant to be limiting and any signal peptide that is known in the art to facilitate targeting of a protein to ER for processing and/or targeting of a protein to the cell membrane may be used in accordance with the present disclosure.

A signal peptide may have a length of 15-60 amino acids. For example, a signal peptide may have a length of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 amino acids. In some embodiments, a signal peptide may have a length of 20-60, 25-60, 30-60, 35-60, 40-60, 45-60, 50-60, 55-60, 15-55, 20-55, 25-55, 30-55, 35-55, 40-55, 45-55, 50-55, 15-50, 20-50, 25-50, 30-50, 35-50, 40-50, 45-50, 15-45, 20-45, 25-45, 30-45, 35-45, 40-45, 15-40, 20-40, 25-40, 30-40, 35-40, 15-35, 20-35, 25-35, 30-35, 15-30, 20-30, 25-30, 15-25, 20-25, or 15-20 amino acids.

A signal peptide is typically cleaved from the nascent polypeptide at the cleavage junction during ER processing. The mature RSV antigenic polypeptide produced by RSV RNA vaccine of the present disclosure typically does not comprise a signal peptide.

Chemical Modifications

RNA (*e.g.*, mRNA) vaccines of the present disclosure comprise, in some embodiments, at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding at least one respiratory syncytial virus (RSV) antigenic polypeptide, wherein said RNA comprises at least one chemical modification.

The terms “chemical modification” and “chemically modified” refer to modification with respect to adenosine (A), guanosine (G), uridine (U), thymidine (T) or cytidine (C) ribonucleosides or deoxyribonucleosides in at least one of their position, pattern, percent or

population. Generally, these terms do not refer to the ribonucleotide modifications in naturally occurring 5'-terminal mRNA cap moieties.

Modifications of polynucleotides include, without limitation, those described herein, and include, but are expressly not limited to, those modifications that comprise chemical modifications. Polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides) may comprise modifications that are naturally-occurring, non-naturally-occurring or the polynucleotide may comprise a combination of naturally-occurring and non-naturally-occurring modifications. Polynucleotides may include any useful modification, for example, of a sugar, a nucleobase, or an internucleoside linkage (*e.g.*, to a linking phosphate, to a phosphodiester linkage or to the phosphodiester backbone).

With respect to a polypeptide, the term “modification” refers to a modification relative to the canonical set of 20 amino acids. Polypeptides, as provided herein, are also considered “modified” if they contain amino acid substitutions, insertions or a combination of substitutions and insertions.

Polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides), in some embodiments, comprise various (more than one) different modifications. In some embodiments, a particular region of a polynucleotide contains one, two or more (optionally different) nucleoside or nucleotide modifications. In some embodiments, a modified RNA polynucleotide (*e.g.*, a modified mRNA polynucleotide), introduced to a cell or organism, exhibits reduced degradation in the cell or organism, respectively, relative to an unmodified polynucleotide. In some embodiments, a modified RNA polynucleotide (*e.g.*, a modified mRNA polynucleotide), introduced into a cell or organism, may exhibit reduced immunogenicity in the cell or organism, respectively (*e.g.*, a reduced innate response).

Polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides), in some embodiments, comprise non-natural modified nucleotides that are introduced during synthesis or post-synthesis of the polynucleotides to achieve desired functions or properties. The modifications may be present on internucleotide linkages, purine or pyrimidine bases, or sugars. The modification may be introduced with chemical synthesis or with a polymerase enzyme at the terminal of a chain or anywhere else in the chain. Any of the regions of a polynucleotide may be chemically modified.

The present disclosure provides for modified nucleosides and nucleotides of a polynucleotide (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides). A “nucleoside” refers to a compound containing a sugar molecule (*e.g.*, a pentose or ribose) or a derivative thereof in combination with an organic base (*e.g.*, a purine or pyrimidine) or a derivative

thereof (also referred to herein as “nucleobase”). A nucleotide” refers to a nucleoside, including a phosphate group. Modified nucleotides may be synthesized by any useful method, such as, for example, chemically, enzymatically, or recombinantly, to include one or more modified or non-natural nucleosides. Polynucleotides may comprise a region or regions of linked nucleosides. Such regions may have variable backbone linkages. The linkages may be standard phosphodiester linkages, in which case the polynucleotides would comprise regions of nucleotides.

Modified nucleotide base pairing encompasses not only the standard adenosine-thymine, adenosine-uracil, or guanosine-cytosine base pairs, but also base pairs formed between nucleotides and/or modified nucleotides comprising non-standard or modified bases, wherein the arrangement of hydrogen bond donors and hydrogen bond acceptors permits hydrogen bonding between a non-standard base and a standard base or between two complementary non-standard base structures, such as, for example, in those polynucleotides having at least one chemical modification. One example of such non-standard base pairing is the base pairing between the modified nucleotide inosine and adenine, cytosine or uracil. Any combination of base/sugar or linker may be incorporated into polynucleotides of the present disclosure.

Modifications of polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides), including but not limited to chemical modification, that are useful in the compositions, vaccines, methods and synthetic processes of the present disclosure include, but are not limited to the following: 2-methylthio-N6-(*cis*-hydroxyisopentenyl)adenosine; 2-methylthio-N6-methyladenosine; 2-methylthio-N6-threonyl carbamoyladenosine; N6-glycylcarbamoyladenosine; N6-isopentenyladenosine; N6-methyladenosine; N6-threonylcarbamoyladenosine; 1,2'-O-dimethyladenosine; 1-methyladenosine; 2'-O-methyladenosine; 2'-O-ribosyladenosine (phosphate); 2-methyladenosine; 2-methylthio-N6 isopentenyladenosine; 2-methylthio-N6-hydroxynorvalyl carbamoyladenosine; 2'-O-methyladenosine; 2'-O-ribosyladenosine (phosphate); Isopentenyladenosine; N6-(*cis*-hydroxyisopentenyl)adenosine; N6,2'-O-dimethyladenosine; N6,2'-O-dimethyladenosine; N6,N6,2'-O-trimethyladenosine; N6,N6-dimethyladenosine; N6-acetyladenosine; N6-hydroxynorvalylcarbamoyladenosine; N6-methyl-N6-threonylcarbamoyladenosine; 2-methyladenosine; 2-methylthio-N6-isopentenyladenosine; 7-deaza-adenosine; N1-methyladenosine; N6, N6 (dimethyl)adenine; N6-*cis*-hydroxy-isopentenyl-adenosine; α -thio-adenosine; 2 (amino)adenine; 2 (aminopropyl)adenine; 2 (methylthio) N6 (isopentenyl)adenine; 2-(alkyl)adenine; 2-(aminoalkyl)adenine; 2-(aminopropyl)adenine; 2-

(halo)adenine; 2-(halo)adenine; 2-(propyl)adenine; 2'-Amino-2'-deoxy-ATP; 2'-Azido-2'-deoxy-ATP; 2'-Deoxy-2'-a-aminoadenosine TP; 2'-Deoxy-2'-a-azidoadenosine TP; 6 (alkyl)adenine; 6 (methyl)adenine; 6-(alkyl)adenine; 6-(methyl)adenine; 7 (deaza)adenine; 8 (alkenyl)adenine; 8 (alkynyl)adenine; 8 (amino)adenine; 8 (thioalkyl)adenine; 8-
5 (alkenyl)adenine; 8-(alkyl)adenine; 8-(alkynyl)adenine; 8-(amino)adenine; 8-(halo)adenine; 8-(hydroxyl)adenine; 8-(thioalkyl)adenine; 8-(thiol)adenine; 8-azido-adenosine; aza adenine; deaza adenine; N6 (methyl)adenine; N6-(isopentyl)adenine; 7-deaza-8-aza-adenosine; 7-methyladenine; 1-Deazaadenosine TP; 2'Fluoro-N6-Bz-deoxyadenosine TP; 2'-OMe-2-Amino-ATP; 2'O-methyl-N6-Bz-deoxyadenosine TP; 2'-a-Ethynyladenosine TP; 2-
10 aminoadenine; 2-Aminoadenosine TP; 2-Amino-ATP; 2'-a-Trifluoromethyladenosine TP; 2-Azidoadenosine TP; 2'-b-Ethynyladenosine TP; 2-Bromoadenosine TP; 2'-b-Trifluoromethyladenosine TP; 2-Chloroadenosine TP; 2'-Deoxy-2',2'-difluoroadenosine TP; 2'-Deoxy-2'-a-mercaptoadenosine TP; 2'-Deoxy-2'-a-thiomethoxyadenosine TP; 2'-Deoxy-2'-b-aminoadenosine TP; 2'-Deoxy-2'-b-azidoadenosine TP; 2'-Deoxy-2'-b-bromoadenosine TP;
15 2'-Deoxy-2'-b-chloroadenosine TP; 2'-Deoxy-2'-b-fluoroadenosine TP; 2'-Deoxy-2'-b-iodoadenosine TP; 2'-Deoxy-2'-b-mercaptoadenosine TP; 2'-Deoxy-2'-b-thiomethoxyadenosine TP; 2-Fluoroadenosine TP; 2-Iodoadenosine TP; 2-Mercaptadenosine TP; 2-methoxy-adenine; 2-methylthio-adenine; 2-Trifluoromethyladenosine TP; 3-Deaza-3-bromoadenosine TP; 3-Deaza-3-chloroadenosine
20 TP; 3-Deaza-3-fluoroadenosine TP; 3-Deaza-3-iodoadenosine TP; 3-Deazaadenosine TP; 4'-Azidoadenosine TP; 4'-Carbocyclic adenosine TP; 4'-Ethynyladenosine TP; 5'-Homo-adenosine TP; 8-Aza-ATP; 8-bromo-adenosine TP; 8-Trifluoromethyladenosine TP; 9-Deazaadenosine TP; 2-aminopurine; 7-deaza-2,6-diaminopurine; 7-deaza-8-aza-2,6-diaminopurine; 7-deaza-8-aza-2-aminopurine; 2,6-diaminopurine; 7-deaza-8-aza-adenine, 7-
25 deaza-2-aminopurine; 2-thiocytidine; 3-methylcytidine; 5-formylcytidine; 5-hydroxymethylcytidine; 5-methylcytidine; N4-acetylcytidine; 2'-O-methylcytidine; 2'-O-methylcytidine; 5,2'-O-dimethylcytidine; 5-formyl-2'-O-methylcytidine; Lysidine; N4,2'-O-dimethylcytidine; N4-acetyl-2'-O-methylcytidine; N4-methylcytidine; N4,N4-Dimethyl-2'-OMe-Cytidine TP; 4-methylcytidine; 5-aza-cytidine; Pseudo-iso-cytidine; pyrrolo-cytidine;
30 α -thio-cytidine; 2-(thio)cytosine; 2'-Amino-2'-deoxy-CTP; 2'-Azido-2'-deoxy-CTP; 2'-Deoxy-2'-a-aminocytidine TP; 2'-Deoxy-2'-a-azidocytidine TP; 3 (deaza) 5 (aza)cytosine; 3 (methyl)cytosine; 3-(alkyl)cytosine; 3-(deaza) 5 (aza)cytosine; 3-(methyl)cytidine; 4,2'-O-dimethylcytidine; 5 (halo)cytosine; 5 (methyl)cytosine; 5 (propynyl)cytosine; 5 (trifluoromethyl)cytosine; 5-(alkyl)cytosine; 5-(alkynyl)cytosine; 5-(halo)cytosine; 5-

(propynyl)cytosine; 5-(trifluoromethyl)cytosine; 5-bromo-cytidine; 5-iodo-cytidine; 5-propynyl cytosine; 6-(azo)cytosine; 6-aza-cytidine; aza cytosine; deaza cytosine; N4 (acetyl)cytosine; 1-methyl-1-deaza-pseudoisocytidine; 1-methyl-pseudoisocytidine; 2-methoxy-5-methyl-cytidine; 2-methoxy-cytidine; 2-thio-5-methyl-cytidine; 4-methoxy-1-methyl-pseudoisocytidine; 4-methoxy-pseudoisocytidine; 4-thio-1-methyl-1-deaza-pseudoisocytidine; 4-thio-1-methyl-pseudoisocytidine; 4-thio-pseudoisocytidine; 5-azazebularine; 5-methyl-zebularine; pyrrolo-pseudoisocytidine; Zebularine; (E)-5-(2-Bromovinyl)cytidine TP; 2,2'-anhydro-cytidine TP hydrochloride; 2'Fluor-N4-Bz-cytidine TP; 2'Fluoro-N4-Acetyl-cytidine TP; 2'-O-Methyl-N4-Acetyl-cytidine TP; 2'-O-methyl-N4-Bz-cytidine TP; 2'-a-Ethynylcytidine TP; 2'-a-Trifluoromethylcytidine TP; 2'-b-Ethynylcytidine TP; 2'-b-Trifluoromethylcytidine TP; 2'-Deoxy-2',2'-difluorocytidine TP; 2'-Deoxy-2'-a-mercaptocytidine TP; 2'-Deoxy-2'-a-thiomethoxycytidine TP; 2'-Deoxy-2'-b-aminocytidine TP; 2'-Deoxy-2'-b-azidocytidine TP; 2'-Deoxy-2'-b-bromocytidine TP; 2'-Deoxy-2'-b-chlorocytidine TP; 2'-Deoxy-2'-b-fluorocytidine TP; 2'-Deoxy-2'-b-iodocytidine TP; 2'-Deoxy-2'-b-mercaptocytidine TP; 2'-Deoxy-2'-b-thiomethoxycytidine TP; 2'-O-Methyl-5-(1-propynyl)cytidine TP; 3'-Ethynylcytidine TP; 4'-Azidocytidine TP; 4'-Carbocyclic cytidine TP; 4'-Ethynylcytidine TP; 5-(1-Propynyl)ara-cytidine TP; 5-(2-Chloro-phenyl)-2-thiocytidine TP; 5-(4-Amino-phenyl)-2-thiocytidine TP; 5-Aminoallyl-CTP; 5-Cyanocytidine TP; 5-Ethynylara-cytidine TP; 5-Ethynylcytidine TP; 5'-Homo-cytidine TP; 5-Methoxycytidine TP; 5-Trifluoromethyl-Cytidine TP; N4-Amino-cytidine TP; N4-Benzoyl-cytidine TP; Pseudoisocytidine; 7-methylguanosine; N2,2'-O-dimethylguanosine; N2-methylguanosine; Wyosine; 1,2'-O-dimethylguanosine; 1-methylguanosine; 2'-O-methylguanosine; 2'-O-ribosylguanosine (phosphate); 2'-O-methylguanosine; 2'-O-ribosylguanosine (phosphate); 7-aminomethyl-7-deazaguanosine; 7-cyano-7-deazaguanosine; Archaeosine; Methylwyosine; N2,7-dimethylguanosine; N2,N2,2'-O-trimethylguanosine; N2,N2,7-trimethylguanosine; N2,N2-dimethylguanosine; N2,7,2'-O-trimethylguanosine; 6-thio-guanosine; 7-deaza-guanosine; 8-oxo-guanosine; N1-methyl-guanosine; α -thioguanosine; 2 (propyl)guanine; 2-(alkyl)guanine; 2'-Amino-2'-deoxy-GTP; 2'-Azido-2'-deoxy-GTP; 2'-Deoxy-2'-a-aminoguanosine TP; 2'-Deoxy-2'-a-azidoguanosine TP; 6 (methyl)guanine; 6-(alkyl)guanine; 6-(methyl)guanine; 6-methyl-guanosine; 7 (alkyl)guanine; 7 (deaza)guanine; 7 (methyl)guanine; 7-(alkyl)guanine; 7-(deaza)guanine; 7-(methyl)guanine; 8 (alkyl)guanine; 8 (alkynyl)guanine; 8 (halo)guanine; 8 (thioalkyl)guanine; 8-(alkenyl)guanine; 8-(alkyl)guanine; 8-(alkynyl)guanine; 8-(amino)guanine; 8-(halo)guanine; 8-(hydroxyl)guanine; 8-(thioalkyl)guanine; 8-(thiol)guanine; aza guanine;

deaza guanine; N (methyl)guanine; N-(methyl)guanine; 1-methyl-6-thio-guanosine; 6-methoxy-guanosine; 6-thio-7-deaza-8-aza-guanosine; 6-thio-7-deaza-guanosine; 6-thio-7-methyl-guanosine; 7-deaza-8-aza-guanosine; 7-methyl-8-oxo-guanosine; N2,N2-dimethyl-6-thio-guanosine; N2-methyl-6-thio-guanosine; 1-Me-GTP; 2'Fluoro-N2-isobutyl-guanosine TP; 2'O-methyl-N2-isobutyl-guanosine TP; 2'-a-Ethynylguanosine TP; 2'-a-Trifluoromethylguanosine TP; 2'-b-Ethynylguanosine TP; 2'-b-Trifluoromethylguanosine TP; 2'-Deoxy-2',2'-difluoroguanosine TP; 2'-Deoxy-2'-a-mercaptopguanosine TP; 2'-Deoxy-2'-a-thiomethoxyguanosine TP; 2'-Deoxy-2'-b-aminoguanosine TP; 2'-Deoxy-2'-b-azidoguanosine TP; 2'-Deoxy-2'-b-bromoguanosine TP; 2'-Deoxy-2'-b-chloroguanosine TP; 2'-Deoxy-2'-b-fluoroguanosine TP; 2'-Deoxy-2'-b-iodoguanosine TP; 2'-Deoxy-2'-b-mercaptopguanosine TP; 2'-Deoxy-2'-b-thiomethoxyguanosine TP; 4'-Azidoguanosine TP; 4'-Carbocyclic guanosine TP; 4'-Ethynylguanosine TP; 5'-Homo-guanosine TP; 8-bromo-guanosine TP; 9-Deazaguanosine TP; N2-isobutyl-guanosine TP; 1-methylinosine; Inosine; 1,2'-O-dimethylinosine; 2'-O-methylinosine; 7-methylinosine; 2'-O-methylinosine; Epoxyqueuosine; galactosyl-queuosine; Mannosylqueuosine; Queuosine; allyamino-thymidine; aza thymidine; deaza thymidine; deoxy-thymidine; 2'-O-methyluridine; 2-thiouridine; 3-methyluridine; 5-carboxymethyluridine; 5-hydroxyuridine; 5-methyluridine; 5-taurinomethyl-2-thiouridine; 5-taurinomethyluridine; Dihydrouridine; Pseudouridine; (3-(3-amino-3-carboxypropyl)uridine; 1-methyl-3-(3-amino-5-carboxypropyl)pseudouridine; 1-methylpseudouridine; 1-ethyl-pseudouridine; 2'-O-methyluridine; 2'-O-methylpseudouridine; 2'-O-methyluridine; 2-thio-2'-O-methyluridine; 3-(3-amino-3-carboxypropyl)uridine; 3,2'-O-dimethyluridine; 3-Methylpseudo-Uridine TP; 4-thiouridine; 5-(carboxyhydroxymethyl)uridine; 5-(carboxyhydroxymethyl)uridine methyl ester; 5,2'-O-dimethyluridine; 5,6-dihydro-uridine; 5-aminomethyl-2-thiouridine; 5-carbamoylmethyl-2'-O-methyluridine; 5-carbamoylmethyluridine; 5-carboxyhydroxymethyluridine; 5-carboxyhydroxymethyluridine methyl ester; 5-carboxymethylaminomethyl-2'-O-methyluridine; 5-carboxymethylaminomethyl-2-thiouridine; 5-carboxymethylaminomethyluridine; 5-carboxymethylaminomethyluridine TP; 5-methoxycarbonylmethyl-2'-O-methyluridine; 5-methoxycarbonylmethyl-2-thiouridine; 5-methoxycarbonylmethyluridine; 5-methyluridine), 5-methoxyuridine; 5-methyl-2-thiouridine; 5-methylaminomethyl-2-selenouridine; 5-methylaminomethyl-2-thiouridine; 5-methylaminomethyluridine; 5-Methyldihydrouridine; 5-Oxyacetic acid- Uridine TP; 5-Oxyacetic acid-methyl ester-Uridine TP; N1-methyl-pseudo-uracil; N1-ethyl-pseudo-uracil; uridine 5-oxyacetic acid; uridine 5-oxyacetic acid methyl ester; 3-(3-Amino-3-

carboxypropyl)-Uridine TP; 5-(iso-Pentenylaminomethyl)- 2-thiouridine TP; 5-(iso-Pentenylaminomethyl)-2'-O-methyluridine TP; 5-(iso-Pentenylaminomethyl)uridine TP; 5-propynyl uracil; α -thio-uridine; 1 (aminoalkylamino-carbonylethylenyl)-2(thio)-pseudouracil; 1 (aminoalkylaminocarbonylethylenyl)-2,4-(dithio)pseudouracil;

5 1 (aminoalkylaminocarbonylethylenyl)-4 (thio)pseudouracil; 1 (aminoalkylaminocarbonylethylenyl)-pseudouracil; 1 (aminocarbonylethylenyl)-2(thio)-pseudouracil; 1 (aminocarbonylethylenyl)-2,4-(dithio)pseudouracil; 1 (aminocarbonylethylenyl)-4 (thio)pseudouracil; 1 (aminocarbonylethylenyl)-pseudouracil; 1 substituted 2(thio)-pseudouracil; 1 substituted 2,4-(dithio)pseudouracil; 1 substituted 4

10 (thio)pseudouracil; 1 substituted pseudouracil; 1-(aminoalkylamino-carbonylethylenyl)-2-(thio)-pseudouracil; 1-Methyl-3-(3-amino-3-carboxypropyl) pseudouridine TP; 1-Methyl-3-(3-amino-3-carboxypropyl)pseudo-UTP; 1-Methyl-pseudo-UTP; 1-Ethyl-pseudo-UTP; 2 (thio)pseudouracil; 2' deoxy uridine; 2' fluorouridine; 2-(thio)uracil; 2,4-(dithio)pseudouracil; 2' methyl, 2'amino, 2'azido, 2'fluoro-guanosine; 2'-Amino-2'-deoxy-UTP; 2'-Azido-2'-deoxy-

15 UTP; 2'-Azido-deoxyuridine TP; 2'-O-methylpseudouridine; 2' deoxy uridine; 2' fluorouridine; 2'-Deoxy-2'-a-aminouridine TP; 2'-Deoxy-2'-a-azidouridine TP; 2-methylpseudouridine; 3 (3 amino-3 carboxypropyl)uracil; 4 (thio)pseudouracil; 4-(thio)pseudouracil; 4-(thio)uracil; 4-thiouracil; 5 (1,3-diazole-1-alkyl)uracil; 5 (2-aminopropyl)uracil; 5 (aminoalkyl)uracil; 5 (dimethylaminoalkyl)uracil; 5

20 (guanidiniumalkyl)uracil; 5 (methoxycarbonylmethyl)-2-(thio)uracil; 5 (methoxycarbonylmethyl)uracil; 5 (methyl) 2 (thio)uracil; 5 (methyl) 2,4 (dithio)uracil; 5 (methyl) 4 (thio)uracil; 5 (methylaminomethyl)-2 (thio)uracil; 5 (methylaminomethyl)-2,4 (dithio)uracil; 5 (methylaminomethyl)-4 (thio)uracil; 5 (propynyl)uracil; 5 (trifluoromethyl)uracil; 5-(2-aminopropyl)uracil; 5-(alkyl)-2-(thio)pseudouracil; 5-(alkyl)-2,4 (dithio)pseudouracil; 5-

25 (alkyl)-4 (thio)pseudouracil; 5-(alkyl)pseudouracil; 5-(alkyl)uracil; 5-(alkynyl)uracil; 5-(allylamino)uracil; 5-(cyanoalkyl)uracil; 5-(dialkylaminoalkyl)uracil; 5-(dimethylaminoalkyl)uracil; 5-(guanidiniumalkyl)uracil; 5-(halo)uracil; 5-(1,3-diazole-1-alkyl)uracil; 5-(methoxy)uracil; 5-(methoxycarbonylmethyl)-2-(thio)uracil; 5-

(methoxycarbonylmethyl)uracil; 5-(methyl) 2(thio)uracil; 5-(methyl) 2,4 (dithio)uracil; 5-

30 (methyl) 4 (thio)uracil; 5-(methyl)-2-(thio)pseudouracil; 5-(methyl)-2,4 (dithio)pseudouracil; 5-(methyl)-4 (thio)pseudouracil; 5-(methyl)pseudouracil; 5-(methylaminomethyl)-2 (thio)uracil; 5-(methylaminomethyl)-2,4(dithio)uracil; 5-(methylaminomethyl)-4-(thio)uracil; 5-(propynyl)uracil; 5-(trifluoromethyl)uracil; 5-aminoallyl-uridine; 5-bromo-uridine; 5-iodo-uridine; 5-uracil; 6 (azo)uracil; 6-(azo)uracil; 6-aza-uridine; allylamino-uracil; aza uracil;

deaza uracil; N3 (methyl)uracil; P pseudo-UTP-1-2-ethanoic acid; Pseudouracil; 4-Thio-pseudo-UTP; 1-carboxymethyl-pseudouridine; 1-methyl-1-deaza-pseudouridine; 1-propynyl-uridine; 1-taurinomethyl-1-methyl-uridine; 1-taurinomethyl-4-thio-uridine; 1-taurinomethyl-pseudouridine; 2-methoxy-4-thio-pseudouridine; 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; ($\pm$)-1-(2-Hydroxypropyl)pseudouridine TP; (2R)-1-(2-Hydroxypropyl)pseudouridine TP; (2S)-1-(2-Hydroxypropyl)pseudouridine TP; (E)-5-(2-Bromo-vinyl)ara-uridine TP; (E)-5-(2-Bromo-vinyl)uridine TP; (Z)-5-(2-Bromo-vinyl)ara-uridine TP; (Z)-5-(2-Bromo-vinyl)uridine TP; 1-(2,2,2-Trifluoroethyl)-pseudo-UTP; 1-(2,2,3,3,3-Pentafluoropropyl)pseudouridine TP; 1-(2,2-Diethoxyethyl)pseudouridine TP; 1-(2,4,6-Trimethylbenzyl)pseudouridine TP; 1-(2,4,6-Trimethyl-benzyl)pseudo-UTP; 1-(2,4,6-Trimethyl-phenyl)pseudo-UTP; 1-(2-Amino-2-carboxyethyl)pseudo-UTP; 1-(2-Amino-ethyl)pseudo-UTP; 1-(2-Hydroxyethyl)pseudouridine TP; 1-(2-Methoxyethyl)pseudouridine TP; 1-(3,4-Bis-trifluoromethoxybenzyl)pseudouridine TP; 1-(3,4-Dimethoxybenzyl)pseudouridine TP; 1-(3-Amino-3-carboxypropyl)pseudo-UTP; 1-(3-Amino-propyl)pseudo-UTP; 1-(3-Cyclopropyl-prop-2-ynyl)pseudouridine TP; 1-(4-Amino-4-carboxybutyl)pseudo-UTP; 1-(4-Amino-benzyl)pseudo-UTP; 1-(4-Amino-butyl)pseudo-UTP; 1-(4-Amino-phenyl)pseudo-UTP; 1-(4-Azidobenzyl)pseudouridine TP; 1-(4-Bromobenzyl)pseudouridine TP; 1-(4-Chlorobenzyl)pseudouridine TP; 1-(4-Fluorobenzyl)pseudouridine TP; 1-(4-Iodobenzyl)pseudouridine TP; 1-(4-Methanesulfonylbenzyl)pseudouridine TP; 1-(4-Methoxybenzyl)pseudouridine TP; 1-(4-Methoxy-benzyl)pseudo-UTP; 1-(4-Methoxy-phenyl)pseudo-UTP; 1-(4-Methylbenzyl)pseudouridine TP; 1-(4-Methyl-benzyl)pseudo-UTP; 1-(4-Nitrobenzyl)pseudouridine TP; 1-(4-Nitro-benzyl)pseudo-UTP; 1-(4-Nitro-phenyl)pseudo-UTP; 1-(4-Thiomethoxybenzyl)pseudouridine TP; 1-(4-Trifluoromethoxybenzyl)pseudouridine TP; 1-(4-Trifluoromethylbenzyl)pseudouridine TP; 1-(5-Amino-pentyl)pseudo-UTP; 1-(6-Amino-hexyl)pseudo-UTP; 1,6-Dimethyl-pseudo-UTP; 1-[3-(2-{2-[2-(2-Aminoethoxy)-ethoxy]-ethoxy}-ethoxy)-propionyl]pseudouridine TP; 1-{3-[2-(2-Aminoethoxy)-ethoxy]-propionyl} pseudouridine TP; 1-Acetyl-pseudouridine TP; 1-Alkyl-6-(1-propynyl)-pseudo-UTP; 1-Alkyl-6-(2-propynyl)-pseudo-UTP; 1-Alkyl-6-allyl-pseudo-UTP; 1-Alkyl-6-ethynyl-pseudo-UTP; 1-Alkyl-6-homoallyl-pseudo-UTP; 1-Alkyl-6-vinyl-pseudo-UTP; 1-Allylpseudouridine TP; 1-Aminomethyl-pseudo-UTP; 1-

Benzoylpseudouridine TP; 1-Benzyloxymethylpseudouridine TP; 1-Benzyl-pseudo-UTP; 1-Biotinyl-PEG2-pseudouridine TP; 1-Biotinylpseudouridine TP; 1-Butyl-pseudo-UTP; 1-Cyanomethylpseudouridine TP; 1-Cyclobutylmethyl-pseudo-UTP; 1-Cyclobutyl-pseudo-UTP; 1-Cycloheptylmethyl-pseudo-UTP; 1-Cycloheptyl-pseudo-UTP; 1-Cyclohexylmethyl-pseudo-UTP; 1-Cyclohexyl-pseudo-UTP; 1-Cyclooctylmethyl-pseudo-UTP; 1-Cyclooctyl-pseudo-UTP; 1-Cyclopentylmethyl-pseudo-UTP; 1-Cyclopentyl-pseudo-UTP; 1-Cyclopropylmethyl-pseudo-UTP; 1-Cyclopropyl-pseudo-UTP; 1-Ethyl-pseudo-UTP; 1-Hexyl-pseudo-UTP; 1-Homoallylpseudouridine TP; 1-Hydroxymethylpseudouridine TP; 1-iso-propyl-pseudo-UTP; 1-Me-2-thio-pseudo-UTP; 1-Me-4-thio-pseudo-UTP; 1-Me-alpha-thio-pseudo-UTP; 1-Methanesulfonylmethylpseudouridine TP; 1-Methoxymethylpseudouridine TP; 1-Methyl-6-(2,2,2-Trifluoroethyl)pseudo-UTP; 1-Methyl-6-(4-morpholino)-pseudo-UTP; 1-Methyl-6-(4-thiomorpholino)-pseudo-UTP; 1-Methyl-6-(substituted phenyl)pseudo-UTP; 1-Methyl-6-amino-pseudo-UTP; 1-Methyl-6-azido-pseudo-UTP; 1-Methyl-6-bromo-pseudo-UTP; 1-Methyl-6-butyl-pseudo-UTP; 1-Methyl-6-chloro-pseudo-UTP; 1-Methyl-6-cyano-pseudo-UTP; 1-Methyl-6-dimethylamino-pseudo-UTP; 1-Methyl-6-ethoxy-pseudo-UTP; 1-Methyl-6-ethylcarboxylate-pseudo-UTP; 1-Methyl-6-ethyl-pseudo-UTP; 1-Methyl-6-fluoro-pseudo-UTP; 1-Methyl-6-formyl-pseudo-UTP; 1-Methyl-6-hydroxyamino-pseudo-UTP; 1-Methyl-6-hydroxy-pseudo-UTP; 1-Methyl-6-iodo-pseudo-UTP; 1-Methyl-6-iso-propyl-pseudo-UTP; 1-Methyl-6-methoxy-pseudo-UTP; 1-Methyl-6-methylamino-pseudo-UTP; 1-Methyl-6-phenyl-pseudo-UTP; 1-Methyl-6-propyl-pseudo-UTP; 1-Methyl-6-tert-butyl-pseudo-UTP; 1-Methyl-6-trifluoromethoxy-pseudo-UTP; 1-Methyl-6-trifluoromethyl-pseudo-UTP; 1-Morpholinomethylpseudouridine TP; 1-Pentyl-pseudo-UTP; 1-Phenyl-pseudo-UTP; 1-Pivaloylpseudouridine TP; 1-Propargylpseudouridine TP; 1-Propyl-pseudo-UTP; 1-propynyl-pseudouridine; 1-p-tolyl-pseudo-UTP; 1-tert-Butyl-pseudo-UTP; 1-Thiomethoxymethylpseudouridine TP; 1-Thiomorpholinomethylpseudouridine TP; 1-Trifluoroacetylpsudouridine TP; 1-Trifluoromethyl-pseudo-UTP; 1-Vinylpseudouridine TP; 2,2'-anhydro-uridine TP; 2'-bromo-deoxyuridine TP; 2'-F-5-Methyl-2'-deoxy-UTP; 2'-OMe-5-Me-UTP; 2'-OMe-pseudo-UTP; 2'-a-Ethynyluridine TP; 2'-a-Trifluoromethyluridine TP; 2'-b-Ethynyluridine TP; 2'-b-Trifluoromethyluridine TP; 2'-Deoxy-2',2'-difluorouridine TP; 2'-Deoxy-2'-a-mercaptopuridine TP; 2'-Deoxy-2'-a-thiomethoxyuridine TP; 2'-Deoxy-2'-b-aminouridine TP; 2'-Deoxy-2'-b-azidouridine TP; 2'-Deoxy-2'-b-bromouridine TP; 2'-Deoxy-2'-b-chlorouridine TP; 2'-Deoxy-2'-b-fluorouridine TP; 2'-Deoxy-2'-b-iodouridine TP; 2'-Deoxy-2'-b-mercaptopuridine TP; 2'-Deoxy-2'-b-thiomethoxyuridine TP; 2-methoxy-4-thio-uridine; 2-methoxyuridine; 2'-O-

Methyl-5-(1-propynyl)uridine TP; 3-Alkyl-pseudo-UTP; 4'-Azidouridine TP; 4'-Carbocyclic uridine TP; 4'-Ethynyluridine TP; 5-(1-Propynyl)ara-uridine TP; 5-(2-Furanyl)uridine TP; 5-Cyanouridine TP; 5-Dimethylaminouridine TP; 5'-Homo-uridine TP; 5-iodo-2'-fluoro-deoxyuridine TP; 5-Phenylethynyluridine TP; 5-Trideuteromethyl-6-deuterouridine TP; 5-Trifluoromethyl-Uridine TP; 5-Vinylarauridine TP; 6-(2,2,2-Trifluoroethyl)-pseudo-UTP; 6-(4-Morpholino)-pseudo-UTP; 6-(4-Thiomorpholino)-pseudo-UTP; 6-(Substituted-Phenyl)-pseudo-UTP; 6-Amino-pseudo-UTP; 6-Azido-pseudo-UTP; 6-Bromo-pseudo-UTP; 6-Butyl-pseudo-UTP; 6-Chloro-pseudo-UTP; 6-Cyano-pseudo-UTP; 6-Dimethylamino-pseudo-UTP; 6-Ethoxy-pseudo-UTP; 6-Ethylcarboxylate-pseudo-UTP; 6-Ethyl-pseudo-UTP; 6-Fluoro-pseudo-UTP; 6-Formyl-pseudo-UTP; 6-Hydroxyamino-pseudo-UTP; 6-Hydroxy-pseudo-UTP; 6-Iodo-pseudo-UTP; 6-iso-Propyl-pseudo-UTP; 6-Methoxy-pseudo-UTP; 6-Methylamino-pseudo-UTP; 6-Methyl-pseudo-UTP; 6-Phenyl-pseudo-UTP; 6-Phenyl-pseudo-UTP; 6-Propyl-pseudo-UTP; 6-tert-Butyl-pseudo-UTP; 6-Trifluoromethoxy-pseudo-UTP; 6-Trifluoromethyl-pseudo-UTP; Alpha-thio-pseudo-UTP; Pseudouridine 1-(4-methylbenzenesulfonic acid) TP; Pseudouridine 1-(4-methylbenzoic acid) TP; Pseudouridine TP 1-[3-(2-ethoxy)]propionic acid; Pseudouridine TP 1-[3-{2-(2-[2-(2-ethoxy)-ethoxy]-ethoxy)-ethoxy}]propionic acid; Pseudouridine TP 1-[3-{2-(2-[2-{2(2-ethoxy)-ethoxy}-ethoxy]-ethoxy)-ethoxy}]propionic acid; Pseudouridine TP 1-[3-{2-(2-[2-ethoxy]-ethoxy)-ethoxy}]propionic acid; Pseudouridine TP 1-[3-{2-(2-ethoxy)-ethoxy}] propionic acid; Pseudouridine TP 1-methylphosphonic acid; Pseudouridine TP 1-methylphosphonic acid diethyl ester; Pseudo-UTP-N1-3-propionic acid; Pseudo-UTP-N1-4-butanoic acid; Pseudo-UTP-N1-5-pentanoic acid; Pseudo-UTP-N1-6-hexanoic acid; Pseudo-UTP-N1-7-heptanoic acid; Pseudo-UTP-N1-methyl-p-benzoic acid; Pseudo-UTP-N1-p-benzoic acid; Wybutosine; Hydroxywybutosine; Isowyosine; Peroxywybutosine; undermodified hydroxywybutosine; 4-demethylwyosine; 2,6-(diamino)purine; 1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl; 1,3-(diaz)-2-(oxo)-phenthiazin-1-yl; 1,3-(diaz)-2-(oxo)-phenoxazin-1-yl; 1,3,5-(triaz)-2,6-(diox)-naphthalene; 2 (amino)purine; 2,4,5-(trimethyl)phenyl; 2' methyl, 2' amino, 2' azido, 2' fluoro-cytidine; 2' methyl, 2' amino, 2' azido, 2' fluoro-adenine; 2' methyl, 2' amino, 2' azido, 2' fluoro-uridine; 2'-amino-2'-deoxyribose; 2-amino-6-Chloro-purine; 2-aza-inosinyl; 2'-azido-2'-deoxyribose; 2'fluoro-2'-deoxyribose; 2'-fluoro-modified bases; 2'-O-methyl-ribose; 2-oxo-7-aminopyridopyrimidin-3-yl; 2-oxo-pyridopyrimidine-3-yl; 2-pyridinone; 3 nitropyrrole; 3-(methyl)-7-(propynyl)isocarbostyryl; 3-(methyl)isocarbostyryl; 4-(fluoro)-6-(methyl)benzimidazole; 4-(methyl)benzimidazole; 4-(methyl)indolyl; 4,6-(dimethyl)indolyl; 5 nitroindole; 5 substituted pyrimidines; 5-(methyl)isocarbostyryl; 5-nitroindole; 6-

(aza)pyrimidine; 6-(azo)thymine; 6-(methyl)-7-(aza)indolyl; 6-chloro-purine; 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; 7-(aminoalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl; 7-(aminoalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl; 7-(aminoalkylhydroxy)-1,3-(diaz)-2-(oxo)-phenoxazin-1-yl; 7-(aminoalkylhydroxy)-1,3-(diaz)-2-(oxo)-phenthiazin-1-yl; 7-(aminoalkylhydroxy)-1,3-(diaz)-2-(oxo)-phenoxazin-1-yl; 7-(aza)indolyl; 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl; 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl; 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl; 7-(guanidiniumalkylhydroxy)-1,3-(diaz)-2-(oxo)-phenoxazin-1-yl; 7-(guanidiniumalkylhydroxy)-1,3-(diaz)-2-(oxo)-phenthiazin-1-yl; 7-(guanidiniumalkylhydroxy)-1,3-(diaz)-2-(oxo)-phenoxazin-1-yl; 7-(propynyl)isocarbostyryl; 7-(propynyl)isocarbostyryl, propynyl-7-(aza)indolyl; 7-deaza-inosinyl; 7-substituted 1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl; 7-substituted 1,3-(diaz)-2-(oxo)-phenoxazin-1-yl; 9-(methyl)-imidizopyridinyl; Aminoindolyl; Anthracenyl; bis-ortho-(aminoalkylhydroxy)-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; bis-ortho-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; Difluorotolyl; Hypoxanthine; Imidizopyridinyl; Inosinyl; Isocarbostyryl; Isoguanisine; N2-substituted purines; N6-methyl-2-amino-purine; N6-substituted purines; N-alkylated derivative; Napthalenyl; Nitrobenzimidazolyl; Nitroimidazolyl; Nitroindazolyl; Nitropyrazolyl; Nubularine; O6-substituted purines; O-alkylated derivative; ortho-(aminoalkylhydroxy)-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; ortho-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; Oxoformycin TP; para-(aminoalkylhydroxy)-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; para-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; Pentacenyl; Phenanthracenyl; Phenyl; propynyl-7-(aza)indolyl; Pyrenyl; pyridopyrimidin-3-yl; pyridopyrimidin-3-yl, 2-oxo-7-amino-pyridopyrimidin-3-yl; pyrrolo-pyrimidin-2-on-3-yl; Pyrrolopyrimidinyl; Pyrrolopyrizinyl; Stilbenzyl; substituted 1,2,4-triazoles; Tetracenyl; Tubercidine; Xanthine; Xanthosine-5'-TP; 2-thio-zebularine; 5-aza-2-thio-zebularine; 7-deaza-2-amino-purine; pyridin-4-one ribonucleoside; 2-Amino-riboside-TP; Formycin A TP; Formycin B TP; Pyrrolosine TP; 2'-OH-ara-adenosine TP; 2'-OH-ara-cytidine TP; 2'-OH-ara-uridine TP; 2'-OH-ara-guanosine TP; 5-(2-carbomethoxyvinyl)uridine TP; and N6-(19-Amino-pentaoxanonadecyl)adenosine TP.

In some embodiments, polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides) include a combination of at least two (*e.g.*, 2, 3, 4 or more) of the aforementioned modified nucleobases.

In some embodiments, modified nucleobases in polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides) are selected from the group consisting of pseudouridine (ψ), 2-thiouridine (s2U), 4'-thiouridine, 5-methylcytosine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-
5 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-methyluridine, 5-methoxyuridine, 2'-O-methyl uridine, 1-methyl-pseudouridine (m1 ψ), 1-ethyl-pseudouridine (e1 ψ), 5-methoxy-uridine (mo5U), 5-methyl-cytidine (m5C), α -thio-guanosine, α -thio-adenosine, 5-cyano
10 uridine, 4'-thio uridine 7-deaza-adenine, 1-methyl-adenosine (m1A), 2-methyl-adenine (m2A), N6-methyl-adenosine (m6A), and 2,6-Diaminopurine, (I), 1-methyl-inosine (m1I), wyosine (imG), methylwyosine (mimG), 7-deaza-guanosine, 7-cyano-7-deaza-guanosine (preQ0), 7-aminomethyl-7-deaza-guanosine (preQ1), 7-methyl-guanosine (m7G), 1-methyl-guanosine (m1G), 8-oxo-guanosine, 7-methyl-8-oxo-guanosine, 2,8-dimethyladenosine, 2-
15 geranylthiouridine, 2-lysidine, 2-selenouridine, 3-(3-amino-3-carboxypropyl)-5,6-dihydrouridine, 3-(3-amino-3-carboxypropyl)pseudouridine, 3-methylpseudouridine, 5-(carboxyhydroxymethyl)-2'-O-methyluridine methyl ester, 5-aminomethyl-2-geranylthiouridine, 5-aminomethyl-2-selenouridine, 5-aminomethyluridine, 5-carbamoylhydroxymethyluridine, 5-carbamoylmethyl-2-thiouridine, 5-carboxymethyl-2-
20 thiouridine, 5-carboxymethylaminomethyl-2-geranylthiouridine, 5-carboxymethylaminomethyl-2-selenouridine, 5-cyanomethyluridine, 5-hydroxycytidine, 5-methylaminomethyl-2-geranylthiouridine, 7-aminocarboxypropyl-demethylwyosine, 7-aminocarboxypropylwyosine, 7-aminocarboxypropylwyosine methyl ester, 8-methyladenosine, N4,N4-dimethylcytidine, N6-formyladenosine, N6-
25 hydroxymethyladenosine, agmatidine, cyclic N6-threonylcarbamoyladenosine, glutamyl-queuosine, methylated undermodified hydroxywybutosine, N4,N4,2'-O-trimethylcytidine, geranylated 5-methylaminomethyl-2-thiouridine, geranylated 5-carboxymethylaminomethyl-2-thiouridine, Qbase, preQ0base, preQ1base, and combinations of two or more thereof. In some embodiments, the at least one chemically modified nucleoside is selected from the
30 group consisting of pseudouridine, 1-methyl-pseudouridine, 1-ethyl-pseudouridine, 5-methylcytosine, 5-methoxyuridine, and a combination thereof. In some embodiments, the polyribonucleotide (*e.g.*, RNA polyribonucleotide, such as mRNA polyribonucleotide) includes a combination of at least two (*e.g.*, 2, 3, 4 or more) of the aforementioned modified nucleobases. In some embodiments, polynucleotides (*e.g.*, RNA polynucleotides, such as

mRNA polynucleotides) include a combination of at least two (*e.g.*, 2, 3, 4 or more) of the aforementioned modified nucleobases.

In some embodiments, modified nucleobases in polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides) are selected from the group consisting of 1-methyl-pseudouridine (m1 ψ), 1-ethyl-pseudouridine (e1 ψ), 5-methoxy-uridine (mo5U), 5-methyl-cytidine (m5C), pseudouridine (ψ), α -thio-guanosine and α -thio-adenosine. In some embodiments, the polyribonucleotide includes a combination of at least two (*e.g.*, 2, 3, 4 or more) of the aforementioned modified nucleobases.

In some embodiments, polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides) comprise pseudouridine (ψ) and 5-methyl-cytidine (m5C). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 1-methyl-pseudouridine (m1 ψ). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 1-ethyl-pseudouridine (e1 ψ). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 1-methyl-pseudouridine (m1 ψ) and 5-methyl-cytidine (m5C). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 1-ethyl-pseudouridine (e1 ψ) and 5-methyl-cytidine (m5C). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 2-thiouridine (s2U). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 2-thiouridine and 5-methyl-cytidine (m5C). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise methoxy-uridine (mo5U). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 5-methoxy-uridine (mo5U) and 5-methyl-cytidine (m5C). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 2'-O-methyl uridine. In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 2'-O-methyl uridine and 5-methyl-cytidine (m5C). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise N6-methyl-adenosine (m6A). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise N6-methyl-adenosine (m6A) and 5-methyl-cytidine (m5C).

In some embodiments, polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides) are uniformly modified (*e.g.*, fully modified, modified throughout the entire sequence) for a particular modification. For example, a polynucleotide can be uniformly modified with 1-methyl-pseudouridine, meaning that all uridine residues in the mRNA sequence are replaced with 1-methyl-pseudouridine. Similarly, a polynucleotide can be uniformly modified for any type of nucleoside residue present in the sequence by replacement with a modified residue such as those set forth above.

Exemplary nucleobases and nucleosides having a modified cytosine include N4-acetyl-cytidine (ac4C), 5-methyl-cytidine (m5C), 5-halo-cytidine (*e.g.*, 5-iodo-cytidine), 5-hydroxymethyl-cytidine (hm5C), 1-methyl-pseudoisocytidine, 2-thio-cytidine (s2C), and 2-thio-5-methyl-cytidine.

5 In some embodiments, a modified nucleobase is a modified uridine. Exemplary nucleobases and nucleosides having a modified uridine include 1-methyl-pseudouridine (m1ψ), 1-ethyl-pseudouridine (e1ψ), 5-methoxy uridine, 2-thio uridine, 5-cyano uridine, 2'-O-methyl uridine and 4'-thio uridine.

10 In some embodiments, a modified nucleobase is a modified adenine. Exemplary nucleobases and nucleosides having a modified adenine include 7-deaza-adenine, 1-methyl-adenosine (m1A), 2-methyl-adenine (m2A), and N6-methyl-adenosine (m6A).

In some embodiments, a modified nucleobase is a modified guanine. Exemplary nucleobases and nucleosides having a modified guanine include inosine (I), 1-methyl-inosine (m1I), wyosine (imG), methylwyosine (mimG), 7-deaza-guanosine, 7-cyano-7-deaza-
15 guanosine (preQ0), 7-aminomethyl-7-deaza-guanosine (preQ1), 7-methyl-guanosine (m7G), 1-methyl-guanosine (m1G), 8-oxo-guanosine, and 7-methyl-8-oxo-guanosine.

The polynucleotides of the present disclosure may be partially or fully modified along the entire length of the molecule. For example, one or more or all or a given type of nucleotide (*e.g.*, purine or pyrimidine, or any one or more or all of A, G, U, C) may be
20 uniformly modified in a polynucleotide of the invention, or in a given predetermined sequence region thereof (*e.g.*, in the mRNA including or excluding the polyA tail). In some embodiments, all nucleotides X in a polynucleotide of the present disclosure (or in a given sequence region thereof) are modified nucleotides, wherein X may be any one of nucleotides A, G, U, C, or any one of the combinations A+G, A+U, A+C, G+U, G+C, U+C, A+G+U,
25 A+G+C, G+U+C or A+G+C.

The polynucleotide may contain from about 1% to about 100% modified nucleotides (either in relation to overall nucleotide content, or in relation to one or more types of nucleotide, *i.e.*, any one or more of A, G, U or C) or any intervening percentage (*e.g.*, from 1% to 20%, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1%
30 to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to 100%, from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50%

to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100%). It will be understood that any remaining percentage is accounted for by the presence of unmodified A, G, U, or C.

5 The polynucleotides may contain at a minimum 1% and at maximum 100% modified nucleotides, or any intervening percentage, such as at least 5% modified nucleotides, at least 10% modified nucleotides, at least 25% modified nucleotides, at least 50% modified nucleotides, at least 80% modified nucleotides, or at least 90% modified nucleotides. For example, the polynucleotides may contain a modified pyrimidine such as a modified uracil or
 10 cytosine. In some embodiments, at least 5%, at least 10%, at least 25%, at least 50%, at least 80%, at least 90% or 100% of the uracil in the polynucleotide is replaced with a modified uracil (*e.g.*, a 5-substituted uracil). The modified uracil can be replaced by a compound having a single unique structure, or can be replaced by a plurality of compounds having different structures (*e.g.*, 2, 3, 4 or more unique structures). In some embodiments, at least
 15 5%, at least 10%, at least 25%, at least 50%, at least 80%, at least 90% or 100% of the cytosine in the polynucleotide is replaced with a modified cytosine (*e.g.*, a 5-substituted cytosine). The modified cytosine can be replaced by a compound having a single unique structure, or can be replaced by a plurality of compounds having different structures (*e.g.*, 2, 3, 4 or more unique structures).

20 In some embodiments, the modified nucleobase is a modified uracil. Exemplary nucleobases and nucleosides having a modified uracil include pseudouridine (ψ), pyridin-4-one ribonucleoside, 5-aza-uridine, 6-aza-uridine, 2-thio-5-aza-uridine, 2-thio-uridine (s^2U), 4-thio-uridine (s^4U), 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxy-uridine (ho^5U), 5-aminoallyl-uridine, 5-halo-uridine (*e.g.*, 5-iodo-uridine or 5-bromo-uridine), 3-methyl-uridine
 25 (m^3U), 5-methoxy-uridine (mo^5U), uridine 5-oxyacetic acid (cmo^5U), uridine 5-oxyacetic acid methyl ester ($mcmo^5U$), 5-carboxymethyl-uridine (cm^5U), 1-carboxymethyl-pseudouridine, 5-carboxyhydroxymethyl-uridine (chm^5U), 5-carboxyhydroxymethyl-uridine methyl ester ($mchm^5U$), 5-methoxycarbonylmethyl-uridine (mcm^5U), 5-methoxycarbonylmethyl-2-thio-uridine (mcm^5s^2U), 5-aminomethyl-2-thio-uridine (nm^5s^2U),
 30 5-methylaminomethyl-uridine (mnm^5U), 5-methylaminomethyl-2-thio-uridine (mnm^5s^2U), 5-methylaminomethyl-2-seleno-uridine (mnm^5se^2U), 5-carbamoylmethyl-uridine (ncm^5U), 5-carboxymethylaminomethyl-uridine ($cmnm^5U$), 5-carboxymethylaminomethyl-2-thio-uridine ($cmnm^5s^2U$), 5-propynyl-uridine, 1-propynyl-pseudouridine, 5-aurinomethyl-uridine (τm^5U), 1-aurinomethyl-pseudouridine, 5-aurinomethyl-2-thio-uridine (τm^5s^2U), 1-aurinomethyl-4-

thio-pseudouridine, 5-methyl-uridine (m^5U , i.e., having the nucleobase deoxythymine), 1-methyl-pseudouridine ($m^1\psi$), 1-ethyl-pseudouridine ($e1\psi$), 5-methyl-2-thio-uridine (m^5s^2U), 1-methyl-4-thio-pseudouridine ($m^1s^4\psi$), 4-thio-1-methyl-pseudouridine, 3-methyl-pseudouridine ($m^3\psi$), 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydrouridine (D), dihydropseudouridine, 5,6-dihydrouridine, 5-methyl-dihydrouridine (m^5D), 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxy-uridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, 3-(3-amino-3-carboxypropyl)uridine (acp^3U), 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine ($acp^3\psi$), 5-(isopentenylaminomethyl)uridine (inm^5U), 5-(isopentenylaminomethyl)-2-thio-uridine (inm^5s^2U), α -thio-uridine, 2'-O-methyl-uridine (Um), 5,2'-O-dimethyl-uridine (m^5Um), 2'-O-methyl-pseudouridine (ψm), 2-thio-2'-O-methyl-uridine (s^2Um), 5-methoxycarbonylmethyl-2'-O-methyl-uridine (mcm^5Um), 5-carbamoylmethyl-2'-O-methyl-uridine (ncm^5Um), 5-carboxymethylaminomethyl-2'-O-methyl-uridine ($cmnm^5Um$), 3,2'-O-dimethyl-uridine (m^3Um), and 5-(isopentenylaminomethyl)-2'-O-methyl-uridine (inm^5Um), 1-thio-uridine, deoxythymidine, 2'-F-ara-uridine, 2'-F-uridine, 2'-OH-ara-uridine, 5-(2-carbomethoxyvinyl)uridine, and 5-[3-(1-E-propenylamino)]uridine.

In some embodiments, the modified nucleobase is a modified cytosine. Exemplary nucleobases and nucleosides having a modified cytosine include 5-aza-cytidine, 6-azacytidine, pseudoisocytidine, 3-methyl-cytidine (m^3C), N4-acetyl-cytidine (ac^4C), 5-formyl-cytidine (f^5C), N4-methyl-cytidine (m^4C), 5-methyl-cytidine (m^5C), 5-halo-cytidine (e.g., 5-iodo-cytidine), 5-hydroxymethyl-cytidine (hm^5C), 1-methyl-pseudoisocytidine, pyrrolo-cytidine, pyrrolo-pseudoisocytidine, 2-thio-cytidine (s^2C), 2-thio-5-methyl-cytidine, 4-thio-pseudoisocytidine, 4-thio-1-methyl-pseudoisocytidine, 4-thio-1-methyl-1-deaza-pseudoisocytidine, 1-methyl-1-deaza-pseudoisocytidine, zebularine, 5-aza-zebularine, 5-methyl-zebularine, 5-aza-2-thio-zebularine, 2-thio-zebularine, 2-methoxy-cytidine, 2-methoxy-5-methyl-cytidine, 4-methoxy-pseudoisocytidine, 4-methoxy-1-methyl-pseudoisocytidine, lysidine (k_2C), α -thio-cytidine, 2'-O-methyl-cytidine (Cm), 5,2'-O-dimethyl-cytidine (m^5Cm), N4-acetyl-2'-O-methyl-cytidine (ac^4Cm), N4,2'-O-dimethyl-cytidine (m^4Cm), 5-formyl-2'-O-methyl-cytidine (f^5Cm), N4,N4,2'-O-trimethyl-cytidine (m^4_2Cm), 1-thio-cytidine, 2'-F-ara-cytidine, 2'-F-cytidine, and 2'-OH-ara-cytidine.

In some embodiments, the modified nucleobase is a modified adenine. Exemplary nucleobases and nucleosides having a modified adenine include 2-amino-purine, 2, 6-diaminopurine, 2-amino-6-halo-purine (e.g., 2-amino-6-chloro-purine), 6-halo-purine (e.g., 6-

chloro-purine), 2-amino-6-methyl-purine, 8-azido-adenosine, 7-deaza-adenine, 7-deaza-8-aza-adenine, 7-deaza-2-amino-purine, 7-deaza-8-aza-2-amino-purine, 7-deaza-2,6-diaminopurine, 7-deaza-8-aza-2,6-diaminopurine, 1-methyl-adenosine (m^1A), 2-methyl-adenine (m^2A), N6-methyl-adenosine (m^6A), 2-methylthio-N6-methyl-adenosine (ms^2m^6A),
 5 N6-isopentenyl-adenosine (i^6A), 2-methylthio-N6-isopentenyl-adenosine (ms^2i^6A), N6-(cis-hydroxyisopentenyl)adenosine (io^6A), 2-methylthio-N6-(cis-hydroxyisopentenyl)adenosine (ms^2io^6A), N6-glycylcarbamoyl-adenosine (g^6A), N6-threonylcarbamoyl-adenosine (t^6A), N6-methyl-N6-threonylcarbamoyl-adenosine (m^6t^6A), 2-methylthio-N6-threonylcarbamoyl-adenosine (ms^2g^6A), N6,N6-dimethyl-adenosine (m^6_2A), N6-hydroxynorvalylcarbamoyl-adenosine (hn^6A), 2-methylthio-N6-hydroxynorvalylcarbamoyl-adenosine (ms^2hn^6A), N6-acetyl-adenosine (ac^6A), 7-methyl-adenine, 2-methylthio-adenine, 2-methoxy-adenine, α -thio-adenosine, 2'-O-methyl-adenosine (Am), N6,2'-O-dimethyl-adenosine (m^6Am), N6,N6,2'-O-trimethyl-adenosine (m^6_2Am), 1,2'-O-dimethyl-adenosine (m^1Am), 2'-O-ribosyladenosine (phosphate) ($Ar(p)$), 2-amino-N6-methyl-purine, 1-thio-adenosine, 8-azido-adenosine, 2'-F-ara-adenosine, 2'-F-adenosine, 2'-OH-ara-adenosine, and N6-(19-amino-pentaoxanonadecyl)-adenosine.

In some embodiments, the modified nucleobase is a modified guanine. Exemplary nucleobases and nucleosides having a modified guanine include inosine (I), 1-methyl-inosine (m^1I), wyosine (imG), methylwyosine ($mimG$), 4-demethyl-wyosine ($imG-14$), isowyosine ($imG2$), wybutosine (yW), peroxywybutosine (o_2yW), hydroxywybutosine ($OhyW$), undermodified hydroxywybutosine ($OhyW^*$), 7-deaza-guanosine, queuosine (Q), epoxyqueuosine (oQ), galactosyl-queuosine ($galQ$), mannosyl-queuosine ($manQ$), 7-cyano-7-deaza-guanosine ($preQ_0$), 7-aminomethyl-7-deaza-guanosine ($preQ_1$), archaeosine (G^+), 7-deaza-8-aza-guanosine, 6-thio-guanosine, 6-thio-7-deaza-guanosine, 6-thio-7-deaza-8-aza-guanosine, 7-methyl-guanosine (m^7G), 6-thio-7-methyl-guanosine, 7-methyl-inosine, 6-methoxy-guanosine, 1-methyl-guanosine (m^1G), N2-methyl-guanosine (m^2G), N2,N2-dimethyl-guanosine (m^2_2G), N2,7-dimethyl-guanosine ($m^{2,7}G$), N2, N2,7-dimethyl-guanosine ($m^{2,2,7}G$), 8-oxo-guanosine, 7-methyl-8-oxo-guanosine, 1-methyl-6-thio-guanosine, N2-methyl-6-thio-guanosine, N2,N2-dimethyl-6-thio-guanosine, α -thio-guanosine, 2'-O-methyl-guanosine (Gm), N2-methyl-2'-O-methyl-guanosine (m^2Gm), N2,N2-dimethyl-2'-O-methyl-guanosine (m^2_2Gm), 1-methyl-2'-O-methyl-guanosine (m^1Gm), N2,7-dimethyl-2'-O-methyl-guanosine ($m^{2,7}Gm$), 2'-O-methyl-inosine (Im), 1,2'-O-dimethyl-inosine (m^1Im), 2'-O-ribosylguanosine (phosphate) ($Gr(p)$), 1-thio-guanosine, O6-methyl-guanosine, 2'-F-ara-guanosine, and 2'-F-guanosine.

In some embodiments, the RNA vaccines comprise a 5'UTR element, an optionally codon optimized open reading frame, and a 3'UTR element, a poly(A) sequence and/or a polyadenylation signal, wherein the RNA is not chemically modified.

5 *RSV RNA Vaccines - In Vitro Transcription of RNA (e.g., mRNA)*

RSV vaccines of the present disclosure comprise at least one RNA polynucleotide, such as a mRNA (*e.g.*, modified mRNA). mRNA, for example, is transcribed *in vitro* from template DNA, referred to as an “*in vitro* transcription template.” In some embodiments, the at least one RNA polynucleotide has at least one chemical modification. The at least one
10 chemical modification may include, but is expressly not limited to, any modification described herein.

In vitro transcription of RNA is known in the art and is described in International Publication WO2014/152027, which is incorporated by reference herein in its entirety. For example, in some embodiments, the RNA transcript is generated using a non-amplified,
15 linearized DNA template in an *in vitro* transcription reaction to generate the RNA transcript. In some embodiments the RNA transcript is capped *via* enzymatic capping. In some embodiments the RNA transcript is purified via chromatographic methods, *e.g.*, use of an oligo dT substrate. Some embodiments exclude the use of DNase. In some embodiments the RNA transcript is synthesized from a non-amplified, linear DNA template coding for the
20 gene of interest via an enzymatic *in vitro* transcription reaction utilizing a T7 phage RNA polymerase and nucleotide triphosphates of the desired chemistry. Any number of RNA polymerases or variants may be used in the method of the present invention. The polymerase may be selected from, but is not limited to, a phage RNA polymerase, *e.g.*, a T7 RNA polymerase, a T3 RNA polymerase, a SP6 RNA polymerase, and/or mutant polymerases such
25 as, but not limited to, polymerases able to incorporate modified nucleic acids and/or modified nucleotides, including chemically modified nucleic acids and/or nucleotides.

In some embodiments a non-amplified, linearized plasmid DNA is utilized as the template DNA for *in vitro* transcription. In some embodiments, the template DNA is isolated DNA. In some embodiments, the template DNA is cDNA. In some embodiments, the cDNA
30 is formed by reverse transcription of a RNA polynucleotide, for example, but not limited to RSV RNA, *e.g.* RSV mRNA. In some embodiments, Cells, *e.g.*, bacterial cells, *e.g.*, *E. coli*, *e.g.*, DH-1 cells are transfected with the plasmid DNA template. In some embodiments, the transfected cells are cultured to replicate the plasmid DNA which is then isolated and

purified. In some embodiments, the DNA template includes a RNA polymerase promoter, *e.g.*, a T7 promoter located 5' to and operably linked to the gene of interest.

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

A "5' untranslated region" (UTR) refers to a region of an mRNA that is directly upstream (*i.e.*, 5') from the start codon (*i.e.*, the first codon of an mRNA transcript translated by a ribosome) that does not encode a polypeptide.

A "3' untranslated region" (UTR) refers to a region of an mRNA that is directly downstream (*i.e.*, 3') from the stop codon (*i.e.*, the codon of an mRNA transcript that signals a termination of translation) that does not encode a polypeptide.

An "open reading frame" is a continuous stretch of DNA or RNA beginning with a start codon (*e.g.*, methionine (ATG or AUG)), and ending with a stop codon (*e.g.*, TAA, TAG or TGA, or UAA, UAG or UGA) and typically encodes a polypeptide (*e.g.*, protein). It will be understood that the sequences disclosed herein may further comprise additional elements, *e.g.*, 5' and 3' UTRs, but that those elements, unlike the ORF, need not necessarily be present in a vaccine of the present disclosure.

A "polyA tail" is a region of mRNA that is downstream, *e.g.*, directly downstream (*i.e.*, 3'), from the 3' UTR that contains multiple, consecutive adenosine monophosphates. A polyA tail may contain 10 to 300 adenosine monophosphates. For example, a polyA tail may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290 or 300 adenosine monophosphates. In some embodiments, a polyA tail contains 50 to 250 adenosine monophosphates. In a relevant biological setting (*e.g.*, in cells, *in vivo*) the poly(A) tail functions to protect mRNA from enzymatic degradation, *e.g.*, in the cytoplasm, and aids in transcription termination, and/or export of the mRNA from the nucleus and translation.

In some embodiments, a polynucleotide includes 200 to 3,000 nucleotides. For example, a polynucleotide may include 200 to 500, 200 to 1000, 200 to 1500, 200 to 3000, 500 to 1000, 500 to 1500, 500 to 2000, 500 to 3000, 1000 to 1500, 1000 to 2000, 1000 to 3000, 1500 to 3000, or 2000 to 3000 nucleotides).

Methods of Treatment

Provided herein are compositions (*e.g.*, pharmaceutical compositions), methods, kits and reagents for prevention and/or treatment of RSV in humans and other mammals. RSV RNA (*e.g.* mRNA) vaccines can be used as therapeutic or prophylactic agents. They may be used in medicine to prevent and/or treat infectious disease. In exemplary aspects, the RSV RNA vaccines of the present disclosure are used to provide prophylactic protection from RSV. Prophylactic protection from RSV can be achieved following administration of a RSV RNA vaccine of the present disclosure. Vaccines can be administered once, twice, three times, four times or more but it is likely sufficient to administer the vaccine once (optionally followed by a single booster). It is possible, although less desirable, to administer the vaccine to an infected individual to achieve a therapeutic response. Dosing may need to be adjusted accordingly.

A method of eliciting an immune response in a subject against a RSV is provided in aspects of the invention. The method involves administering to the subject a RSV RNA vaccine comprising at least one RNA polynucleotide having an open reading frame encoding at least one RSV antigenic polypeptide, thereby inducing in the subject an immune response specific to RSV antigenic polypeptide, wherein anti-antigenic polypeptide antibody titer in the subject is increased following vaccination relative to anti-antigenic polypeptide antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional (*e.g.*, non-nucleic acid) vaccine against the RSV. An “anti-antigenic polypeptide antibody” is a serum antibody the binds specifically to the antigenic polypeptide.

A prophylactically effective dose is a therapeutically effective dose that prevents infection with the virus at a clinically acceptable level. In some embodiments the therapeutically effective dose is a dose listed in a package insert for the vaccine. A traditional vaccine, as used herein, refers to a vaccine other than the mRNA vaccines of the invention. For instance, a traditional vaccine includes but is not limited to live microorganism vaccines, killed microorganism vaccines, subunit vaccines, protein antigen vaccines, DNA vaccines, *etc.*

In some embodiments the anti-antigenic polypeptide antibody titer in the subject is increased 1 log to 10 log following vaccination relative to anti-antigenic polypeptide antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the RSV.

In some embodiments the anti-antigenic polypeptide antibody titer in the subject is increased 1 log following vaccination relative to anti-antigenic polypeptide antibody titer in a

subject vaccinated with a prophylactically effective dose of a traditional vaccine against the RSV.

In some embodiments the anti-antigenic polypeptide antibody titer in the subject is increased 2 log following vaccination relative to anti-antigenic polypeptide antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the RSV.

In some embodiments the anti-antigenic polypeptide antibody titer in the subject is increased 3 log following vaccination relative to anti-antigenic polypeptide antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the RSV.

In some embodiments the anti-antigenic polypeptide antibody titer in the subject is increased 5 log following vaccination relative to anti-antigenic polypeptide antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the RSV.

In some embodiments the anti-antigenic polypeptide antibody titer in the subject is increased 10 log following vaccination relative to anti-antigenic polypeptide antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the RSV.

A method of eliciting an immune response in a subject against a RSV is provided in other aspects of the invention. The method involves administering to the subject a RSV RNA vaccine comprising at least one RNA polynucleotide having an open reading frame encoding at least one RSV antigenic polypeptide, thereby inducing in the subject an immune response specific to RSV antigenic polypeptide, wherein the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine against the RSV at 2 times to 100 times the dosage level relative to the RNA vaccine.

In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at twice the dosage level relative to the RSV RNA vaccine.

In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at three times the dosage level relative to the RSV RNA vaccine.

In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 4 times the dosage level relative to the RSV RNA vaccine.

In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 5 times the dosage level relative to the RSV RNA vaccine.

5 In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 10 times the dosage level relative to the RSV RNA vaccine.

In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 50 times the dosage level relative to the RSV RNA vaccine.

10 In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 100 times the dosage level relative to the RSV RNA vaccine.

In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 10 times to 1000 times the dosage level relative to the RSV RNA vaccine.

15 In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 100 times to 1000 times the dosage level relative to the RSV RNA vaccine.

In other embodiments the immune response is assessed by determining [protein] antibody titer in the subject.

20 In other aspects the invention is a method of eliciting an immune response in a subject against a RSV by administering to the subject a RSV RNA vaccine comprising at least one RNA polynucleotide having an open reading frame encoding at least one RSV antigenic polypeptide, thereby inducing in the subject an immune response specific to RSV antigenic polypeptide, wherein the immune response in the subject is induced 2 days to 10 weeks earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the RSV. In some embodiments the immune response in the subject is induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine at 2 times to 100 times the dosage level relative to the RNA vaccine.

30 In some embodiments the immune response in the subject is induced 2 days earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine.

In some embodiments the immune response in the subject is induced 3 days earlier relative to an immune response induced in a subject vaccinated a prophylactically effective dose of a traditional vaccine.

5 In some embodiments the immune response in the subject is induced 1 week earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine.

In some embodiments the immune response in the subject is induced 2 weeks earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine.

10 In some embodiments the immune response in the subject is induced 3 weeks earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine.

In some embodiments the immune response in the subject is induced 5 weeks earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine.

15 In some embodiments the immune response in the subject is induced 10 weeks earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine.

20 *Multiprotein and Multicomponent Vaccines*

The present disclosure encompasses RSV vaccines comprising multiple RNA (*e.g.*, mRNA) polynucleotides, each encoding a single antigenic polypeptide, as well as RSV vaccines comprising a single RNA polynucleotide encoding more than one antigenic polypeptide (*e.g.*, as a fusion polypeptide). Thus, it should be understood that a vaccine composition comprising a RNA polynucleotide having an open reading frame encoding a first RSV antigenic polypeptide and a RNA polynucleotide having an open reading frame encoding a second RSV antigenic polypeptide encompasses (a) vaccines that comprise a first RNA polynucleotide encoding a first RSV antigenic polypeptide and a second RNA polynucleotide encoding a second RSV antigenic polypeptide, and (b) vaccines that comprise a single RNA polynucleotide encoding a first and second RSV antigenic polypeptide (*e.g.*, as a fusion polypeptide). RSV RNA vaccines of the present disclosure, in some embodiments, comprise 2-10 (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9 or 10), or more, RNA polynucleotides having an open reading frame, each of which encodes a different RSV antigenic polypeptide (or a single RNA polynucleotide encoding 2-10, or more, different RSV antigenic polypeptides). In

some embodiments, a RSV RNA vaccine comprises a RNA polynucleotide having an open reading frame encoding a RSV Fusion (F) glycoprotein, a RNA polynucleotide having an open reading frame encoding a RSV attachment (G) protein, a RNA polynucleotide having an open reading frame encoding a RSV nucleoprotein (N), a RNA polynucleotide having an open reading frame encoding a RSV phosphoprotein (P), a RNA polynucleotide having an open reading frame encoding a RSV large polymerase protein (L), a RNA polynucleotide having an open reading frame encoding a RSV matrix protein (M), a RNA polynucleotide having an open reading frame encoding a RSV small hydrophobic protein (SH), a RNA polynucleotide having an open reading frame encoding a RSV nonstructural protein 1 (NS1), and a RNA polynucleotide having an open reading frame encoding a RSV nonstructure protein 2 (NS2). In some embodiments, a RSV RNA vaccine comprises a RNA polynucleotide having an open reading frame encoding a RSV fusion (F) protein and a RNA polynucleotide having an open reading frame encoding a RSV attachment protein (G). In some embodiments, a RSV RNA vaccine comprises a RNA polynucleotide having an open reading frame encoding a RSV F protein. In some embodiments, a RSV RNA vaccine comprises a RNA polynucleotide having an open reading frame encoding a RSV N protein. In some embodiments, a RSV RNA vaccine comprises a RNA polynucleotide having an open reading frame encoding a RSV M protein. In some embodiments, a RSV RNA vaccine comprises a RNA polynucleotide having an open reading frame encoding a RSV L protein. In some embodiments, a RSV RNA vaccine comprises a RNA polynucleotide having an open reading frame encoding a RSV P protein. In some embodiments, a RSV RNA vaccine comprises a RNA polynucleotide having an open reading frame encoding a RSV SH protein. In some embodiments, a RSV RNA vaccine comprises a RNA polynucleotide having an open reading frame encoding a RSV NS1 protein. In some embodiments, a RSV RNA vaccine comprises a RNA polynucleotide having an open reading frame encoding a RSV NS2 protein.

In some embodiments, a RNA polynucleotide encodes a RSV antigenic polypeptide fused to a signal peptide (*e.g.*, SEQ ID NO: 281 or SEQ ID NO:282). Thus, RSV vaccines comprising at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding a signal peptide linked to a RSV antigenic peptide are provided.

Further provided herein are RSV vaccines comprising any RSV antigenic polypeptides disclosed herein (*e.g.*, F, G, M, N, L, P, SH, NS1, NS2, or any antigenic fragment thereof) fused to signal peptides. The signal peptide may be fused to the N- or C-terminus of the RSV antigenic polypeptides.

Broad spectrum RSV vaccines

It is envisioned that there may be situations where persons are at risk for infection with more than one strain of RSV. RNA (e.g., mRNA) therapeutic vaccines are particularly amenable to combination vaccination approaches due to a number of factors including, but not limited to, speed of manufacture, ability to rapidly tailor vaccines to accommodate perceived geographical threat, and the like. Moreover, because the vaccines utilize the human body to produce the antigenic protein, the vaccines are amenable to the production of larger, more complex antigenic proteins, allowing for proper folding, surface expression, antigen presentation, *etc.* in the human subject. To protect against more than one strain of RSV, a combination vaccine can be administered that includes RNA encoding at least one antigenic polypeptide protein (or antigenic portion thereof) of a first RSV and further includes RNA encoding at least one antigenic polypeptide protein (or antigenic portion thereof) of a second RSV. RNAs (mRNAs) can be co-formulated, for example, in a single lipid nanoparticle (LNP) or can be formulated in separate LNPs destined for co-administration.

Flagellin Adjuvants

Flagellin is an approximately 500 amino acid monomeric protein that polymerizes to form the flagella associated with bacterial motion. Flagellin is expressed by a variety of flagellated bacteria (*Salmonella typhimurium* for example) as well as non-flagellated bacteria (such as *Escherichia coli*). Sensing of flagellin by cells of the innate immune system (dendritic cells, macrophages, *etc.*) is mediated by the Toll-like receptor 5 (TLR5) as well as by Nod-like receptors (NLRs) Ipaf and Naip5. TLRs and NLRs have been identified as playing a role in the activation of innate immune response and adaptive immune response. As such, flagellin provides an adjuvant effect in a vaccine.

The nucleotide and amino acid sequences encoding known flagellin polypeptides are publicly available in the NCBI GenBank database. The flagellin sequences from *S. Typhimurium*, *H. Pylori*, *V. Cholera*, *S. marcesens*, *S. flexneri*, *T. Pallidum*, *L. pneumophila*, *B. burgdorferi*, *C. difficile*, *R. meliloti*, *A. tumefaciens*, *R. lupini*, *B. clarridgeiae*, *P. Mirabilis*, *B. subtilis*, *L. monocytogenes*, *P. aeruginosa*, and *E. coli*, among others are known.

A flagellin polypeptide, as used herein, refers to a full length flagellin protein, immunogenic fragments thereof, and peptides having at least 50% sequence identity to a flagellin protein or immunogenic fragments thereof. Exemplary flagellin proteins include

flagellin from *Salmonella typhi* (UniPro Entry number: Q56086), *Salmonella typhimurium* (A0A0C9DG09), *Salmonella enteritidis* (A0A0C9BAB7), and *Salmonella choleraesuis* (Q6V2X8), and SEQ ID NO: 173-175. In some embodiments, the flagellin polypeptide has at least 60%, 70%, 75%, 80%, 90%, 95%, 97%, 98%, or 99% sequence identity to a flagellin protein or immunogenic fragments thereof.

In some embodiments, the flagellin polypeptide is an immunogenic fragment. An immunogenic fragment is a portion of a flagellin protein that provokes an immune response. In some embodiments, the immune response is a TLR5 immune response. An example of an immunogenic fragment is a flagellin protein in which all or a portion of a hinge region has been deleted or replaced with other amino acids. For example, an antigenic polypeptide may be inserted in the hinge region. Hinge regions are the hypervariable regions of a flagellin. Hinge regions of a flagellin are also referred to as “D3 domain or region,” “propeller domain or region,” “hypervariable domain or region” and “variable domain or region.” “At least a portion of a hinge region,” as used herein, refers to any part of the hinge region of the flagellin, or the entirety of the hinge region. In other embodiments an immunogenic fragment of flagellin is a 20, 25, 30, 35, or 40 amino acid C-terminal fragment of flagellin.

The flagellin monomer is formed by domains D0 through D3. D0 and D1, which form the stem, are composed of tandem long alpha helices and are highly conserved among different bacteria. The D1 domain includes several stretches of amino acids that are useful for TLR5 activation. The entire D1 domain or one or more of the active regions within the domain are immunogenic fragments of flagellin. Examples of immunogenic regions within the D1 domain include residues 88-114 and residues 411-431 (in *Salmonella typhimurium* FliC flagellin. Within the 13 amino acids in the 88-100 region, at least 6 substitutions are permitted between *Salmonella* flagellin and other flagellins that still preserve TLR5 activation. Thus, immunogenic fragments of flagellin include flagellin like sequences that activate TLR5 and contain a 13 amino acid motif that is 53% or more identical to the *Salmonella* sequence in 88-100 of FliC (LQRVRELAVQSAN; SEQ ID NO: 286).

In some embodiments, the RNA (e.g., mRNA) vaccine includes an RNA that encodes a fusion protein of flagellin and one or more antigenic polypeptides. A “fusion protein” as used herein, refers to a linking of two components of the construct. In some embodiments, a carboxy-terminus of the antigenic polypeptide is fused or linked to an amino terminus of the flagellin polypeptide. In other embodiments, an amino-terminus of the antigenic polypeptide is fused or linked to a carboxy-terminus of the flagellin polypeptide. The fusion protein may include, for example, one, two, three, four, five, six or more flagellin polypeptides linked to

one, two, three, four, five, six or more antigenic polypeptides. When two or more flagellin polypeptides and/or two or more antigenic polypeptides are linked such a construct may be referred to as a “multimer.”

Each of the components of a fusion protein may be directly linked to one another or they may be connected through a linker. For instance, the linker may be an amino acid linker. The amino acid linker encoded for by the RNA (*e.g.*, mRNA) vaccine to link the components of the fusion protein may include, for instance, at least one member selected from the group consisting of a lysine residue, a glutamic acid residue, a serine residue and an arginine residue. In some embodiments the linker is 1-30, 1-25, 1-25, 5-10, 5, 15, or 5-20 amino acids in length.

In other embodiments the RNA (*e.g.*, mRNA) vaccine includes at least two separate RNA polynucleotides, one encoding one or more antigenic polypeptides and the other encoding the flagellin polypeptide. The at least two RNA polynucleotides may be co-formulated in a carrier such as a lipid nanoparticle.

Therapeutic and Prophylactic Compositions

Provided herein are compositions (*e.g.*, pharmaceutical compositions), methods, kits and reagents for prevention, treatment or diagnosis of RSV in humans and other mammals, for example. RSV RNA (*e.g.*, mRNA) vaccines can be used as therapeutic or prophylactic agents. They may be used in medicine to prevent and/or treat infectious disease. In some embodiments, the RSV vaccines of the invention can be envisioned for use in the priming of immune effector cells, for example, to activate peripheral blood mononuclear cells (PBMCs) *ex vivo*, which are then infused (re-infused) into a subject.

In exemplary embodiments, a RSV vaccine containing RNA polynucleotides as described herein can be administered to a subject (*e.g.*, a mammalian subject, such as a human subject), and the RNA polynucleotides are translated *in vivo* to produce an antigenic polypeptide.

The RSV RNA vaccines may be induced for translation of a polypeptide (*e.g.*, antigen or immunogen) in a cell, tissue or organism. In exemplary embodiments, such translation occurs *in vivo*, although there can be envisioned embodiments where such translation occurs *ex vivo*, in culture or *in vitro*. In exemplary embodiments, the cell, tissue or organism is contacted with an effective amount of a composition containing a RSV RNA vaccine that contains a polynucleotide that has at least one a translatable region encoding an antigenic polypeptide.

An "effective amount" of the RSV RNA vaccine is provided based, at least in part, on the target tissue, target cell type, means of administration, physical characteristics of the polynucleotide (*e.g.*, size, and extent of modified nucleosides) and other components of the RSV RNA vaccine, and other determinants. In general, an effective amount of the RSV RNA vaccine composition provides an induced or boosted immune response as a function of antigen production in the cell. In general, an effective amount of the RSV RNA vaccine containing RNA polynucleotides having at least one chemical modifications are preferably more efficient than a composition containing a corresponding unmodified polynucleotide encoding the same antigen or a peptide antigen. Increased antigen production may be demonstrated by increased cell transfection (the percentage of cells transfected with the RNA vaccine), increased protein translation from the polynucleotide, decreased nucleic acid degradation (as demonstrated, for example, by increased duration of protein translation from a modified polynucleotide), or altered antigen specific immune response of the host cell.

The term "pharmaceutical composition" refers to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use *in vivo* or *ex vivo*. A "pharmaceutically acceptable carrier," after administered to or upon a subject, does not cause undesirable physiological effects. The carrier in the pharmaceutical composition must be "acceptable" also in the sense that it is compatible with the active ingredient and can be capable of stabilizing it. One or more solubilizing agents can be utilized as pharmaceutical carriers for delivery of an active agent. Examples of a pharmaceutically acceptable carrier include, but are not limited to, biocompatible vehicles, adjuvants, additives, and diluents to achieve a composition usable as a dosage form. Examples of other carriers include colloidal silicon oxide, magnesium stearate, cellulose, and sodium lauryl sulfate. Additional suitable pharmaceutical carriers and diluents, as well as pharmaceutical necessities for their use, are described in Remington's Pharmaceutical Sciences.

In some embodiments, RNA vaccines (including polynucleotides and their encoded polypeptides) in accordance with the present disclosure may be used for treatment or prevention of RSV.

RSV RNA vaccines may be administered prophylactically or therapeutically as part of an active immunization scheme to healthy individuals or early in infection during the incubation phase or during active infection after onset of symptoms. In some embodiments, the amount of RNA vaccines of the present disclosure provided to a cell, a tissue or a subject may be an amount effective for immune prophylaxis.

RSV RNA (*e.g.*, mRNA) vaccines may be administered with other prophylactic or therapeutic compounds. As a non-limiting example, a prophylactic or therapeutic compound may be an adjuvant or a booster. As used herein, when referring to a prophylactic composition, such as a vaccine, the term “booster” refers to an extra administration of the prophylactic (vaccine) composition. A booster (or booster vaccine) may be given after an earlier administration of the prophylactic composition. The time of administration between the initial administration of the prophylactic composition and the booster may be, but is not limited to, 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 15 minutes, 20 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 1 day, 36 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 10 days, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 18 months, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, 12 years, 13 years, 14 years, 15 years, 16 years, 17 years, 18 years, 19 years, 20 years, 25 years, 30 years, 35 years, 40 years, 45 years, 50 years, 55 years, 60 years, 65 years, 70 years, 75 years, 80 years, 85 years, 90 years, 95 years or more than 99 years. In exemplary embodiments, the time of administration between the initial administration of the prophylactic composition and the booster may be, but is not limited to, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 6 months or 1 year.

In some embodiments, RSV RNA vaccines may be administered intramuscularly, intranasally or intradermally, similarly to the administration of inactivated vaccines known in the art.

The RSV RNA vaccines may be utilized in various settings depending on the prevalence of the infection or the degree or level of unmet medical need. As a non-limiting example, the RNA vaccines may be utilized to treat and/or prevent a variety of infectious disease. RNA vaccines, in many instances, have superior properties in that they produce much larger antibody titers and produce responses early than commercially available anti-virals.

Provided herein are pharmaceutical compositions including RSV RNA vaccines and RNA vaccine compositions and/or complexes optionally in combination with one or more pharmaceutically acceptable excipients.

RSV RNA (*e.g.*, mRNA) vaccines may be formulated or administered alone or in conjunction with one or more other components. For instance, RSV RNA vaccines (vaccine compositions) may comprise other components including, but not limited to, adjuvants.

In some embodiments, RSV RNA vaccines do not include an adjuvant (they are adjuvant free).

RSV RNA (*e.g.*, mRNA) vaccines may be formulated or administered in combination with one or more pharmaceutically-acceptable excipients. In some embodiments, vaccine compositions comprise at least one additional active substances, such as, for example, a therapeutically-active substance, a prophylactically-active substance, or a combination of both. Vaccine compositions may be sterile, pyrogen-free or both sterile and pyrogen-free. General considerations in the formulation and/or manufacture of pharmaceutical agents, such as vaccine compositions, may be found, for example, in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference in its entirety).

In some embodiments, RSV RNA vaccines are administered to humans, human patients or subjects. For the purposes of the present disclosure, the phrase “active ingredient” generally refers to the RNA vaccines or the polynucleotides contained therein, for example, RNA polynucleotides (*e.g.*, mRNA polynucleotides) encoding antigenic polypeptides.

Formulations of the vaccine compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient (*e.g.*, mRNA polynucleotide) into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-dose unit.

Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the disclosure will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100%, *e.g.*, between 0.5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient.

RSV RNA vaccines can be formulated using one or more excipients to: (1) increase stability; (2) increase cell transfection; (3) permit the sustained or delayed release (*e.g.*, from a depot formulation); (4) alter the biodistribution (*e.g.*, target to specific tissues or cell types); (5) increase the translation of encoded protein *in vivo*; and/or (6) alter the release profile of

encoded protein (antigen) *in vivo*. In addition to traditional excipients such as any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, excipients can include, without limitation, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, cells transfected with RSV RNA vaccines (*e.g.*, for transplantation into a subject), hyaluronidase, nanoparticle mimics and combinations thereof.

Stabilizing Elements

Naturally-occurring eukaryotic mRNA molecules have been found to contain stabilizing elements, including, but not limited to untranslated regions (UTR) at their 5'-end (5'UTR) and/or at their 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 typically transcribed from the genomic DNA and are elements of the premature mRNA. Characteristic structural features of mature mRNA, such as the 5'-cap and the 3'-poly(A) tail are usually added to the transcribed (premature) mRNA during mRNA processing. The 3'-poly(A) tail is typically a stretch of adenine nucleotides added to the 3'-end of the transcribed mRNA. It can comprise up to about 400 adenine nucleotides. In some embodiments the length of the 3'-poly(A) tail may be an essential element with respect to the stability of the individual mRNA.

In some embodiments the RNA vaccine may include one or more stabilizing elements. Stabilizing elements may include for instance a histone stem-loop. A stem-loop binding protein (SLBP), a 32 kDa protein has been identified. It is associated with the histone stem-loop at the 3'-end of the histone messages in both the nucleus and the cytoplasm. Its expression level is regulated by the cell cycle; it peaks during the S-phase, when histone mRNA levels are also elevated. The protein has been shown to be essential for efficient 3'-end processing of histone pre-mRNA by the U7 snRNP. SLBP continues to be associated with the stem-loop after processing, and then stimulates the translation of mature histone mRNAs into histone proteins in the cytoplasm. The RNA binding domain of SLBP is conserved through metazoa and protozoa; its binding to the histone stem-loop depends on the structure of the loop. The minimum binding site includes at least three nucleotides 5' and two nucleotides 3' relative to the stem-loop.

In some embodiments, the RNA vaccines include a coding region, at least one histone stem-loop, and optionally, a poly(A) sequence or polyadenylation signal. The poly(A) sequence or polyadenylation signal generally should enhance the expression level of the

encoded protein. The encoded protein, in some embodiments, is not a histone protein, a reporter protein (*e.g.* Luciferase, GFP, EGFP, β -Galactosidase, EGFP), or a marker or selection protein (*e.g.* alpha-Globin, Galactokinase and Xanthine:guanine phosphoribosyl transferase (GPT)).

5 In some embodiments, the combination of a poly(A) sequence or polyadenylation signal and at least one histone stem-loop, even though both represent alternative mechanisms in nature, acts synergistically to increase the protein expression beyond the level observed with either of the individual elements. It has been found that the synergistic effect of the combination of poly(A) and at least one histone stem-loop does not depend on the order of
10 the elements or the length of the poly(A) sequence.

In some embodiments, the RNA vaccine does not comprise a histone downstream element (HDE). "Histone downstream element" (HDE) includes a purine-rich polynucleotide stretch of approximately 15 to 20 nucleotides 3' of naturally occurring stem-loops, representing the binding site for the U7 snRNA, which is involved in processing of histone
15 pre-mRNA into mature histone mRNA. In some embodiments, the nucleic acid does not include an intron.

In some embodiments, the RNA vaccine may or may not contain a enhancer and/or promoter sequence, which may be modified or unmodified or which may be activated or inactivated. In some embodiments, the histone stem-loop is generally derived from histone
20 genes, and includes an intramolecular base pairing of two neighbored partially or entirely reverse complementary sequences separated by a spacer, consisting of a short sequence, which forms the loop of the structure. The unpaired loop region is typically unable to base pair with either of the stem loop elements. It occurs more often in RNA, as is a key component of many RNA secondary structures, but may be present in single-stranded DNA
25 as well. Stability of the stem-loop structure generally depends on the length, number of mismatches or bulges, and base composition of the paired region. In some embodiments, wobble base pairing (non-Watson-Crick base pairing) may result. In some embodiments, the at least one histone stem-loop sequence comprises a length of 15 to 45 nucleotides.

In other embodiments the RNA vaccine may have one or more AU-rich sequences removed. These sequences, sometimes referred to as AURES are destabilizing sequences
30 found in the 3'UTR. The AURES may be removed from the RNA vaccines. Alternatively the AURES may remain in the RNA vaccine.

In some embodiments, the RNA polynucleotide does not include a stabilization element.

Nanoparticle Formulations

In some embodiments, RSV RNA (*e.g.*, mRNA) vaccines are formulated in a nanoparticle. In some embodiments, RSV RNA vaccines are formulated in a lipid nanoparticle. In some embodiments, RSV RNA vaccines are formulated in a lipid-polycation complex, referred to as a cationic lipid nanoparticle. The formation of the lipid nanoparticle may be accomplished by methods known in the art and/or as described in U.S. Publication No. 20120178702, herein incorporated by reference in its entirety. As a non-limiting example, the polycation may include a cationic peptide or a polypeptide such as, but not limited to, polylysine, polyornithine and/or polyarginine and the cationic peptides described in International Publication No. WO2012013326 or U.S. Publication No. US20130142818; each of which is herein incorporated by reference in its entirety. In some embodiments, RSV RNA vaccines are formulated in a lipid nanoparticle that includes a non-cationic lipid such as, but not limited to, cholesterol or dioleoyl phosphatidylethanolamine (DOPE).

A lipid nanoparticle formulation may be influenced by, but not limited to, the selection of the cationic lipid component, the degree of cationic lipid saturation, the nature of the PEGylation, ratio of all components and biophysical parameters such as size. In one example by Semple *et al.* (*Nature Biotech.* 2010 28:172-176; herein incorporated by reference in its entirety), the lipid nanoparticle formulation is composed of 57.1 % cationic lipid, 7.1% dipalmitoylphosphatidylcholine, 34.3 % cholesterol, and 1.4% PEG-c-DMA. As another example, changing the composition of the cationic lipid was shown to more effectively deliver siRNA to various antigen presenting cells (Basha *et al.* *Mol Ther.* 2011 19:2186-2200; herein incorporated by reference in its entirety).

In some embodiments, lipid nanoparticle formulations may comprise 35 to 45% cationic lipid, 40% to 50% cationic lipid, 50% to 60% cationic lipid and/or 55% to 65% cationic lipid. In some embodiments, the ratio of lipid to RNA (*e.g.*, mRNA) in lipid nanoparticles may be 5:1 to 20:1, 10:1 to 25:1, 15:1 to 30:1 and/or at least 30:1.

In some embodiments, the ratio of PEG in the lipid nanoparticle formulations may be increased or decreased and/or the carbon chain length of the PEG lipid may be modified from C14 to C18 to alter the pharmacokinetics and/or biodistribution of the lipid nanoparticle formulations. As a non-limiting example, lipid nanoparticle formulations may contain 0.5% to 3.0%, 1.0% to 3.5%, 1.5% to 4.0%, 2.0% to 4.5%, 2.5% to 5.0% and/or 3.0% to 6.0% of the lipid molar ratio of PEG-c-DOMG (R-3-[(ω -methoxy-poly(ethyleneglycol)2000)carbamoyl]-1,2-dimyristyloxypropyl-3-amine) (also referred to

herein as PEG-DOMG) as compared to the cationic lipid, DSPC and cholesterol. In some embodiments, the PEG-c-DOMG may be replaced with a PEG lipid such as, but not limited to, PEG-DSG (1,2-Distearoyl-sn-glycerol, methoxypolyethylene glycol), PEG-DMG (1,2-Dimyristoyl-sn-glycerol) and/or PEG-DPG (1,2-Dipalmitoyl-sn-glycerol, methoxypolyethylene glycol). The cationic lipid may be selected from any lipid known in the art such as, but not limited to, DLin-MC3-DMA, DLin-DMA, C12-200 and DLin-KC2-DMA (*see, e.g.*, U.S. Publication No. 20130245107 A1).

In some embodiments, a RSV RNA (*e.g.*, mRNA) vaccine formulation is a nanoparticle that comprises at least one lipid. The lipid may be selected from, but is not limited to, DLin-DMA, DLin-K-DMA, 98N12-5, C12-200, DLin-MC3-DMA, DLin-KC2-DMA, DODMA, PLGA, PEG, PEG-DMG, PEGylated lipids and amino alcohol lipids. In some embodiments, the lipid may be a cationic lipid such as, but not limited to, DLin-DMA, DLin-D-DMA, DLin-MC3-DMA, DLin-KC2-DMA, DODMA and amino alcohol lipids. The amino alcohol cationic lipid may be the lipids described in and/or made by the methods described in U.S. Publication No. US20130150625, herein incorporated by reference in its entirety. As a non-limiting example, the cationic lipid may be 2-amino-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-2-{[(9Z,2Z)-octadeca-9,12-dien-1-yloxy]methyl}propan-1-ol (Compound 1 in US20130150625); 2-amino-3-[(9Z)-octadec-9-en-1-yloxy]-2-{[(9Z)-octadec-9-en-1-yloxy]methyl}propan-1-ol (Compound 2 in US20130150625); 2-amino-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-2-[(octyloxy)methyl]propan-1-ol (Compound 3 in US20130150625); and 2-(dimethylamino)-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-2-{[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]methyl}propan-1-ol (Compound 4 in US20130150625); or any pharmaceutically acceptable salt or stereoisomer thereof.

Lipid nanoparticle formulations typically comprise a lipid, in particular, an ionizable cationic lipid, for example, 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, or N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine and further comprise a neutral lipid, a sterol and a molecule capable of reducing particle aggregation, for example a PEG or PEG-modified lipid.

In some embodiments, a lipid nanoparticle formulation consists essentially of (i) at least one lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-

DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine; (ii) a neutral lipid selected from DSPC, DPPC, POPC, DOPE and SM; (iii) a sterol, *e.g.*, cholesterol; and (iv) a PEG-lipid, *e.g.*, PEG-DMG or PEG-cDMA, in a molar ratio of 20-60% cationic lipid: 5-25% neutral lipid (non-cationic lipid): 25-55% sterol; 0.5-15% PEG-lipid.

In some embodiments, a lipid nanoparticle formulation includes 25% to 75% on a molar basis of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine, *e.g.*, 35 to 65%, 45 to 65%, 60%, 57.5%, 50% or 40% on a molar basis.

In some embodiments, a lipid nanoparticle formulation includes 0.5% to 15% on a molar basis of the neutral lipid, *e.g.*, 3 to 12%, 5 to 10% or 15%, 10%, or 7.5% on a molar basis. Examples of neutral lipids include, without limitation, DSPC, POPC, DPPC, DOPE and SM. In some embodiments, the formulation includes 5% to 50% on a molar basis of the sterol (*e.g.*, 15 to 45%, 20 to 40%, 40%, 38.5%, 35%, or 31% on a molar basis. A non-limiting example of a sterol is cholesterol. In some embodiments, a lipid nanoparticle formulation includes 0.5% to 20% on a molar basis of the PEG or PEG-modified lipid (*e.g.*, 0.5 to 10%, 0.5 to 5%, 1.5%, 0.5%, 1.5%, 3.5%, or 5% on a molar basis. In some embodiments, a PEG or PEG modified lipid comprises a PEG molecule of an average molecular weight of 2,000 Da. In some embodiments, a PEG or PEG modified lipid comprises a PEG molecule of an average molecular weight of less than 2,000, for example around 1,500 Da, around 1,000 Da, or around 500 Da. Non-limiting examples of PEG-modified lipids include PEG-distearoyl glycerol (PEG-DMG) (also referred herein as PEG-C14 or C14-PEG), PEG-cDMA (further discussed in Reyes *et al. J. Controlled Release*, 107, 276-287 (2005) the content of which is herein incorporated by reference in its entirety).

In some embodiments, lipid nanoparticle formulations include 25-75% of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, and N,N-dimethyl-1-

[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine, 0.5-15% of the neutral lipid, 5-50% of the sterol, and 0.5-20% of the PEG or PEG-modified lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 35-65% of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine, 3-12% of the neutral lipid, 15-45% of the sterol, and 0.5-10% of the PEG or PEG-modified lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 45-65% of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine, 5-10% of the neutral lipid, 25-40% of the sterol, and 0.5-10% of the PEG or PEG-modified lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 60% of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine, 7.5% of the neutral lipid, 31 % of the sterol, and 1.5% of the PEG or PEG-modified lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 50% of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine, 10% of the neutral lipid, 38.5 % of the sterol, and 1.5% of the PEG or PEG-modified lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 50% of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-

dimethyl-2-nonylhenicosa-12,15-dien-1-amine, and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine, 10% of the neutral lipid, 35 % of the sterol, 4.5% or 5% of the PEG or PEG-modified lipid, and 0.5% of the targeting lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 40% of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine, 15% of the neutral lipid, 40% of the sterol, and 5% of the PEG or PEG-modified lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 57.2% of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine, 7.1% of the neutral lipid, 34.3% of the sterol, and 1.4% of the PEG or PEG-modified lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 57.5% of a cationic lipid selected from the PEG lipid is PEG-cDMA (PEG-cDMA is further discussed in Reyes et al. (J. Controlled Release, 107, 276-287 (2005), the content of which is herein incorporated by reference in its entirety), 7.5% of the neutral lipid, 31.5 % of the sterol, and 3.5% of the PEG or PEG-modified lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations consists essentially of a lipid mixture in molar ratios of 20-70% cationic lipid: 5-45% neutral lipid: 20-55% cholesterol: 0.5-15% PEG-modified lipid. In some embodiments, lipid nanoparticle formulations consists essentially of a lipid mixture in a molar ratio of 20-60% cationic lipid: 5-25% neutral lipid (non-cationic lipid): 25-55% cholesterol: 0.5-15% PEG-modified lipid.

In some embodiments, the molar lipid ratio is 50/10/38.5/1.5 (mol% cationic lipid/neutral lipid, *e.g.*, DSPC/Chol/PEG-modified lipid, *e.g.*, PEG-DMG, PEG-DSG or PEG-DPG), 57.2/7.1/34.3/1.4 (mol% cationic lipid/ neutral lipid, *e.g.*, DPPC/Chol/ PEG-modified lipid, *e.g.*, PEG-cDMA), 40/15/40/5 (mol% cationic lipid/ neutral lipid, *e.g.*, DSPC/Chol/ PEG-modified lipid, *e.g.*, PEG-DMG), 50/10/35/4.5/0.5 (mol% cationic lipid/ neutral lipid, *e.g.*, DSPC/Chol/ PEG-modified lipid, *e.g.*, PEG-DSG), 50/10/35/5 (cationic lipid/ neutral lipid, *e.g.*, DSPC/Chol/ PEG-modified lipid, *e.g.*, PEG-DMG), 40/10/40/10 (mol% cationic

lipid/ neutral lipid, *e.g.*, DSPC/Chol/ PEG-modified lipid, *e.g.*, PEG-DMG or PEG-cDMA), 35/15/40/10 (mol% cationic lipid/ neutral lipid, *e.g.*, DSPC/Chol/ PEG-modified lipid, *e.g.*, PEG-DMG or PEG-cDMA) or 52/13/30/5 (mol% cationic lipid/ neutral lipid, *e.g.*, DSPC/Chol/ PEG-modified lipid, *e.g.*, PEG-DMG or PEG-cDMA).

Non-limiting examples of lipid nanoparticle compositions and methods of making them are described, for example, in Semple *et al.* (2010) *Nat. Biotechnol.* 28:172-176; Jayarama *et al.* (2012), *Angew. Chem. Int. Ed.*, 51: 8529–8533; and Maier *et al.* (2013) *Molecular Therapy* 21, 1570-1578 (the contents of each of which are incorporated herein by reference in their entirety).

In some embodiments, lipid nanoparticle formulations may comprise a cationic lipid, a PEG lipid and a structural lipid and optionally comprise a non-cationic lipid. As a non-limiting example, a lipid nanoparticle may comprise 40-60% of cationic lipid, 5-15% of a non-cationic lipid, 1-2% of a PEG lipid and 30-50% of a structural lipid. As another non-limiting example, the lipid nanoparticle may comprise 50% cationic lipid, 10% non-cationic lipid, 1.5% PEG lipid and 38.5% structural lipid. As yet another non-limiting example, a lipid nanoparticle may comprise 55% cationic lipid, 10% non-cationic lipid, 2.5% PEG lipid and 32.5% structural lipid. In some embodiments, the cationic lipid may be any cationic lipid described herein such as, but not limited to, 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine.

In some embodiments, the lipid nanoparticle formulations described herein may be 4 component lipid nanoparticles. The lipid nanoparticle may comprise a cationic lipid, a non-cationic lipid, a PEG lipid and a structural lipid. As a non-limiting example, the lipid nanoparticle may comprise 40-60% of cationic lipid, 5-15% of a non-cationic lipid, 1-2% of a PEG lipid and 30-50% of a structural lipid. As another non-limiting example, the lipid nanoparticle may comprise 50% cationic lipid, 10% non-cationic lipid, 1.5% PEG lipid and 38.5% structural lipid. As yet another non-limiting example, the lipid nanoparticle may comprise 55% cationic lipid, 10% non-cationic lipid, 2.5% PEG lipid and 32.5% structural lipid. In some embodiments, the cationic lipid may be any cationic lipid described herein such as, but not limited to, 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-dimethyl-2-

nonylhenicosa-12,15-dien-1-amine, and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine.

In some embodiments, the lipid nanoparticle formulations described herein may comprise a cationic lipid, a non-cationic lipid, a PEG lipid and a structural lipid. As a non-limiting example, the lipid nanoparticle comprise 50% of the cationic lipid DLin-KC2-DMA, 10% of the non-cationic lipid DSPC, 1.5% of the PEG lipid PEG-DOMG and 38.5% of the structural lipid cholesterol. As a non-limiting example, the lipid nanoparticle comprise 50% of the cationic lipid DLin-MC3-DMA, 10% of the non-cationic lipid DSPC, 1.5% of the PEG lipid PEG-DOMG and 38.5% of the structural lipid cholesterol. As a non-limiting example, the lipid nanoparticle comprise 50% of the cationic lipid DLin-MC3-DMA, 10% of the non-cationic lipid DSPC, 1.5% of the PEG lipid PEG-DMG and 38.5% of the structural lipid cholesterol. As yet another non-limiting example, the lipid nanoparticle comprise 55% of the cationic lipid di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, or N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine, 10% of the non-cationic lipid DSPC, 2.5% of the PEG lipid PEG-DMG and 32.5% of the structural lipid cholesterol.

Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a vaccine composition may vary, depending upon the identity, size, and/or condition of the subject being treated and further depending upon the route by which the composition is to be administered. For example, the composition may comprise between 0.1% and 99% (w/w) of the active ingredient. By way of example, the composition may comprise between 0.1% and 100%, *e.g.*, between 0.5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient.

In some embodiments, the RNA vaccine composition may comprise the polynucleotide described herein, formulated in a lipid nanoparticle comprising DLin-MC3-DMA, Cholesterol, DSPC and PEG2000-DMG, the buffer trisodium citrate, sucrose and water for injection. As a non-limiting example, the composition comprises: 2.0 mg/mL of drug substance (*e.g.*, polynucleotides encoding RSV), 21.8 mg/mL of MC3, 10.1 mg/mL of cholesterol, 5.4 mg/mL of DSPC, 2.7 mg/mL of PEG2000-DMG, 5.16 mg/mL of trisodium citrate, 71 mg/mL of sucrose and 1.0 mL of water for injection.

In some embodiments, a nanoparticle (*e.g.*, a lipid nanoparticle) has a mean diameter of 10-500 nm, 20-400 nm, 30-300 nm, 40-200 nm. In some embodiments, a nanoparticle (*e.g.*, a lipid nanoparticle) has a mean diameter of 50-150 nm, 50-200 nm, 80-100 nm or 80-200 nm.

Liposomes, Lipoplexes, and Lipid Nanoparticles

In some embodiments, the RNA vaccine pharmaceutical compositions may be formulated in liposomes such as, but not limited to, DiLa2 liposomes (Marina Biotech, Bothell, WA), SMARTICLES® (Marina Biotech, Bothell, WA), neutral DOPC (1,2-
5 dioleoyl-sn-glycero-3-phosphocholine) based liposomes (*e.g.*, siRNA delivery for ovarian cancer (Landen et al. Cancer Biology & Therapy 2006 5(12)1708-1713); herein incorporated by reference in its entirety) and hyaluronan-coated liposomes (Quiet Therapeutics, Israel).

In some embodiments, the RNA vaccines may be formulated in a lyophilized gel-phase liposomal composition as described in U.S. Publication No. US2012/0060293, herein
10 incorporated by reference in its entirety.

The nanoparticle formulations may comprise a phosphate conjugate. The phosphate conjugate may increase *in vivo* circulation times and/or increase the targeted delivery of the nanoparticle. Phosphate conjugates for use with the present invention may be made by the methods described in International Publication No. WO2013/033438 or U.S. Publication No.
15 US2013/0196948, the content of each of which is herein incorporated by reference in its entirety. As a non-limiting example, the phosphate conjugates may include a compound of any one of the formulas described in International Publication No. WO2013/033438, herein incorporated by reference in its entirety.

The nanoparticle formulation may comprise a polymer conjugate. The polymer
20 conjugate may be a water soluble conjugate. The polymer conjugate may have a structure as described in U.S. Publication No. 2013/0059360, the content of which is herein incorporated by reference in its entirety. In some aspects, polymer conjugates with the polynucleotides of the present invention may be made using the methods and/or segmented polymeric reagents described in U.S. Publication No. 2013/0072709, herein incorporated by reference in its entirety.
25 In other aspects, the polymer conjugate may have pendant side groups comprising ring moieties such as, but not limited to, the polymer conjugates described in U.S. Publication No. US2013/0196948, the contents of which is herein incorporated by reference in its entirety.

The nanoparticle formulations may comprise a conjugate to enhance the delivery of nanoparticles of the present invention in a subject. Further, the conjugate may inhibit
30 phagocytic clearance of the nanoparticles in a subject. In some aspects, the conjugate may be a “self” peptide designed from the human membrane protein CD47 (*e.g.*, the “self” particles described by Rodriguez *et al* (*Science* 2013, 339, 971-975), herein incorporated by reference in its entirety). As shown by Rodriguez *et al.* the self peptides delayed macrophage-mediated clearance of nanoparticles which enhanced delivery of the nanoparticles. In other aspects, the

conjugate may be the membrane protein CD47 (*e.g., see Rodriguez et al. Science* 2013, 339, 971-975, herein incorporated by reference in its entirety). Rodriguez et al. showed that, similarly to “self” peptides, CD47 can increase the circulating particle ratio in a subject as compared to scrambled peptides and PEG coated nanoparticles.

5 In some embodiments, the RNA vaccines of the present invention are formulated in nanoparticles which comprise a conjugate to enhance the delivery of the nanoparticles of the present invention in a subject. The conjugate may be the CD47 membrane or the conjugate may be derived from the CD47 membrane protein, such as the “self” peptide described previously. In other embodiments, the nanoparticle may comprise PEG and a conjugate of CD47 or a
10 derivative thereof. In yet other embodiments, the nanoparticle may comprise both the “self” peptide described above and the membrane protein CD47.

In some embodiments, a “self” peptide and/or CD47 protein may be conjugated to a virus-like particle or pseudovirion, as described herein for delivery of the RNA vaccines of the present invention.

15 In other embodiments, RNA vaccine pharmaceutical compositions comprising the polynucleotides of the present invention and a conjugate, which may have a degradable linkage. Non-limiting examples of conjugates include an aromatic moiety comprising an ionizable hydrogen atom, a spacer moiety, and a water-soluble polymer. As a non-limiting example, pharmaceutical compositions comprising a conjugate with a degradable linkage and
20 methods for delivering such pharmaceutical compositions are described in U.S. Publication No. US2013/0184443, the content of which is herein incorporated by reference in its entirety.

The nanoparticle formulations may be a carbohydrate nanoparticle comprising a carbohydrate carrier and a RNA vaccine. As a non-limiting example, the carbohydrate carrier may include, but is not limited to, an anhydride-modified phytoglycogen or glycogen-type
25 material, phytoglycogen octenyl succinate, phytoglycogen beta-dextrin, anhydride-modified phytoglycogen beta-dextrin. (*See e.g., International Publication No. WO2012/109121, the content of which is herein incorporated by reference in its entirety*).

Nanoparticle formulations of the present invention may be coated with a surfactant or polymer in order to improve the delivery of the particle. In some embodiments, the
30 nanoparticle may be coated with a hydrophilic coating such as, but not limited to, PEG coatings and/or coatings that have a neutral surface charge. The hydrophilic coatings may help to deliver nanoparticles with larger payloads such as, but not limited to, RNA vaccines within the central nervous system. As a non-limiting example nanoparticles comprising a hydrophilic coating and

methods of making such nanoparticles are described in U.S. Publication No.

US2013/0183244, the content of which is herein incorporated by reference in its entirety.

In some embodiments, the lipid nanoparticles of the present invention may be hydrophilic polymer particles. Non-limiting examples of hydrophilic polymer particles and methods of making hydrophilic polymer particles are described in U.S. Publication No. US2013/0210991, the content of which is herein incorporated by reference in its entirety.

In other embodiments, the lipid nanoparticles of the present invention may be hydrophobic polymer particles.

Lipid nanoparticle formulations may be improved by replacing the cationic lipid with a biodegradable cationic lipid which is known as a rapidly eliminated lipid nanoparticle (reLNP). Ionizable cationic lipids, such as, but not limited to, DLinDMA, DLin-KC2-DMA, and DLin-MC3-DMA, have been shown to accumulate in plasma and tissues over time and may be a potential source of toxicity. The rapid metabolism of the rapidly eliminated lipids can improve the tolerability and therapeutic index of the lipid nanoparticles by an order of magnitude from a 1 mg/kg dose to a 10 mg/kg dose in rat. Inclusion of an enzymatically degraded ester linkage can improve the degradation and metabolism profile of the cationic component, while still maintaining the activity of the reLNP formulation. The ester linkage can be internally located within the lipid chain or it may be terminally located at the terminal end of the lipid chain. The internal ester linkage may replace any carbon in the lipid chain.

In some embodiments, the internal ester linkage may be located on either side of the saturated carbon.

In some embodiments, an immune response may be elicited by delivering a lipid nanoparticle which may include a nanospecies, a polymer and an immunogen. (U.S. Publication No. 2012/0189700 and International Publication No. WO2012/099805, each of which is herein incorporated by reference in its entirety).

The polymer may encapsulate the nanospecies or partially encapsulate the nanospecies. The immunogen may be a recombinant protein, a modified RNA and/or a polynucleotide described herein. In some embodiments, the lipid nanoparticle may be formulated for use in a vaccine such as, but not limited to, against a pathogen.

Lipid nanoparticles may be engineered to alter the surface properties of particles so the lipid nanoparticles may penetrate the mucosal barrier. Mucus is located on mucosal tissue such as, but not limited to, oral (*e.g.*, the buccal and esophageal membranes and tonsil tissue), ophthalmic, gastrointestinal (*e.g.*, stomach, small intestine, large intestine, colon, rectum), nasal, respiratory (*e.g.*, nasal, pharyngeal, tracheal and bronchial membranes), genital (*e.g.*, vaginal,

cervical and urethral membranes). Nanoparticles larger than 10-200 nm which are preferred for higher drug encapsulation efficiency and the ability to provide the sustained delivery of a wide array of drugs have been thought to be too large to rapidly diffuse through mucosal barriers.

Mucus is continuously secreted, shed, discarded or digested and recycled so most of the trapped particles may be removed from the mucosal tissue within seconds or within a few hours. Large polymeric nanoparticles (200 nm to 500 nm in diameter) which have been coated densely with a low molecular weight polyethylene glycol (PEG) diffused through mucus only 4 to 6-fold lower than the same particles diffusing in water (Lai *et al. PNAS* 2007 104(5):1482-487; Lai *et al. Adv Drug Deliv Rev.* 2009 61(2): 158-171; each of which is herein incorporated by reference in its entirety). The transport of nanoparticles may be determined using rates of permeation and/or fluorescent microscopy techniques including, but not limited to, fluorescence recovery after photobleaching (FRAP) and high resolution multiple particle tracking (MPT). As a non-limiting example, compositions which can penetrate a mucosal barrier may be made as described in U.S. Patent No. 8,241,670 or International Publication No. WO2013/110028, the content of each of which is herein incorporated by reference in its entirety.

The lipid nanoparticle engineered to penetrate mucus may comprise a polymeric material (*e.g.*, a polymeric core) and/or a polymer-vitamin conjugate and/or a tri-block co-polymer. The polymeric material may include, but is not limited to, polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, poly(styrenes), polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyleneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. The polymeric material may be biodegradable and/or biocompatible. Non-limiting examples of biocompatible polymers are described in International Publication No. WO2013/116804, the content of which is herein incorporated by reference in its entirety. The polymeric material may additionally be irradiated.

As a non-limiting example, the polymeric material may be gamma irradiated (*see e.g.*, International Publication No. WO201282165, herein incorporated by reference in its entirety). Non-limiting examples of specific polymers include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-co-glycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacrylate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol, poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides),

polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone, polysiloxanes, polystyrene (PS), polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fumarate, polyoxymethylene, poloxamers, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), PEG-PLGA-PEG and trimethylene carbonate, polyvinylpyrrolidone. The lipid nanoparticle may be coated or associated with a copolymer such as, but not limited to, a block co-polymer (such as a branched polyether-polyamide block copolymer described in International Publication No. WO2013/012476, herein incorporated by reference in its entirety), and (poly(ethylene glycol))-(poly(propylene oxide))-(poly(ethylene glycol)) triblock copolymer (*see e.g.*, U.S. Publication 2012/0121718, U.S. Publication 2010/0003337 and U.S. Patent No. 8,263,665, each of which is herein incorporated by reference in its entirety). The co-polymer may be a polymer that is generally regarded as safe (GRAS) and the formation of the lipid nanoparticle may be in such a way that no new chemical entities are created. For example, the lipid nanoparticle may comprise poloxamers coating PLGA nanoparticles without forming new chemical entities which are still able to rapidly penetrate human mucus (Yang *et al. Angew. Chem. Int. Ed.* 2011 50:25972600, the content of which is herein incorporated by reference in its entirety). A non-limiting scalable method to produce nanoparticles which can penetrate human mucus is described by Xu *et al. (see e.g., J Control Release* 2013, 170(2):279-86, the content of which is herein incorporated by reference in its entirety).

The vitamin of the polymer-vitamin conjugate may be vitamin E. The vitamin portion of the conjugate may be substituted with other suitable components such as, but not limited to, vitamin A, vitamin E, other vitamins, cholesterol, a hydrophobic moiety, or a hydrophobic

component of other surfactants (*e.g.*, sterol chains, fatty acids, hydrocarbon chains and alkylene oxide chains).

In some embodiments, the RNA (*e.g.*, mRNA) vaccine pharmaceutical compositions may be formulated in liposomes such as, but not limited to, DiLa2 liposomes (Marina Biotech, Bothell, WA), SMARTICLES® (Marina Biotech, Bothell, WA), neutral DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) based liposomes (*e.g.*, siRNA delivery for ovarian cancer (Landen *et al. Cancer Biology & Therapy* 2006 5(12)1708-1713, herein incorporated by reference in its entirety)) and hyaluronan-coated liposomes (Quiet Therapeutics, Israel).

In some embodiments, the RNA vaccines may be formulated in a lyophilized gel- phase liposomal composition as described in U.S. Publication No. US2012/0060293, herein incorporated by reference in its entirety.

The nanoparticle formulations may comprise a phosphate conjugate. The phosphate conjugate may increase *in vivo* circulation times and/or increase the targeted delivery of the nanoparticle. Phosphate conjugates for use with the present invention may be made by the methods described in International Publication No. WO2013/033438 or U.S. Publication No. 2013/0196948, the content of each of which is herein incorporated by reference in its entirety. As a non-limiting example, the phosphate conjugates may include a compound of any one of the formulas described in International Publication No. WO2013/033438, herein incorporated by reference in its entirety.

The nanoparticle formulation may comprise a polymer conjugate. The polymer conjugate may be a water soluble conjugate. The polymer conjugate may have a structure as described in U.S. Application No. 2013/0059360, the content of which is herein incorporated by reference in its entirety. In some aspects, polymer conjugates with the polynucleotides of the present invention may be made using the methods and/or segmented polymeric reagents described in U.S. Patent Application No. 2013/0072709, herein incorporated by reference in its entirety. In other aspects, the polymer conjugate may have pendant side groups comprising ring moieties such as, but not limited to, the polymer conjugates described in U.S. Publication No. US2013/0196948, the content of which is herein incorporated by reference in its entirety.

The nanoparticle formulations may comprise a conjugate to enhance the delivery of nanoparticles of the present invention in a subject. Further, the conjugate may inhibit phagocytic clearance of the nanoparticles in a subject. In some aspects, the conjugate may be a “self” peptide designed from the human membrane protein CD47 (*e.g.*, the “self” particles described by Rodriguez *et al. (Science* 2013, 339, 971-975), herein incorporated by reference in its entirety). As shown by Rodriguez *et al.* the self peptides delayed macrophage-mediated

clearance of nanoparticles which enhanced delivery of the nanoparticles. In other aspects, the conjugate may be the membrane protein CD47 (*e.g.*, see Rodriguez *et al. Science* 2013, 339, 971-975, herein incorporated by reference in its entirety). Rodriguez *et al.* showed that, similarly to “self” peptides, CD47 can increase the circulating particle ratio in a subject as compared to scrambled peptides and PEG coated nanoparticles.

In some embodiments, the RNA vaccines of the present invention are formulated in nanoparticles that comprise a conjugate to enhance the delivery of the nanoparticles of the present disclosure in a subject. The conjugate may be the CD47 membrane or the conjugate may be derived from the CD47 membrane protein, such as the “self” peptide described previously. In other aspects the nanoparticle may comprise PEG and a conjugate of CD47 or a derivative thereof. In yet other aspects, the nanoparticle may comprise both the “self” peptide described above and the membrane protein CD47.

In other aspects, a “self” peptide and/or CD47 protein may be conjugated to a virus-like particle or pseudovirion, as described herein for delivery of the RNA vaccines of the present invention.

In other embodiments, RNA vaccine pharmaceutical compositions comprising the polynucleotides of the present invention and a conjugate which may have a degradable linkage. Non-limiting examples of conjugates include an aromatic moiety comprising an ionizable hydrogen atom, a spacer moiety, and a water-soluble polymer. As a non-limiting example, pharmaceutical compositions comprising a conjugate with a degradable linkage and methods for delivering such pharmaceutical compositions are described in U.S. Publication No. US2013/0184443, the content of which is herein incorporated by reference in its entirety.

The nanoparticle formulations may be a carbohydrate nanoparticle comprising a carbohydrate carrier and a RNA (*e.g.*, mRNA) vaccine. As a non-limiting example, the carbohydrate carrier may include, but is not limited to, an anhydride-modified phytoglycogen or glycogen-type material, phytoglycogen octenyl succinate, phytoglycogen beta-dextrin, anhydride-modified phytoglycogen beta-dextrin. (See *e.g.*, International Publication No. WO2012/109121; the content of which is herein incorporated by reference in its entirety).

Nanoparticle formulations of the present invention may be coated with a surfactant or polymer in order to improve the delivery of the particle. In some embodiments, the nanoparticle may be coated with a hydrophilic coating such as, but not limited to, PEG coatings and/or coatings that have a neutral surface charge. The hydrophilic coatings may help to deliver nanoparticles with larger payloads such as, but not limited to, RNA vaccines within the central nervous system. As a non-limiting example nanoparticles comprising a hydrophilic coating and

methods of making such nanoparticles are described in U.S. Publication No.

US2013/0183244, the content of which is herein incorporated by reference in its entirety.

In some embodiments, the lipid nanoparticles of the present invention may be hydrophilic polymer particles. Non-limiting examples of hydrophilic polymer particles and methods of making hydrophilic polymer particles are described in U.S. Publication No. US2013/0210991, the content of which is herein incorporated by reference in its entirety.

In other embodiments, the lipid nanoparticles of the present invention may be hydrophobic polymer particles.

Lipid nanoparticle formulations may be improved by replacing the cationic lipid with a biodegradable cationic lipid which is known as a rapidly eliminated lipid nanoparticle (reLNP). Ionizable cationic lipids, such as, but not limited to, DLinDMA, DLin-KC2-DMA, and DLin-MC3-DMA, have been shown to accumulate in plasma and tissues over time and may be a potential source of toxicity. The rapid metabolism of the rapidly eliminated lipids can improve the tolerability and therapeutic index of the lipid nanoparticles by an order of magnitude from a 1 mg/kg dose to a 10 mg/kg dose in rat. Inclusion of an enzymatically degraded ester linkage can improve the degradation and metabolism profile of the cationic component, while still maintaining the activity of the reLNP formulation. The ester linkage can be internally located within the lipid chain or it may be terminally located at the terminal end of the lipid chain. The internal ester linkage may replace any carbon in the lipid chain.

In some embodiments, the internal ester linkage may be located on either side of the saturated carbon.

In some embodiments, an immune response may be elicited by delivering a lipid nanoparticle which may include a nanospecies, a polymer and an immunogen. (U.S. Publication No. 2012/0189700 and International Publication No. WO2012/099805, each of which is herein incorporated by reference in its entirety).

Lipid nanoparticles may be engineered to alter the surface properties of particles so the lipid nanoparticles may penetrate the mucosal barrier. Mucus is located on mucosal tissue such as, but not limited to, oral (*e.g.*, the buccal and esophageal membranes and tonsil tissue), ophthalmic, gastrointestinal (*e.g.*, stomach, small intestine, large intestine, colon, rectum), nasal, respiratory (*e.g.*, nasal, pharyngeal, tracheal and bronchial membranes), genital (*e.g.*, vaginal, cervical and urethral membranes). Nanoparticles larger than 10-200 nm which are preferred for higher drug encapsulation efficiency and the ability to provide the sustained delivery of a wide array of drugs have been thought to be too large to rapidly diffuse through mucosal barriers. Mucus is continuously secreted, shed, discarded or digested and recycled so most of the trapped

particles may be removed from the mucosal tissue within seconds or within a few hours. Large polymeric nanoparticles (200nm -500nm in diameter) which have been coated densely with a low molecular weight polyethylene glycol (PEG) diffused through mucus only 4 to 6-fold lower than the same particles diffusing in water (Lai *et al. PNAS* 2007 104(5):1482-487; Lai *et al. Adv Drug Deliv Rev.* 2009 61(2): 158-171; each of which is herein incorporated by reference in its entirety). The transport of nanoparticles may be determined using rates of permeation and/or fluorescent microscopy techniques including, but not limited to, fluorescence recovery after photobleaching (FRAP) and high resolution multiple particle tracking (MPT). As a non-limiting example, compositions which can penetrate a mucosal barrier may be made as described in U.S. Patent No. 8,241,670 or International Publication No. WO2013/110028, the content of each of which is herein incorporated by reference in its entirety.

The lipid nanoparticle engineered to penetrate mucus may comprise a polymeric material (i.e. a polymeric core) and/or a polymer-vitamin conjugate and/or a tri-block co-polymer. The polymeric material may include, but is not limited to, polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, poly(styrenes), polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyleneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. The polymeric material may be biodegradable and/or biocompatible. Non-limiting examples of biocompatible polymers are described in International Publication No. WO2013/116804, the content of which is herein incorporated by reference in its entirety. The polymeric material may additionally be irradiated. As a non-limiting example, the polymeric material may be gamma irradiated (*see e.g.*, International Publication No. WO2012/082165, herein incorporated by reference in its entirety). Non-limiting examples of specific polymers include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-co-glycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacrylate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol, poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl

halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone, polysiloxanes, polystyrene (PS), polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fumarate, polyoxymethylene, poloxamers, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), PEG-PLGA-PEG and trimethylene carbonate, polyvinylpyrrolidone. The lipid nanoparticle may be coated or associated with a copolymer such as, but not limited to, a block co-polymer (such as a branched polyether-polyamide block copolymer described in International Publication No. WO2013/012476, herein incorporated by reference in its entirety), and (poly(ethylene glycol))-(poly(propylene oxide))-(poly(ethylene glycol)) triblock copolymer (see *e.g.*, U.S. Publication 2012/0121718 and U.S. Publication 2010/0003337 and U.S. Pat. No. 8,263,665; each of which is herein incorporated by reference in its entirety). The co-polymer may be a polymer that is generally regarded as safe (GRAS) and the formation of the lipid nanoparticle may be in such a way that no new chemical entities are created. For example, the lipid nanoparticle may comprise poloxamers coating PLGA nanoparticles without forming new chemical entities which are still able to rapidly penetrate human mucus (Yang *et al. Angew. Chem. Int. Ed.* 2011 50:25972600; the content of which is herein incorporated by reference in its entirety). A non-limiting scalable method to produce nanoparticles which can penetrate human mucus is described by Xu *et al.* (see *e.g.*, *J Control Release* 2013, 170(2):279-86, the content of which is herein incorporated by reference in its entirety).

The vitamin of the polymer-vitamin conjugate may be vitamin E. The vitamin portion of the conjugate may be substituted with other suitable components such as, but not limited to, vitamin A, vitamin E, other vitamins, cholesterol, a hydrophobic moiety, or a hydrophobic component of other surfactants (*e.g.*, sterol chains, fatty acids, hydrocarbon chains and alkylene oxide chains).

The lipid nanoparticle engineered to penetrate mucus may include surface altering agents such as, but not limited to, polynucleotides, anionic proteins (*e.g.*, bovine serum albumin), surfactants (*e.g.*, cationic surfactants such as for example dimethyldioctadecyl-

ammonium bromide), sugars or sugar derivatives (*e.g.*, cyclodextrin), nucleic acids, polymers (*e.g.*, heparin, polyethylene glycol and poloxamer), mucolytic agents (*e.g.*, N-acetylcysteine, mugwort, bromelain, papain, clerodendrum, acetylcysteine, bromhexine, carbocysteine, eprazinone, mesna, ambroxol, sobrerol, domiodol, letosteine, stepronin, tiopronin, gelsolin, thymosin β 4 dornase alfa, neltenexine, erdosteine) and various DNases including rhDNase. The surface altering agent may be embedded or enmeshed in the particle's surface or disposed (*e.g.*, by coating, adsorption, covalent linkage, or other process) on the surface of the lipid nanoparticle (see *e.g.*, U.S. Publication 2010/0215580 and U.S. Publication 2008/0166414 and US2013/0164343 the content of each of which is herein incorporated by reference in its entirety).

In some embodiments, the mucus penetrating lipid nanoparticles may comprise at least one polynucleotide described herein. The polynucleotide may be encapsulated in the lipid nanoparticle and/or disposed on the surface of the particle. The polynucleotide may be covalently coupled to the lipid nanoparticle. Formulations of mucus penetrating lipid nanoparticles may comprise a plurality of nanoparticles. Further, the formulations may contain particles which may interact with the mucus and alter the structural and/or adhesive properties of the surrounding mucus to decrease mucoadhesion which may increase the delivery of the mucus penetrating lipid nanoparticles to the mucosal tissue.

In other embodiments, the mucus penetrating lipid nanoparticles may be a hypotonic formulation comprising a mucosal penetration enhancing coating. The formulation may be hypotonic for the epithelium to which it is being delivered.

Non-limiting examples of hypotonic formulations may be found in International Publication No. WO2013/110028, the content of which is herein incorporated by reference in its entirety.

In some embodiments, in order to enhance the delivery through the mucosal barrier the RNA vaccine formulation may comprise or be a hypotonic solution. Hypotonic solutions were found to increase the rate at which mucoinert particles such as, but not limited to, mucus-penetrating particles, were able to reach the vaginal epithelial surface (*see e.g.*, Ensign *et al. Biomaterials* 2013, 34(28):6922-9, the content of which is herein incorporated by reference in its entirety).

In some embodiments, the RNA vaccine is formulated as a lipoplex, such as, without limitation, the ATUPLEXTM system, the DACC system, the DBTC system and other siRNA-lipoplex technology from Silence Therapeutics (London, United Kingdom), STEMFECTTM from STEMGENT® (Cambridge, MA), and polyethylenimine (PEI) or protamine-based

targeted and non-targeted delivery of nucleic acids (Aleku *et al.* *Cancer Res.* 2008 68:9788-9798; Strumberg *et al.* *Int J Clin Pharmacol Ther* 2012 50:76-78; Santel *et al.*, *Gene Ther* 2006 13:1222-1234; Santel *et al.*, *Gene Ther* 2006 13:1360-1370; Gutbier *et al.*, *Pulm Pharmacol. Ther.* 2010 23:334-344; Kaufmann *et al.* *Microvasc Res* 2010 80:286-293; Weide *et al.* *J Immunother.* 2009 32:498-507; Weide *et al.* *J Immunother.* 2008 31:180-188; Pascolo, *Expert Opin. Biol. Ther.* 4:1285-1294; Fotin-Mleczek *et al.*, 2011 *J. Immunother.* 34:1-15; Song *et al.*, *Nature Biotechnol.* 2005, 23:709-717; Peer *et al.*, *Proc Natl Acad Sci U S A.* 2007 6;104:4095-4100; deFougerolles *Hum Gene Ther.* 2008 19:125-132; each of which is incorporated herein by reference in its entirety).

10 In some embodiments, such formulations may also be constructed or compositions altered such that they passively or actively are directed to different cell types *in vivo*, including but not limited to hepatocytes, immune cells, tumor cells, endothelial cells, antigen presenting cells, and leukocytes (Akinc *et al.* *Mol Ther.* 2010 18:1357-1364; Song *et al.*, *Nat Biotechnol.* 2005 23:709-717; Judge *et al.*, *J Clin Invest.* 2009 119:661-673; Kaufmann *et al.*, *Microvasc*
15 *Res* 2010 80:286-293; Santel *et al.*, *Gene Ther* 2006 13:1222-1234; Santel *et al.*, *Gene Ther* 2006 13:1360-1370; Gutbier *et al.*, *Pulm Pharmacol. Ther.* 2010 23:334-344; Basha *et al.*, *Mol. Ther.* 2011 19:2186-2200; Fenske and Cullis, *Expert Opin Drug Deliv.* 2008 5:25-44; Peer *et al.*, *Science.* 2008 319:627-630; Peer and Lieberman, *Gene Ther.* 2011 18:1127-1133; each of which is incorporated herein by reference in its entirety). One example of passive targeting of
20 formulations to liver cells includes the DLin-DMA, DLin-KC2-DMA and DLin-MC3-DMA-based lipid nanoparticle formulations which have been shown to bind to apolipoprotein E and promote binding and uptake of these formulations into hepatocytes *in vivo* (Akinc *et al.* *Mol Ther.* 2010 18:1357-1364; herein incorporated by reference in its entirety). Formulations can also be selectively targeted through expression of different ligands on their surface as
25 exemplified by, but not limited by, folate, transferrin, N-acetylgalactosamine (GalNAc), and antibody targeted approaches (Kolhatkar *et al.*, *Curr Drug Discov Technol.* 2011 8:197-206; Musacchio and Torchilin, *Front Biosci.* 2011 16:1388-1412; Yu *et al.*, *Mol Membr Biol.* 2010 27:286-298; Patil *et al.*, *Crit Rev Ther Drug Carrier Syst.* 2008 25:1-61; Benoit *et al.*, *Biomacromolecules.* 2011 12:2708-2714; Zhao *et al.*, *Expert Opin Drug Deliv.* 2008 5:309-
30 319; Akinc *et al.*, *Mol Ther.* 2010 18:1357-1364; Srinivasan *et al.*, *Methods Mol Biol.* 2012 820:105-116; Ben-Arie *et al.*, *Methods Mol Biol.* 2012 757:497-507; Peer 2010 *J Control Release.* 20:63-68; Peer *et al.*, *Proc Natl Acad Sci U S A.* 2007 104:4095-4100; Kim *et al.*, *Methods Mol Biol.* 2011 721:339-353; Subramanya *et al.*, *Mol Ther.* 2010 18:2028-2037; Song *et al.*, *Nat Biotechnol.* 2005 23:709-717; Peer *et al.*, *Science.* 2008 319:627-630; Peer

and Lieberman, *Gene Ther.* 2011 18:1127-1133; each of which is incorporated herein by reference in its entirety).

In some embodiments, the RNA (*e.g.*, mRNA) vaccine is formulated as a solid lipid nanoparticle. A solid lipid nanoparticle (SLN) may be spherical with an average diameter
5 between 10 to 1000 nm. SLN possess a solid lipid core matrix that can solubilize lipophilic molecules and may be stabilized with surfactants and/or emulsifiers. In other embodiments, the lipid nanoparticle may be a self-assembly lipid-polymer nanoparticle (*see* Zhang *et al.*, *ACS Nano*, 2008, 2 (8), pp 1696–1702; the content of which is herein incorporated by reference in its entirety). As a non-limiting example, the SLN may be the SLN described in International
10 Publication No. WO2013/105101, the content of which is herein incorporated by reference in its entirety. As another non-limiting example, the SLN may be made by the methods or processes described in International Publication No. WO2013/105101, the content of which is herein incorporated by reference in its entirety.

Liposomes, lipoplexes, or lipid nanoparticles may be used to improve the efficacy of
15 polynucleotides directed protein production as these formulations may be able to increase cell transfection by the RNA vaccine; and/or increase the translation of encoded protein. One such example involves the use of lipid encapsulation to enable the effective systemic delivery of polyplex plasmid DNA (Heyes *et al.*, *Mol Ther.* 2007 15:713-720; herein incorporated by reference in its entirety). The liposomes, lipoplexes, or lipid nanoparticles may also be used
20 to increase the stability of the polynucleotide.

In some embodiments, the RNA (*e.g.*, mRNA) vaccines of the present invention can be formulated for controlled release and/or targeted delivery. As used herein, “controlled release” refers to a pharmaceutical composition or compound release profile that conforms to a particular pattern of release to effect a therapeutic outcome. In some embodiments, the RNA vaccines
25 may be encapsulated into a delivery agent described herein and/or known in the art for controlled release and/or targeted delivery. As used herein, the term “encapsulate” means to enclose, surround or encase. As it relates to the formulation of the compounds of the invention, encapsulation may be substantial, complete or partial. The term “substantially encapsulated” means that at least greater than 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9,
30 99.9 or greater than 99.999% of the pharmaceutical composition or compound of the invention may be enclosed, surrounded or encased within the delivery agent. “Partially encapsulation” means that less than 10, 10, 20, 30, 40 50 or less of the pharmaceutical composition or compound of the invention may be enclosed, surrounded or encased within the delivery agent. Advantageously, encapsulation may be determined by measuring the escape or

the activity of the pharmaceutical composition or compound of the invention using fluorescence and/or electron micrograph. For example, at least 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the pharmaceutical composition or compound of the present disclosure are encapsulated in the delivery agent.

5 In some embodiments, the controlled release formulation may include, but is not limited to, tri-block co-polymers. As a non-limiting example, the formulation may include two different types of tri-block co-polymers (International Pub. No. WO2012/131104 and WO2012/131106; the contents of each of which is herein incorporated by reference in its entirety).

10 In other embodiments, the RNA vaccines may be encapsulated into a lipid nanoparticle or a rapidly eliminated lipid nanoparticle and the lipid nanoparticles or a rapidly eliminated lipid nanoparticle may then be encapsulated into a polymer, hydrogel and/or surgical sealant described herein and/or known in the art. As a non-limiting example, the polymer, hydrogel or surgical sealant may be PLGA, ethylene vinyl acetate (EVAc), poloxamer, GELSITE® (Nanotherapeutics, Inc. Alachua, FL), HYLENEX® (Halozyme Therapeutics, San Diego CA), 15 surgical sealants such as fibrinogen polymers (Ethicon Inc. Cornelia, GA), TISSELL® (Baxter International, Inc. Deerfield, IL), PEG-based sealants, and COSEAL® (Baxter International, Inc. Deerfield, IL).

In other embodiments, the lipid nanoparticle may be encapsulated into any polymer known in the art which may form a gel when injected into a subject. As another non-limiting 20 example, the lipid nanoparticle may be encapsulated into a polymer matrix which may be biodegradable.

In some embodiments, the RNA vaccine formulation for controlled release and/or targeted delivery may also include at least one controlled release coating. Controlled release coatings include, but are not limited to, OPADRY®, polyvinylpyrrolidone/vinyl acetate 25 copolymer, polyvinylpyrrolidone, hydroxypropyl methylcellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, EUDRAGIT RL®, EUDRAGIT RS® and cellulose derivatives such as ethylcellulose aqueous dispersions (AQUACOAT® and SURELEASE®).

In some embodiments, the RNA (*e.g.*, mRNA) vaccine controlled release and/or targeted delivery formulation may comprise at least one degradable polyester which may 30 contain polycationic side chains. Degradable polyesters include, but are not limited to, poly(*serine ester*), poly(*L-lactide-co-L-lysine*), poly(*4-hydroxy-L-proline ester*), and combinations thereof. In other embodiments, the degradable polyesters may include a PEG conjugation to form a PEGylated polymer.

In some embodiments, the RNA vaccine controlled release and/or targeted delivery formulation comprising at least one polynucleotide may comprise at least one PEG and/or PEG related polymer derivatives as described in U.S. Patent No. 8,404,222, herein incorporated by reference in its entirety.

5 In other embodiments, the RNA vaccine controlled release delivery formulation comprising at least one polynucleotide may be the controlled release polymer system described in U.S. Publication No. 2013/0130348, herein incorporated by reference in its entirety.

In some embodiments, the RNA (*e.g.*, mRNA) vaccines of the present invention may be encapsulated in a therapeutic nanoparticle, referred to herein as “therapeutic nanoparticle RNA vaccines.” Therapeutic nanoparticles may be formulated by methods described herein and
10 known in the art such as, but not limited to, International Publication Nos. WO2010/005740, WO2010/030763, WO2010/005721, WO2010/005723, WO2012/054923, U.S. Publication Nos. US2011/0262491, US2010/0104645, US2010/0087337, US2010/0068285, US2011/0274759, US2010/0068286, US2012/0288541, US2013/0123351 and
15 US2013/0230567 and US Patent Nos. 8,206,747, 8,293,276, 8,318,208 and 8,318,211, the content of each of which is herein incorporated by reference in its entirety. In other embodiments, therapeutic polymer nanoparticles may be identified by the methods described in U.S. Publication No. US2012/0140790, the content of which is herein incorporated by reference in its entirety.

20 In some embodiments, the therapeutic nanoparticle RNA vaccine may be formulated for sustained release. As used herein, “sustained release” refers to a pharmaceutical composition or compound that conforms to a release rate over a specific period of time. The period of time may include, but is not limited to, hours, days, weeks, months and years. As a non-limiting example, the sustained release nanoparticle may comprise a polymer and a therapeutic agent
25 such as, but not limited to, the polynucleotides of the present invention (*see* International Publication No. WO2010/075072 and U.S. Publication Nos. US2010/0216804, US2011/0217377 and US2012/0201859, each of which is herein incorporated by reference in its entirety). In another non-limiting example, the sustained release formulation may comprise agents which permit persistent bioavailability such as, but not limited to, crystals,
30 macromolecular gels and/or particulate suspensions (*see* U.S. Publication No. US2013/0150295, the content of which is herein incorporated by reference in its entirety).

In some embodiments, the therapeutic nanoparticle RNA vaccines may be formulated to be target specific. As a non-limiting example, the therapeutic nanoparticles may include a corticosteroid (*see* International Publication No. WO2011/084518, herein incorporated by

reference in its entirety). As a non-limiting example, the therapeutic nanoparticles may be formulated in nanoparticles described in International Publication Nos. WO2008/121949, WO2010/005726, WO2010/005725, WO2011/084521 and U.S. Publication Nos. US2010/0069426, US2012/0004293 and US2010/0104655, each of which is herein incorporated by reference in its entirety.

In some embodiments, the nanoparticles of the present invention may comprise a polymeric matrix. As a non-limiting example, the nanoparticle may comprise two or more polymers such as, but not limited to, polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polylysine, poly(ethylene imine), poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester) or combinations thereof.

In some embodiments, the therapeutic nanoparticle comprises a diblock copolymer. In some embodiments, the diblock copolymer may include PEG in combination with a polymer such as, but not limited to, polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polylysine, poly(ethylene imine), poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester) or combinations thereof. In yet other embodiments, the diblock copolymer may be a high-X diblock copolymer such as those described in International Publication No. WO2013120052, the content of which is herein incorporated by reference in its entirety.

As a non-limiting example, the therapeutic nanoparticle comprises a PLGA-PEG block copolymer (*see* U.S. Publication No. US2012/0004293 and U.S. Patent No. 8,236,330, each of which is herein incorporated by reference in its entirety). In another non-limiting example, the therapeutic nanoparticle is a stealth nanoparticle comprising a diblock copolymer of PEG and PLA or PEG and PLGA (*see* U.S. Patent No. 8,246,968 and International Publication No. WO2012/166923, the content of each of which is herein incorporated by reference in its entirety). In yet another non-limiting example, the therapeutic nanoparticle is a stealth nanoparticle or a target-specific stealth nanoparticle as described in U.S. Publication No. 2013/0172406, the content of which is herein incorporated by reference in its entirety.

In some embodiments, the therapeutic nanoparticle may comprise a multiblock copolymer (*see e.g.*, U.S. Patent Nos. 8,263,665 and 8,287,910 and U.S. Publication No. 2013/0195987, the content of each of which is herein incorporated by reference in its entirety).

5 In yet another non-limiting example, the lipid nanoparticle comprises the block copolymer PEG-PLGA-PEG (*see e.g.*, the thermosensitive hydrogel (PEG-PLGA-PEG) used as a TGF-beta1 gene delivery vehicle in Lee *et al.* "Thermosensitive Hydrogel as a TGF- β 1 Gene Delivery Vehicle Enhances Diabetic Wound Healing." *Pharmaceutical Research*, 2003 20(12): 1995-2000; and used as a controlled gene delivery system in Li *et al.* "Controlled Gene
10 Delivery System Based on Thermosensitive Biodegradable Hydrogel" *Pharmaceutical Research* 2003 20(6):884- 888; and Chang *et al.*, "Non-ionic amphiphilic biodegradable PEG-PLGA-PEG copolymer enhances gene delivery efficiency in rat skeletal muscle." *J Controlled Release*. 2007 118:245-253; each of which is herein incorporated by reference in its entirety). The RNA (*e.g.*, mRNA) vaccines of the present disclosure may be formulated in
15 lipid nanoparticles comprising the PEG-PLGA-PEG block copolymer.

In some embodiments, the block copolymers described herein may be included in a polyion complex comprising a non-polymeric micelle and the block copolymer. (*see e.g.*, U.S. Publication No. 2012/0076836, herein incorporated by reference in its entirety).

20 In some embodiments, the therapeutic nanoparticle may comprise at least one acrylic polymer. Acrylic polymers include but are not limited to, acrylic acid, methacrylic acid, acrylic acid and methacrylic acid copolymers, methyl methacrylate copolymers, ethoxyethyl methacrylates, cyanoethyl methacrylate, amino alkyl methacrylate copolymer, poly(acrylic acid), poly(methacrylic acid), polycyanoacrylates and combinations thereof.

25 In some embodiments, the therapeutic nanoparticles may comprise at least one poly(vinyl ester) polymer. The poly(vinyl ester) polymer may be a copolymer such as a random copolymer. As a non-limiting example, the random copolymer may have a structure such as those described in International Publication No. WO2013/032829 or U.S. Publication No. 2013/0121954, the content of which is herein incorporated by reference in its entirety. In some aspects, the poly(vinyl ester) polymers may be conjugated to the polynucleotides described
30 herein..

In some embodiments, the therapeutic nanoparticle may comprise at least one diblock copolymer. The diblock copolymer may be, but it not limited to, a poly(lactic) acid-poly(ethylene)glycol copolymer (*see e.g.*, International Publication No. WO2013/044219; herein incorporated by reference in its entirety). As a non-limiting example, the therapeutic

nanoparticle may be used to treat cancer (*see* International publication No. WO2013/044219, herein incorporated by reference in its entirety).

In some embodiments, the therapeutic nanoparticles may comprise at least one cationic polymer described herein and/or known in the art.

5 In some embodiments, the therapeutic nanoparticles may comprise at least one amine-containing polymer such as, but not limited to polylysine, polyethyleneimine, poly(amidoamine) dendrimers, poly(beta-amino esters) (*see e.g.*, U.S. Patent No. 8,287,849, herein incorporated by reference in its entirety) and combinations thereof. In other embodiments, the nanoparticles described herein may comprise an amine cationic lipid such as those described in
10 International Publication No. WO2013/059496, the content of which is herein incorporated by reference in its entirety. In some aspects the cationic lipids may have an amino-amine or an amino-amide moiety.

In some embodiments, the therapeutic nanoparticles may comprise at least one degradable polyester, which may contain polycationic side chains. Degradable polyesters
15 include, but are not limited to, poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester), and combinations thereof. In other embodiments, the degradable polyesters may include a PEG conjugation to form a PEGylated polymer.

In other embodiments, the therapeutic nanoparticle may include a conjugation of at least one targeting ligand. The targeting ligand may be any ligand known in the art such as, but not
20 limited to, a monoclonal antibody (Kirpotin *et al*, *Cancer Res.* 2006 66:6732-6740, herein incorporated by reference in its entirety).

In some embodiments, the therapeutic nanoparticle may be formulated in an aqueous solution, which may be used to target cancer (*see* International Publication No. WO2011/084513 and U.S. Publication No. 2011/0294717, each of which is herein
25 incorporated by reference in its entirety).

In some embodiments, the therapeutic nanoparticle RNA vaccines, *e.g.*, therapeutic nanoparticles comprising at least one RNA vaccine may be formulated using the methods described by Podobinski *et al* in U.S. Patent No. 8,404,799, the content of which is herein incorporated by reference in its entirety.

30 In some embodiments, the RNA (*e.g.*, mRNA) vaccines may be encapsulated in, linked to and/or associated with synthetic nanocarriers. Synthetic nanocarriers include, but are not limited to, those described in International Publication Nos. WO2010/005740, WO2012/149454 and WO2013/019669, and U.S. Publication Nos. US2011/0262491, US2010/0104645, US2010/0087337 and US2012/0244222, each of which is herein

incorporated by reference in its entirety. The synthetic nanocarriers may be formulated using methods known in the art and/or described herein. As a non-limiting example, the synthetic nanocarriers may be formulated by the methods described in International Publication Nos. WO2010/005740, WO2010/030763 and WO2012/13501, and U.S. Publication Nos.

5 US2011/0262491, US2010/0104645, US2010/0087337 and US2012/024422, each of which is herein incorporated by reference in its entirety. In other embodiments, the synthetic nanocarrier formulations may be lyophilized by methods described in International Publication No. WO2011/072218 and U.S. Patent No. 8,211,473, the content of each of which is herein incorporated by reference in its entirety. In yet other embodiments, formulations of the present
10 invention, including, but not limited to, synthetic nanocarriers, may be lyophilized or reconstituted by the methods described in U.S. Publication No. 2013/0230568, the content of which is herein incorporated by reference in its entirety.

In some embodiments, the synthetic nanocarriers may contain reactive groups to release the polynucleotides described herein (*see* International Publication No. WO2012/092552 and
15 U.S. Publication No. US2012/0171229, each of which is herein incorporated by reference in its entirety).

In some embodiments, the synthetic nanocarriers may contain an immunostimulatory agent to enhance the immune response from delivery of the synthetic nanocarrier. As a non-limiting example, the synthetic nanocarrier may comprise a Th1 immunostimulatory agent
20 which may enhance a Th1-based response of the immune system (*see* International Publication No. WO2010/123569 and U.S. Publication No. 2011/0223201, each of which is herein incorporated by reference in its entirety).

In some embodiments, the synthetic nanocarriers may be formulated for targeted release. In some embodiments, the synthetic nanocarrier is formulated to release the
25 polynucleotides at a specified pH and/or after a desired time interval. As a non-limiting example, the synthetic nanoparticle may be formulated to release the RNA vaccines after 24 hours and/or at a pH of 4.5 (*see* International Publication Nos. WO2010/138193 and WO2010/138194 and U.S. Publication Nos. US2011/0020388 and US2011/0027217, each of which is herein incorporated by reference in their entireties).

30 In some embodiments, the synthetic nanocarriers may be formulated for controlled and/or sustained release of the polynucleotides described herein. As a non-limiting example, the synthetic nanocarriers for sustained release may be formulated by methods known in the art, described herein and/or as described in International Publication No. WO2010/138192 and

U.S. Publication No. 2010/0303850, each of which is herein incorporated by reference in its entirety.

In some embodiments, the RNA vaccine may be formulated for controlled and/or sustained release wherein the formulation comprises at least one polymer that is a crystalline side chain (CYSC) polymer. CYSC polymers are described in U.S. Patent No. 8,399,007, herein incorporated by reference in its entirety.

In some embodiments, the synthetic nanocarrier may be formulated for use as a vaccine. In some embodiments, the synthetic nanocarrier may encapsulate at least one polynucleotide which encode at least one antigen. As a non-limiting example, the synthetic nanocarrier may include at least one antigen and an excipient for a vaccine dosage form (*see* International Publication No. WO2011/150264 and U.S. Publication No. 2011/0293723, each of which is herein incorporated by reference in its entirety). As another non-limiting example, a vaccine dosage form may include at least two synthetic nanocarriers with the same or different antigens and an excipient (*see* International Publication No. WO2011/150249 and U.S. Publication No. 2011/0293701, each of which is herein incorporated by reference in its entirety). The vaccine dosage form may be selected by methods described herein, known in the art and/or described in International Publication No. WO2011/150258 and U.S. Publication No. US2012/0027806, each of which is herein incorporated by reference in its entirety).

In some embodiments, the synthetic nanocarrier may comprise at least one polynucleotide which encodes at least one adjuvant (*e.g.*, a flagellin protein). In some embodiments, the synthetic nanocarrier may comprise at least one adjuvant. As non-limiting example, the adjuvant may comprise dimethyldioctadecylammonium-bromide, dimethyldioctadecylammonium-chloride, dimethyldioctadecylammonium-phosphate or dimethyldioctadecylammonium-acetate (DDA) and an apolar fraction or part of said apolar fraction of a total lipid extract of a mycobacterium (See *e.g.*, U.S. Patent No. 8,241,610; herein incorporated by reference in its entirety). In other embodiments, the synthetic nanocarrier may comprise at least one polynucleotide and an adjuvant. As a non-limiting example, the synthetic nanocarrier comprising, optionally comprising an adjuvant, may be formulated by the methods described in International Publication No. WO2011/150240 and U.S. Publication No. US2011/0293700, each of which is herein incorporated by reference in its entirety.

In some embodiments, the synthetic nanocarrier may encapsulate at least one polynucleotide which encodes a peptide, fragment or region from a virus. As a non-limiting example, the synthetic nanocarrier may include, but is not limited to, the nanocarriers described in International Publication Nos. WO2012/024621, WO2012/02629, WO2012/024632 and

U.S. Publication No. US2012/0064110, US2012/0058153 and US2012/0058154, each of which is herein incorporated by reference in its entirety.

In some embodiments, the synthetic nanocarrier may be coupled to a polynucleotide which may be able to trigger a humoral and/or cytotoxic T lymphocyte (CTL) response (See *e.g.*, International Publication No. WO2013/019669, herein incorporated by reference in its entirety).

In some embodiments, the RNA vaccine may be encapsulated in, linked to and/or associated with zwitterionic lipids. Non-limiting examples of zwitterionic lipids and methods of using zwitterionic lipids are described in U.S. Publication No. 2013/0216607, the content of which is herein incorporated by reference in its entirety. In some aspects, the zwitterionic lipids may be used in the liposomes and lipid nanoparticles described herein.

In some embodiments, the RNA vaccine may be formulated in colloid nanocarriers as described in U.S. Publication No. 2013/0197100, the content of which is herein incorporated by reference in its entirety.

In some embodiments, the nanoparticle may be optimized for oral administration. The nanoparticle may comprise at least one cationic biopolymer such as, but not limited to, chitosan or a derivative thereof. As a non-limiting example, the nanoparticle may be formulated by the methods described in U.S. Publication No. 2012/0282343; herein incorporated by reference in its entirety.

In some embodiments, LNPs comprise the lipid KL52 (an amino-lipid disclosed in U.S. Application Publication No. 2012/0295832 expressly incorporated herein by reference in its entirety). Activity and/or safety (as measured by examining one or more of ALT/AST, white blood cell count and cytokine induction) of LNP administration may be improved by incorporation of such lipids. LNPs comprising KL52 may be administered intravenously and/or in one or more doses. In some embodiments, administration of LNPs comprising KL52 results in equal or improved mRNA and/or protein expression as compared to LNPs comprising MC3.

In some embodiments, RNA vaccine may be delivered using smaller LNPs. Such particles may comprise a diameter from below 0.1 μm up to 100 nm such as, but not limited to, less than 0.1 μm , less than 1.0 μm , less than 5 μm , less than 10 μm , less than 15 μm , less than 20 μm , less than 25 μm , less than 30 μm , less than 35 μm , less than 40 μm , less than 50 μm , less than 55 μm , less than 60 μm , less than 65 μm , less than 70 μm , less than 75 μm , less than 80 μm , less than 85 μm , less than 90 μm , less than 95 μm , less than 100 μm , less than 125 μm , less than 150 μm , less than 175 μm , less than 200 μm , less than 225 μm , less than

250 μm , less than 275 μm , less than 300 μm , less than 325 μm , less than 350 μm , less than 375 μm , less than 400 μm , less than 425 μm , less than 450 μm , less than 475 μm , less than 500 μm , less than 525 μm , less than 550 μm , less than 575 μm , less than 600 μm , less than 625 μm , less than 650 μm , less than 675 μm , less than 700 μm , less than 725 μm , less than 750 μm , less than 775 μm , less than 800 μm , less than 825 μm , less than 850 μm , less than 875 μm , less than 900 μm , less than 925 μm , less than 950 μm , or less than 975 μm .

In other embodiments, RNA (*e.g.*, mRNA) vaccines may be delivered using smaller LNPs which may comprise a diameter from about 1 nm to about 100 nm, from about 1 nm to about 10 nm, about 1 nm to about 20 nm, from about 1 nm to about 30 nm, from about 1 nm to about 40 nm, from about 1 nm to about 50 nm, from about 1 nm to about 60 nm, from about 1 nm to about 70 nm, from about 1 nm to about 80 nm, from about 1 nm to about 90 nm, from about 5 nm to about 100 nm, from about 5 nm to about 10 nm, about 5 nm to about 20 nm, from about 5 nm to about 30 nm, from about 5 nm to about 40 nm, from about 5 nm to about 50 nm, from about 5 nm to about 60 nm, from about 5 nm to about 70 nm, from about 5 nm to about 80 nm, from about 5 nm to about 90 nm, about 10 to about 50 nm, from about 20 to about 50 nm, from about 30 to about 50 nm, from about 40 to about 50 nm, from about 20 to about 60 nm, from about 30 to about 60 nm, from about 40 to about 60 nm, from about 20 to about 70 nm, from about 30 to about 70 nm, from about 40 to about 70 nm, from about 50 to about 70 nm, from about 60 to about 70 nm, from about 20 to about 80 nm, from about 30 to about 80 nm, from about 40 to about 80 nm, from about 50 to about 80 nm, from about 60 to about 80 nm, from about 20 to about 90 nm, from about 30 to about 90 nm, from about 40 to about 90 nm, from about 50 to about 90 nm, from about 60 to about 90 nm and/or from about 70 to about 90 nm.

In some embodiments, such LNPs are synthesized using methods comprising microfluidic mixers. Exemplary microfluidic mixers may include, but are not limited to a slit interdigital micromixer including, but not limited to those manufactured by Microinnova (Allerheiligen bei Wildon, Austria) and/or a staggered herringbone micromixer (SHM) (Zhigaltsev, I.V. *et al.*, Bottom-up design and synthesis of limit size lipid nanoparticle systems with aqueous and triglyceride cores using millisecond microfluidic mixing have been published (Langmuir. 2012. 28:3633-40; Belliveau, N.M. *et al.*, Microfluidic synthesis of highly potent limit-size lipid nanoparticles for *in vivo* delivery of siRNA. *Molecular Therapy-Nucleic Acids*. 2012. 1:e37; Chen, D. *et al.*, Rapid discovery of potent siRNA-containing lipid nanoparticles enabled by controlled microfluidic formulation. *J Am Chem Soc*. 2012. 134(16):6948-51; each of which is herein incorporated by reference in its entirety).

In some embodiments, methods of LNP generation comprising SHM, further comprise the mixing of at least two input streams wherein mixing occurs by microstructure-induced chaotic advection (MICA). According to this method, fluid streams flow through channels present in a herringbone pattern causing rotational flow and folding the fluids around each other. This method may also comprise a surface for fluid mixing wherein the surface changes orientations during fluid cycling. Methods of generating LNPs using SHM include those disclosed in U.S. Application Publication Nos. 2004/0262223 and 2012/0276209, each of which is expressly incorporated herein by reference in their entirety.

In some embodiments, the RNA vaccine of the present invention may be formulated in lipid nanoparticles created using a micromixer such as, but not limited to, a Slit Interdigital Microstructured Mixer (SIMM-V2) or a Standard Slit Interdigital Micro Mixer (SSIMM) or Caterpillar (CPMM) or Impinging-jet (IJMM) from the Institut für Mikrotechnik Mainz GmbH, Mainz Germany).

In some embodiments, the RNA (*e.g.*, mRNA) vaccines of the present disclosure may be formulated in lipid nanoparticles created using microfluidic technology (*see* Whitesides, George M. The Origins and the Future of Microfluidics. *Nature*, 2006 442: 368-373; and Abraham *et al.* Chaotic Mixer for Microchannels. *Science*, 2002 295: 647-651; each of which is herein incorporated by reference in its entirety). As a non-limiting example, controlled microfluidic formulation includes a passive method for mixing streams of steady pressure-driven flows in micro channels at a low Reynolds number (*see e.g.*, Abraham *et al.* Chaotic Mixer for Microchannels. *Science*, 2002 295: 647-651; which is herein incorporated by reference in its entirety).

In some embodiments, the RNA (*e.g.*, mRNA) vaccines of the present invention may be formulated in lipid nanoparticles created using a micromixer chip such as, but not limited to, those from Harvard Apparatus (Holliston, MA) or Dolomite Microfluidics (Royston, UK). A micromixer chip can be used for rapid mixing of two or more fluid streams with a split and recombine mechanism.

In some embodiments, the RNA (*e.g.*, mRNA) vaccines of the invention may be formulated for delivery using the drug encapsulating microspheres described in International Publication No. WO2013/063468 or U.S. Patent No. 8,440,614, each of which is herein incorporated by reference in its entirety. The microspheres may comprise a compound of the formula (I), (II), (III), (IV), (V) or (VI) as described in International Publication No. WO2013/063468, the content of which is herein incorporated by reference in its entirety. In other aspects, the amino acid, peptide, polypeptide, lipids (APPL) are useful in delivering the

RNA vaccines of the invention to cells (*see* International Publication No. WO2013/063468, the contents of which is herein incorporated by reference in its entirety).

In some embodiments, the RNA (*e.g.*, mRNA) vaccines of the present disclosure may be formulated in lipid nanoparticles having a diameter from about 10 to about 100 nm such as, but not limited to, about 10 to about 20 nm, about 10 to about 30 nm, about 10 to about 40 nm, about 10 to about 50 nm, about 10 to about 60 nm, about 10 to about 70 nm, about 10 to about 80 nm, about 10 to about 90 nm, about 20 to about 30 nm, about 20 to about 40 nm, about 20 to about 50 nm, about 20 to about 60 nm, about 20 to about 70 nm, about 20 to about 80 nm, about 20 to about 90 nm, about 20 to about 100 nm, about 30 to about 40 nm, about 30 to about 50 nm, about 30 to about 60 nm, about 30 to about 70 nm, about 30 to about 80 nm, about 30 to about 90 nm, about 30 to about 100 nm, about 40 to about 50 nm, about 40 to about 60 nm, about 40 to about 70 nm, about 40 to about 80 nm, about 40 to about 90 nm, about 40 to about 100 nm, about 50 to about 60 nm, about 50 to about 70 nm, about 50 to about 80 nm, about 50 to about 90 nm, about 50 to about 100 nm, about 60 to about 70 nm, about 60 to about 80 nm, about 60 to about 90 nm, about 60 to about 100 nm, about 70 to about 80 nm, about 70 to about 90 nm, about 70 to about 100 nm, about 80 to about 90 nm, about 80 to about 100 nm and/or about 90 to about 100 nm.

In some embodiments, the lipid nanoparticles may have a diameter from about 10 to 500 nm.

In some embodiments, the lipid nanoparticle may have a diameter greater than 100 nm, greater than 150 nm, greater than 200 nm, greater than 250 nm, greater than 300 nm, greater than 350 nm, greater than 400 nm, greater than 450 nm, greater than 500 nm, greater than 550 nm, greater than 600 nm, greater than 650 nm, greater than 700 nm, greater than 750 nm, greater than 800 nm, greater than 850 nm, greater than 900 nm, greater than 950 nm or greater than 1000 nm.

In some aspects, the lipid nanoparticle may be a limit size lipid nanoparticle described in International Publication No. WO2013/059922, the content of which is herein incorporated by reference in its entirety. The limit size lipid nanoparticle may comprise a lipid bilayer surrounding an aqueous core or a hydrophobic core; where the lipid bilayer may comprise a phospholipid such as, but not limited to, diacylphosphatidylcholine, a diacylphosphatidylethanolamine, a ceramide, a sphingomyelin, a dihydrosphingomyelin, a cephalin, a cerebroside, a C8-C20 fatty acid diacylphosphatidylcholine, and 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC). In other aspects the limit size lipid nanoparticle may comprise a polyethylene glycol-lipid such as, but not limited to, DLPE-PEG, DMPE-PEG, DPPC-PEG and DSPE-PEG.

In some embodiments, the RNA vaccines may be delivered, localized and/or concentrated in a specific location using the delivery methods described in International Publication No. WO2013/063530, the content of which is herein incorporated by reference in its entirety. As a non-limiting example, a subject may be administered an empty polymeric particle prior to, simultaneously with or after delivering the RNA vaccines to the subject. The empty polymeric particle undergoes a change in volume once in contact with the subject and becomes lodged, embedded, immobilized or entrapped at a specific location in the subject.

In some embodiments, the RNA vaccines may be formulated in an active substance release system (*see e.g.*, U.S. Publication No. US2013/0102545, the contents of which is herein incorporated by reference in its entirety). The active substance release system may comprise 1) at least one nanoparticle bonded to an oligonucleotide inhibitor strand which is hybridized with a catalytically active nucleic acid and 2) a compound bonded to at least one substrate molecule bonded to a therapeutically active substance (*e.g.*, polynucleotides described herein), where the therapeutically active substance is released by the cleavage of the substrate molecule by the catalytically active nucleic acid.

In some embodiments, the RNA (*e.g.*, mRNA) vaccines may be formulated in a nanoparticle comprising an inner core comprising a non-cellular material and an outer surface comprising a cellular membrane. The cellular membrane may be derived from a cell or a membrane derived from a virus. As a non-limiting example, the nanoparticle may be made by the methods described in International Publication No. WO2013/052167, herein incorporated by reference in its entirety. As another non-limiting example, the nanoparticle described in International Publication No. WO2013/052167, herein incorporated by reference in its entirety, may be used to deliver the RNA vaccines described herein.

In some embodiments, the RNA vaccines may be formulated in porous nanoparticle-supported lipid bilayers (protocells). Protocells are described in International Publication No. WO2013/056132, the content of which is herein incorporated by reference in its entirety.

In some embodiments, the RNA vaccines described herein may be formulated in polymeric nanoparticles as described in or made by the methods described in US Patent Nos. 8,420,123 and 8,518,963 and European Patent No. EP2073848B1, the contents of each of which are herein incorporated by reference in their entirety. As a non-limiting example, the polymeric nanoparticle may have a high glass transition temperature such as the nanoparticles described in or nanoparticles made by the methods described in US Patent No. 8,518,963, the content of which is herein incorporated by reference in its entirety. As another non-limiting example,

the polymer nanoparticle for oral and parenteral formulations may be made by the methods described in European Patent No. EP2073848B1, the content of which is herein incorporated by reference in its entirety.

In other embodiments, the RNA (*e.g.*, mRNA) vaccines described herein may be formulated in nanoparticles used in imaging. The nanoparticles may be liposome nanoparticles such as those described in U.S. Publication No. 2013/0129636, herein incorporated by reference in its entirety. As a non-limiting example, the liposome may comprise gadolinium(III)-2-{4,7-bis-carboxymethyl-10-[(N,N-distearylamidomethyl-N'-amido-methyl)-1,4,7,10-tetra-azacyclododec-1-yl]}-acetic acid and a neutral, fully saturated phospholipid component (*see e.g.*, U.S. Publication No. US2013/0129636, the contents of which is herein incorporated by reference in its entirety).

In some embodiments, the nanoparticles which may be used in the present invention are formed by the methods described in U.S. Patent Application No. 2013/0130348, the contents of which is herein incorporated by reference in its entirety.

The nanoparticles of the present invention may further include nutrients such as, but not limited to, those which deficiencies can lead to health hazards from anemia to neural tube defects (*see e.g.*, the nanoparticles described in International Patent Publication No. WO2013/072929, the contents of which is herein incorporated by reference in its entirety). As a non-limiting example, the nutrient may be iron in the form of ferrous, ferric salts or elemental iron, iodine, folic acid, vitamins or micronutrients.

In some embodiments, the RNA (*e.g.*, mRNA) vaccines of the present invention may be formulated in a swellable nanoparticle. The swellable nanoparticle may be, but is not limited to, those described in U.S. Patent No. 8,440,231, the contents of which is herein incorporated by reference in its entirety. As a non-limiting embodiment, the swellable nanoparticle may be used for delivery of the RNA (*e.g.*, mRNA) vaccines of the present invention to the pulmonary system (*see e.g.*, U.S. Patent No. 8,440,231, the contents of which is herein incorporated by reference in its entirety).

The RNA (*e.g.*, mRNA) vaccines of the present invention may be formulated in polyanhydride nanoparticles such as, but not limited to, those described in U.S. Patent No. 8,449,916, the contents of which is herein incorporated by reference in its entirety. The nanoparticles and microparticles of the present invention may be geometrically engineered to modulate macrophage and/or the immune response. In some aspects, the geometrically engineered particles may have varied shapes, sizes and/or surface charges in order to incorporated the polynucleotides of the present invention for targeted delivery such as, but not limited to, pulmonary delivery (*see e.g.*,

International Publication No. WO2013/082111, the content of which is herein incorporated by reference in its entirety). Other physical features the geometrically engineering particles may have include, but are not limited to, fenestrations, angled arms, asymmetry and surface roughness, charge which can alter the interactions with cells and tissues. As a non-limiting example, nanoparticles of the present invention may be made by the methods described in International Publication No. WO2013/082111, the contents of which is herein incorporated by reference in its entirety.

In some embodiments, the nanoparticles of the present invention may be water soluble nanoparticles such as, but not limited to, those described in International Publication No. WO2013/090601, the content of which is herein incorporated by reference in its entirety. The nanoparticles may be inorganic nanoparticles which have a compact and zwitterionic ligand in order to exhibit good water solubility. The nanoparticles may also have small hydrodynamic diameters (HD), stability with respect to time, pH, and salinity and a low level of non-specific protein binding.

In some embodiments the nanoparticles of the present invention may be developed by the methods described in U.S. Publication No. US2013/0172406, the content of which is herein incorporated by reference in its entirety.

In some embodiments, the nanoparticles of the present invention are stealth nanoparticles or target-specific stealth nanoparticles such as, but not limited to, those described in U.S. Publication No. 2013/0172406, the content of which is herein incorporated by reference in its entirety. The nanoparticles of the present invention may be made by the methods described in U.S. Publication No. 2013/0172406, the content of which is herein incorporated by reference in its entirety.

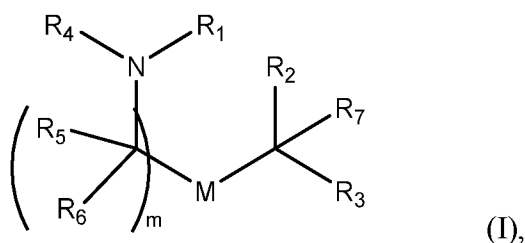
In other embodiments, the stealth or target-specific stealth nanoparticles may comprise a polymeric matrix. The polymeric matrix may comprise two or more polymers such as, but not limited to, polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polyesters, polyanhydrides, polyethers, polyurethanes, polymethacrylates, polyacrylates, polycyanoacrylates or combinations thereof.

In some embodiments, the nanoparticle may be a nanoparticle-nucleic acid hybrid structure having a high density nucleic acid layer. As a non-limiting example, the nanoparticle-nucleic acid hybrid structure may made by the methods described in U.S. Publication No.

2013/0171646, the content of which is herein incorporated by reference in its entirety. The nanoparticle may comprise a nucleic acid such as, but not limited to, polynucleotides described herein and/or known in the art.

At least one of the nanoparticles of the present invention may be embedded in the core a nanostructure or coated with a low density porous 3-D structure or coating which is capable of carrying or associating with at least one payload within or on the surface of the nanostructure. Non-limiting examples of the nanostructures comprising at least one nanoparticle are described in International Publication No. WO2013/123523, the content of which is herein incorporated by reference in its entirety.

In some embodiments, a nanoparticle comprises compounds of Formula (I):



or a salt or isomer thereof, wherein:

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a carbocycle, heterocycle, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -N(R)₂, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(R)N(R)₂C(O)OR, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

5 R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

10 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13.

In some embodiments, a subset of compounds of Formula (I) includes those in which
20 when R₄ is -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, or -CQ(R)₂, then (i) Q is not -N(R)₂ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

In some embodiments, another subset of compounds of Formula (I) includes those in which

25 R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

30 R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂,

-OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR,
 -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂,
 -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and a 5- to 14-membered heterocycloalkyl
 having one or more heteroatoms selected from N, O, and S which is substituted with one or
 5 more substituents selected from oxo (=O), OH, amino, mono- or di-alkylamino, and C₁₋₃
 alkyl, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl,
 and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl,
 10 and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-,
 -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an
 aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

15 R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R,
 -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl,
 and H;

20 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈
 alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄
 alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂
 25 alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and
 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

30 In some embodiments, another subset of compounds of Formula (I) includes those in
 which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'',
 and -R''M'R';

R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, $-R^*YR''$, $-YR''$, and $-R^*OR''$, or R_2 and R_3 , together with the atom to which they are attached, form a heterocycle or carbocycle;

R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$,

- 5 $-(CH_2)_nCHQR$, $-CHQR$, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a 5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S, $-OR$, $-O(CH_2)_nN(R)_2$, $-C(O)OR$, $-OC(O)R$, $-CX_3$, $-CX_2H$, $-CXH_2$, $-CN$, $-C(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-CRN(R)_2C(O)OR$, $-N(R)R_8$, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)C(=CHR_9)N(R)_2$,
 10 $-OC(O)N(R)_2$, $-N(R)C(O)OR$, $-N(OR)C(O)R$, $-N(OR)S(O)_2R$, $-N(OR)C(O)OR$, $-N(OR)C(O)N(R)_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, $-C(=NR_9)R$, $-C(O)N(R)OR$, and $-C(=NR_9)N(R)_2$, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a 5- to 14-membered heterocycle and (i) R_4 is $-(CH_2)_nQ$ in which n is 1 or 2, or (ii) R_4 is $-(CH_2)_nCHQR$ in which n is 1, or (iii) R_4 is $-CHQR$, and
 15 $-CQ(R)_2$, then Q is either a 5- to 14-membered heteroaryl or 8- to 14-membered heterocycloalkyl;

each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

- 20 each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

M and M' are independently selected from $-C(O)O-$, $-OC(O)-$, $-C(O)N(R')$, $-N(R')C(O)-$, $-C(O)-$, $-C(S)-$, $-C(S)S-$, $-SC(S)-$, $-CH(OH)-$, $-P(O)(OR')O-$, $-S(O)_2-$, $-S-S-$, an aryl group, and a heteroaryl group;

R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

- 25 R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle;

R_9 is selected from the group consisting of H, CN, NO_2 , C_{1-6} alkyl, $-OR$, $-S(O)_2R$, $-S(O)_2N(R)_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle;

each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

- 30 each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, $-R^*YR''$, $-YR''$, and H;

each R'' is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

5 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

In some embodiments, another subset of compounds of Formula (I) includes those in which

10 R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

15 R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂,
20 -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5;

25 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

30 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

5 each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

10 each X is independently selected from the group consisting of F, Cl, Br, and I; and
m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
or salts or isomers thereof.

In some embodiments, another subset of compounds of Formula (I) includes those in which

15 R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₂₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

20 R₄ is -(CH₂)_nQ or -(CH₂)_nCHQR, where Q is -N(R)₂, and n is selected from 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

25 each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

30 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₁₋₁₂ alkenyl;

5 each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

10 In some embodiments, another subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

15 R₂ and R₃ are independently selected from the group consisting of C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, and -CQ(R)₂, where Q is -N(R)₂, and n is selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

20 each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

25 R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

30 each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

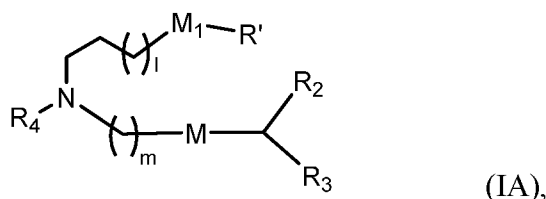
each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₁₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and
 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
 or salts or isomers thereof.

In some embodiments, a subset of compounds of Formula (I) includes those of

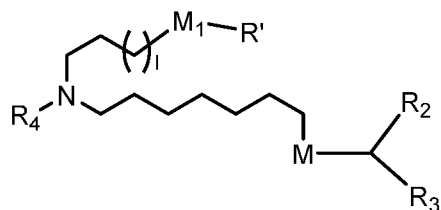
5 Formula (IA):



or a salt or isomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6, 7, 8, and 9; M₁ is a bond or M'; R₄ is unsubstituted C₁₋₃ alkyl, or -(CH₂)_nQ, in which Q is OH, -NHC(S)N(R)₂, -NHC(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)R₈,
 10 -NHC(=NR₉)N(R)₂, -NHC(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, heteroaryl or heterocycloalkyl; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, -S-S-, an aryl group, and a heteroaryl group; and R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, and C₂₋₁₄ alkenyl.

In some embodiments, a subset of compounds of Formula (I) includes those of

15 Formula (II):

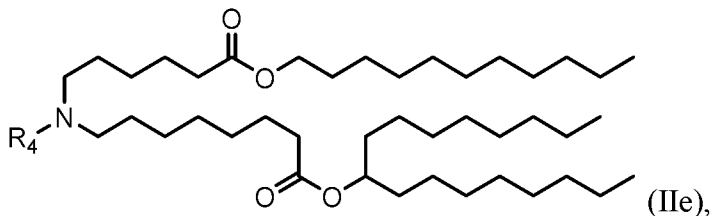
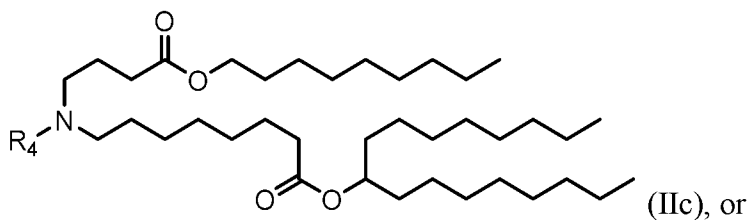
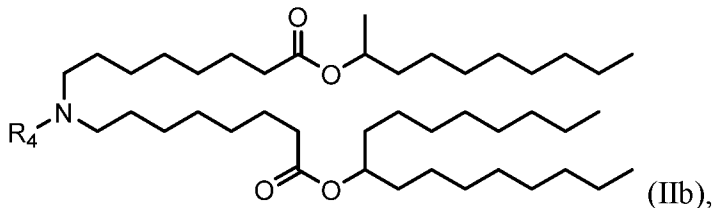
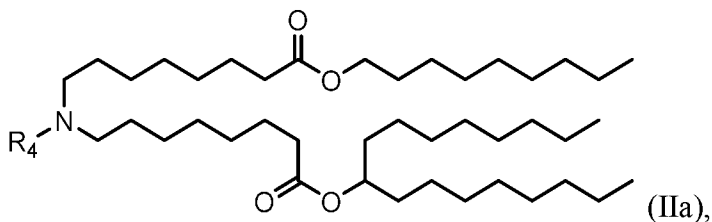


is selected from 1, 2, 3, 4, and 5; M₁ is a bond or M'; R₄ is unsubstituted C₁₋₃ alkyl, or -(CH₂)_nQ, in which n is 2, 3, or 4, and Q is OH, -NHC(S)N(R)₂, -NHC(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)R₈, -NHC(=NR₉)N(R)₂, -NHC(=CHR₉)N(R)₂,
 20 -OC(O)N(R)₂, -N(R)C(O)OR, heteroaryl or heterocycloalkyl; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, -S-S-, an aryl group, and a heteroaryl group; and R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, and C₂₋₁₄ alkenyl.

In some embodiments, a subset of compounds of Formula (I) includes those of

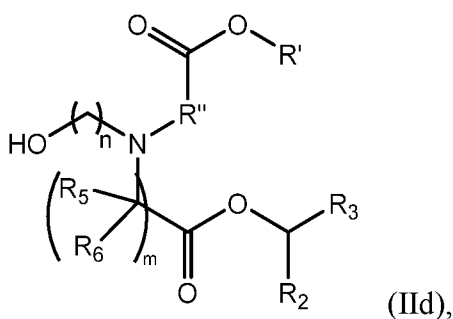
25 Formula (IIa), (IIb), (IIc), or (IIe):

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5 or a salt or isomer thereof, wherein R_4 is as described herein.

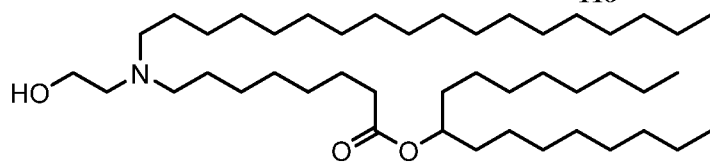
In some embodiments, a subset of compounds of Formula (I) includes those of Formula (II):



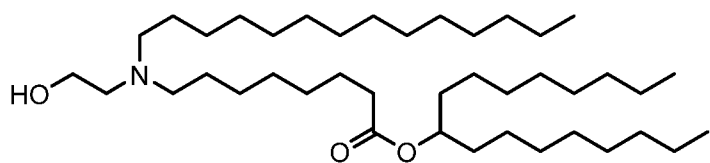
10 or a salt or isomer thereof, wherein n is 2, 3, or 4; and m , R' , R'' , and R_2 through R_6 are as described herein. For example, each of R_2 and R_3 may be independently selected from the group consisting of C_{5-14} alkyl and C_{5-14} alkenyl.

In some embodiments, the compound of Formula (I) is selected from the group consisting of:

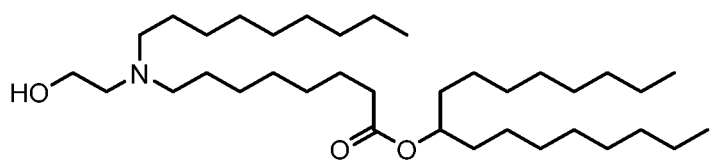
118



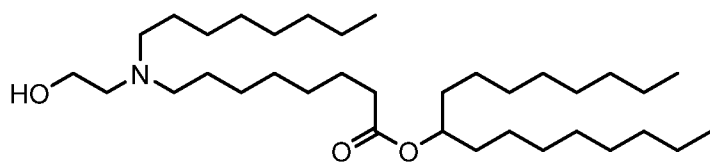
(Compound 1),



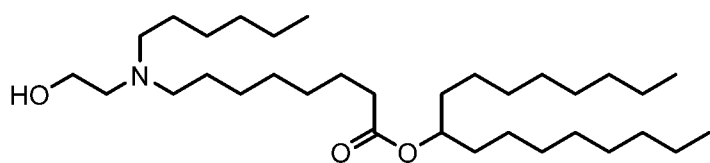
(Compound 2),



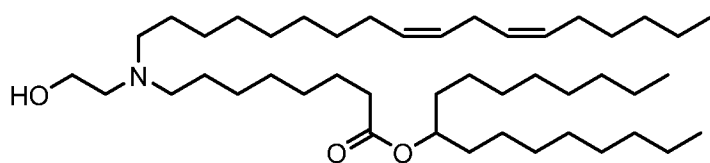
(Compound 3),



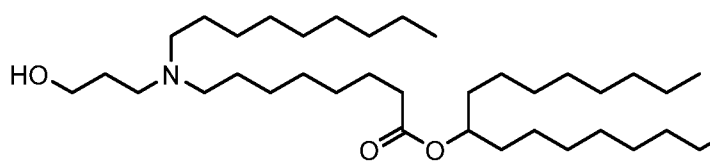
(Compound 4),



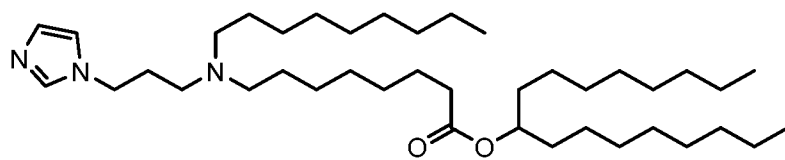
(Compound 5),



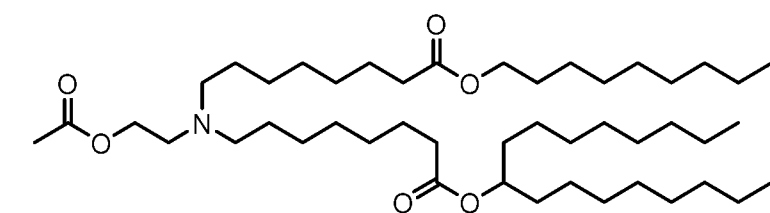
(Compound 6),



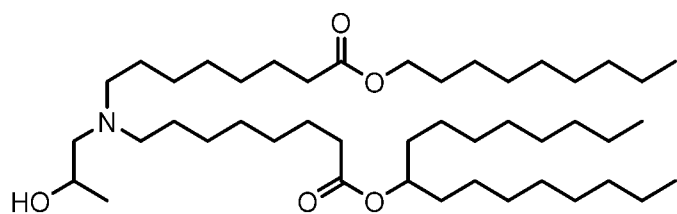
(Compound 7),



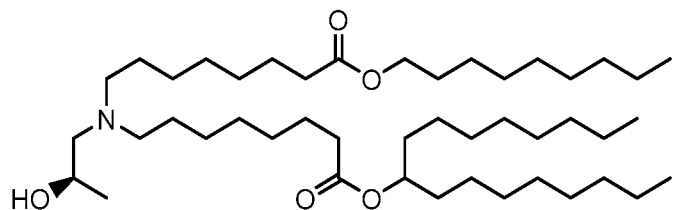
(Compound 8),



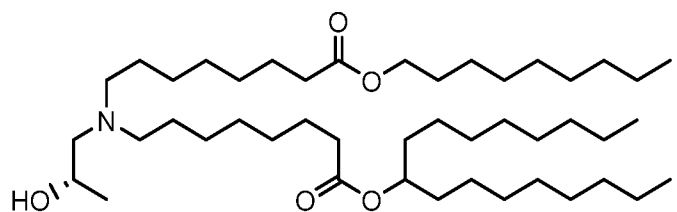
(Compound 9),



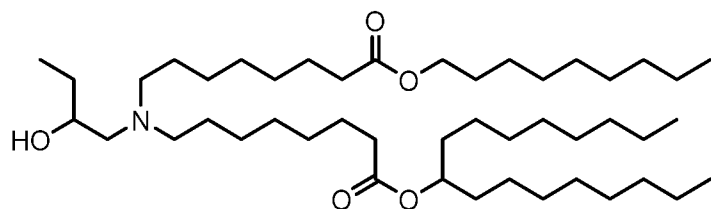
(Compound 10),



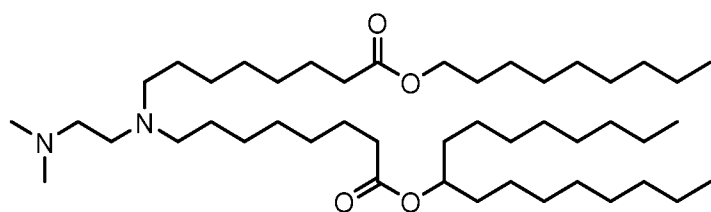
(Compound 11),



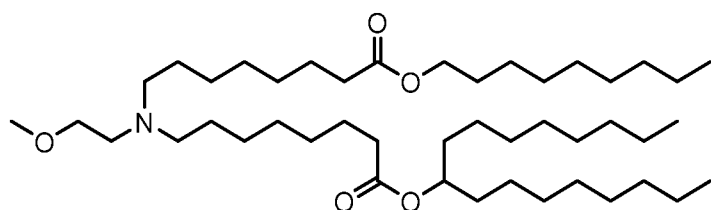
(Compound 12),



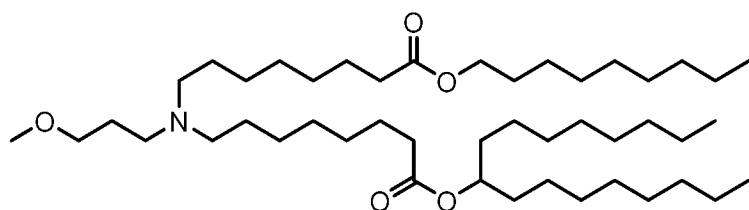
(Compound 13),



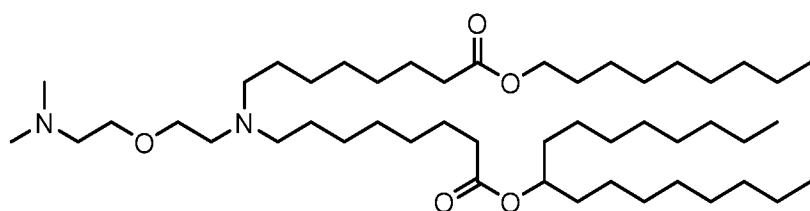
(Compound 14).



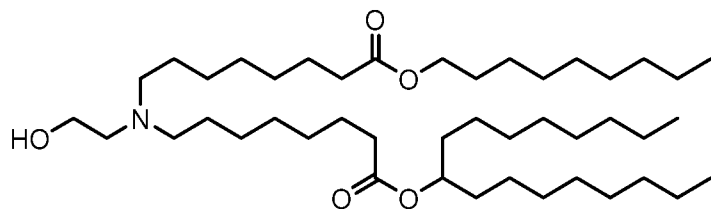
(Compound 15),



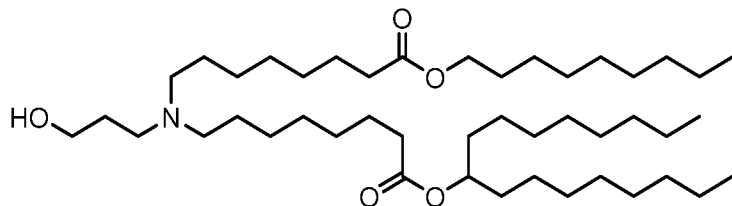
(Compound 16),



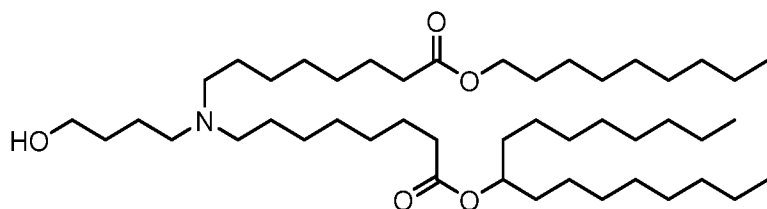
(Compound 17),



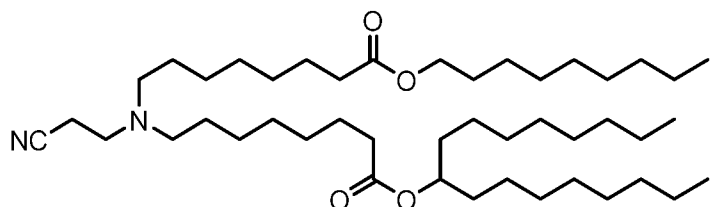
(Compound 18),



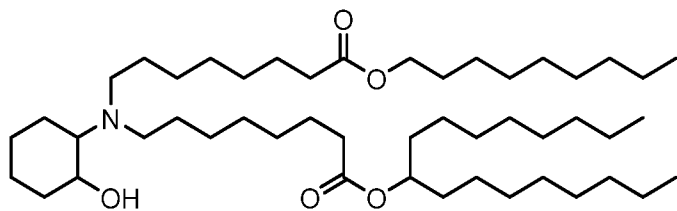
(Compound 19),



(Compound 20),

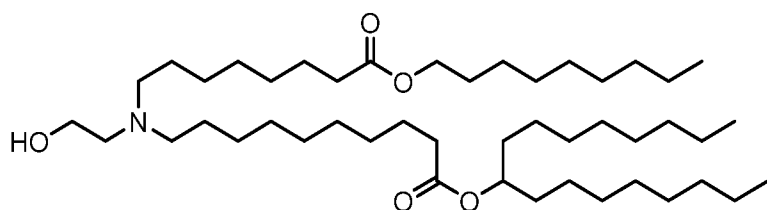


(Compound 21),

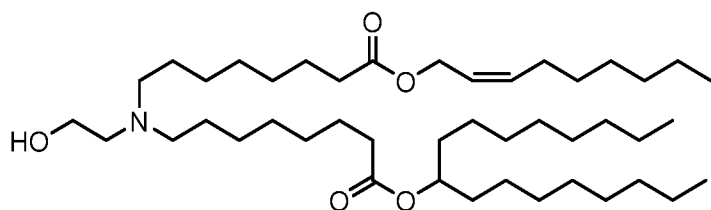


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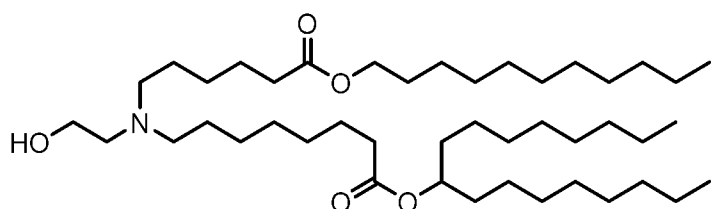
(Compound 22),



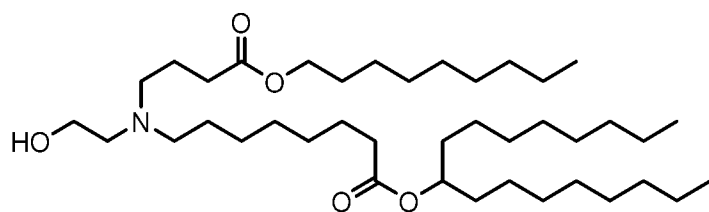
(Compound 23),



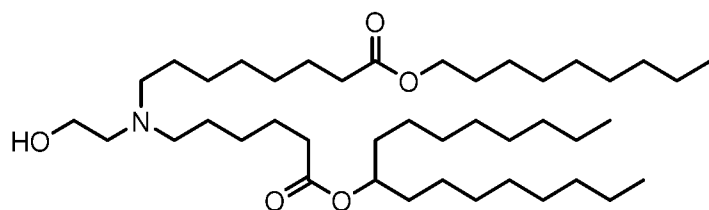
(Compound 24),



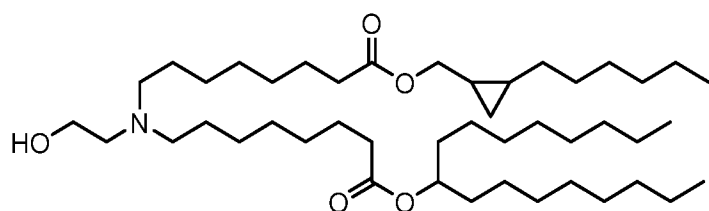
(Compound 25),



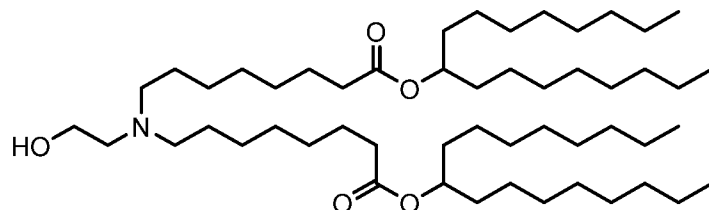
(Compound 26),



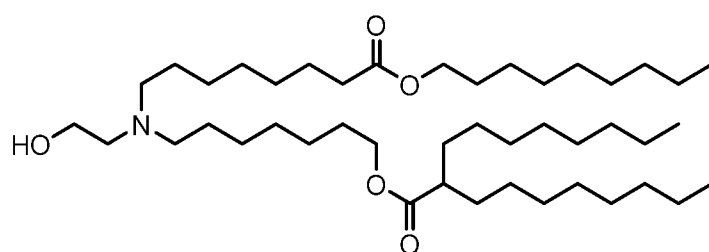
(Compound 27),



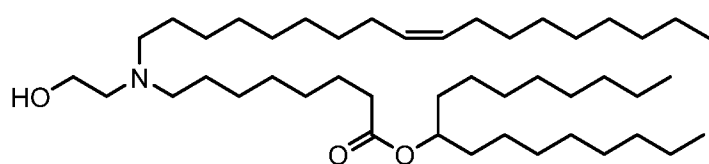
(Compound 28),



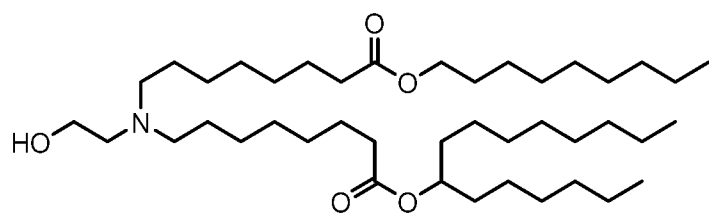
(Compound 29),



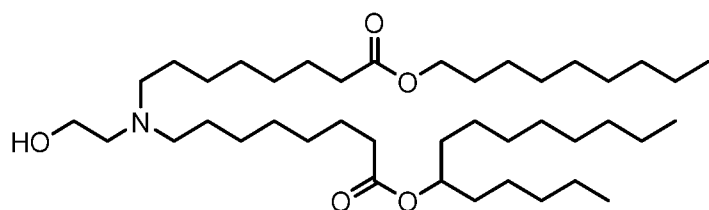
(Compound 30),



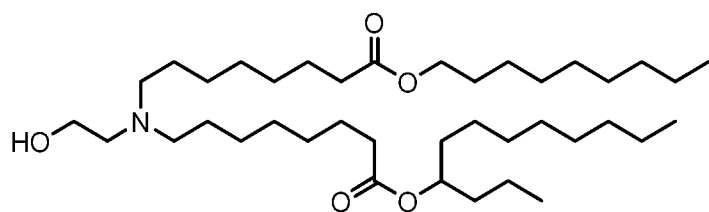
(Compound 31),



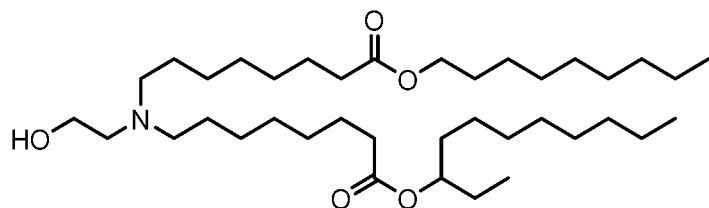
(Compound 32),



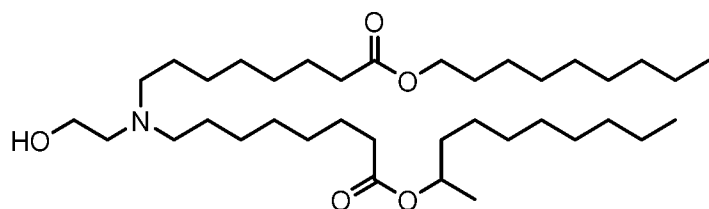
(Compound 33),



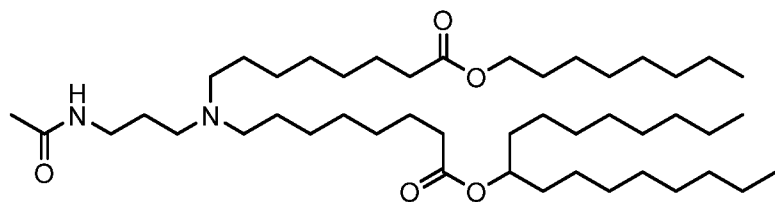
(Compound 34),



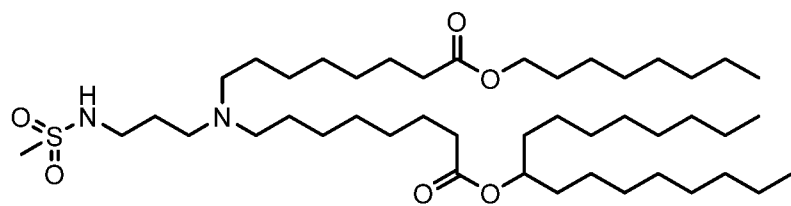
(Compound 35),



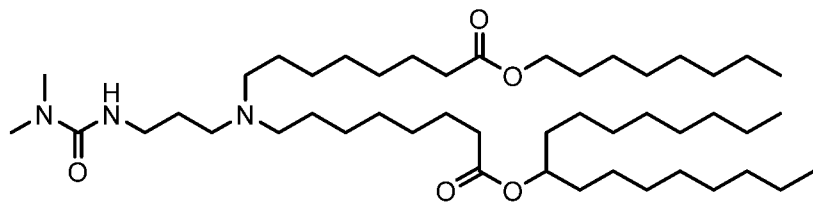
(Compound 36),



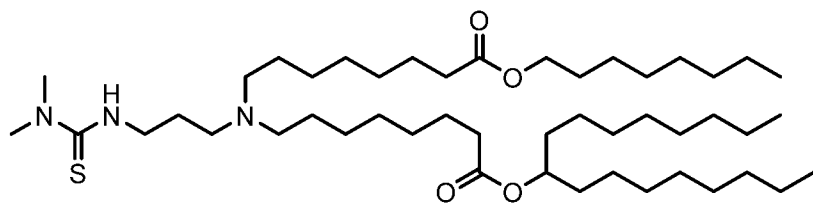
(Compound 37),



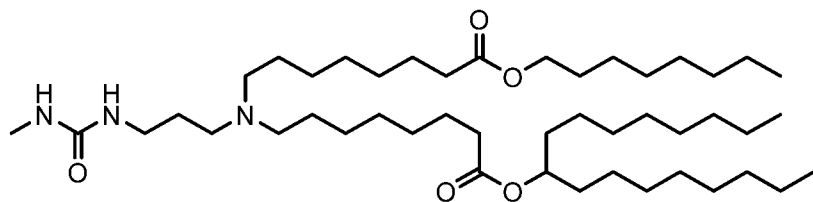
(Compound 38),



(Compound 39),

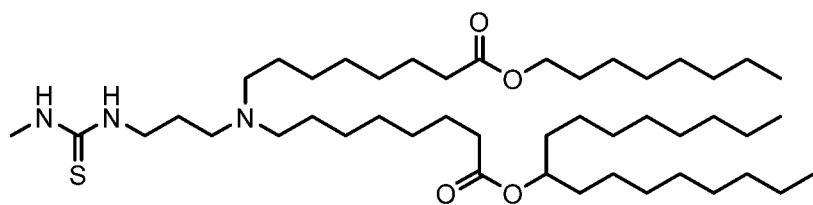


(Compound 40),

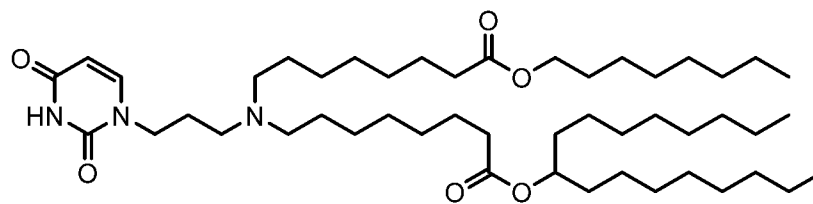


(Compound 41),

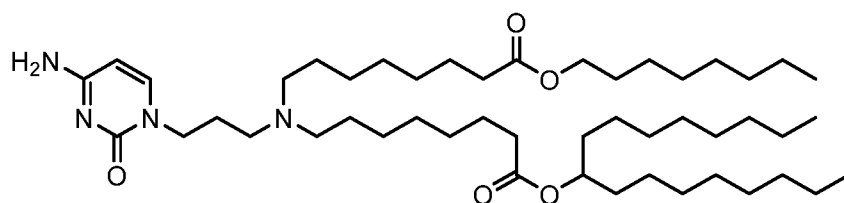
123



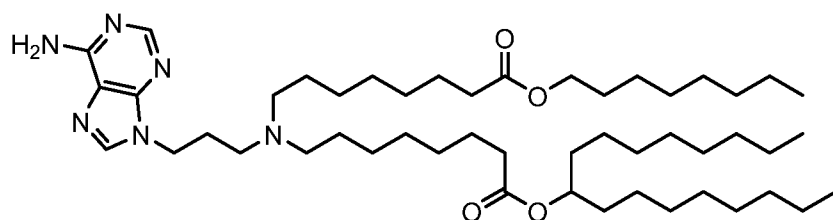
(Compound 42),



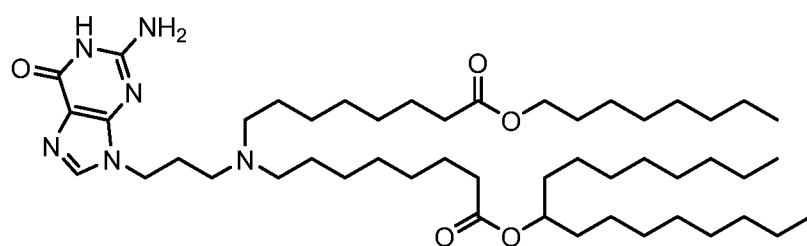
(Compound 43),



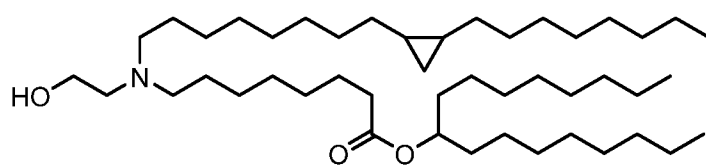
(Compound 44),



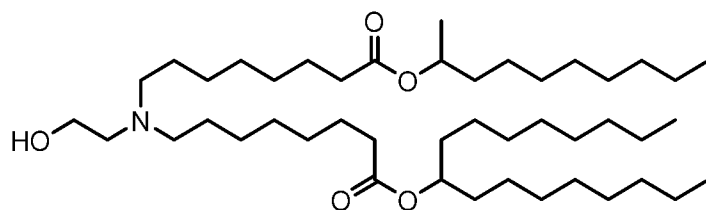
(Compound 45),



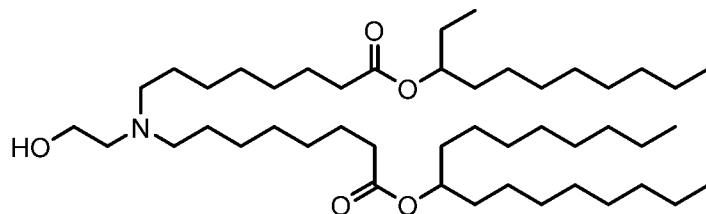
(Compound 46),



(Compound 47),

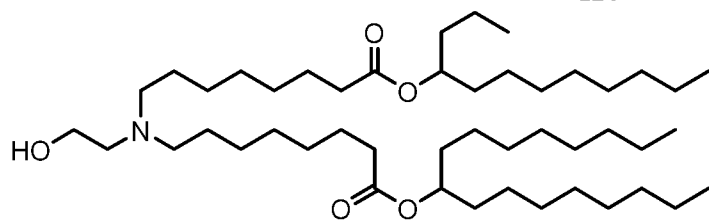


(Compound 48),

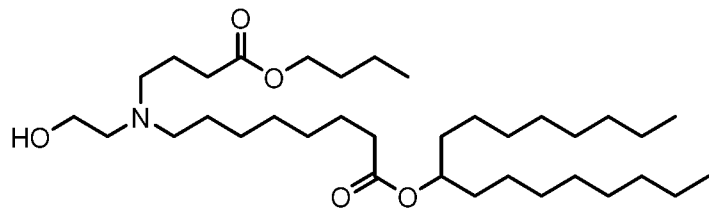


(Compound 49),

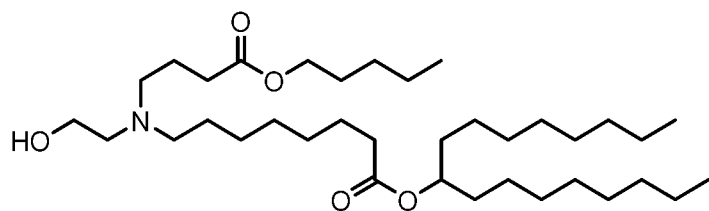
124



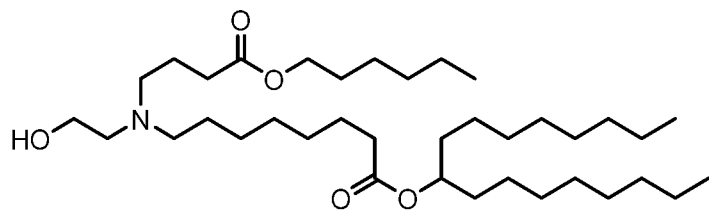
(Compound 50),



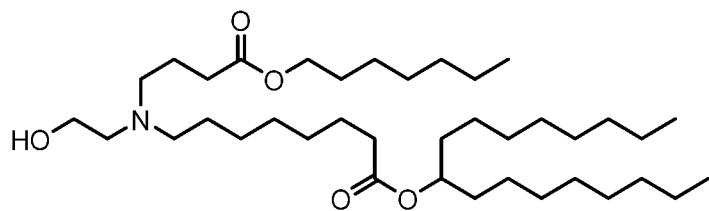
(Compound 51),



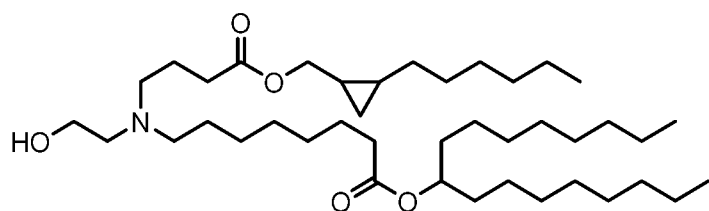
(Compound 52),



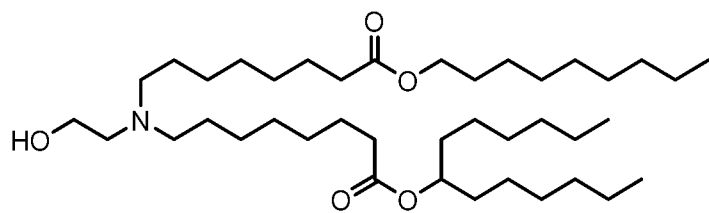
(Compound 53),



(Compound 54),

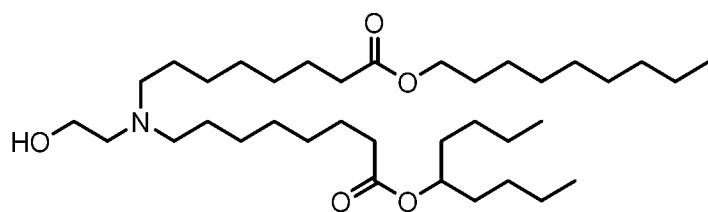


(Compound 55),

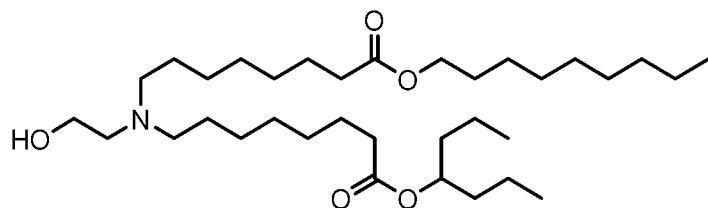


(Compound 56),

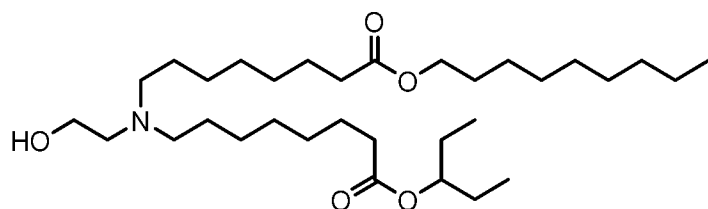
125



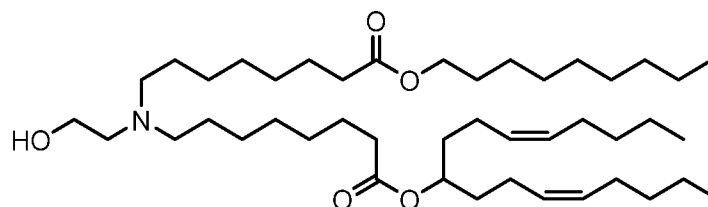
(Compound 57),



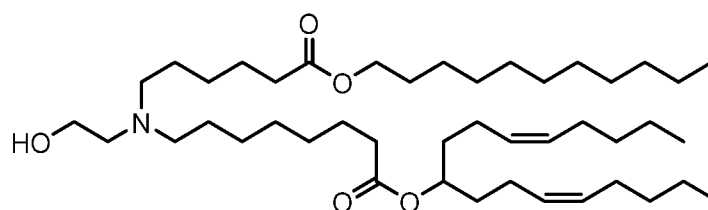
(Compound 58),



(Compound 59),



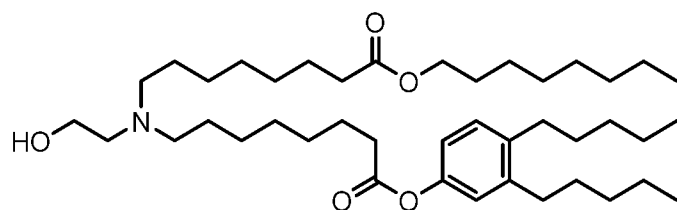
(Compound 60), and



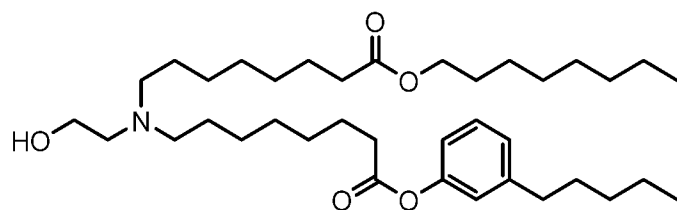
(Compound 61).

5

[0001] In further embodiments, the compound of Formula (I) is selected from the group consisting of:



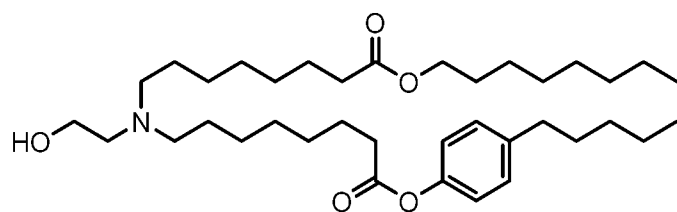
(Compound 62),



(Compound 63), and

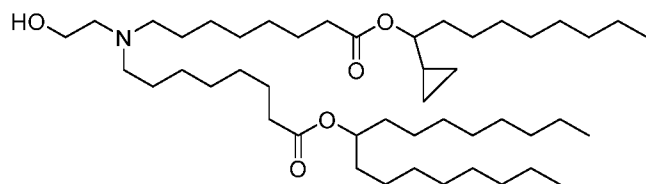
10

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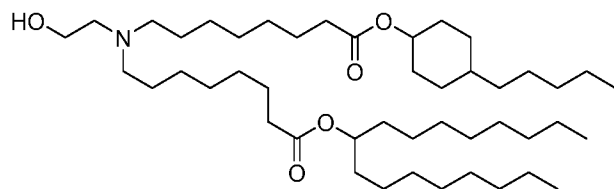
(Compound 64).

[0002] In some embodiments, the compound of Formula (I) is selected from the group consisting of:

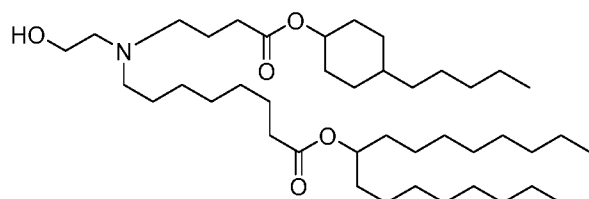


5

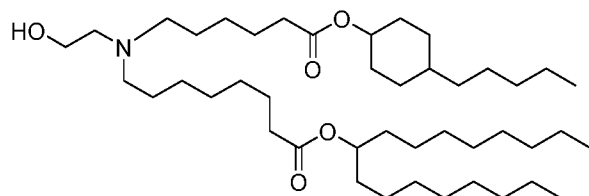
(Compound 65),



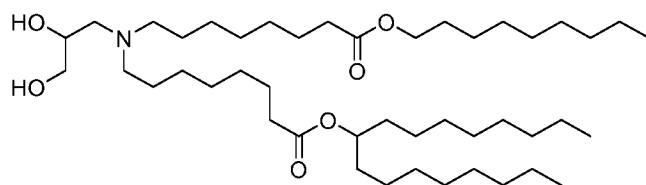
(Compound 66),



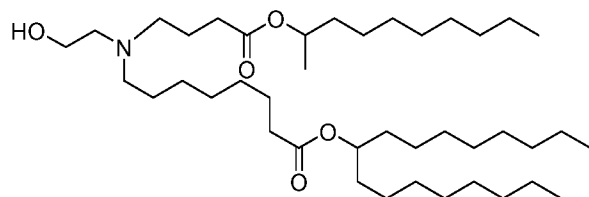
(Compound 67),



(Compound 68),

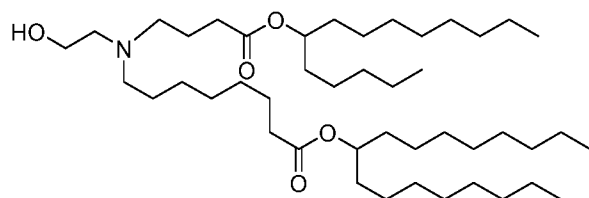


(Compound 69),

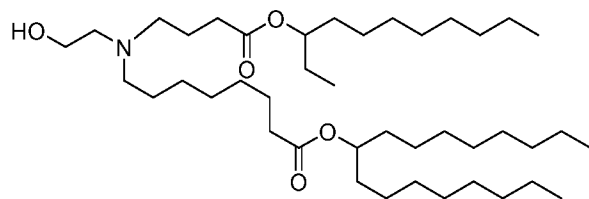


(Compound 70),

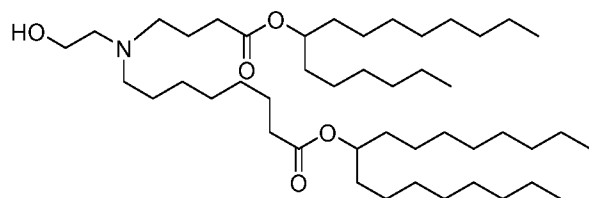
10



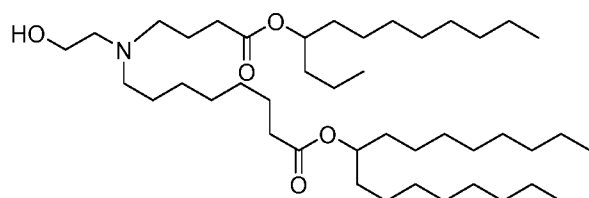
(Compound 71),



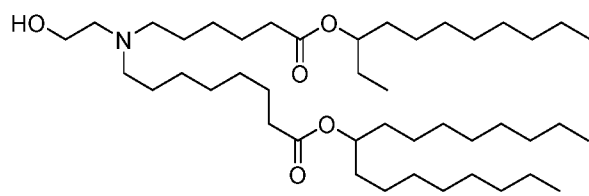
(Compound 72),



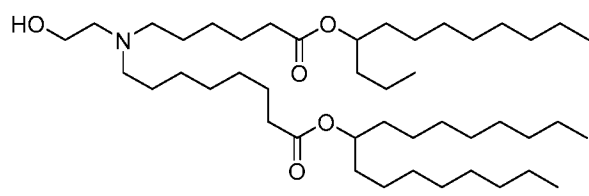
(Compound 73),



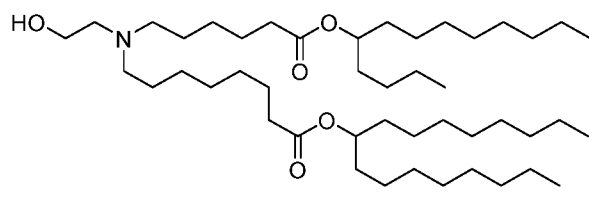
(Compound 74),



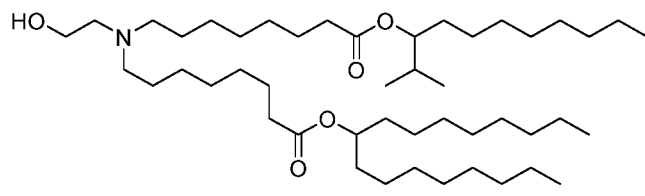
(Compound 75),



(Compound 76),

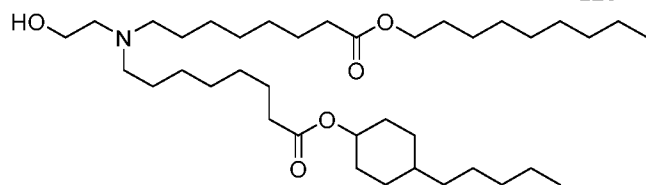


(Compound 77),

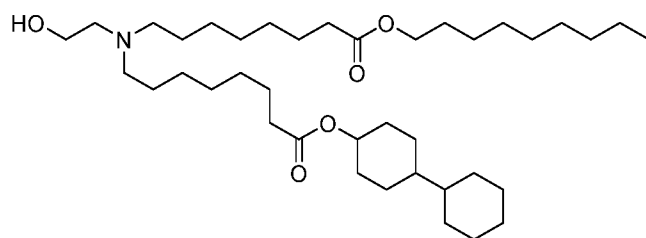


(Compound 78),

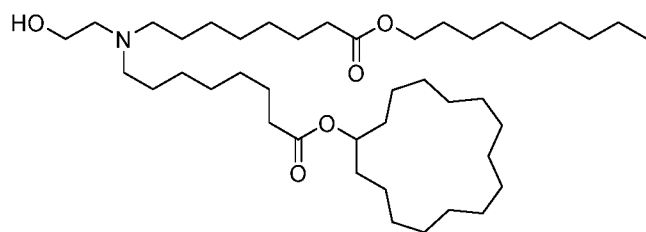
128



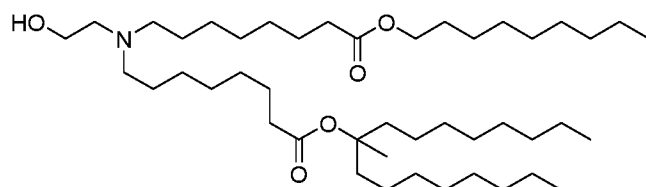
(Compound 79),



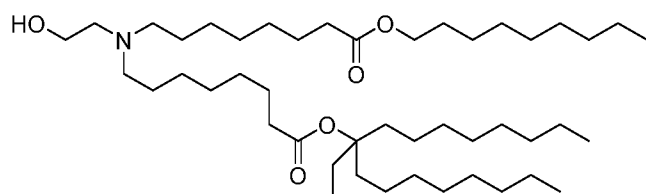
(Compound 80),



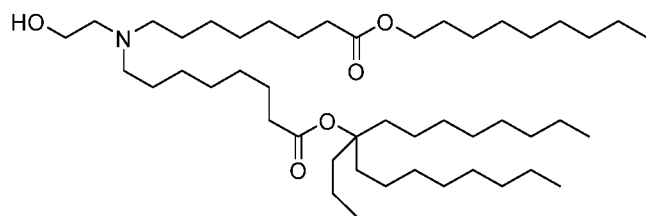
(Compound 81),



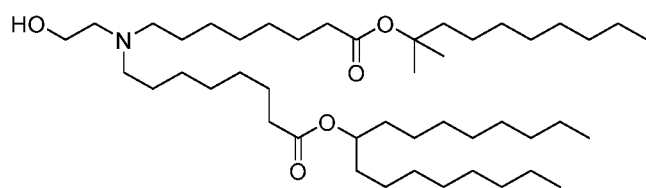
(Compound 82),



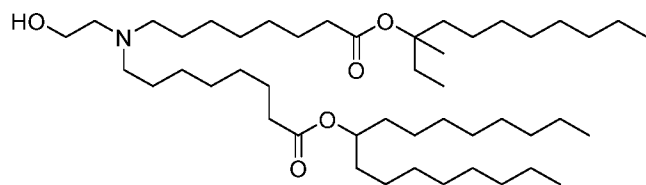
(Compound 83),



(Compound 84),

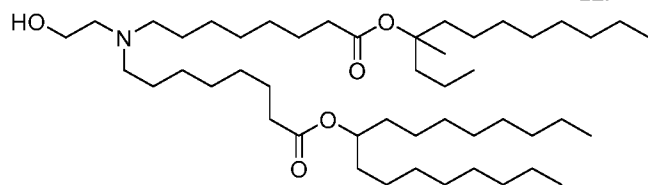


(Compound 85),

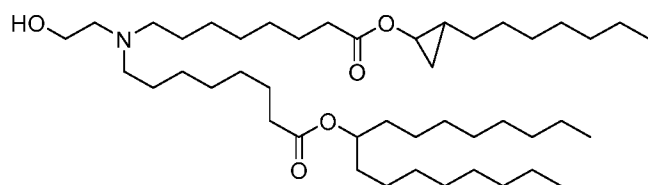


(Compound 86),

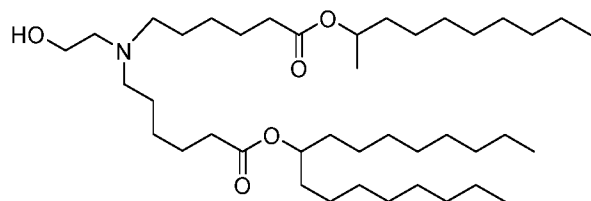
129



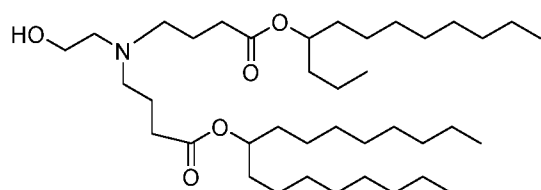
(Compound 87),



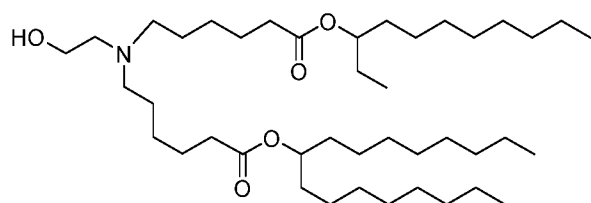
(Compound 88),



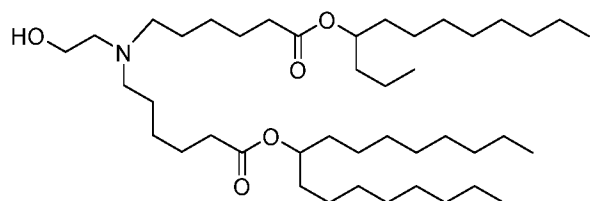
(Compound 89),



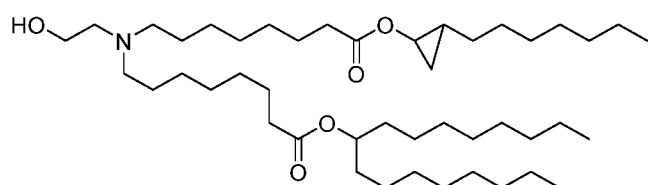
(Compound 90),



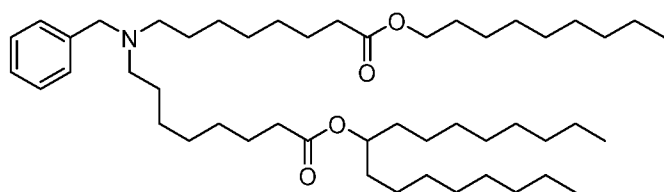
(Compound 91),



(Compound 92),

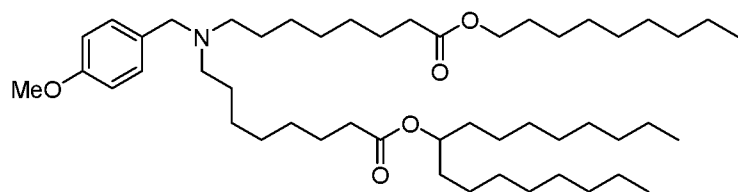


(Compound 93),

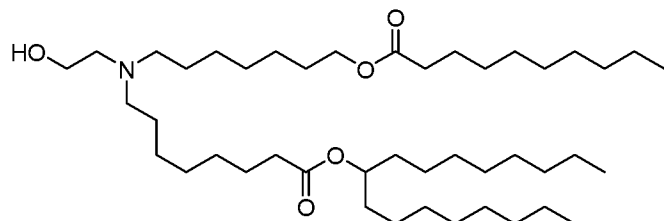


(Compound 94),

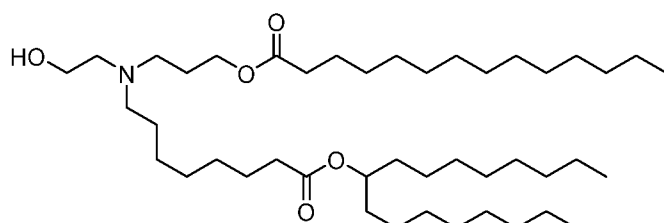
130



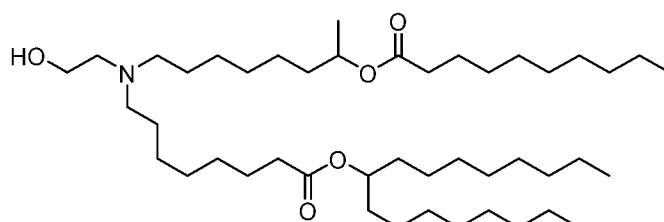
(Compound 95),



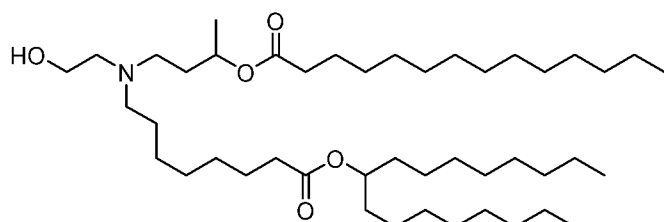
(Compound 96),



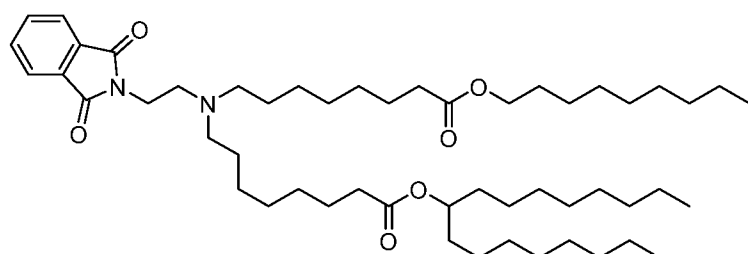
(Compound 97),



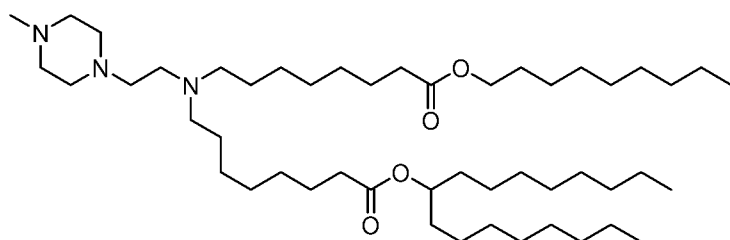
(Compound 98),



(Compound 99),

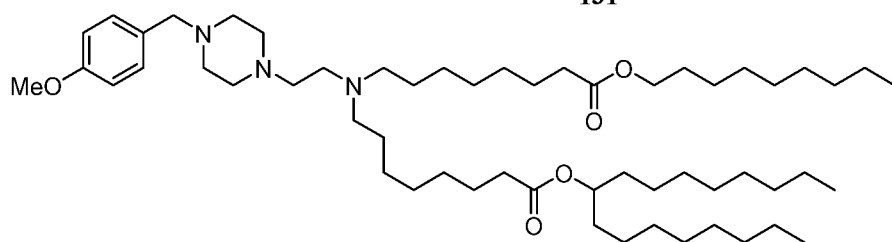


(Compound 100),

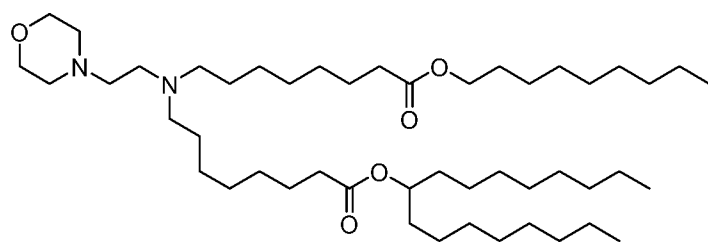


(Compound 101),

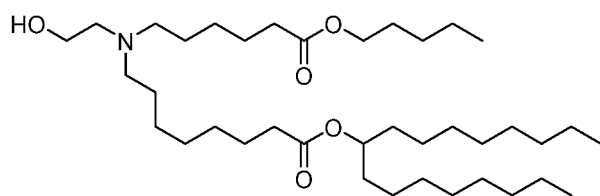
131



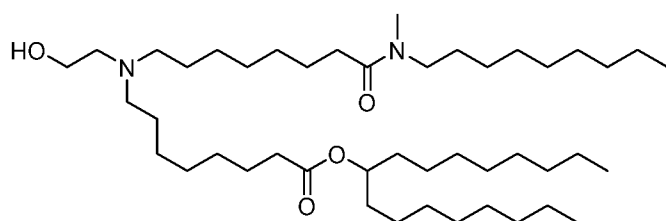
(Compound 102),



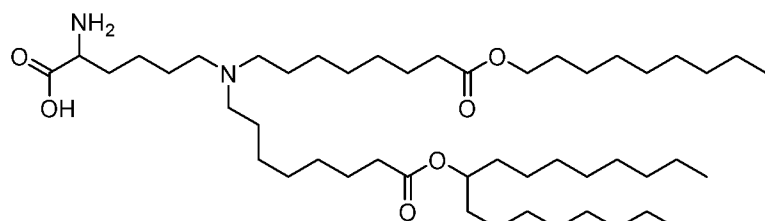
(Compound 103),



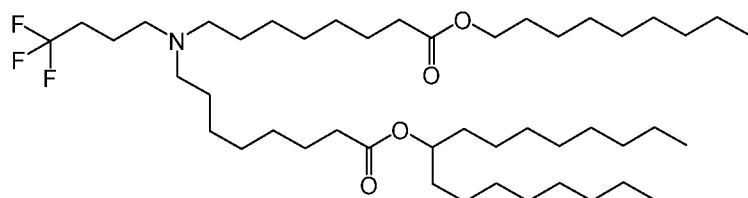
(Compound 104),



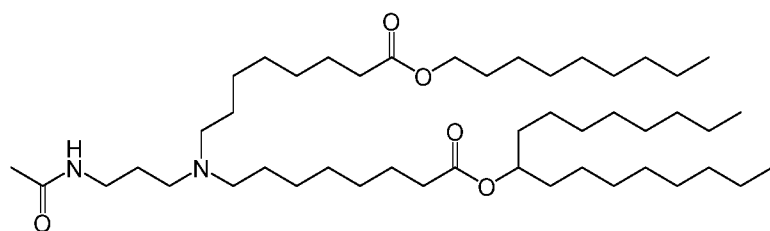
(Compound 105),



(Compound 106),

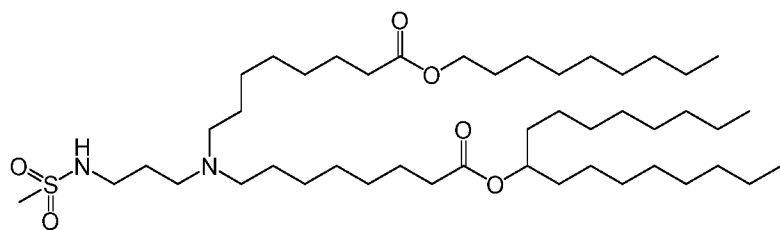


(Compound 107),

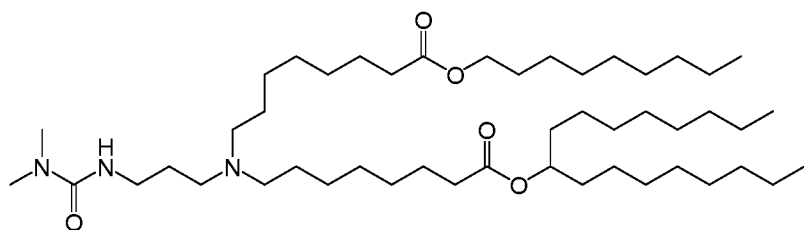


(Compound 108),

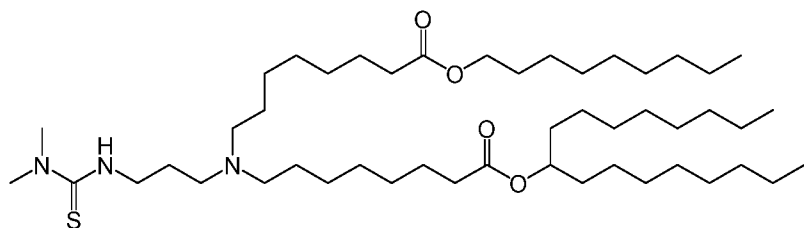
132



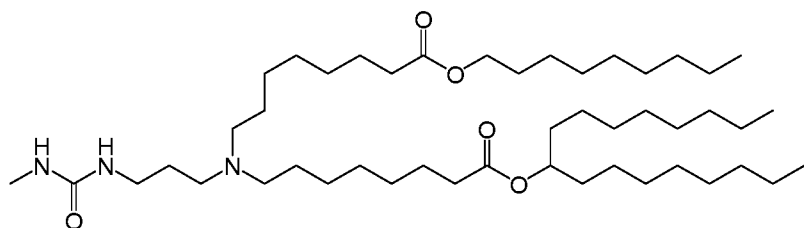
(Compound 109),



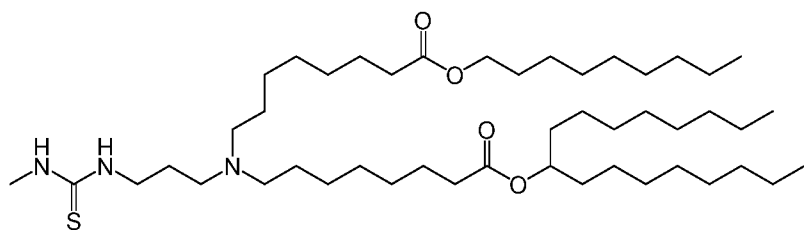
(Compound 110),



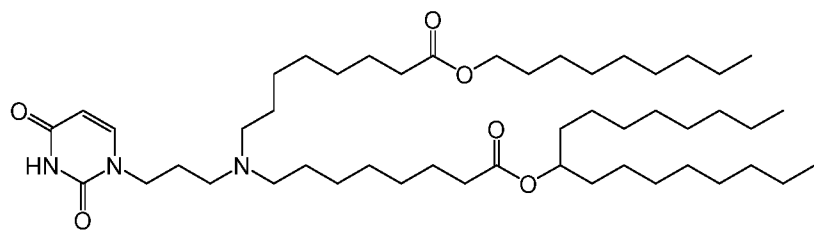
(Compound 111),



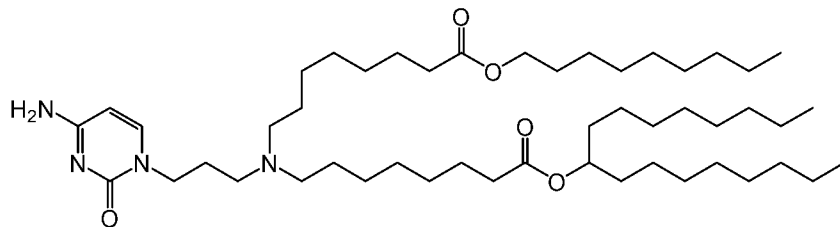
(Compound 112),



(Compound 113),

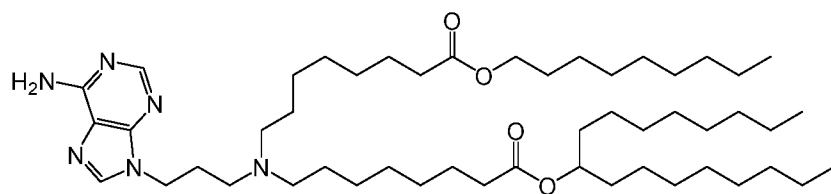


(Compound 114),

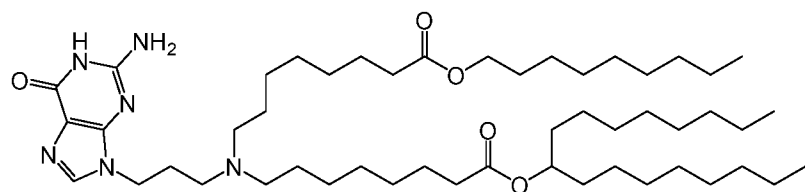


(Compound 115),

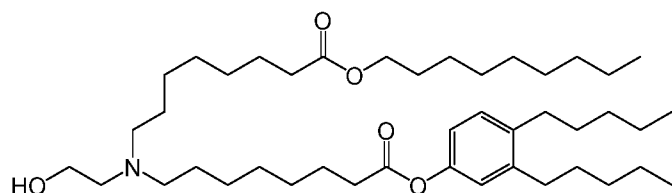
133



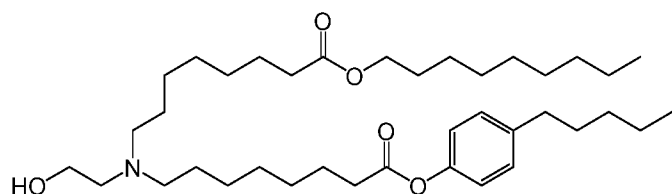
(Compound 116),



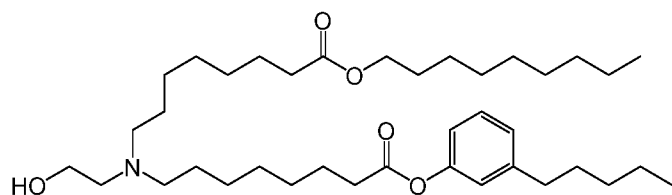
(Compound 117),



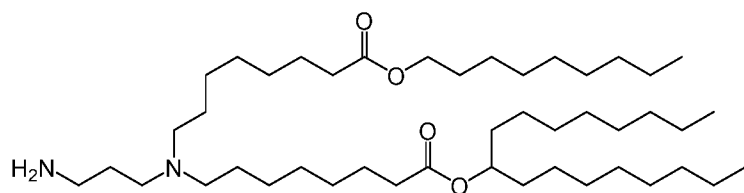
(Compound 118),



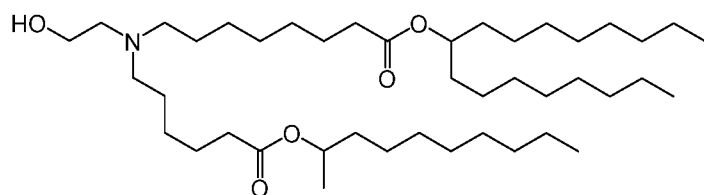
(Compound 119),



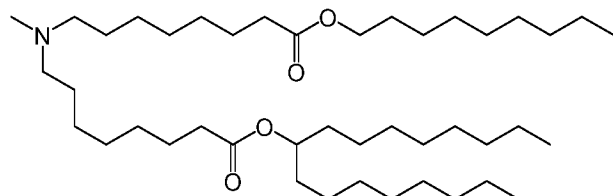
(Compound 120),



(Compound 121),

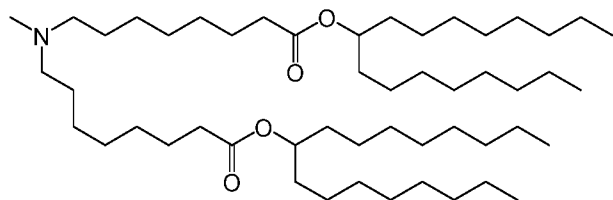


(Compound 122),

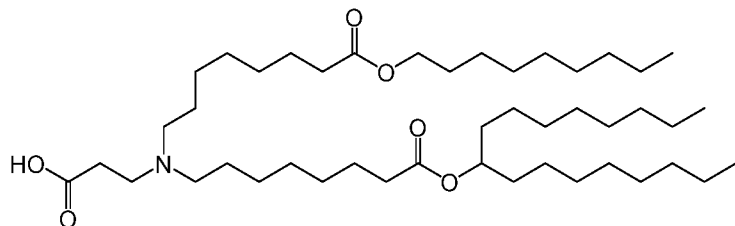


(Compound 123),

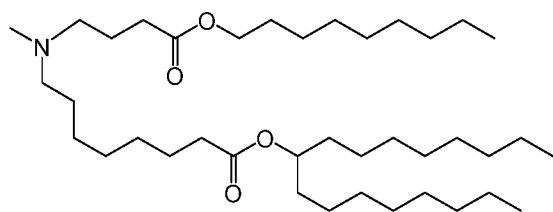
134



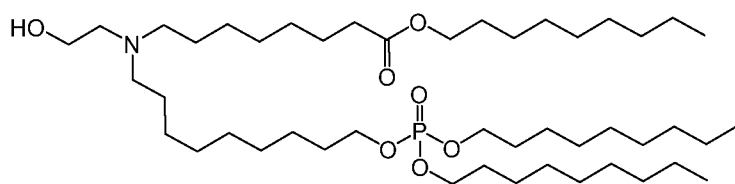
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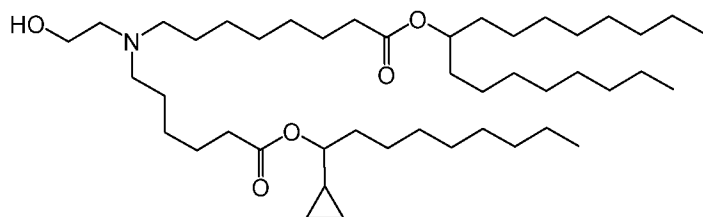
(Compound 125),



(Compound 126),

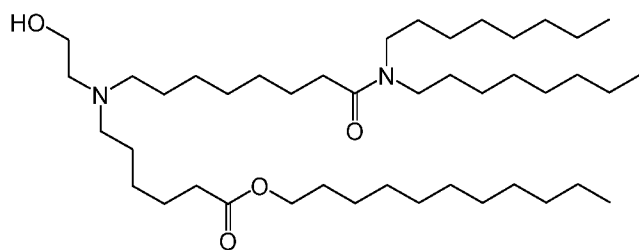


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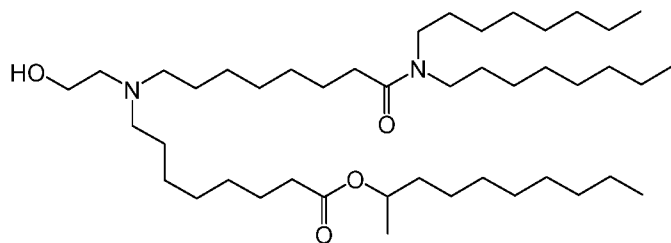


(Compound 128),

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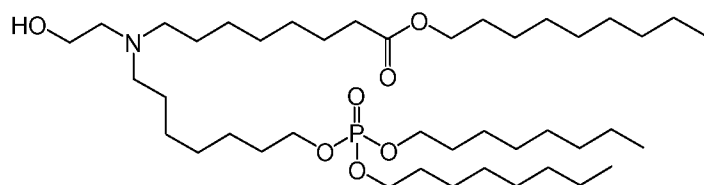


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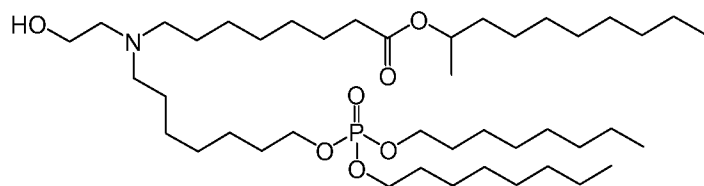


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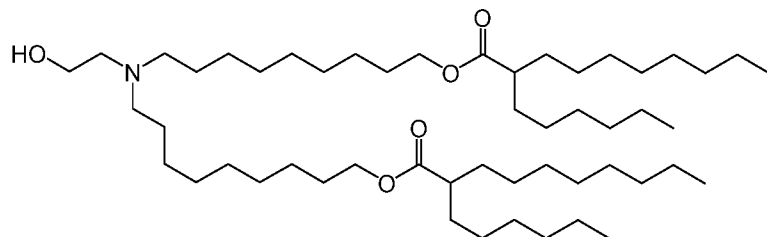
135



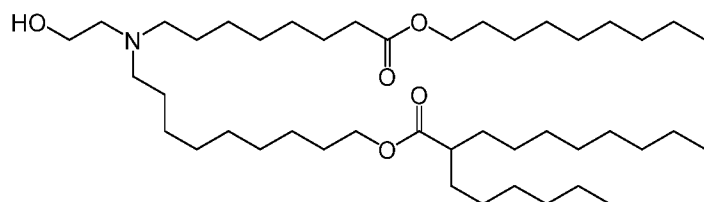
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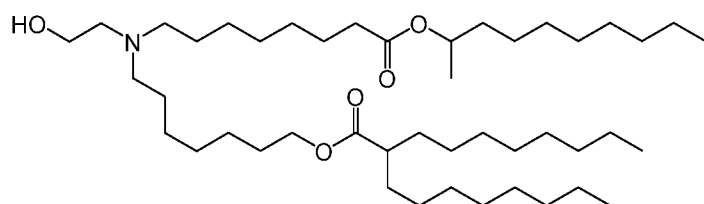
(Compound 132),



(Compound 133),

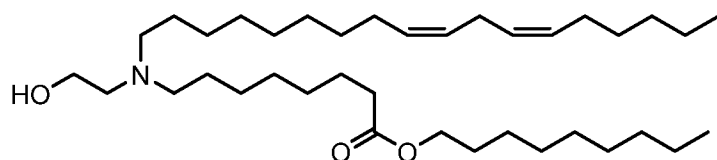


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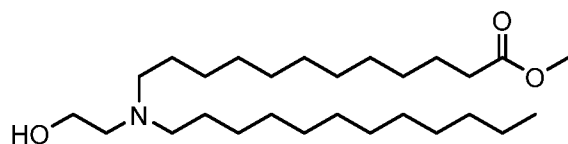


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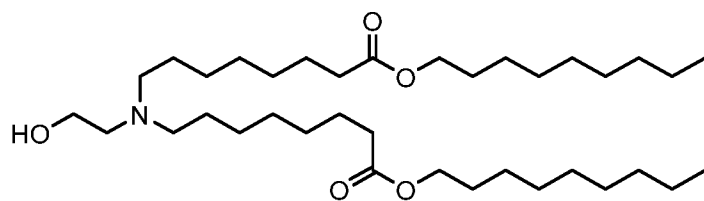
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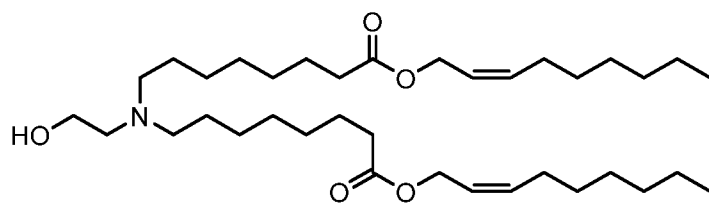


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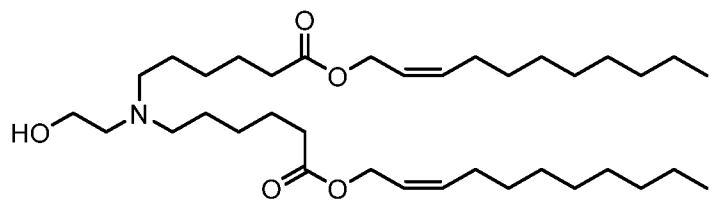


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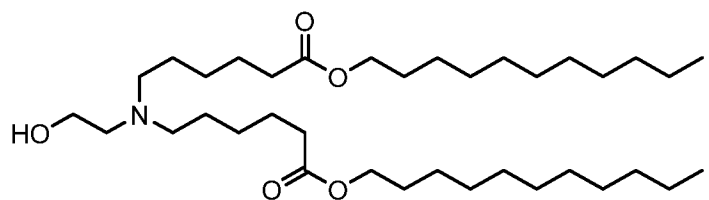
136



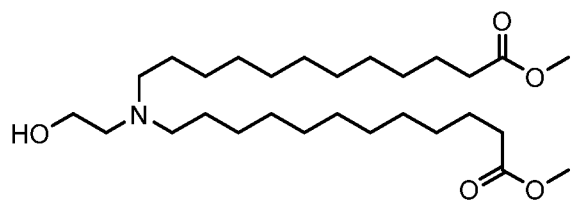
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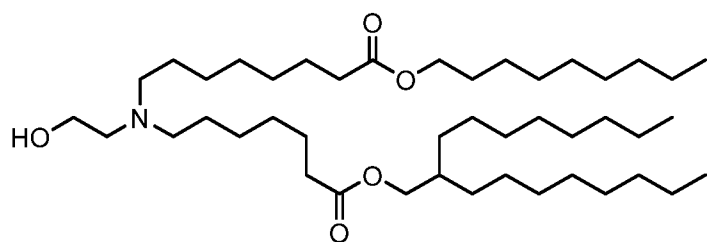
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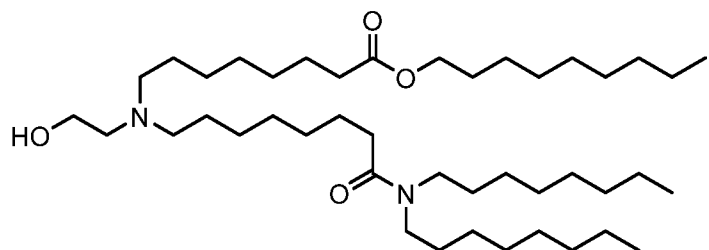
(Compound 141),



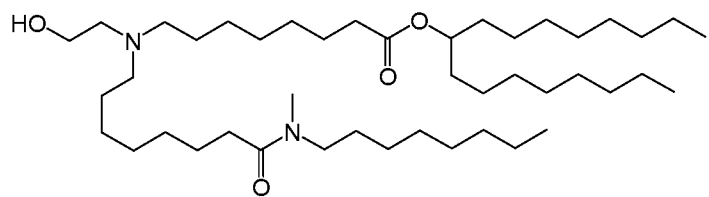
(Compound 142),



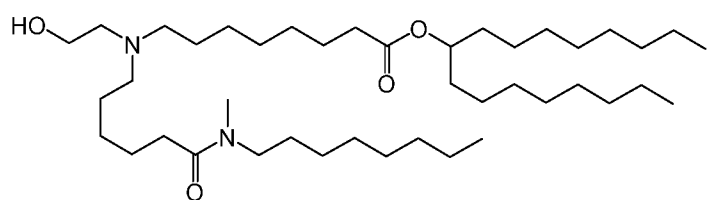
(Compound 143),



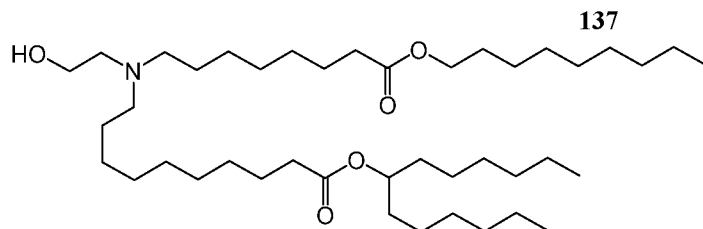
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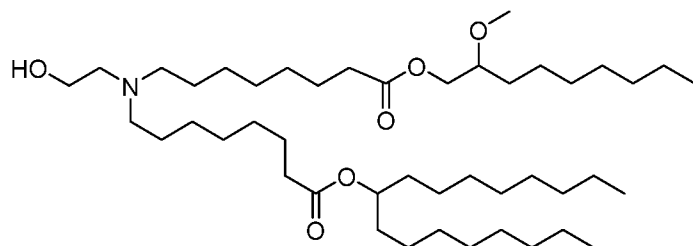
(Compound 145),



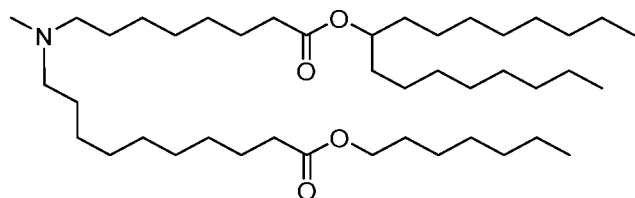
(Compound 146),



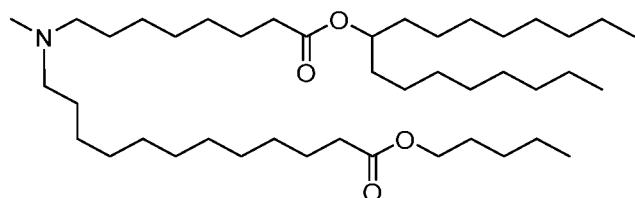
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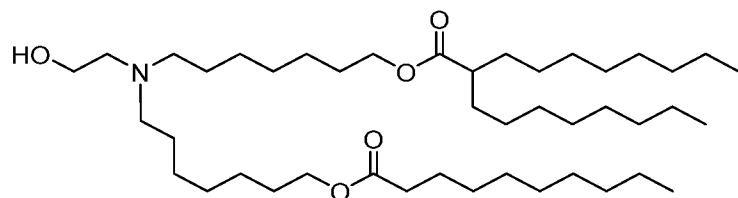
(Compound 148),



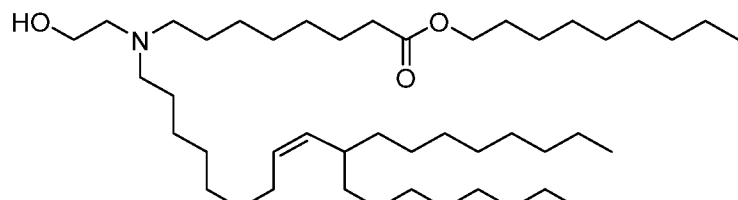
(Compound 149),



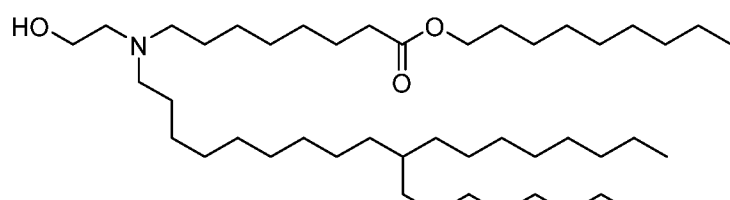
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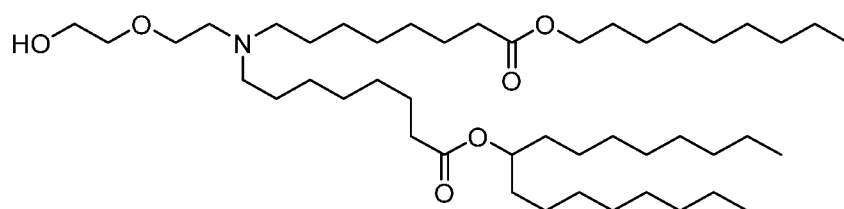
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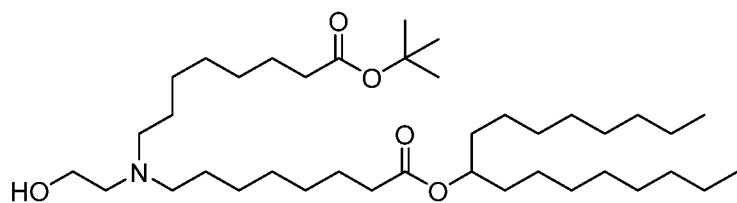
(Compound 152),



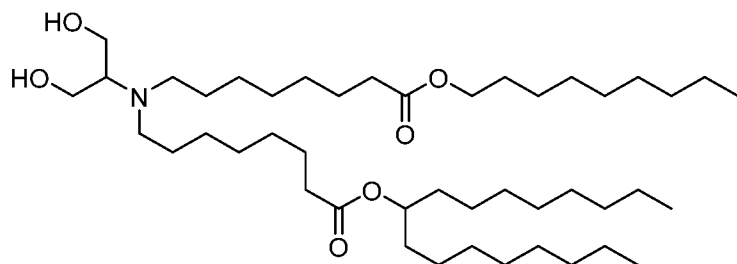
(Compound 153),



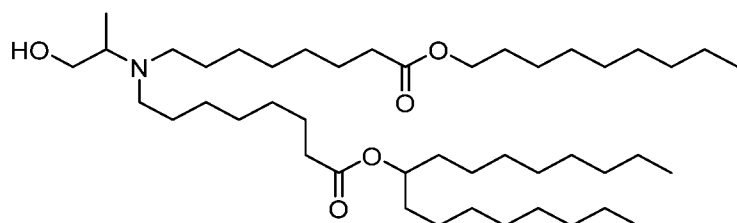
(Compound 154),



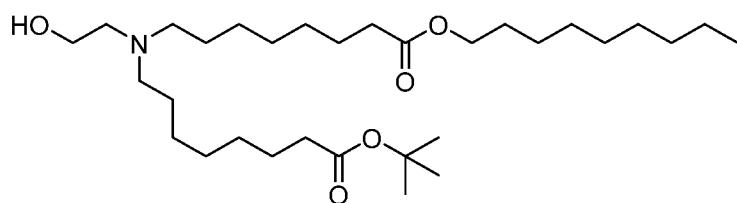
(Compound 155),



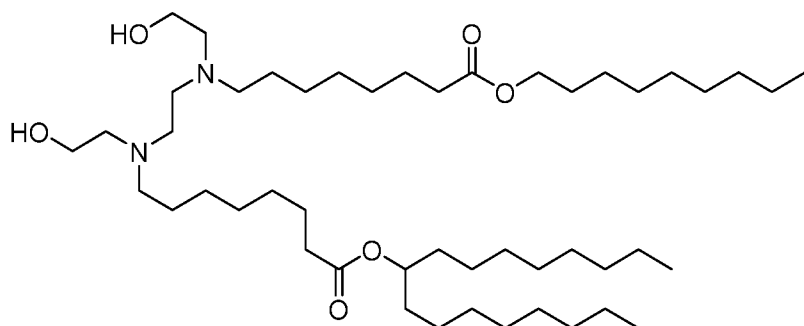
(Compound 156),



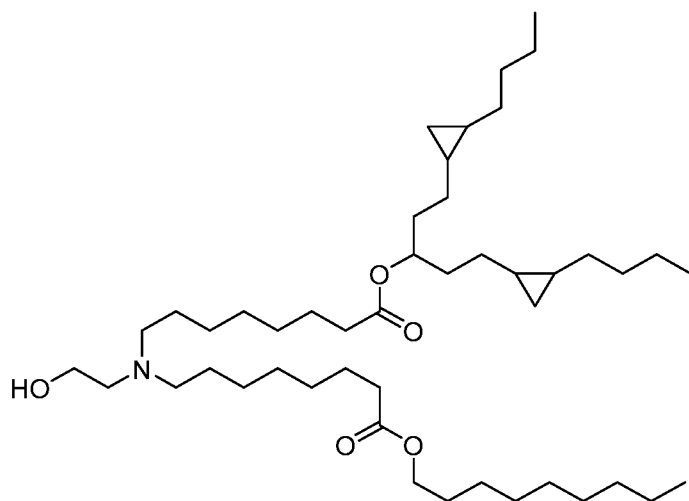
(Compound 157),



(Compound 158),

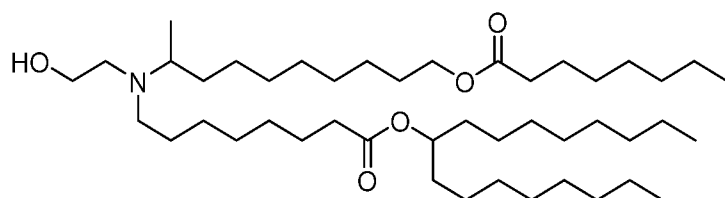


(Compound 159),

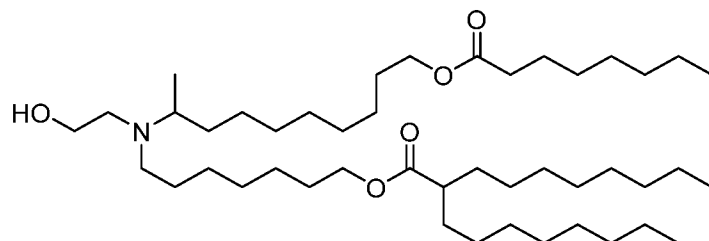


(Compound 160),

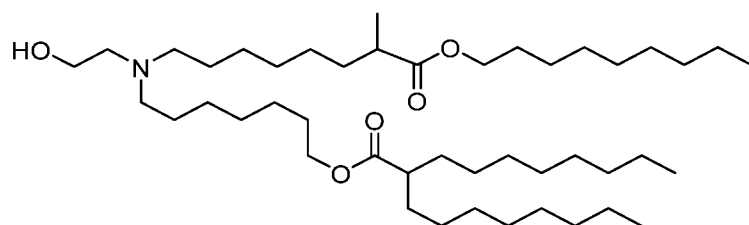
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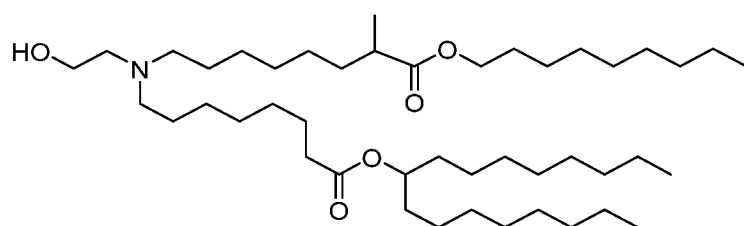
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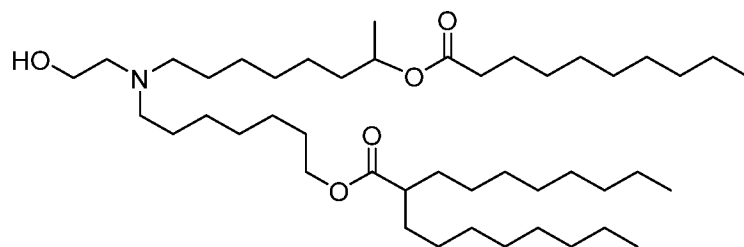
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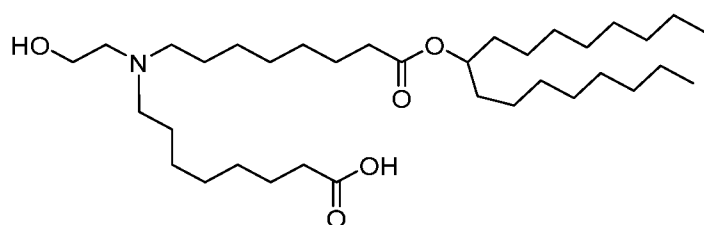
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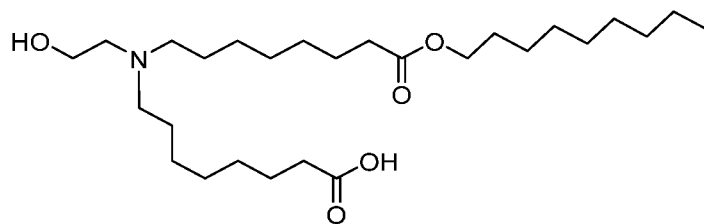
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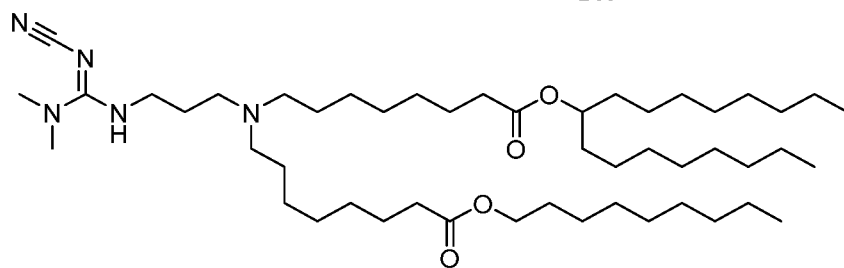
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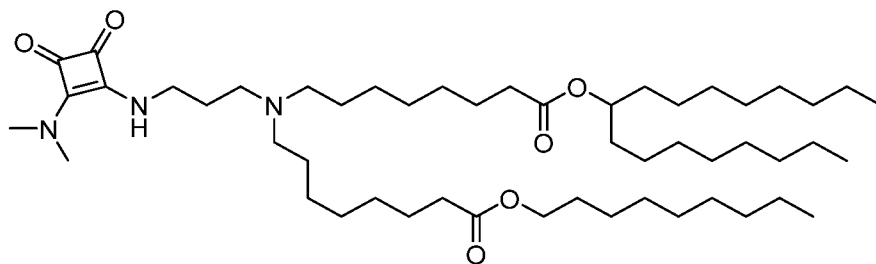
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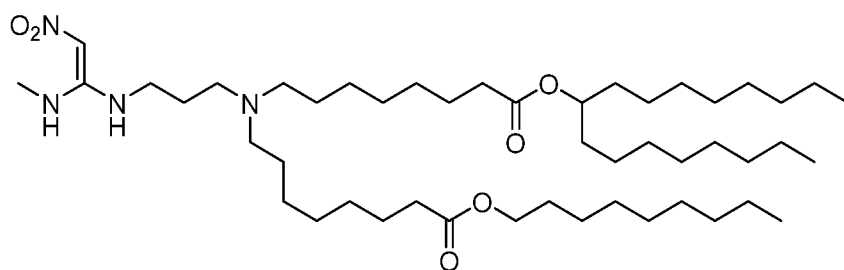
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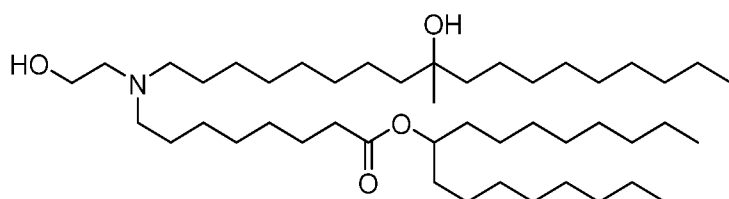
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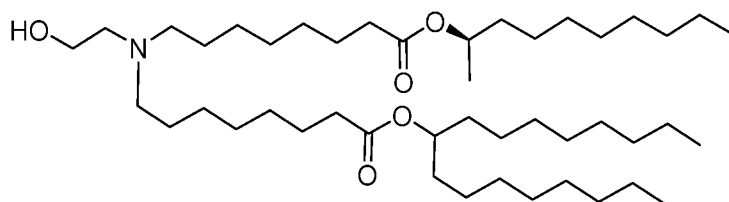
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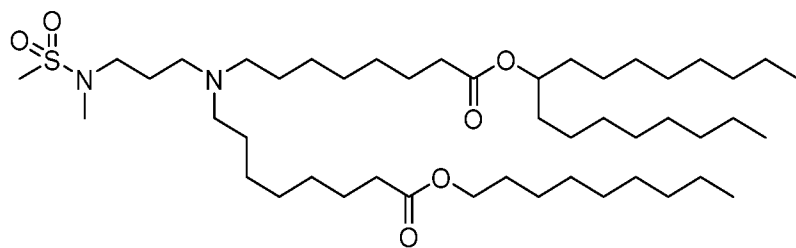
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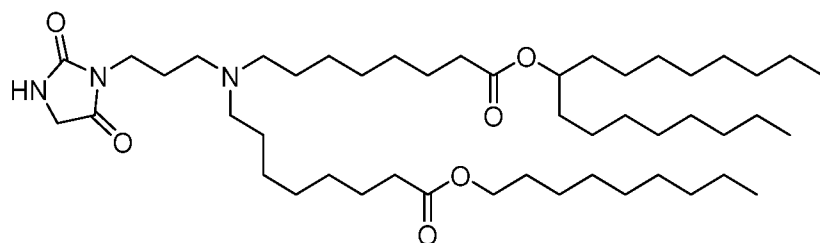
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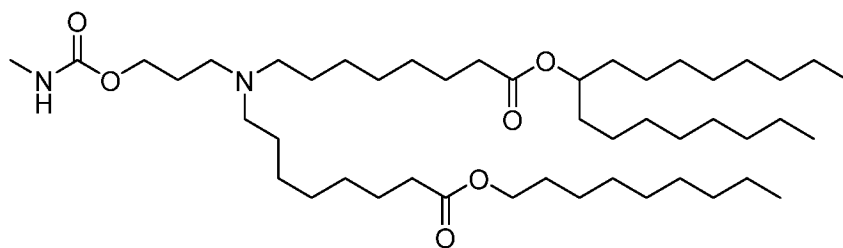


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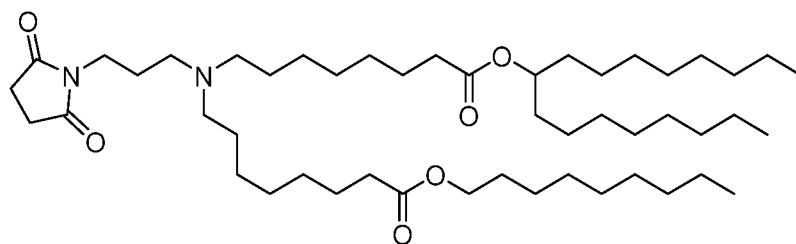


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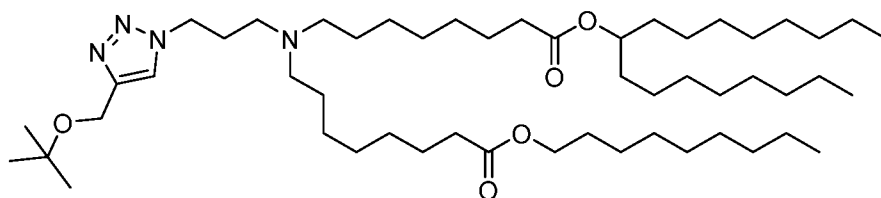
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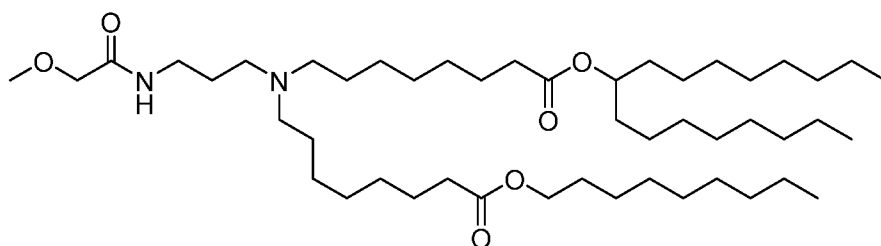
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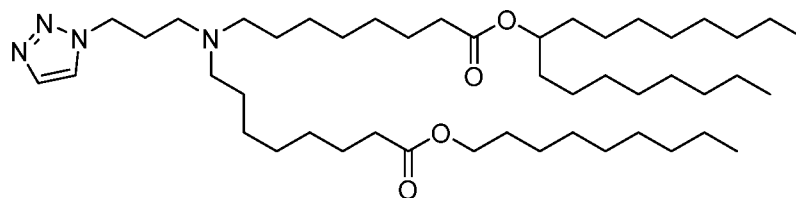
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(Compound 177),

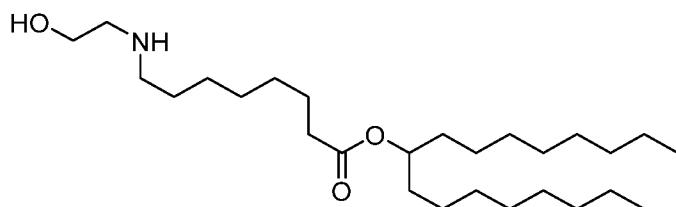


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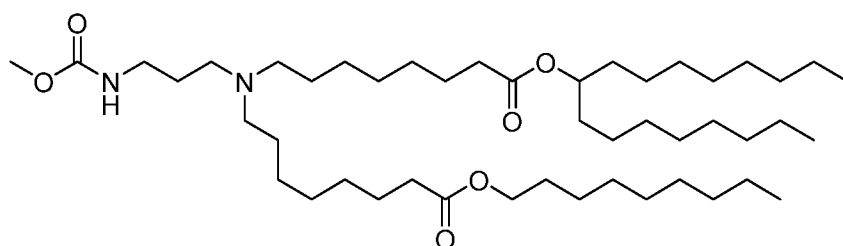


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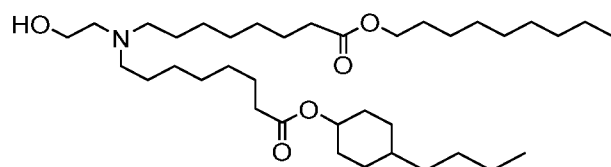
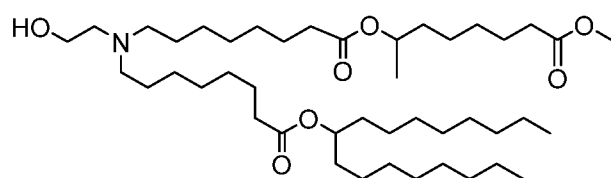
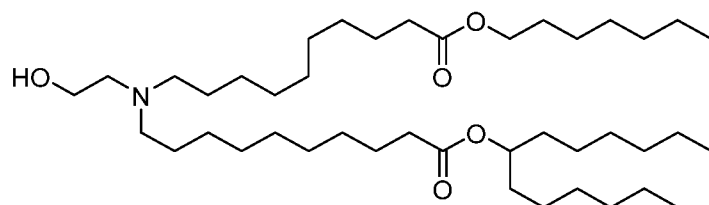
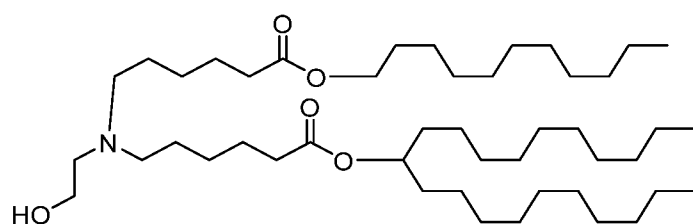
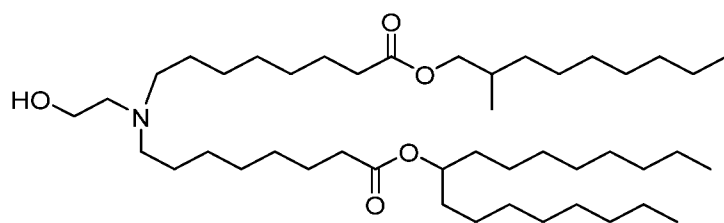
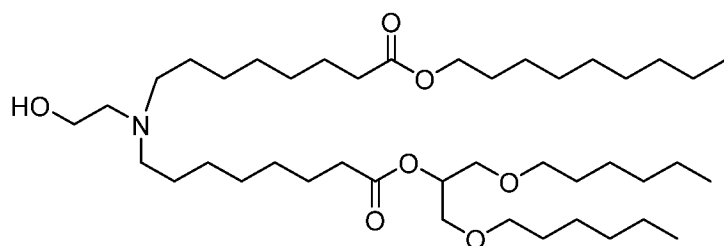
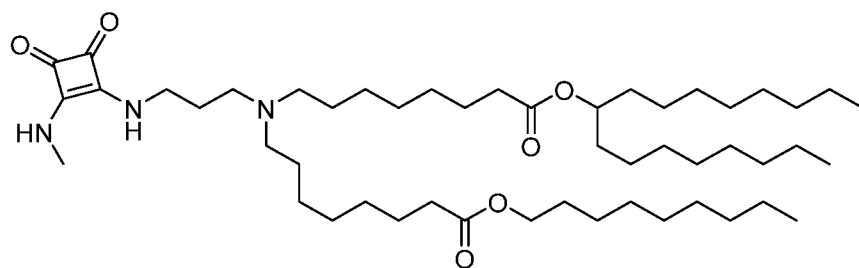
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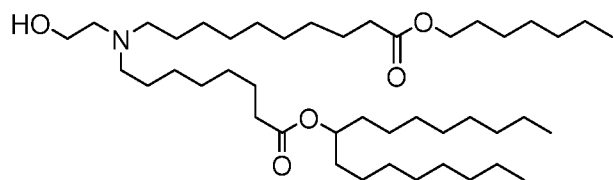
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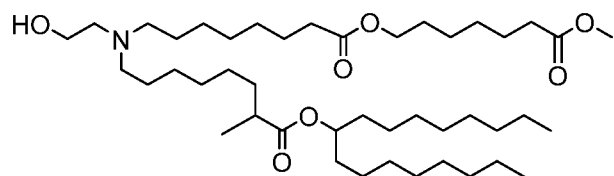
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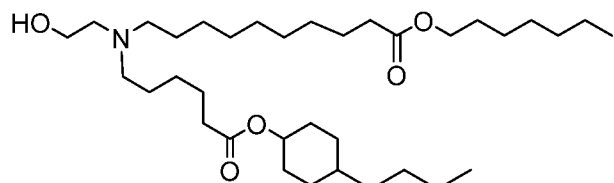
143



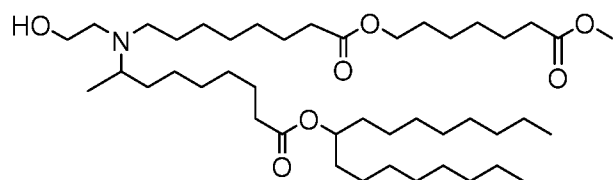
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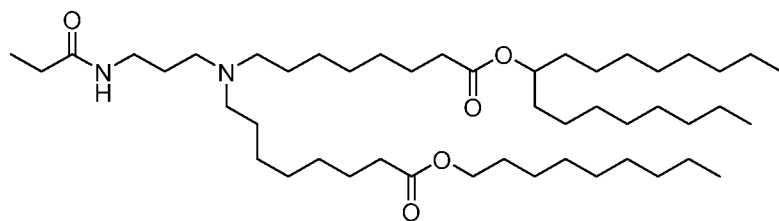
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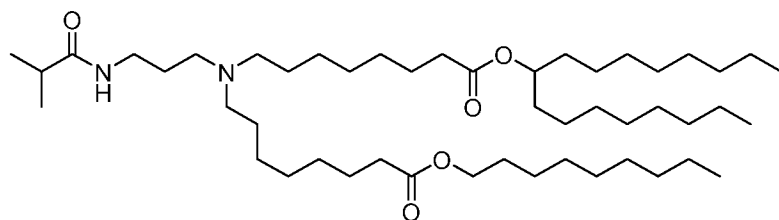
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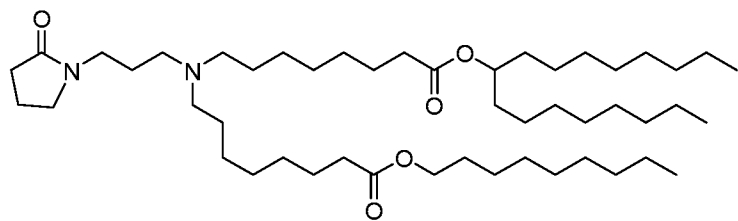
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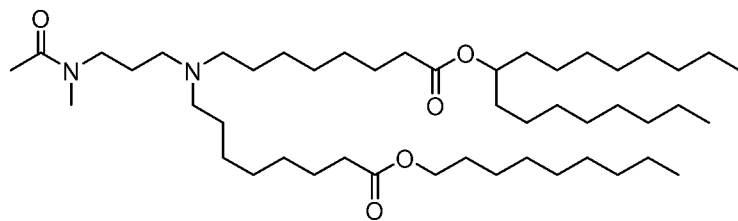
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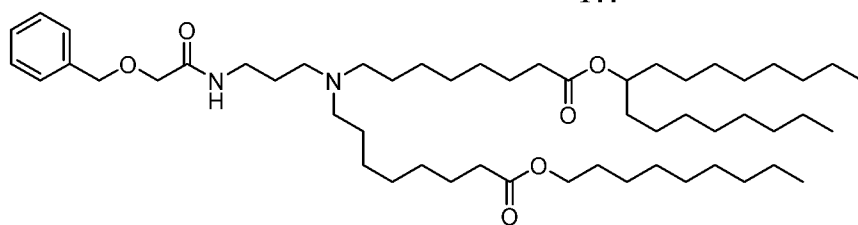


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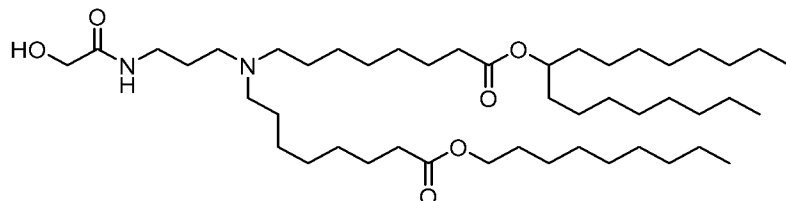


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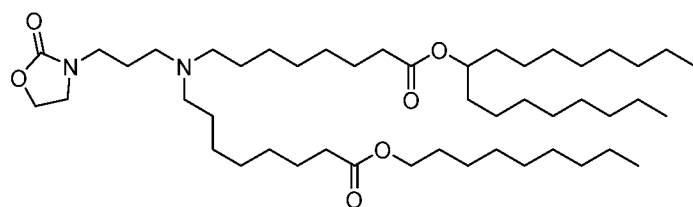
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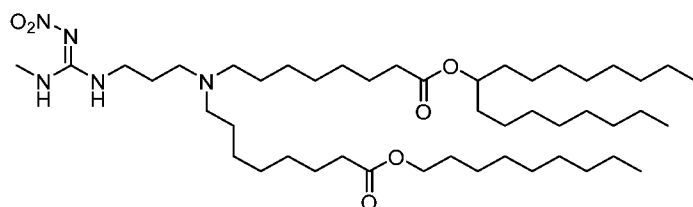
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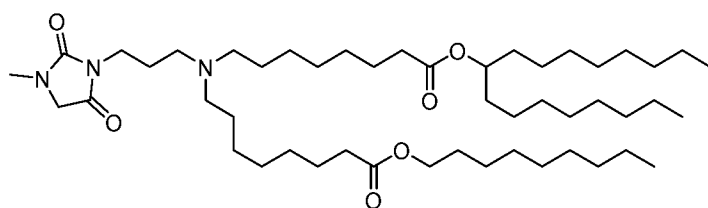
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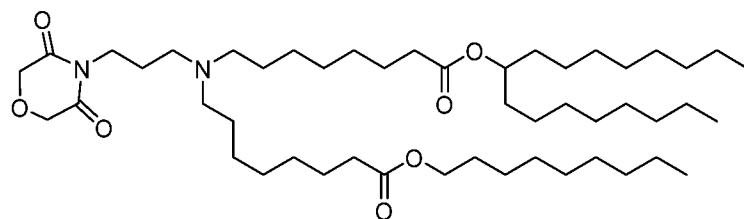
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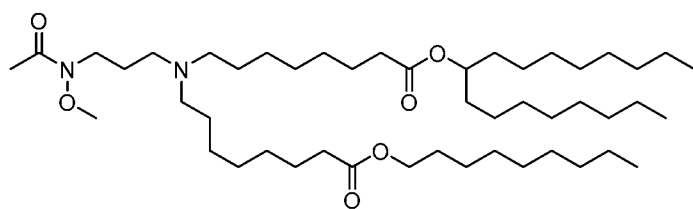
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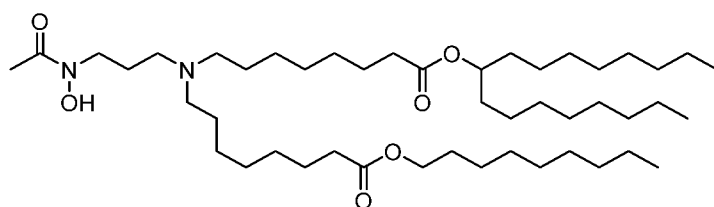
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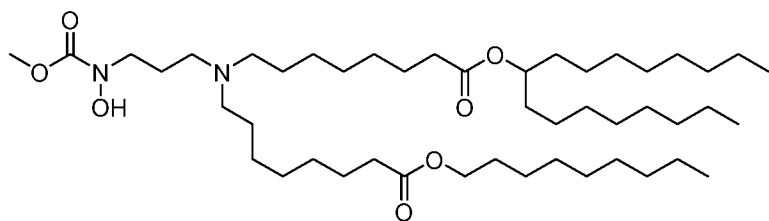
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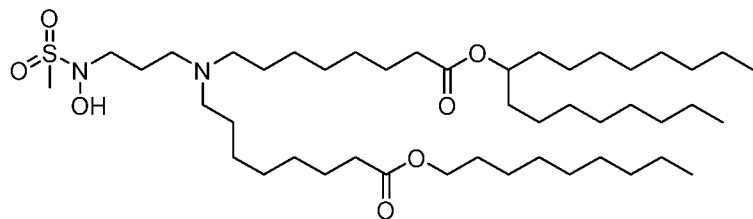
(Compound 203),



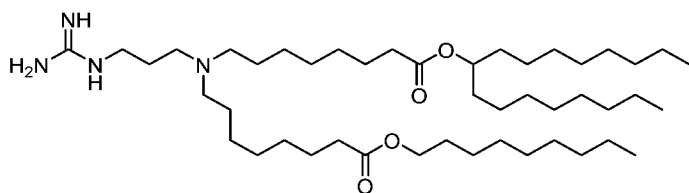
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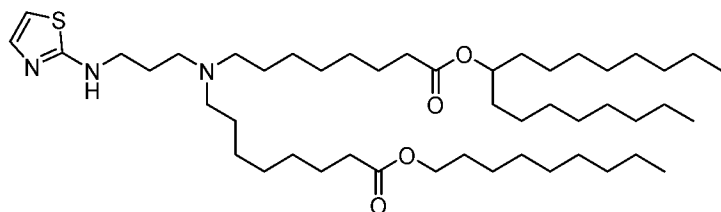
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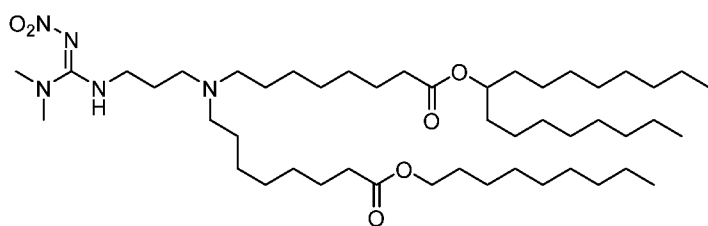
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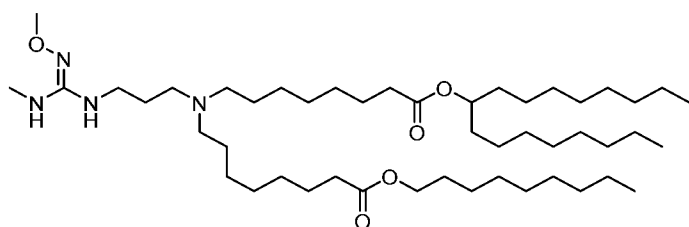
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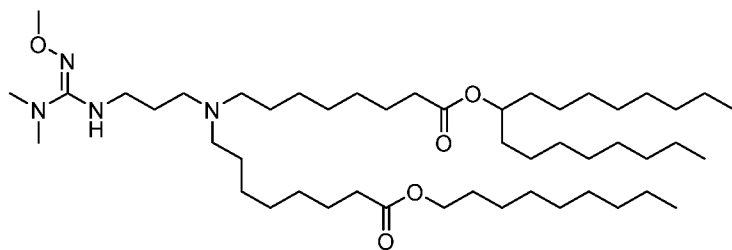
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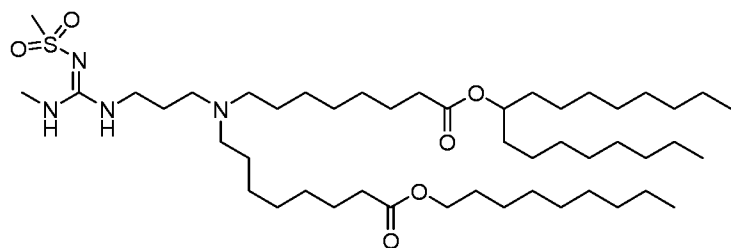
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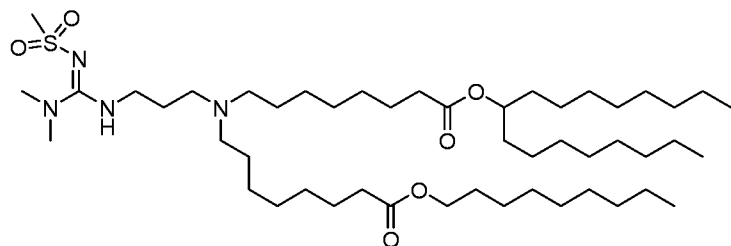
(Compound 210),



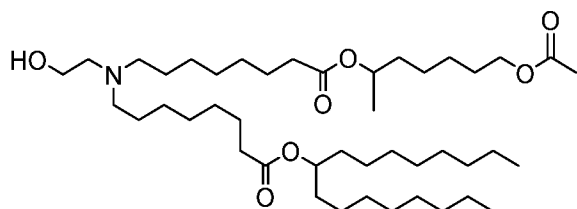
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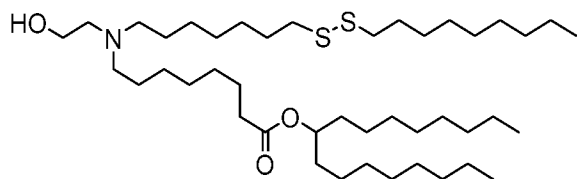
(Compound 212),



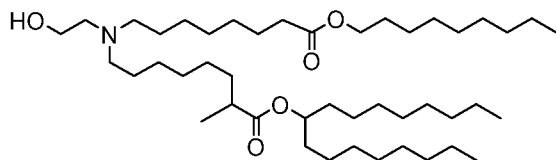
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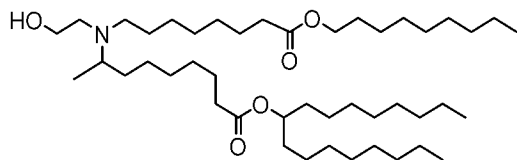
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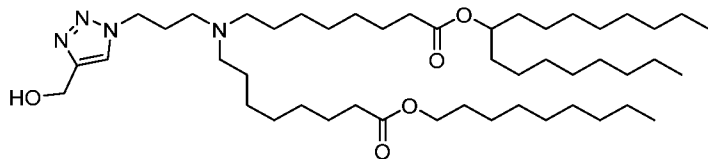
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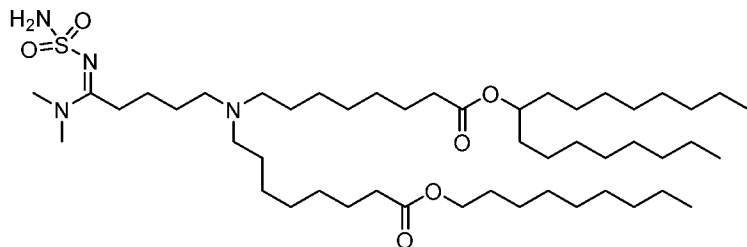
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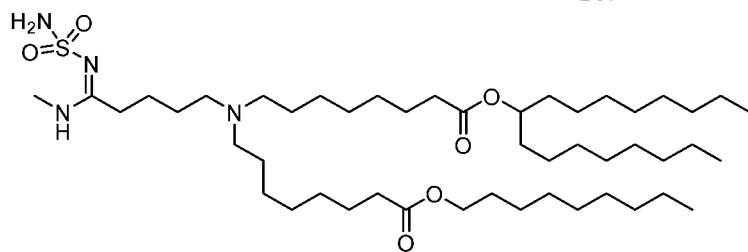
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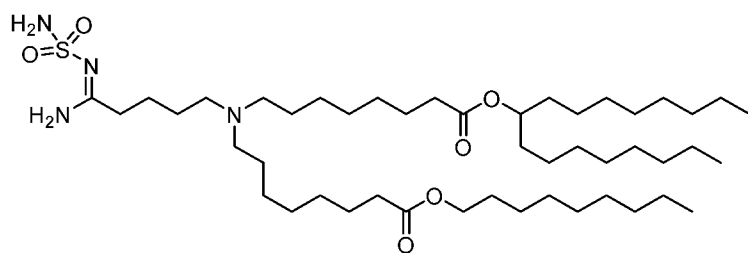
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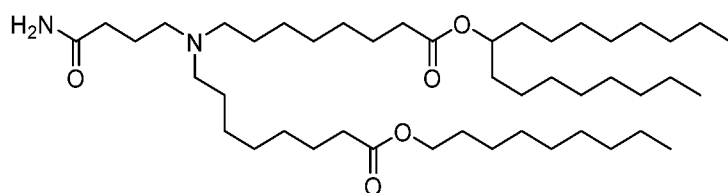
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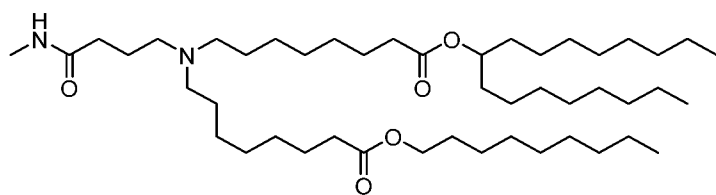
(Compound 220),



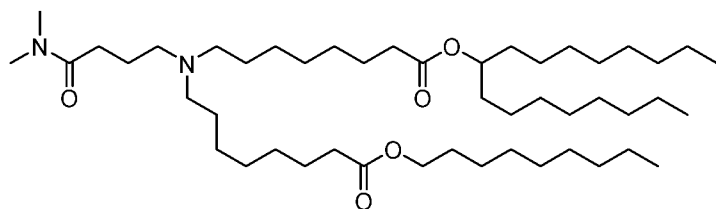
(Compound 221),



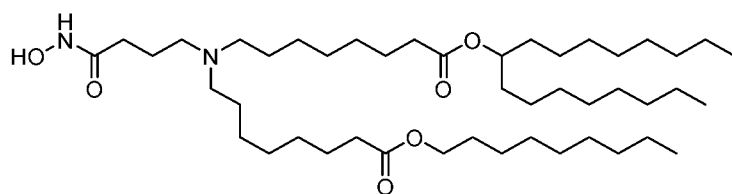
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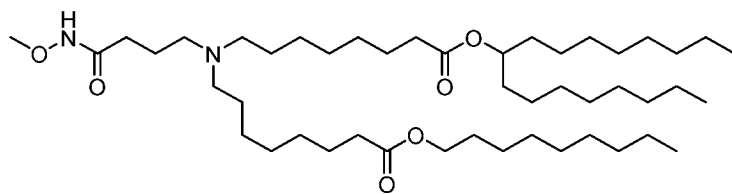
(Compound 223),



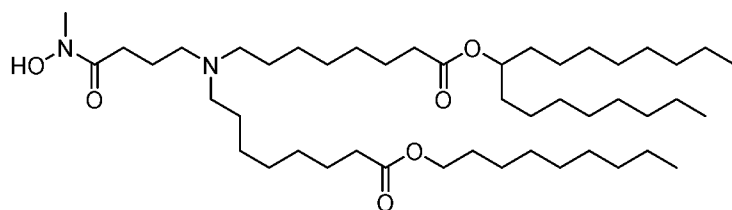
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(Compound 225),

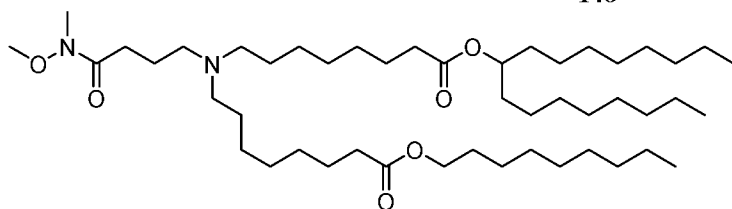


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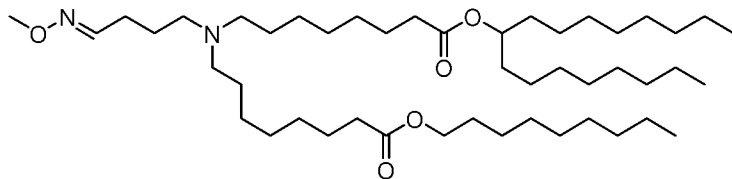


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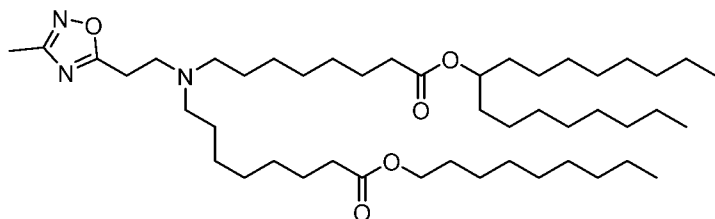
148



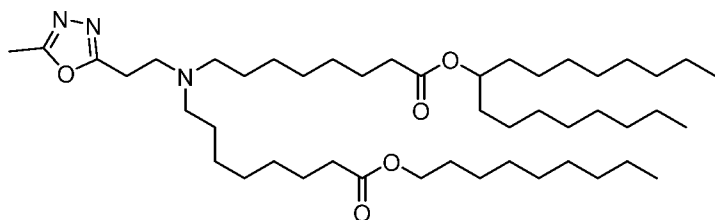
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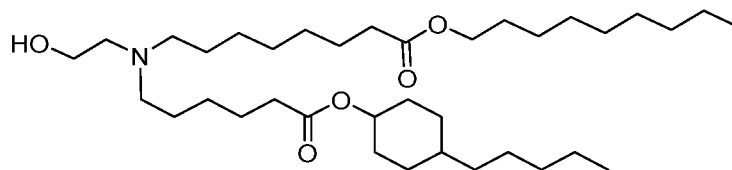
(Compound 229),



(Compound 230),



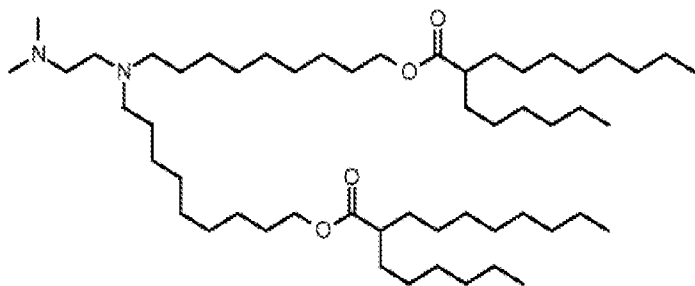
(Compound 231),



(Compound 232), and

salts and isomers thereof.

In some embodiments, a nanoparticle comprises the following compound:



(Compound 233) or salts

and isomers thereof.

In some embodiments, the disclosure features a nanoparticle composition including a lipid component comprising a compound as described herein (*e.g.*, a compound according to Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IIc) or (IIe)).

In some embodiments, the disclosure features a pharmaceutical composition comprising a nanoparticle composition according to the preceding embodiments and a

pharmaceutically acceptable carrier. For example, the pharmaceutical composition is refrigerated or frozen for storage and/or shipment (*e.g.*, being stored at a temperature of 4 °C or lower, such as a temperature between about -150 °C and about 0 °C or between about -80 °C and about -20 °C (*e.g.*, about -5 °C, -10 °C, -15 °C, -20 °C, -25 °C, -30 °C, -40 °C, -50 °C, -60 °C, -70 °C, -80 °C, -90 °C, -130 °C or -150 °C). For example, the pharmaceutical composition is a solution that is refrigerated for storage and/or shipment at, for example, about -20° C, -30 °C, -40 °C, -50 °C, -60 °C, -70 °C, or -80 °C.

In some embodiments, the disclosure provides a method of delivering a therapeutic and/or prophylactic (*e.g.*, RNA, such as mRNA) to a cell (*e.g.*, a mammalian cell). This method includes the step of administering to a subject (*e.g.*, a mammal, such as a human) a nanoparticle composition including (i) a lipid component including a phospholipid (such as a polyunsaturated lipid), a PEG lipid, a structural lipid, and a compound of Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe) and (ii) a therapeutic and/or prophylactic, in which administering involves contacting the cell with the nanoparticle composition, whereby the therapeutic and/or prophylactic is delivered to the cell.

In some embodiments, the disclosure provides a method of producing a polypeptide of interest in a cell (*e.g.*, a mammalian cell). The method includes the step of contacting the cell with a nanoparticle composition including (i) a lipid component including a phospholipid (such as a polyunsaturated lipid), a PEG lipid, a structural lipid, and a compound of Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe) and (ii) an mRNA encoding the polypeptide of interest, whereby the mRNA is capable of being translated in the cell to produce the polypeptide.

In some embodiments, the disclosure provides a method of treating a disease or disorder in a mammal (*e.g.*, a human) in need thereof. The method includes the step of administering to the mammal a therapeutically effective amount of a nanoparticle composition including (i) a lipid component including a phospholipid (such as a polyunsaturated lipid), a PEG lipid, a structural lipid, and a compound of Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe) and (ii) a therapeutic and/or prophylactic (*e.g.*, an mRNA). In some embodiments, the disease or disorder is characterized by dysfunctional or aberrant protein or polypeptide activity. For example, the disease or disorder is selected from the group consisting of rare diseases, infectious diseases, cancer and proliferative diseases, genetic diseases (*e.g.*, cystic fibrosis), autoimmune diseases, diabetes, neurodegenerative diseases, cardio- and reno-vascular diseases, and metabolic diseases.

In some embodiments, the disclosure provides a method of delivering (*e.g.*, specifically delivering) a therapeutic and/or prophylactic to a mammalian organ (*e.g.*, a liver, spleen, lung, or femur). This method includes the step of administering to a subject (*e.g.*, a mammal) a nanoparticle composition including (i) a lipid component including a phospholipid, a PEG lipid, a structural lipid, and a compound of Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe) and (ii) a therapeutic and/or prophylactic (*e.g.*, an mRNA), in which administering involves contacting the cell with the nanoparticle composition, whereby the therapeutic and/or prophylactic is delivered to the target organ (*e.g.*, a liver, spleen, lung, or femur).

In some embodiments, the disclosure features a method for the enhanced delivery of a therapeutic and/or prophylactic (*e.g.*, an mRNA) to a target tissue (*e.g.*, a liver, spleen, lung, or femur). This method includes administering to a subject (*e.g.*, a mammal) a nanoparticle composition, the composition including (i) a lipid component including a compound of Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe), a phospholipid, a structural lipid, and a PEG lipid; and (ii) a therapeutic and/or prophylactic, the administering including contacting the target tissue with the nanoparticle composition, whereby the therapeutic and/or prophylactic is delivered to the target tissue.

In some embodiments, the disclosure features a method of lowering immunogenicity comprising introducing the nanoparticle composition of the disclosure into cells, wherein the nanoparticle composition reduces the induction of the cellular immune response of the cells to the nanoparticle composition, as compared to the induction of the cellular immune response in cells induced by a reference composition which comprises a reference lipid instead of a compound of Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe). For example, the cellular immune response is an innate immune response, an adaptive immune response, or both.

The disclosure also includes methods of synthesizing a compound of Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe) and methods of making a nanoparticle composition including a lipid component comprising the compound of Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe).

Modes of Vaccine Administration

RSV RNA (*e.g.*, mRNA) vaccines may be administered by any route which results in a therapeutically effective outcome. These include, but are not limited, to intradermal, intramuscular, intranasal, and/or subcutaneous administration. The present disclosure

provides methods comprising administering RNA vaccines to a subject in need thereof. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like. RSV RNA (*e.g.*, mRNA) vaccines compositions are typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of RSV RNA (*e.g.*, mRNA) vaccines compositions may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate imaging dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

In some embodiments, RSV RNA (*e.g.*, mRNA) vaccines compositions may be administered at dosage levels sufficient to deliver 0.0001 mg/kg to 100 mg/kg, 0.001 mg/kg to 0.05 mg/kg, 0.005 mg/kg to 0.05 mg/kg, 0.001 mg/kg to 0.005 mg/kg, 0.05 mg/kg to 0.5 mg/kg, 0.01 mg/kg to 50 mg/kg, 0.1 mg/kg to 40 mg/kg, 0.5 mg/kg to 30 mg/kg, 0.01 mg/kg to 10 mg/kg, 0.1 mg/kg to 10 mg/kg, or 1 mg/kg to 25 mg/kg, of subject body weight per day, one or more times a day, per week, per month, etc. to obtain the desired therapeutic, diagnostic, prophylactic, or imaging effect (*see e.g.*, the range of unit doses described in International Publication No. WO2013078199, herein incorporated by reference in its entirety). The desired dosage may be delivered three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, every four weeks, every 2 months, every three months, every 6 months, *etc.* In certain embodiments, the desired dosage may be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). When multiple administrations are employed, split dosing regimens such as those described herein may be used. In exemplary embodiments, RSV RNA (*e.g.*, mRNA) vaccines compositions may be administered at dosage levels sufficient to deliver 0.0005 mg/kg to 0.01 mg/kg, *e.g.*, about 0.0005 mg/kg to about 0.0075 mg/kg, *e.g.*, about 0.0005 mg/kg, about 0.001 mg/kg, about 0.002 mg/kg, about 0.003 mg/kg, about 0.004 mg/kg or about 0.005 mg/kg.

In some embodiments, RSV RNA (*e.g.*, mRNA) vaccine compositions may be administered once or twice (or more) at dosage levels sufficient to deliver 0.025 mg/kg to 0.250 mg/kg, 0.025 mg/kg to 0.500 mg/kg, 0.025 mg/kg to 0.750 mg/kg, or 0.025 mg/kg to 1.0 mg/kg.

5 In some embodiments, RSV RNA (*e.g.*, mRNA) vaccine compositions may be administered twice (*e.g.*, Day 0 and Day 7, Day 0 and Day 14, Day 0 and Day 21, Day 0 and Day 28, Day 0 and Day 60, Day 0 and Day 90, Day 0 and Day 120, Day 0 and Day 150, Day 0 and Day 180, Day 0 and 3 months later, Day 0 and 6 months later, Day 0 and 9 months later, Day 0 and 12 months later, Day 0 and 18 months later, Day 0 and 2 years later, Day 0 and 5 years later, or Day 0 and 10 years later) at a total dose of or at dosage levels sufficient to deliver a total dose of 0.0100 mg, 0.025 mg, 0.050 mg, 0.075 mg, 0.100 mg, 0.125 mg, 10 0.150 mg, 0.175 mg, 0.200 mg, 0.225 mg, 0.250 mg, 0.275 mg, 0.300 mg, 0.325 mg, 0.350 mg, 0.375 mg, 0.400 mg, 0.425 mg, 0.450 mg, 0.475 mg, 0.500 mg, 0.525 mg, 0.550 mg, 0.575 mg, 0.600 mg, 0.625 mg, 0.650 mg, 0.675 mg, 0.700 mg, 0.725 mg, 0.750 mg, 0.775 15 mg, 0.800 mg, 0.825 mg, 0.850 mg, 0.875 mg, 0.900 mg, 0.925 mg, 0.950 mg, 0.975 mg, or 1.0 mg. Higher and lower dosages and frequency of administration are encompassed by the present disclosure. For example, a RSV RNA (*e.g.*, mRNA) vaccine composition may be administered three or four times.

In some embodiments, RSV RNA (*e.g.*, mRNA) vaccine compositions may be administered twice (*e.g.*, Day 0 and Day 7, Day 0 and Day 14, Day 0 and Day 21, Day 0 and Day 28, Day 0 and Day 60, Day 0 and Day 90, Day 0 and Day 120, Day 0 and Day 150, Day 0 and Day 180, Day 0 and 3 months later, Day 0 and 6 months later, Day 0 and 9 months later, Day 0 and 12 months later, Day 0 and 18 months later, Day 0 and 2 years later, Day 0 and 5 years later, or Day 0 and 10 years later) at a total dose of or at dosage levels sufficient 20 to deliver a total dose of 0.010 mg, 0.025 mg, 0.100 mg or 0.400 mg.

In some embodiments the RSV RNA (*e.g.*, mRNA) vaccine for use in a method of vaccinating a subject is administered the subject a single dosage of between 10 µg/kg and 400 µg/kg of the nucleic acid vaccine in an effective amount to vaccinate the subject. In some 25 embodiments the RNA vaccine for use in a method of vaccinating a subject is administered the subject a single dosage of between 10 µg and 400 µg of the nucleic acid vaccine in an effective amount to vaccinate the subject. In some embodiments, a RSV RNA (*e.g.*, mRNA) vaccine for use in a method of vaccinating a subject is administered to the subject as a single dosage of 25-1000 µg (*e.g.*, a single dosage of mRNA encoding an RSV antigen). In some 30 embodiments, a RSV RNA vaccine is administered to the subject as a single dosage of 25,

50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 µg. For example, a RSV RNA vaccine may be administered to a subject as a single dose of 25-100, 25-500, 50-100, 50-500, 50-1000, 100-500, 100-1000, 250-500, 250-1000, or 500-1000 µg. In some embodiments, a RSV RNA (*e.g.*, mRNA) vaccine for use in a method of vaccinating a subject is administered to the subject as two dosages, the combination of which equals 25-1000 µg of the RSV RNA (*e.g.*, mRNA) vaccine.

A RSV RNA (*e.g.*, mRNA) vaccine pharmaceutical composition described herein can be formulated into a dosage form described herein, such as an intranasal, intratracheal, or injectable (*e.g.*, intravenous, intraocular, intravitreal, intramuscular, intradermal, intracardiac, intraperitoneal, and subcutaneous).

RSV RNA vaccine formulations and methods of use

Some aspects of the present disclosure provide formulations of the RSV RNA (*e.g.*, mRNA) vaccine, wherein the RSV RNA vaccine is formulated in an effective amount to produce an antigen specific immune response in a subject (*e.g.*, production of antibodies specific to an anti-RSV antigenic polypeptide). “An effective amount” is a dose of an RSV RNA (*e.g.*, mRNA) vaccine effective to produce an antigen-specific immune response. Also provided herein are methods of inducing an antigen-specific immune response in a subject.

In some embodiments, the antigen-specific immune response is characterized by measuring an anti-RSV antigenic polypeptide antibody titer produced in a subject administered a RSV RNA (*e.g.*, mRNA) vaccine as provided herein. An antibody titer is a measurement of the amount of antibodies within a subject, for example, antibodies that are specific to a particular antigen (*e.g.*, an anti-RSV antigenic polypeptide) or epitope of an antigen. Antibody titer is typically expressed as the inverse of the greatest dilution that provides a positive result. Enzyme-linked immunosorbent assay (ELISA) is a common assay for determining antibody titers, for example.

In some embodiments, an antibody titer is used to assess whether a subject has had an infection or to determine whether immunizations are required. In some embodiments, an antibody titer is used to determine the strength of an autoimmune response, to determine whether a booster immunization is needed, to determine whether a previous vaccine was effective, and to identify any recent or prior infections. In accordance with the present disclosure, an antibody titer may be used to determine the strength of an immune response induced in a subject by the RSV RNA (*e.g.*, mRNA) vaccine.

In some embodiments, an anti-RSV antigenic polypeptide antibody titer produced in a subject is increased by at least 1 log relative to a control (*e.g.*, a control vaccine). For example, anti-RSV antigenic polypeptide antibody titer produced in a subject may be increased by at least 1.5, at least 2, at least 2.5, or at least 3 log relative to a control (*e.g.*, a control vaccine). In some embodiments, the anti-RSV antigenic polypeptide antibody titer produced in the subject is increased by 1, 1.5, 2, 2.5 or 3 log relative to a control (*e.g.*, a control vaccine). In some embodiments, the anti-RSV antigenic polypeptide antibody titer produced in the subject is increased by 1-3 log relative to a control (*e.g.*, a control vaccine). For example, the anti-RSV antigenic polypeptide antibody titer produced in a subject may be increased by 1-1.5, 1-2, 1-2.5, 1-3, 1.5-2, 1.5-2.5, 1.5-3, 2-2.5, 2-3, or 2.5-3 log relative to a control (*e.g.*, a control vaccine).

In some embodiments, the anti-RSV antigenic polypeptide antibody titer produced in a subject is increased at least 2 times relative to a control (*e.g.*, a control vaccine). For example, the anti-RSV antigenic polypeptide antibody titer produced in a subject may be increased at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, or at least 10 times relative to a control (*e.g.*, a control vaccine). In some embodiments, the anti-RSV antigenic polypeptide antibody titer produced in the subject is increased 2, 3, 4, 5, 6, 7, 8, 9, or 10 times relative to a control (*e.g.*, a control vaccine). In some embodiments, the anti-RSV antigenic polypeptide antibody titer produced in a subject is increased 2-10 times relative to a control (*e.g.*, a control vaccine). For example, the anti-RSV antigenic polypeptide antibody titer produced in a subject may be increased 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-10, 5-9, 5-8, 5-7, 5-6, 6-10, 6-9, 6-8, 6-7, 7-10, 7-9, 7-8, 8-10, 8-9, or 9-10 times relative to a control (*e.g.*, a control vaccine).

A control, in some embodiments, is the anti-RSV antigenic polypeptide antibody titer produced in a subject who has not been administered a RSV RNA (*e.g.*, mRNA) vaccine. In some embodiments, a control is an anti-RSV antigenic polypeptide antibody titer produced in a subject who has been administered a live attenuated RSV vaccine. An attenuated vaccine is a vaccine produced by reducing the virulence of a viable (live). An attenuated virus is altered in a manner that renders it harmless or less virulent relative to live, unmodified virus. In some embodiments, a control is an anti-RSV antigenic polypeptide antibody titer produced in a subject administered inactivated RSV vaccine. In some embodiments, a control is an anti-RSV antigenic polypeptide antibody titer produced in a subject administered a recombinant or purified RSV protein vaccine. Recombinant protein vaccines typically include protein

antigens that either have been produced in a heterologous expression system (*e.g.*, bacteria or yeast) or purified from large amounts of the pathogenic organism. In some embodiments, a control is an anti-RSV antigenic polypeptide antibody titer produced in a subject who has been administered a RSV virus-like particle (VLP) vaccine (*e.g.*, particles that contain viral capsid protein but lack a viral genome and, therefore, cannot replicate/produce progeny virus). In some embodiments, the control is a VLP RSV vaccine that comprises prefusion or postfusion F proteins, or that comprises a combination of the two.

In some embodiments, an effective amount of a RSV RNA (*e.g.*, mRNA) vaccine is a dose that is reduced compared to the standard of care dose of a recombinant RSV protein vaccine. A “standard of care,” as provided herein, refers to a medical or psychological treatment guideline and can be general or specific. “Standard of care” specifies appropriate treatment based on scientific evidence and collaboration between medical professionals involved in the treatment of a given condition. It is the diagnostic and treatment process that a physician/ clinician should follow for a certain type of patient, illness or clinical circumstance. A “standard of care dose,” as provided herein, refers to the dose of a recombinant or purified RSV protein vaccine, or a live attenuated or inactivated RSV vaccine, or a RSV VLP vaccine, that a physician/clinician or other medical professional would administer to a subject to treat or prevent RSV, or a RSV-related condition, while following the standard of care guideline for treating or preventing RSV, or a RSV-related condition.

In some embodiments, the anti-RSV antigenic polypeptide antibody titer produced in a subject administered an effective amount of a RSV RNA vaccine is equivalent to an anti-RSV antigenic polypeptide antibody titer produced in a control subject administered a standard of care dose of a recombinant or purified RSV protein vaccine, or a live attenuated or inactivated RSV vaccine, or a RSV VLP vaccine.

In some embodiments, an effective amount of a RSV RNA (*e.g.*, mRNA) vaccine is a dose equivalent to an at least 2-fold reduction in a standard of care dose of a recombinant or purified RSV protein vaccine. For example, an effective amount of a RSV RNA vaccine may be a dose equivalent to an at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold reduction in a standard of care dose of a recombinant or purified RSV protein vaccine. In some embodiments, an effective amount of a RSV RNA vaccine is a dose equivalent to an at least at least 100-fold, at least 500-fold, or at least 1000-fold reduction in a standard of care dose of a recombinant or purified RSV protein vaccine. In some embodiments, an effective amount of a RSV RNA vaccine is a dose

equivalent to a 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 20-, 50-, 100-, 250-, 500-, or 1000-fold reduction in a standard of care dose of a recombinant or purified RSV protein vaccine. In some embodiments, the anti-RSV antigenic polypeptide antibody titer produced in a subject administered an effective amount of a RSV RNA vaccine is equivalent to an anti-RSV

5 antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified RSV protein vaccine, or a live attenuated or inactivated RSV vaccine, or a RSV VLP vaccine. In some embodiments, an effective amount of a RSV RNA (*e.g.*, mRNA) vaccine is a dose equivalent to a 2-fold to 1000-fold (*e.g.*, 2-fold to 100-fold, 10-fold to 1000-fold) reduction in the standard of care dose of a recombinant
10 or purified RSV protein vaccine, wherein the anti-RSV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-RSV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified RSV protein vaccine, or a live attenuated or inactivated RSV vaccine, or a RSV VLP vaccine.

15 In some embodiments, the effective amount of a RSV RNA (*e.g.*, mRNA) vaccine is a dose equivalent to a 2 to 1000-, 2 to 900-, 2 to 800-, 2 to 700-, 2 to 600-, 2 to 500-, 2 to 400-, 2 to 300-, 2 to 200-, 2 to 100-, 2 to 90-, 2 to 80-, 2 to 70-, 2 to 60-, 2 to 50-, 2 to 40-, 2 to 30-, 2 to 20-, 2 to 10-, 2 to 9-, 2 to 8-, 2 to 7-, 2 to 6-, 2 to 5-, 2 to 4-, 2 to 3-, 3 to 1000-, 3 to 900-, 3 to 800-, 3 to 700-, 3 to 600-, 3 to 500-, 3 to 400-, 3 to 300-, 3 to 200-, 3 to 100-, 3 to 90-,
20 3 to 80-, 3 to 70-, 3 to 60-, 3 to 50-, 3 to 40-, 3 to 30-, 3 to 20-, 3 to 10-, 3 to 9-, 3 to 8-, 3 to 7-, 3 to 6-, 3 to 5-, 3 to 4-, 4 to 1000-, 4 to 900-, 4 to 800-, 4 to 700-, 4 to 600-, 4 to 500-, 4 to 400-, 4 to 300-, 4 to 200-, 4 to 100-, 4 to 90-, 4 to 80-, 4 to 70-, 4 to 60-, 4 to 50-, 4 to 40-, 4 to 30-, 4 to 20-, 4 to 10-, 4 to 9-, 4 to 8-, 4 to 7-, 4 to 6-, 4 to 5-, 4 to 4-, 5 to 1000-, 5 to 900-, 5 to 800-, 5 to 700-, 5 to 600-, 5 to 500-, 5 to 400-, 5 to 300-, 5 to 200-, 5 to 100-, 5 to 90-, 5
25 to 80-, 5 to 70-, 5 to 60-, 5 to 50-, 5 to 40-, 5 to 30-, 5 to 20-, 5 to 10-, 5 to 9-, 5 to 8-, 5 to 7-, 5 to 6-, 6 to 1000-, 6 to 900-, 6 to 800-, 6 to 700-, 6 to 600-, 6 to 500-, 6 to 400-, 6 to 300-, 6 to 200-, 6 to 100-, 6 to 90-, 6 to 80-, 6 to 70-, 6 to 60-, 6 to 50-, 6 to 40-, 6 to 30-, 6 to 20-, 6 to 10-, 6 to 9-, 6 to 8-, 6 to 7-, 7 to 1000-, 7 to 900-, 7 to 800-, 7 to 700-, 7 to 600-, 7 to 500-, 7 to 400-, 7 to 300-, 7 to 200-, 7 to 100-, 7 to 90-, 7 to 80-, 7 to 70-, 7 to 60-, 7 to 50-, 7 to 40-
30 -, 7 to 30-, 7 to 20-, 7 to 10-, 7 to 9-, 7 to 8-, 8 to 1000-, 8 to 900-, 8 to 800-, 8 to 700-, 8 to 600-, 8 to 500-, 8 to 400-, 8 to 300-, 8 to 200-, 8 to 100-, 8 to 90-, 8 to 80-, 8 to 70-, 8 to 60-, 8 to 50-, 8 to 40-, 8 to 30-, 8 to 20-, 8 to 10-, 8 to 9-, 9 to 1000-, 9 to 900-, 9 to 800-, 9 to 700-, 9 to 600-, 9 to 500-, 9 to 400-, 9 to 300-, 9 to 200-, 9 to 100-, 9 to 90-, 9 to 80-, 9 to 70-, 9 to 60-, 9 to 50-, 9 to 40-, 9 to 30-, 9 to 20-, 9 to 10-, 10 to 1000-, 10 to 900-, 10 to 800-, 10 to

700-, 10 to 600-, 10 to 500-, 10 to 400-, 10 to 300-, 10 to 200-, 10 to 100-, 10 to 90-, 10 to 80-, 10 to 70-, 10 to 60-, 10 to 50-, 10 to 40-, 10 to 30-, 10 to 20-, 20 to 1000-, 20 to 900-, 20 to 800-, 20 to 700-, 20 to 600-, 20 to 500-, 20 to 400-, 20 to 300-, 20 to 200-, 20 to 100-, 20 to 90-, 20 to 80-, 20 to 70-, 20 to 60-, 20 to 50-, 20 to 40-, 20 to 30-, 30 to 1000-, 30 to 900-, 30 to 800-, 30 to 700-, 30 to 600-, 30 to 500-, 30 to 400-, 30 to 300-, 30 to 200-, 30 to 100-, 30 to 90-, 30 to 80-, 30 to 70-, 30 to 60-, 30 to 50-, 30 to 40-, 40 to 1000-, 40 to 900-, 40 to 800-, 40 to 700-, 40 to 600-, 40 to 500-, 40 to 400-, 40 to 300-, 40 to 200-, 40 to 100-, 40 to 90-, 40 to 80-, 40 to 70-, 40 to 60-, 40 to 50-, 50 to 1000-, 50 to 900-, 50 to 800-, 50 to 700-, 50 to 600-, 50 to 500-, 50 to 400-, 50 to 300-, 50 to 200-, 50 to 100-, 50 to 90-, 50 to 80-, 50 to 70-, 50 to 60-, 60 to 1000-, 60 to 900-, 60 to 800-, 60 to 700-, 60 to 600-, 60 to 500-, 60 to 400-, 60 to 300-, 60 to 200-, 60 to 100-, 60 to 90-, 60 to 80-, 60 to 70-, 70 to 1000-, 70 to 900-, 70 to 800-, 70 to 700-, 70 to 600-, 70 to 500-, 70 to 400-, 70 to 300-, 70 to 200-, 70 to 100-, 70 to 90-, 70 to 80-, 80 to 1000-, 80 to 900-, 80 to 800-, 80 to 700-, 80 to 600-, 80 to 500-, 80 to 400-, 80 to 300-, 80 to 200-, 80 to 100-, 80 to 90-, 90 to 1000-, 90 to 900-, 90 to 800-, 90 to 700-, 90 to 600-, 90 to 500-, 90 to 400-, 90 to 300-, 90 to 200-, 90 to 100-, 100 to 1000-, 100 to 900-, 100 to 800-, 100 to 700-, 100 to 600-, 100 to 500-, 100 to 400-, 100 to 300-, 100 to 200-, 200 to 1000-, 200 to 900-, 200 to 800-, 200 to 700-, 200 to 600-, 200 to 500-, 200 to 400-, 200 to 300-, 300 to 1000-, 300 to 900-, 300 to 800-, 300 to 700-, 300 to 600-, 300 to 500-, 300 to 400-, 400 to 1000-, 400 to 900-, 400 to 800-, 400 to 700-, 400 to 600-, 400 to 500-, 500 to 1000-, 500 to 900-, 500 to 800-, 500 to 700-, 500 to 600-, 600 to 1000-, 600 to 900-, 600 to 800-, 600 to 700-, 700 to 1000-, 700 to 900-, 700 to 800-, 800 to 1000-, 800 to 900-, or 900 to 1000-fold reduction in the standard of care dose of a recombinant RSV protein vaccine. In some embodiments, such as the foregoing, the anti-RSV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-RSV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified RSV protein vaccine, or a live attenuated or inactivated RSV vaccine, or a RSV VLP vaccine. In some embodiments, the effective amount is a dose equivalent to (or equivalent to an at least) 2-, 3 -,4 -,5 -,6-, 7-, 8-, 9-, 10-, 20-, 30-, 40-, 50-, 60-, 70-, 80-, 90-, 100-, 110-, 120-, 130-, 140-, 150-, 160-, 170-, 1280-, 190-, 200-, 210-, 220-, 230-, 240-, 250-, 260-, 270-, 280-, 290-, 300-, 310-, 320-, 330-, 340-, 350-, 360-, 370-, 380-, 390-, 400-, 410-, 420-, 430-, 440-, 450-, 4360-, 470-, 480-, 490-, 500-, 510-, 520-, 530-, 540-, 550-, 560-, 5760-, 580-, 590-, 600-, 610-, 620-, 630-, 640-, 650-, 660-, 670-, 680-, 690-, 700-, 710-, 720-, 730-, 740-, 750-, 760-, 770-, 780-, 790-, 800-, 810-, 820--, 830-, 840-, 850-, 860-, 870-, 880-, 890-, 900-, 910-, 920-, 930-, 940-, 950-, 960-, 970-, 980-,

990-, or 1000-fold reduction in the standard of care dose of a recombinant RSV protein vaccine. In some embodiments, such as the foregoing, an anti-RSV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-RSV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified RSV protein vaccine, or a live attenuated or inactivated RSV vaccine, or a RSV VLP vaccine.

In some embodiments, the effective amount of a RSV RNA (*e.g.*, mRNA) vaccine is a total dose of 50-1000 μ g. In some embodiments, the effective amount of a RSV RNA (*e.g.*, mRNA) vaccine is a total dose of 50-1000, 50- 900, 50-800, 50-700, 50-600, 50-500, 50-400, 50-300, 50-200, 50-100, 50-90, 50-80, 50-70, 50-60, 60-1000, 60- 900, 60-800, 60-700, 60-600, 60-500, 60-400, 60-300, 60-200, 60-100, 60-90, 60-80, 60-70, 70-1000, 70- 900, 70-800, 70-700, 70-600, 70-500, 70-400, 70-300, 70-200, 70-100, 70-90, 70-80, 80-1000, 80-900, 80-800, 80-700, 80-600, 80-500, 80-400, 80-300, 80-200, 80-100, 80-90, 90-1000, 90-900, 90-800, 90-700, 90-600, 90-500, 90-400, 90-300, 90-200, 90-100, 100-1000, 100- 900, 100-800, 100-700, 100-600, 100-500, 100-400, 100-300, 100-200, 200-1000, 200-900, 200-800, 200-700, 200-600, 200-500, 200-400, 200-300, 300-1000, 300-900, 300-800, 300-700, 300-600, 300-500, 300-400, 400-1000, 400-900, 400-800, 400-700, 400-600, 400-500, 500-1000, 500-900, 500-800, 500-700, 500-600, 600-1000, 600-900, 600-800, 600-700, 700-1000, 700-900, 700-800, 800-1000, 800-900, or 900-1000 μ g. In some embodiments, the effective amount of a RSV RNA (*e.g.*, mRNA) vaccine is a total dose of 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 μ g. In some embodiments, the effective amount is a dose of 25-500 μ g administered to the subject a total of two times. In some embodiments, the effective amount of a RSV RNA (*e.g.*, mRNA) vaccine is a dose of 25-500, 25-400, 25-300, 25-200, 25-100, 25-50, 50-500, 50-400, 50-300, 50-200, 50-100, 100-500, 100-400, 100-300, 100-200, 150-500, 150-400, 150-300, 150-200, 200-500, 200-400, 200-300, 250-500, 250-400, 250-300, 300-500, 300-400, 350-500, 350-400, 400-500 or 450-500 μ g administered to the subject a total of two times. In some embodiments, the effective amount of a RSV RNA (*e.g.*, mRNA) vaccine is a total dose of 25, 50, 100, 150, 200, 250, 300, 350, 400, 450, or 500 μ g administered to the subject a total of two times.

Additional Embodiments

1. A respiratory syncytial virus (RSV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap, an open reading frame encoding at least one RSV antigenic polypeptide, and a 3' polyA tail.

2. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 257.
3. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 258.
4. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 259.
5. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 278.
6. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 279.
7. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 280.
8. The vaccine of any one of paragraphs 1-7, wherein the 5' terminal cap is or comprises 7mG(5')ppp(5')NlmpNp.
9. The vaccine of any one of paragraphs 1-8, wherein 100% of the uracil in the open reading frame is modified to include N1-methyl pseudouridine at the 5-position of the uracil.
10. The vaccine of any one of paragraphs 1-9, wherein the vaccine is formulated in a lipid nanoparticle comprising: DLin-MC3-DMA; cholesterol; 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC); and polyethylene glycol (PEG)2000-DMG.
11. The vaccine of paragraph 10, wherein the lipid nanoparticle further comprises trisodium citrate buffer, sucrose and water.
12. A respiratory syncytial virus (RSV) vaccine, comprising:
at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 278 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 278 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide, optionally wherein the vaccine is formulated in a lipid nanoparticle comprising DLin-MC3-DMA, cholesterol, 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), and polyethylene glycol (PEG)2000-DMG.
13. A respiratory syncytial virus (RSV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 279 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 279 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide, optionally

wherein the vaccine is formulated in a lipid nanoparticle comprising DLin-MC3-DMA, cholesterol, 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), and polyethylene glycol (PEG)2000-DMG.

14. A respiratory syncytial virus (RSV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 280 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 280 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide, optionally wherein the vaccine is formulated in a lipid nanoparticle comprising DLin-MC3-DMA, cholesterol, 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), and polyethylene glycol (PEG)2000-DMG.

15. A respiratory syncytial virus (RSV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap, an open reading frame encoding at least one RSV antigenic polypeptide, and a 3' polyA tail.

16. The vaccine of paragraph 15, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 5.

17. The vaccine of paragraph 15, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 262.

18. The vaccine of paragraph 15, wherein the at least one RSV antigenic polypeptide comprises a sequence identified by SEQ ID NO: 6.

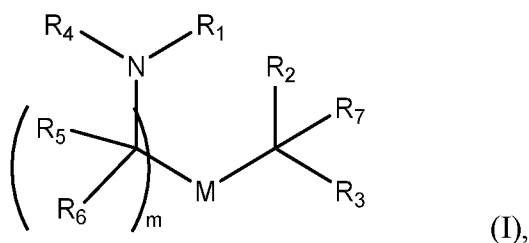
19. The vaccine of paragraph 15, wherein the at least one RSV antigenic polypeptide comprises a sequence identified by SEQ ID NO: 290.

20. The vaccine of paragraph 15, wherein the mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 7.

21. The vaccine of paragraph 15, wherein the mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 263.

22. The vaccine of paragraph 15, wherein the at least one RSV antigenic polypeptide comprises a sequence identified by SEQ ID NO: 8.

23. The vaccine of paragraph 15, wherein the at least one RSV antigenic polypeptide comprises a sequence identified by SEQ ID NO: 291.
24. The vaccine of any one of paragraphs 15-23, wherein the 5' terminal cap is or comprises 7mG(5')ppp(5')NlmpNp.
- 5 25. The vaccine of any one of paragraphs 15-24, wherein 100% of the uracil in the open reading frame is modified to include N1-methyl pseudouridine at the 5-position of the uracil.
26. The vaccine of any one of paragraphs 15-25, wherein the vaccine is formulated in a lipid nanoparticle comprising: DLin-MC3-DMA; cholesterol; 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC); and polyethylene glycol (PEG)2000-DMG.
- 10 27. The vaccine of paragraph 26, wherein the lipid nanoparticle further comprises trisodium citrate buffer, sucrose and water.
28. A respiratory syncytial virus (RSV) vaccine, comprising:
at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 262, and a 3' polyA tail,
15 wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 262 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide, optionally wherein the vaccine is formulated in a lipid nanoparticle comprising DLin-MC3-DMA, cholesterol, 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), and polyethylene glycol (PEG)2000-DMG.
- 20 29. A respiratory syncytial virus (RSV) vaccine, comprising:
at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 263, and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 263 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide, optionally
25 wherein the vaccine is formulated in a lipid nanoparticle comprising DLin-MC3-DMA, cholesterol, 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), and polyethylene glycol (PEG)2000-DMG.
30. The vaccine of any one of paragraphs 1-29 formulated in a lipid nanoparticle comprising at least one cationic lipid selected from compounds of Formula (I):



or a salt or isomer thereof, wherein:

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

- 5 R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a carbocycle,

- 10 heterocycle, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -N(R)₂, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R,
- 15 -C(O)N(R)OR, and -C(R)N(R)₂C(O)OR, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

- M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group,
- 20 and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R,

- 25 -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

- 30 each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13.

31. The vaccine of paragraph 29, wherein a subset of compounds of Formula (I) includes those in which when R₄ is -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, or -CQ(R)₂, then (i) Q is not -N(R)₂ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

32. The vaccine of paragraph 19, wherein a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms selected from N, O, and S which is substituted with one or more substituents selected from oxo (=O), OH, amino, mono- or di-alkylamino, and C₁₋₃ alkyl, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-,

-C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R,

-S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl,

-R*YR'', -YR'', and H;

5 each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

10 or salts or isomers thereof.

33. The vaccine of paragraph 29, wherein a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and

-R''M'R';

15 R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a

20 5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂,
25 -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a 5- to 14-membered heterocycle and (i) R₄ is -(CH₂)_nQ in which n is 1 or 2, or (ii) R₄ is -(CH₂)_nCHQR in which n is 1, or (iii) R₄ is -CHQR, and -CQ(R)₂, then Q is either a 5- to 14-membered heteroaryl or 8- to 14-membered heterocycloalkyl;

30 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

5 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

10 each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

34. The vaccine of paragraph 29, wherein a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5;

30 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

5 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

10 each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

35. The vaccine of paragraph 29, wherein a subset of compounds of Formula (I) includes
15 those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₂₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are
20 attached, form a heterocycle or carbocycle;

R₄ is -(CH₂)_nQ or -(CH₂)_nCHQR, where Q is -N(R)₂, and n is selected from 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group,
25 and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl,
30 -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₁₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

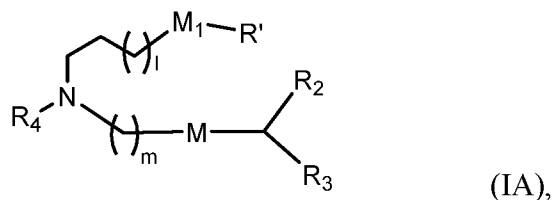
each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
or salts or isomers thereof.

36. The vaccine of paragraph 29, wherein a subset of compounds of Formula (I) includes those in which

- 5 R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, $-R^*YR''$, $-YR''$, and $-R''M'R'$;
 R_2 and R_3 are independently selected from the group consisting of C_{1-14} alkyl, C_{2-14} alkenyl, $-R^*YR''$, $-YR''$, and $-R^*OR''$, or R_2 and R_3 , together with the atom to which they are attached, form a heterocycle or carbocycle;
- 10 R_4 is selected from the group consisting of $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, $-CHQR$, and $-CQ(R)_2$, where Q is $-N(R)_2$, and n is selected from 1, 2, 3, 4, and 5;
each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 M and M' are independently selected from $-C(O)O-$, $-OC(O)-$, $-C(O)N(R')-$, $-N(R')C(O)-$,
15 $-C(O)-$, $-C(S)-$, $-C(S)S-$, $-SC(S)-$, $-CH(OH)-$, $-P(O)(OR')O-$, $-S(O)_2-$, $-S-S-$, an aryl group, and a heteroaryl group;
 R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl,
20 $-R^*YR''$, $-YR''$, and H;
each R'' is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
each R^* is independently selected from the group consisting of C_{1-12} alkyl and C_{1-12} alkenyl;
each Y is independently a C_{3-6} carbocycle;
each X is independently selected from the group consisting of F, Cl, Br, and I; and
25 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
or salts or isomers thereof.

37. The vaccine of paragraph 29, wherein a subset of compounds of Formula (I) includes those of Formula (IA):



- 30 or a salt or isomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6, 7, 8, and 9; M_1 is a bond or M' ; R_4 is unsubstituted C_{1-3} alkyl, or $-(CH_2)_nQ$, in which Q is

OH, -NHC(S)N(R)₂, -NHC(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)R₈,
-NHC(=NR₉)N(R)₂, -NHC(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, heteroaryl or
heterocycloalkyl; M and M' are independently selected from -C(O)O-, -OC(O)-,
-C(O)N(R')-, -P(O)(OR')O-, -S-S-, an aryl group, and a heteroaryl group; and R₂ and R₃ are
5 independently selected from the group consisting of H, C₁₋₁₄ alkyl, and C₂₋₁₄ alkenyl.

This invention is not limited in its application to the details of construction and the
arrangement of components set forth in the following description or illustrated in the
drawings. The invention is capable of other embodiments and of being practiced or of being
carried out in various ways. Also, the phraseology and terminology used herein is for the
10 purpose of description and should not be regarded as limiting. The use of "including,"
"comprising," or "having," "containing," "involving," and variations thereof herein, is meant
to encompass the items listed thereafter and equivalents thereof as well as additional items.

EXAMPLES

Example 1: Manufacture of Polynucleotides

According to the present disclosure, the manufacture of polynucleotides and/or parts
or regions thereof may be accomplished utilizing the methods taught in International
Publication WO2014/152027, entitled "Manufacturing Methods for Production of RNA
Transcripts," the contents of which is incorporated herein by reference in its entirety.

20 Purification methods may include those taught in International Publication
WO2014/152030 and International Publication WO2014/152031, each of which is
incorporated herein by reference in its entirety.

Detection and characterization methods of the polynucleotides may be performed as
taught in International Publication WO2014/144039, which is incorporated herein by
25 reference in its entirety.

Characterization of the polynucleotides of the disclosure may be accomplished using
polynucleotide mapping, reverse transcriptase sequencing, charge distribution analysis,
detection of RNA impurities, or any combination of two or more of the foregoing.
"Characterizing" comprises determining the RNA transcript sequence, determining the purity
30 of the RNA transcript, or determining the charge heterogeneity of the RNA transcript, for
example. Such methods are taught in, for example, International Publication
WO2014/144711 and International Publication WO2014/144767, the content of each of
which is incorporated herein by reference in its entirety.

Example 2: Chimeric polynucleotide synthesis

According to the present disclosure, two regions or parts of a chimeric polynucleotide may be joined or ligated using triphosphate chemistry. A first region or part of 100 nucleotides or less is chemically synthesized with a 5' monophosphate and terminal 3' desOH or blocked OH, for example. If the region is longer than 80 nucleotides, it may be synthesized as two strands for ligation.

If the first region or part is synthesized as a non-positionally modified region or part using *in vitro* transcription (IVT), conversion the 5' monophosphate with subsequent capping of the 3' terminus may follow.

Monophosphate protecting groups may be selected from any of those known in the art.

The second region or part of the chimeric polynucleotide may be synthesized using either chemical synthesis or IVT methods. IVT methods may include an RNA polymerase that can utilize a primer with a modified cap. Alternatively, a cap of up to 130 nucleotides may be chemically synthesized and coupled to the IVT region or part.

For ligation methods, ligation with DNA T4 ligase, followed by treatment with DNase should readily avoid concatenation.

The entire chimeric polynucleotide need not be manufactured with a phosphate-sugar backbone. If one of the regions or parts encodes a polypeptide, then such region or part may comprise a phosphate-sugar backbone.

Ligation is then performed using any known click chemistry, orthoclick chemistry, solulink, or other bioconjugate chemistries known to those in the art.

Synthetic route

The chimeric polynucleotide may be made using a series of starting segments. Such segments include:

- (a) a capped and protected 5' segment comprising a normal 3'OH (SEG. 1)
- (b) a 5' triphosphate segment, which may include the coding region of a polypeptide and a normal 3'OH (SEG. 2)
- (c) a 5' monophosphate segment for the 3' end of the chimeric polynucleotide (*e.g.*, the tail) comprising cordycepin or no 3'OH (SEG. 3)

After synthesis (chemical or IVT), segment 3 (SEG. 3) may be treated with cordycepin and then with pyrophosphatase to create the 5' monophosphate.

Segment 2 (SEG. 2) may then be ligated to SEG. 3 using RNA ligase. The ligated polynucleotide is then purified and treated with pyrophosphatase to cleave the diphosphate. The treated SEG.2-SEG. 3 construct may then be purified and SEG. 1 is ligated to the 5' terminus. A further purification step of the chimeric polynucleotide may be performed.

Where the chimeric polynucleotide encodes a polypeptide, the ligated or joined segments may be represented as: 5'UTR (SEG. 1), open reading frame or ORF (SEG. 2) and 3'UTR+PolyA (SEG. 3).

The yields of each step may be as much as 90-95%.

Example 3: PCR for cDNA Production

PCR procedures for the preparation of cDNA may be performed using 2x KAPA HIFI™ HotStart ReadyMix by Kapa Biosystems (Woburn, MA). This system includes 2x KAPA ReadyMix 12.5 µl; Forward Primer (10 µM) 0.75 µl; Reverse Primer (10 µM) 0.75 µl; Template cDNA 100 ng; and dH₂O diluted to 25.0 µl. The reaction conditions may be at 95 °C for 5 min. The reaction may be performed for 25 cycles of 98 °C for 20 sec, then 58 °C for 15 sec, then 72 °C for 45 sec, then 72 °C for 5 min, then 4 °C to termination.

The reaction may be cleaned up using Invitrogen's PURELINK™ PCR Micro Kit (Carlsbad, CA) per manufacturer's instructions (up to 5 µg). Larger reactions may require a cleanup using a product with a larger capacity. Following the cleanup, the cDNA may be quantified using the NANODROP™ and analyzed by agarose gel electrophoresis to confirm that the cDNA is the expected size. The cDNA may then be submitted for sequencing analysis before proceeding to the *in vitro* transcription reaction.

Example 4: In vitro Transcription (IVT)

The *in vitro* transcription reaction generates RNA polynucleotides. Such polynucleotides may comprise a region or part of the polynucleotides of the disclosure, including chemically modified RNA (e.g., mRNA) polynucleotides. The chemically modified RNA polynucleotides can be uniformly modified polynucleotides. The *in vitro* transcription reaction utilizes a custom mix of nucleotide triphosphates (NTPs). The NTPs may comprise chemically modified NTPs, or a mix of natural and chemically modified NTPs, or natural NTPs.

A typical *in vitro* transcription reaction includes the following:

- | | | |
|----|--------------------------|--------|
| 1) | Template cDNA | 1.0 µg |
| 2) | 10x transcription buffer | 2.0 µl |

(400 mM Tris-HCl pH 8.0, 190 mM
MgCl₂, 50 mM DTT, 10 mM Spermidine)

- | | | |
|-------|-------------------------------------|--------------------|
| 3) | Custom NTPs (25mM each) | 0.2 µl |
| 4) | RNase Inhibitor | 20 U |
| 5) 5) | T7 RNA polymerase | 3000 U |
| 6) | dH ₂ O | up to 20.0 µl. and |
| 7) | Incubation at 37 °C for 3 hr-5 hrs. | |

The crude IVT mix may be stored at 4 °C overnight for cleanup the next day. 1 U of RNase-free DNase may then be used to digest the original template. After 15 minutes of incubation at 37 °C, the mRNA may be purified using Ambion's MEGACLEAR™ Kit (Austin, TX) following the manufacturer's instructions. This kit can purify up to 500 µg of RNA. Following the cleanup, the RNA polynucleotide may be quantified using the NANODROP™ and analyzed by agarose gel electrophoresis to confirm the RNA polynucleotide is the proper size and that no degradation of the RNA has occurred.

Example 5: Enzymatic Capping

Capping of a RNA polynucleotide is performed as follows where the mixture includes: IVT RNA 60 µg-180µg and dH₂O up to 72 µl. The mixture is incubated at 65 °C for 5 minutes to denature RNA, and then is transferred immediately to ice.

The protocol then involves the mixing of 10x Capping Buffer (0.5 M Tris-HCl (pH 8.0), 60 mM KCl, 12.5 mM MgCl₂) (10.0 µl); 20 mM GTP (5.0 µl); 20 mM S-Adenosyl Methionine (2.5 µl); RNase Inhibitor (100 U); 2'-O-Methyltransferase (400U); Vaccinia capping enzyme (Guanylyl transferase) (40 U); dH₂O (Up to 28 µl); and incubation at 37 °C for 30 minutes for 60 µg RNA or up to 2 hours for 180 µg of RNA.

The RNA polynucleotide may then be purified using Ambion's MEGACLEAR™ Kit (Austin, TX) following the manufacturer's instructions. Following the cleanup, the RNA may be quantified using the NANODROP™ (ThermoFisher, Waltham, MA) and analyzed by agarose gel electrophoresis to confirm the RNA polynucleotide is the proper size and that no degradation of the RNA has occurred. The RNA polynucleotide product may also be sequenced by running a reverse-transcription-PCR to generate the cDNA for sequencing.

Example 6: PolyA Tailing Reaction

Without a poly-T in the cDNA, a poly-A tailing reaction must be performed before cleaning the final product. This is done by mixing capped IVT RNA (100 µl); RNase

Inhibitor (20 U); 10x Tailing Buffer (0.5 M Tris-HCl (pH 8.0), 2.5 M NaCl, 100 mM MgCl₂)(12.0 µl); 20 mM ATP (6.0 µl); Poly-A Polymerase (20 U); dH₂O up to 123.5 µl and incubation at 37 °C for 30 min. If the poly-A tail is already in the transcript, then the tailing reaction may be skipped and proceed directly to cleanup with Ambion's MEGACLEAR™ kit (Austin, TX) (up to 500 µg). Poly-A Polymerase may be a recombinant enzyme expressed in yeast.

It should be understood that the processivity or integrity of the polyA tailing reaction may not always result in an exact size polyA tail. Hence, polyA tails of approximately between 40-200 nucleotides, *e.g.*, about 40, 50, 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 150-165, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164 or 165 are within the scope of the present disclosure.

Example 7: Capping Assays

Protein Expression Assay

Polynucleotides (*e.g.*, mRNA) encoding a polypeptide, containing any of the caps taught herein, can be transfected into cells at equal concentrations. The amount of protein secreted into the culture medium can be assayed by ELISA at 6, 12, 24 and/or 36 hours post-transfection. Synthetic polynucleotides that secrete higher levels of protein into the medium correspond to a synthetic polynucleotide with a higher translationally-competent cap structure.

Purity Analysis Synthesis

RNA (*e.g.*, mRNA) polynucleotides encoding a polypeptide, containing any of the caps taught herein can be compared for purity using denaturing Agarose-Urea gel electrophoresis or HPLC analysis. RNA polynucleotides with a single, consolidated band by electrophoresis correspond to the higher purity product compared to polynucleotides with multiple bands or streaking bands. Chemically modified RNA polynucleotides with a single HPLC peak also correspond to a higher purity product. The capping reaction with a higher efficiency provides a more pure polynucleotide population.

Cytokine Analysis

RNA (*e.g.*, mRNA) polynucleotides encoding a polypeptide, containing any of the caps taught herein can be transfected into cells at multiple concentrations. The amount of pro-inflammatory cytokines, such as TNF-alpha and IFN-beta, secreted into the culture

medium can be assayed by ELISA at 6, 12, 24 and/or 36 hours post-transfection. RNA polynucleotides resulting in the secretion of higher levels of pro-inflammatory cytokines into the medium correspond to a polynucleotides containing an immune-activating cap structure.

5 *Capping Reaction Efficiency*

RNA (e.g., mRNA) polynucleotides encoding a polypeptide, containing any of the caps taught herein can be analyzed for capping reaction efficiency by LC-MS after nuclease treatment. Nuclease treatment of capped polynucleotides yield a mixture of free nucleotides and the capped 5'-5-triphosphate cap structure detectable by LC-MS. The amount of capped
10 product on the LC-MS spectra can be expressed as a percent of total polynucleotide from the reaction and correspond to capping reaction efficiency. The cap structure with a higher capping reaction efficiency has a higher amount of capped product by LC-MS.

Example 8: Agarose Gel Electrophoresis of Modified RNA or RT PCR Products

15 Individual RNA polynucleotides (200-400 ng in a 20 µl volume) or reverse transcribed PCR products (200-400 ng) may be loaded into a well on a non-denaturing 1.2% Agarose E-Gel (Invitrogen, Carlsbad, CA) and run for 12-15 minutes, according to the manufacturer protocol.

20 *Example 9: NANODROP™ Modified RNA Quantification and UV Spectral Data*

Chemically modified RNA polynucleotides in TE buffer (1 µl) are used for NANODROP™ UV absorbance readings to quantitate the yield of each polynucleotide from an chemical synthesis or *in vitro* transcription reaction.

25 *Example 10: Formulation of Modified mRNA Using Lipidoids*

RNA (e.g., mRNA) polynucleotides may be formulated for *in vitro* experiments by mixing the polynucleotides with the lipidoid at a set ratio prior to addition to cells. *In vivo* formulation may require the addition of extra ingredients to facilitate circulation throughout the body. To test the ability of these lipidoids to form particles suitable for *in vivo* work, a
30 standard formulation process used for siRNA-lipidoid formulations may be used as a starting point. After formation of the particle, polynucleotide is added and allowed to integrate with the complex. The encapsulation efficiency is determined using a standard dye exclusion assays.

Example 11: RSV RNA Vaccine

A RSV RNA (*e.g.*, mRNA) vaccine may comprise, for example, at least one RNA polynucleotide encoded by at least one of the following sequences, or by at least one fragment of the following sequences, or by derivatives and variants thereof. A RSV RNA vaccine may comprise, for example, at least one RNA (*e.g.*, mRNA) polynucleotide having at least one chemical modification, *e.g.* the RSV vaccine may comprise, for example, at least one chemically modified RNA (*e.g.*, mRNA) polynucleotide encoded by at least one of the following (DNA) sequences or by at least one fragment of the following sequences or by derivatives or variants thereof:

RSV # 1

```
ATGGAGCTGCTCATCTCAAAGCAAATGCCATCACCCTATCCTGACCGCCGTCACCTTTCTGCTTC
GCCTCCGGCCAAAATATCACCGAAGAGTTCTATCAGTCCACCTGCTCTGCCGTTTCTAAAGGTTAC
CTGTCAGCCCTTAGAACAGGGTGGTATACCTCTGTTATTACCATTGAGTTGTCCAACATTAAGAAG
AACAAGTGCAATGGCACAGACGCTAAGGTTAAGCTCATCAAGCAGGAGCTCGACAAATATAAAAA
TGCCGTCACGGAGCTGCAGTTATTGATGCAGAGCACCCAGGCGACAAACAACCGTGCACGACGCG
AGCTACCCCGATTTCATGAATAACACCTCAATAATGCAAAGAAGACAAATGTGACGCTCTCTAAG
AAGCGCAAGCGTCGCTTTCTGGGCTTTCTTCTCGGGGTTGGGAGCGCGATCGCAAGCGGCGTGGCT
GTATCAAAAAGTGCTTCATCTTGAGGGAGAAGTGAATAAAATCAAAAAGTGCTCTGCTATCTACAAA
CAAAGCCGTTGTATCACTGTCCAACGGAGTGTCGTCACGTCCAAAGTGCTAGATTTGAAGAA
TTACATCGATAAGCAGCTGCTCCCTATTGTGAACAAACAATCATGTTCCATCAGTAACATTGAAAC
AGTCATCGAGTTTCAACAGAAAAACAATAGACTGCTGGAGATTACCAGAGAATTTTCGGTTAACG
CCGGCGTGACTACCCCTGTAAGCACCTACATGTTGACAAACTCCGAACCTTTTGTCACTGATAAACG
ATATGCCTATTACTAATGATCAGAAAAAATTGATGTCCAATAATGTCCAATCGTCAGGCAACAGT
CCTACAGTATCATGTCTATTATTAAGGAGGAGGTCCTTGACATACGTGGTGCAACTGCCATTATACG
GAGTCATTGATACTCCCTGTTGGAACTCCATACAAGCCCCCTGTGCACTACTAACACTAAAGAGG
GATCAAAATATTTGTCTCACTCGGACAGATAGAGGTTGGTACTGTGATAATGCTGGCTCAGTGTCAT
TCTTTCCACAGGCTGAAACCTGCAAGGTTTCAGTCAAACAGGGTGTTTTGCGATACCATGAATTCTC
TAACCCTCCCCAGTGAGGTGAACCTGTGTAATGTGGATATATTCAACCCCAAGTATGATTGTAAGA
TCATGACCTCCAAGACGGACGTGAGTAGCAGTGTTATCACCTCCCTGGGGGCCATTGTATCCTGCT
ACGGAAAAACGAAATGTACTGCCTCGAACAAAAATAGGGGAATCATCAAAACTTTTAGTAATGGA
TGCAGTACGTATCTAATAAAGTGTTGACACAGTGTCAGTCGGCAACACACTGTATTACGTGAAT
AAGCAAGAAGGGAAGTCGCTGTATGTCAAAGGGGAGCCTATCATTAAATTTTATGACCCACTGGTT
TTCCCCAGCGATGAGTTCGACGCCAGCATTAGTCAGGTTAATGAGAAAAATCAACAGTCCTTGGCA
TTTATTCGTAAGAGTGATGAATTGCTCCATAATGTGAACGCTGGTAAATCCACTACCAACATTATG
ATAACTACCATCATCATAGTAATAATAGTAATTTTACTGTCTCTGATCGCTGTGGGCCTGTTACTGT
ATTGCAAAGCCCGCAGTACTCCTGTCACCTTATCAAAGGACCAGCTGTCTGGGATAAACAACATCG
CGTTCTCCAAT (SEQ ID NO: 1)
```

RSV # 2

```
ATGGAACTGCTCATTTTGAAGGCAAACGCTATCACGACAATACTCACTGCAGTGACCTTCTGTTTT
GCCTCAGGCCAGAACATAACCGAGGAGTTTATCAATCTACATGCAGCGCTGTATCTAAAGGCTAC
CTGAGTGCGCTCCGCACAGGATGGTACACCTCCGTGATCACCATCGAGCTCAGCAATATTAAGA
GAACAAGTGCAATGGTACCGACGCTAAAGTCAAACCTTATCAAGCAGGAACTCGACAAATATAAAA
ACGCTGTGACCGAGCTGCAGTTATTGATGCAGAGTACACCTGCCACCAATAACAGAGCTAGGAGG
GAGTTGCCTAGGTTTATGAACTACACTCTCAACAACGCGAAAAAAACCAATGTGACGCTATCCAA
GAAACGGAAGAGGAGGTTCCCTGGGGTTTCTTTTAGGGGTGGGCTCTGCCATTGCTTCCGGCGTGCC
TGTATGTAAAGTTCTCCACCTCGAGGGAGAGGTTAATAAGATTAAAGTCGGCCCTGCTGAGTACTAA
CAAAGCAGTGGTGCTGCTGAGTAACGGAGTAAGTGTGTTAACATTTAAGGTGCTGGACCTCAAGA
ATTATATTGACAAACAGTTGCTTCTATTCTAAACAAACAGAGCTGTTCAATAAGTAATATTGAAA
CTGTTATTGAGTTTCAGCAGAAGAACAACAGGCTTCTTGAGATTACACGCGAGTTTCAGTGTCAATG
CCGGCGTTACAACACCCGTGTCTACCTACATGCTGACGAATTCTGAGCTTCTCTCTCATAAACG
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ACATGCCCATACGAATGACCAAAAAAAAACTTATGTCCAACAACGTGCAGATTGTGCGACAGCAA
 TCCTATAGCATTATGTGTATCATCAAGGAAGAGGTACTCGCTTATGTTGTGCAGCTACCACTCTAT
 GGTGTGATTGACCCCCCTGTTGGAAGCTGCATACCAGTCCACTCTGCACCACTAACACAAAGGAA
 GGGAGCAATATTTGCCCTCACTCGAACCGACAGGGGGTGGTATTGCGATAATGCGGGCTCCGTGTCC
 5 TTCTTTCCACAGGCTGAAACTTGTAAAGGTACAGTCAAACCGCGTGTCTGTGATACTATGAATTCTC
 TGACTCTTCCCAGCGAGGTTAATCTCTGCAACGTGCACATTTTCAATCCTAAATATGACTGCAAGA
 TCATGACCAGCAAGACCGACGTCTCCAGCTCAGTAATCACTAGCCTAGGGGCCATTGTAAGCTGCT
 ATGGCAAAACCAAGTGTACTGCCTCTAATAAGAACAGAGGCATAATTAAACCTTTTCAAATGGC
 TGTGACTATGTGTGCAATAAGGGCGTCGACACGGTCTCAGTAGGGAATACCCTCTACTACGTTAAC
 10 AAACAGGAAGGCAAATCCCTTTATGTAAAGGGCGAGCCCATCATAAATTTCTACGACCCACTTGTG
 TTCCCCAGTGATGAATTCGATGCATCAATCTCCAGGTGAACGAAAAGATCAATCAATCCCTTGCT
 TTTATACGAAAGTCAGATGAACTCCTGCATAACGTGAATGCTGGGAAATCTACAACCAACATCATG
 ATCACTACCATCATTATTGTGATTATCGTAATTCTGCTATCCTTGATTGCTGTCGGGCTGCTTCTGT
 ACTGTAAGGCCAGATCGACGCCTGTGACCCTTTCAAAGACCAACTTAGCGGTATCAATAATATTG
 15 CCTTTAGCAAT (SEQ ID NO: 2)

A RSV vaccine may comprise, for example, at least one RNA (*e.g.*, mRNA)
 polynucleotide having an open reading frame that encodes at least one of the following
 antigenic polypeptide sequences or at least one fragment of the following sequences:

20 RSV # 1
MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKNCNG
 TDAKVLIKQELDKYKNAVTELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNTLSKKRKRFL
 GFLLVGSAIASGVAVSKVLHLEGEVNIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPV
 NKQSCSISNIETVIEFQKNNRLLLEITREFSVNAGVTTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNN
 25 VQIVRQSSYSIMSIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA
 GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLNVDFNPKYDCKIMTSKTDVSSSVITSLGAIVSC
 YGKTKCTASNKNRGIKTFNNGCDYVSNKGVDTVSVGNTRYVYVKNQEGKSLYVKGEPIINFYDPLVFPS
 DEFDAISQVNEKINQSLAFIRKSDELLHNVNAGKSTTNIMITTHIIVILLSLIAVGLLLYCKARSTPVT
 SKDQLSGINNIASFN (SEQ ID NO: 3)
 30 The underlined region represents a signal peptide sequence. The underlined regions can be
 substituted with alternative sequences that achieve the same or similar functions, or it can be
 deleted.

RSV # 2
 35 MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKNCNG
 TDAKVLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKKTNTLSKKRKRFL
 GFLLVGSAIASGVAVCKVLHLEGEVNIKSALLSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPIL
 NKQSCSISNIETVIEFQKNNRLLLEITREFSVNAGVTTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNN
 40 VQIVRQSSYSIMCIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA
 GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLNVDFNPKYDCKIMTSKTDVSSSVITSLGAIVSC
 YGKTKCTASNKNRGIKTFNNGCDYVSNKGVDTVSVGNTRYVYVKNQEGKSLYVKGEPIINFYDPLVFPS
 DEFDAISQVNEKINQSLAFIRKSDELLHNVNAGKSTTNIMITTHIIVILLSLIAVGLLLYCKARSTPVT
 SKDQLSGINNIASFN (SEQ ID NO: 4)
 45 The underlined region represents a signal peptide sequence. The underlined regions can be
 substituted with alternative sequences that achieve the same or similar functions, or it can be
 deleted.

Example 12: Mouse immunogenicity

In this example, assays were carried out to evaluate the immune response to RSV
 50 vaccine antigens delivered using an mRNA/LNP platform in comparison to protein antigens.

Female Balb/c (CRL) mice (6-8 weeks old; N= 10 mice per group) were administered
 RSV mRNA vaccines or protein vaccines. The mRNA vaccines were generated and

formulated in MC3 lipid nanoparticles. The mRNA vaccines evaluated in this study included:

MRK-1 membrane-bound RSV F protein (SEQ ID NO: 262)

MRK-4 membrane-bound DS-CAV1 (stabilized prefusion F protein) (SEQ ID NO:

5 263)

MRK-5 RSV F construct (SEQ ID NO: 264)

MRK-6 RSV F construct

MRK-7 RSV F construct (SEQ ID NO: 266)

MRK-8 RSV F construct (SEQ ID NO: 267)

10 MRK-9 membrane-bound RSV G protein (SEQ ID NO: 268)

MRK-11 truncated RSV F protein (ectodomain only); construct modified to include an Ig secretion peptide signal sequence (SEQ ID NO: 269)

MRK-12 DS-CAV1 (non-membrane bound form); modified to include an Ig secretion peptide signal sequence (SEQ ID NO: 270)

15 MRK-13: MRK-5 construct modified to include an Ig secretion peptide signal sequence (SEQ ID NO: 271)

MRK-14: MRK-6 construct modified to include an Ig secretion peptide signal sequence (SEQ ID NO: 272)

20 MRK-16: MRK-8 construct modified to include an Ig secretion peptide signal sequence (SEQ ID NO: 273)

The DNA sequences encoding the above-mentioned 12 mRNAs and related amino acid sequences are listed below.

25 MRK-1 membrane-bound RSV F protein/MRK_01_F (full length, Merck A2 strain)/SQ-030268:

ATGGAGCTGCTCATCCTCAAAGCAAATGCCATCACCACTATCCTGACCGCCGTCACCTTTCTGCTTC
 GCCTCCGGCCAAAATATCACCGAAGAGTTCTATCAGTCCACCTGCTCTGCCGTTTCTAAAGGTTAC
 CTGTCAGCCCTTAGAACAGGGTGGTATACCTCTGTTATTACCATTGAGTTGTCCAACATTAAGAAG
 30 AACAAAGTGCAATGGCACAGACGCTAAGGTTAAGCTCATCAAGCAGGAGCTCGACAAATATAAAAA
 TGCCGTCACGGAGCTGCAGTTATTGATGCAGAGCACCCAGGCGACAAACAACCGTGCACGACGCG
 AGCTACCCCGATTTCATGAACTACACCCTCAATAATGCAAAGAAGACAAATGTGACGCTCTCTAAG
 AAGCGCAAGCGTCGCTTTCTGGGCTTTCTCTCGGGGTTGGGAGCGCGATCGCAAGCGGCGTGGCT
 GTATCAAAAGTGCTTCATCTTGAGGGAGAAGTGAATAAAATCAAAAGTGCTCTGCTATCTACAAA
 35 CAAAGCCGTTGTATCACTGTCCAACGGAGTGTCCGTGCTCACGTCCAAAGTGCTAGATTTGAAGAA
 TTACATCGATAAGCAGCTGCTCCCTATTGTGAACAAACAATCATGTTCCATCAGTAACATTGAAAC
 AGTCATCGAGTTTCAACAGAAAAACAATAGACTGCTGGAGATTACCGAGAGATTTCGGTTAACG
 CCGGCGTGACTACCCCTGTAAGCACCTACATGTTGACAAACTCCGAACCTTTTGCTACTGATAAACG
 ATATGCCTATTACTAATGATCAGAAAAAATTGATGTCCAATAATGTCCAATCGTCAGGCAACAGT
 40 CCTACAGTATCATGTCTATTATTAAGGAGGAGGTCCTTGCATACGTGGTGCAACTGCCATTATACG
 GAGTCATTGATACTCCCTGTTGGAACTCCATACAAGCCCCCTGTGCACTACTAACACTAAAGAGG
 GATCAAATATTTGTCTCACTCGGACAGATAGAGGTTGGTACTGTGATAATGCTGGCTCAGTGTCAT

TCTTTCCACAGGCTGAAACCTGCAAGGTTTCAGTCAAACAGGGTGTTTTGCGATACCATGAATTCTC
 TAACCCTCCCCAGTGAGGTGAACCTGTGTAATGTGGATATATTCAACCCCAAGTATGATTGTAAGA
 TCATGACCTCCAAGACGGACGTGAGTAGCAGTGTTATCACCTCCCTGGGGGCCATTGTATCCTGCT
 ACGGAAAAACGAAATGTACTGCCTCGAACAAAAATAGGGGAATCATCAAACTTTTAGTAATGGA
 5 TCGACTACGTATCTAATAAAGGTGTTGACACAGTGTGAGTCGGCAACACACTGTATTACGTGAAT
 AAGCAAGAAGGGAAGTCGCTGTATGTCAAAGGGGAGCCTATCATTAATTTTTATGACCCACTGGTT
 TTCCCCAGCGATGAGTTCGACGCCAGCATTAGTCAGGTTAATGAGAAAATCAACCAGTCCTTGGA
 TTTATTCGTAAGAGTGATGAATTGCTCCATAATGTGAACGCTGGTAAATCCACTACCAACATTATG
 10 ATA ACTACCATCATCATAGTAATAATAGTAATTTTACTGTCTCTGATCGCTGTGGGCCTGTTACTGT
 ATTGCAAAGCCCGCAGTACTCCTGTCACCTTATCAAAGGACCAGCTGTCTGGGATAAACACATCG
 CGTTCTCCAAT (SEQ ID NO:5)

MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKKNCNG
 TDAKVLIKQELDKYKNAVTELQLLMQSTQATNNRRELPRFMNYTLNNAKKTNTLSKKRKRRL
 15 GFLGVSASIAAGVAVSKVLHLEGEVNIKSALLSTNKAVVSLNNGVSVLTSKVLDLKNYIDKQLLPV
 NKQSCSISNIETVIEFQKNNRLLLEITREFSVNAGVTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNN
 VQIVRQSSYSIMSIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA
 GSVSFFPQAECKVQSNRVFCDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSC
 YGKTKCTASNKNRGIKTFNSGCDYVSNKGVDTVSVGNTRYVYVKNQEGKSLYVKGEPIINFYDPLVFP
 20 DEFDAISQVNEKINQSLAFIRKSDELLHNVNAGKSTTNIMITTHIIVILLSLIAVGLLLYCKARSTPVT
 LSKDQLSGINNIASFN (SEQ ID NO:6)

The underlined region represents a signal peptide sequence. The underlined regions can be substituted with alternative sequences that achieve the same or similar functions, or can be deleted, as shown below.

25 FASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKKNCNGTDAKVLIKQELDKYKNAV
 TELQLLMQSTQATNNRRELPRFMNYTLNNAKKTNTLSKKRKRRLGFLGVSASIAAGVAVSKV
 LHLEGEVNIKSALLSTNKAVVSLNNGVSVLTSKVLDLKNYIDKQLLPVNVKQSCSISNIETVIEFQK
 30 NNRLLLEITREFSVNAGVTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNNVQIVRQSSYSIMSIIKEEVL
 YVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAECKVQSNRVF
 CDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTF
 NGCDYVSNKGVDTVSVGNTRYVYVKNQEGKSLYVKGEPIINFYDPLVFPDEFDAISQVNEKINQSLAFI
 RKDELLHNVNAGKSTTNIMITTHIIVILLSLIAVGLLLYCKARSTPVTLSKDQLSGINNIASFN (SEQ
 ID NO: 290)

35 MRK-4 membrane-bound DS-CAV1 (stabilized prefusion F protein)/MRK_04_Prefusion
 F/DS-CAV1 (Full length, S155C/S290C/S190F/V207L)/SQ-030271:

40 ATGGAAGTCTCATTTTGAAGGCAAACGCTATCACGACAATACTCACTGCAGTGACCTTCTGTTTT
 GCCTCAGGCCAGAACATAACCGAGGAGTTTTATCAATCTACATGCAGCGCTGTATCTAAAGGCTAC
 CTGAGTGCGCTCCGCACAGGATGGTACACCTCCGTGATCACCATCGAGCTCAGCAATATTAAAGA
 GAACAAGTGCAATGGTACCGACGCTAAAGTCAAACCTTATCAAGCAGGAAGCTCGACAAATATAAAA
 ACGCTGTGACCGAGCTGCAGTTATTGATGCAGAGTACACCTGCCACCAATAACAGAGCTAGGAGG
 45 GAGTTGCCTAGGTTTATGAACTACACTCTCAACAACGCGAAAAAACCATGTGACGCTATCCAA
 GAAACGGAAGAGGAGGTTCTCTGGGGTTTCTTTAGGGGTGGGCTCTGCCATTGCTTCCGGCGTGGC
 TGTATGTAAAGTTCTCCACCTCGAGGGAGAGGTTAATAAGATTAAGTCGGCCCTGCTGAGTACTAA
 CAAAGCAGTGGTGTGCTGAGTAACGGAGTAAGTGTGTTAACATTTAAGGTGCTGGACCTCAAGA
 ATTATATTGACAAACAGTTGCTTCTTCTATTCTAAACAAACAGAGCTGTTCAATAAGTAATATTGAAA
 50 CTGTTATTGAGTTTCAGCAGAAGAACAACAGGCTTCTTGAGATTACACGCGAGTTCAGTGTCAATG
 CCGGCGTTACAACACCCGTGTCTACCTACATGCTGACGAATTCTGAGCTTCTCTCTCATAAACG
 ACATGCCCATTAACGAATGACCAAAAAAACTTATGTCCAACAACGTGCAGATTGTGCGACAGCAA
 TCCTATAGCATTATGTGTATCATCAAGGAAGAGGTACTCGCTTATGTTGTGCAGCTACCACTCTAT
 GGTGTGATTGACACCCCTGTTGGAAGCTGCATACCAGTCCACTCTGCACCACTAACACAAAGGAA
 55 GGGAGCAATATTTGCCCTCACTCGAACCGACAGGGGGTGGTATTGCGATAATGCGGGCTCCGTGTCC
 TTCTTTCCACAGGCTGAAACTTGTAAGGTACAGTCAAACCGCGTGTCTGTGATACTATGAATTCTC
 TGACTCTTCCCAGCGAGGTTAATCTCTGCAACGTCGACATTTTCAATCCTAAATATGACTGCAAGA
 TCATGACCAGCAAGACCGACGCTCTCAGCTCAGTAATCACTAGCCTAGGGGCCATTGTAAGCTGCT
 ATGGCAAAACCAAGTGTACTGCCTCTAATAAGAACAGAGGCATAATTAACCTTTTCAAATGGC
 TGTGACTATGTGTCAATAAGGGCGTCGACACGGTCTCAGTAGGGAATACCCTCTACTACGTTAAC

AAACAGGAAGGCAAATCCCTTTATGTAAAGGGCGAGCCCATCATAAATTTCTACGACCCACTTGTG
 TTCCCCAGTGATGAATTCGATGCATCAATCTCCCAGGTGAACGAAAAGATCAATCAATCCCTTGCT
 TTTATACGAAAGTCAGATGAACTCCTGCATAACGTGAATGCTGGGAAATCTACAACCAACATCATG
 ATCACTACCATCATTATTGTGATTATCGTAATTCTGCTATCCTTGATTGCTGTCGGGCTGCTTCTGT
 5 ACTGTAAGGCCAGATCGACGCCTGTGACCCTTTCAAAAGACCAACTTAGCGGTATCAATAATATTG
 CCTTTAGCAAT (SEQ ID NO:7)

MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKCNG
 TDAKVLIKQELDKYKNAVTELQLLMQSTPATNNRARRLPRFMNYTLNNAKKTNTVLSKKRKRRL
 10 GFLGVSASIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPIL
 NKQSCSISNIETVIEFQQKNRRLLEITREFSVNAGVTPPVSTYMLTNSSELLSLINDMPITNDQKKLMSNN
 VQIVRQSSYSIMCIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA
 GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNVDFNPKYDCKIMTSKTDVSSSVITSLGAIVSC
 YGKTKCTASNKNRGIKTFNNGCDYVSNKGVDTVSVGNTRYVVKQEGKSLYVKGEPIINFYDPLVFPS
 15 DEFDAISQVNEKINQSLAFIRKSDELLHNVNAGKSTTNIMITTHIIVILLSLIAVGLLLYCKARSTPVTLSKDQLSGINNIASFN (SEQ ID NO:8)

The underlined region represents a signal peptide sequence. The underlined regions can be substituted with alternative sequences that achieve the same or similar functions, or can be deleted, as shown below.

FASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVLIKQELDKYKNAV
 TELQLLMQSTPATNNRARRLPRFMNYTLNNAKKTNTVLSKKRKRRLGFLGVSASIASGVA VCKVL
 HLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNR
 LLEITREFSVNAGVTPPVSTYMLTNSSELLSLINDMPITNDQKKLMSNNVQIVRQSSYSIMCIIKEEVLAY
 25 VVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFC
 DTMNSLTLPSEVNLCNVDFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFN
 GCDYVSNKGVDTVSVGNTRYVVKQEGKSLYVKGEPIINFYDPLVFPSDEFDAISQVNEKINQSLAFIR
 KSDELLHNVNAGKSTTNIMITTHIIVILLSLIAVGLLLYCKARSTPVTLSKDQLSGINNIASFN (SEQ ID
 NO: 291)

MRK-5 RSV F Construct:

ATGGAAGTCTCATCCTTAAAGCCAACGCGATAACGACCATTCTGACCGCCGTGACCTTCTGCTTC
 GCCAGCGGCCAGAACATTACCGAAGAGTTTTACCAGAGCACGTGCTCTGCCGTGAGCAAAGGTTA
 35 TCTGAGCGCTTTAAGAACTGGCTGGTACACCAGTGTTATTACTATAGAGCTGTCAAATATTAAAAA
 GAATAAATGCAACGGGACCGATGCCAAAGTAAATTAATTAAGCAGGAATTGGACAAGTATAAG
 AATGCAGTGACAGAGTTGCAGCTCCTGATGCAGAGCACACAAGCTACAAACAATCGCGCTCGCCA
 GCAGCAACAGCGGTTTTTAGGGTTCCTGCTAGGGGTGGGGTCAGCCATTGCCTCTGGAGTGGCAGT
 GTCCAAAGTGCTGCATCTGGAAGGGGAAGTTAACAAGATAAAATCCGCACTCCTCAGCACCAATA
 40 AAGCCGTGGTCTCCCTGTCCAATGGAGTATCAGTTTTGACAAGCAAGGTGCTGGACCTGAAGAATT
 ATATAGATAAGCAGTTACTGCCAATAGTGAATAAACAGTCATGCTCAATTAGCAACATTGAGACA
 GTTATCGAATTCCAGCAGAAAAATAATAGGCTTCTGGAAATAACTCGGAATTCTCAGTAAATGCC
 GGAGTGACCACACCCGTATCGACTTATATGCTTACAACTCTGAACTGTTGTCCTTGATTAACGAT
 ATGCCAATAACAAATGACCAGAAGAAGCTAATGAGCAACAATGTGCAGATTGTAAGACAGCAGTC
 45 TTAATCAATAATGTCTATAATAAAGAGGAGGTGTTGGCATATGTGGTGCAACTGCCTCTCTATGG
 CGTGATCGATACTCCTTGCTGGAAGTTACATACATCTCCACTGTGTACAATACTAATAAGGAGGG
 TAGCAATATTTGTCTGACACGCACAGATCGGGGTTGGTATTGCGACAACGCGGGCAGTGTGAGCTT
 TTTCCCTCAGGCCGAAACCTGTAAGGTTCAATCTAATCGGGTATTTTGCACACAATGAACAGCCT
 GACCCTTCCGTCCGAAGTTAATTTGTGCAACGTCGACATCTTCAATCCTAAATATGACTGCAAAAT
 50 CATGACTTCTAAAACCGACGTATCCAGCTCAGTGATAACAAGCCTTGGGGCAATTGTAAGCTGCTA
 TGGCAAGACGAAGTGACCCGCTAGTAACAAGAACCGGGGGATTATTAAGACTTTTTTGAACGGAT
 GCGATTACGTCTCAACAAAGGCGTCGATACTGTGTCCGTGGGAAACACCCTCTACTATGTGAACA
 AGCAGGAAGGCAAAAGCCTCTACGTCAAAGGAGAGCCTATCATCAATTTCTACGACCCTCTAGTA
 TTCCCTTCAGACGAATTTGACGCATCAATTTCCCAGGTGAACGAGAAAATAAATCAAAGCTTAGCC
 55 TTTATCCGCAAGAGTGATGAGTTGCTTCACAACGTCAACGCCGGCAAATCAACCACTAAT (SEQ ID
 NO:9)

MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKNCNG
 TDAKVLIKQELDKYKNAVTELQLLMQSTPATNNRARRQQQRFLGFLGVSASIASGVA VSKVLHLE

GEVNKIKSALLSTNKAVVSLNNGVSVLTSKVLDLKNYIDKQLLPIVINKQSCSISNIETVIEFQQKNRRLLE
ITREFSVNAGVTTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIKEEVLAYVVQ
LPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTM
NSLTLPSSEVNLCNVDFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFSNGCDY
5 VSNKGVDTVSGNTLYYVKNQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDE
LLHNVNAGKSTTN

(SEQ ID NO:10)

The underlined region represents a signal peptide sequence. The underlined regions can be
substituted with alternative sequences that achieve the same or similar functions, or it can be
10 deleted, as shown below.

FASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKKNCNGTDAKVLIKQELDKYKNAV
TELQLLMQSTQATNNRARQQQRFLGFLGVGSAIASGVAVSKVLHLEGEVNKIKSALLSTNKAVVSL
SNGVSVLTSKVLDLKNYIDKQLLPIVINKQSCSISNIETVIEFQQKNRRLLEITREFSVNAGVTTTPVSTYML
15 TNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLC
TTNTKEGSNICLTRDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLPSSEVNLCNVDFNPK
YDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFSNGCDYVSNKGVDTVSGNTLYYV
NKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVNAGKSTTN (SEQ ID
NO: 292)

MRK-6 RSV F Construct:

ATGGAACCTCTTGATCCTGAAGGCTAATGCAATAACAACAATTCTGACAGCAGTCACCTTTTGCTTC
GCCAGCGGACAGAATATTACGGAGGAGTTTATCAATCTACCTGTAGTGCCGTGAGCAAGGGGTA
25 CCTGTCTGCCCTGAGGACGGGATGGTACACATCCGTGATCACCATCGAGTTGTCTAACATTAATAA
GAACAAGTGCAACGGAAGTACGCCAAGGTGAAGCTCATTAAAGCAAGAGCTCGACAAATATAAG
AATGCGGTTACAGAACTACAGCTACTAATGCAGTCCACACAGGCAACCAATAACCGAGCACGTCA
GCAGCAGCAACGCTTCCTTGGCTTCCTGCTCGGGGTTGGCTCGGCAATTGCATCCGGAGTGGCTGT
TTCCAAGGTTTTGCACCTTGAGGGAGAGGTCAATAAGATCAAGAGCGCCCTCCTGTCAACTAATAA
30 GGCCGTGGTCAGCCTTTCCAACGGTGTCTGTGTTAACCTCAAAAGTGCTCGACCTTAAAAACTA
TATCGATAAGCAGCTGCTGCCCATAGTGAACAAACAGTCCCTGTTCTATCAGTAATATCGAGACAGT
GATCGAATTCCAGCAGAAGAACAATCGTCTGCTGGAATTACAAGGGAGTTTCAGCGTAAACGCTG
GAGTCACAACCCCCGTGTCCACTTACATGCTGACCAATTCCGAGCTGCTGAGTTTGATTAATGATA
TGCCCATTAACGAACGATCAGAAGAACTGATGTGCAATAATGTTTCAGATCGTTAGGCAGCAGTCTT
35 ATAGCATCATGAGTATTATCAAAGAGGAGTCTCTCGCTATGTGGTTCAGCTGCCTCTCTACGGCG
TTATAGACACCCCATGCTGGAAGCTTCACACCTCTCTCTGTGTACGACCAATACAAAGGAGGGCT
CAAACATTTGCCTTACCCGCACAGATAGAGGATGGTACTGCGATAATGCTGGCTCTGTGTCTTTCT
TTCCTCAGGCCGAAACATGTAAGGTACAGTCCAATAGGGTATTTTGCACACCATGAACTCCCTAA
CCTTACCAAGTGAAGTGAACCTCTGCAATGTGGACATCTTTAACCCGAAGTATGACTGCAAAATCA
40 TGACTTCCAAGACAGACGTGTCCAGTAGTGTGATTACCTACTGGGCGCAATCGTTTCATGCTATG
GGAAGACAAAGTGCACCGCAAGCAACAAGAATCGGGGCATCATCAAAACCTTCAGTAACGGTTGT
GACTATGTTTCAAACAAGGGAGTGCATACCGTGTGCGGTGGGCAATACTCTTTACTACGTGAATAAA
CAGGAGGGGAAATCACTGTATGTGAAAGGTGAGCCGATCATTAACTTTTACGACCCTCTCGTGTTT
CCCTCCGATGAGTTCGACGCATCCATCAGTCAGGTCAATGAGAAAATCAACCAATCTCTCGCCTTC
45 ATTAGAAAACTGACGAATTACTGAGTGCCATTGGAGGATATATTCCGGAGGCTCCAGGGACGG
GCAGGCTTACGTCCGAAAGGATGGAGAATGGGTCCTACTGAGCACATTTCTA (SEQ ID NO:11)

The underlined region represents a sequence coding for foldon. The underlined region can be
substituted with alternative sequences which achieve a same or similar function, or can be
deleted.

MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKKNCNG
TDAKVLIKQELDKYKNAVTELQLLMQSTQATNNRARQQQRFLGFLGVGSAIASGVAVSKVLHLE
GEVNKIKSALLSTNKAVVSLNNGVSVLTSKVLDLKNYIDKQLLPIVINKQSCSISNIETVIEFQQKNRRLLE
ITREFSVNAGVTTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIKEEVLAYVVQ
55 LPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTM
NSLTLPSSEVNLCNVDFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFSNGCDY
VSNKGVDTVSGNTLYYVKNQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDE
LLSAIGGYIPEAPRDGQAYVRKDGWVLLSTFL (SEQ ID NO:12)

The first underlined region represents a signal peptide sequence. The first underlined regions can be substituted with alternative sequences that achieve the same or similar functions, or it can be deleted, as shown below. The second underlined region represents a foldon. The second underlined region can be substituted with alternative sequences which achieve a same or similar function.

FASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKNCNGTDAKVLIKQELDKYKNAV
TELQLLMQSTPATNNRARQQQRFLGLLVGSAIASGVAVSKVLHLEGEVNIKSALLSTNKAVVSL
SNGVSVLTSKVLDLKNYIDKQLLPVINKQSCSISNIETVIEFQQKNRRLLEITREFSVNAGVTPVSTYML
TNSSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLC
TTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNVDIFNPK
YDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFSNGCDYVSNKGVDTVSVGNTLYYV
NKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELL (SEQ ID NO: 293)

MRK-7 RSV F Construct:

ATGGAGCTCCTGATCTTGAAGGCGAATGCCATTACCACCATCCTCACCGCAGTAACTTTCTGTTTC
GCAAGTGGCCAGAATATAACAGAAGAGTTCTATCAGTCAACCTGTAGCGCAGTCTCAAAGGGGTA
TTTATCAGCACTGAGAACCGGTTGGTATACAGTGTTATTACAATAGAGCTGAGTAACATAAAGGA
GAATAAGTGCAACGGCACTGACGCCAAGGTCAAGCTCATCAAACAGGAACCTCGATAAATACAAGA
ACGCTGTCACTGAACTGCAGCTGCTGATGCAAAGCACCCCGCCACCAACAATAGGGCCCGCAGA
GAGCTTCCTAGATTTATGAACTACACTCTGAACAACGCCAAAAAGACCAATGTAACTGTCAAA
GAAACAGAAACAGCAGGCTATTGCAAGCGGTGTGGCTGTGTCTAAAGTGCTGCATCTCGAGGGGG
AGGTCAACAAGATCAAATCCGCATTGCTCAGCACCAACAAGGCTGTGGTGAGCCTGTCCAATGGT
GTCTCAGTGCTCACCAGCAAAGTGCTGGACCTGAAGAATTATATTGATAAGCAGCTGCTACCCATA
GTCAACAAACAGTCATGCTCCATATCTAATATTGAGACTGTCATCGAGTTCCAACAGAAGAACAAT
CGCCTGCTGGAGATTACCAGGGAGTTCTCAGTCAATGCCGGGGTACAGACACCCGTTAGTACTTAT
ATGCTTACCAACTCCGAGCTTCTCTCTTTGATCAATGACATGCCAATTACTAACGACCAGAAGAAG
TTGATGTCTAACAATGTACAGATCGTTCGCCAGCAGTCTATTCCATTATGTCGATTATTAAAGAG
GAGGTTCTTGCATACGTCGTGCAGTTGCCATTATATGGAGTCATCGACACCCCTGCTGGAACTG
CATACGTCACCATTATGCACCACGAATACAAAGGAGGGCAGTAATATTTGTCTTACACGGACTGAT
CGAGGCTGGTATTGTGATAACGCAGGCTCGGTGTCATTCTTTCCACAGGCTGAAACCTGTAAGGTG
CAATCTAATAGGGTGTTTTGCGATACCATGAATTCTCTGACTCTGCCCAGTGAGGTCAATTTGTGTA
ACGTGGACATCTTCAACCCAAAGTACGACTGCAAGATCATGACATCTAAGACAGATGTGTCATCC
AGCGTTATCACGAGCCTCGGCGCTATAGTCTCCTGTTACGGCAAGACCAAGTGCACCGCTAGCAAC
AAGAATCGGGGAATCATCAAAACCTTTTCTAACGGTTGTGACTACGTGAGCAACAAGGGGGTGA
TACCGTCTCAGTCGGTAACACCCTGTACTACGTGAATAAACAGGAGGGGAAGTCATTGTACGTGA
AGGGTGAACCTATCATCAACTTTTATGACCCCTCGTCTTCCCATCAGACGAGTTTGACGCGTCCAT
CTCTCAGGTGAATGAGAAGATTAACAGAGCCTGGCTTTTATCCGCAAATCAGACGAACTACTGCA
CAATGTCAACGCTGGCAAGAGCACAAACAATATAATGATAACAACCATCATCATCGTCATTATTGT
GATCTTGTTATCACTGATCGCTGTGGGGCTCCTCCTTTATTGCAAGGCTCGTAGCACCCCTGTCACC
CTCAGTAAAGATCAGCTGTCAGGGATCAATAATATCGCGTTTAGCAAC (SEQ ID NO:13)

MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKNCNG
TDAKVLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKKTNTVLSKKQKQQA
ASGVAVSKVLHLEGEVNIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPVINKQSCSISNIE
TVIEFQQKNRRLLEITREFSVNAGVTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSI
MSIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAET
CKVQSNRVFCDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASN
KNRGIKTFSNGCDYVSNKGVDTVSVGNTLYYVKNQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQV
NEKINQSLAFIRKSDELLHNVNAGKSTTNIMITTHIIVILLSLIAVGLLLYCKARSTPVTLSKDQLSGIN
NIAFSN (SEQ ID NO:14)

The underlined region represents a signal peptide sequence. The underlined regions can be substituted with alternative sequences that achieve the same or similar functions, or it can be deleted, as shown below.

FASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKNCNGTDAKVLIKQELDKYKNAV
TELQLLMQSTPATNNRARRELPRFMNYTLNNAKKTNTVLSKKQKQQAASGVAVSKVLHLEGEVNI

KSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPVINKQSCSISNIETVIEFQQKNNRLLLEITREFS
VNAGVTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYVVLPLYG
VIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLP
SEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFSNGCDYVSNKG
5 VDTVSVGNTLYYVKNQEGKSLYVKGEPIINFYDPLVFPSEDFDASISQVNEKINQSLAFIRKSDELLHNV
NAGKSTTNIMITTHIIVHIVILLSLIAVGLLLYCKARSTPVTLSKDQLSGINNIAFSN (SEQ ID NO: 294)

MRK-8 RSV F Construct:

10 ATGGAATTATTAATTTTGAAGACAAATGCTATAACCGCGATACTAGCGGCTGTGACTCTTTGTTTC
GCATCAAGCCAGAATATTACAGAAGAATTTTATCAATCCACCTGCAGCGCTGTATCGAAAGGTTAC
CTCAGCGCGCTTAGGACAGGATGGTATACCTCCGTTATCACGATTGAAGTCTGAGTAATATCAAGGAA
AACAAAGTGTAACGGAACAGACGCCAAGGTCAAACCTTATTAACAAGAAGTGGACAAGTATAAGTC
15 TGCAGTGACCGAATTGCAGCTCCTGATGCAGAGTACCCCTGCAACTAACAACAAGTTTTTGGGCTT
TCTGCAAGGCGTGGGTAGCGCGATCGCTCCGGAATCGCGGTCTCCAAAGTGTTGCACCTGGAGG
GAGAAGTTAACAAGATCAAATCGGCTCTGTTGAGTACCAACAAGGCAGTGGTGTCACTGAGCAAC
GGTGTAAAGCGTGTTAACAAGCAAGGTATTGGACTTAAAGAACTATATTGACAAACAGCTGCTCCC
CATCGTGAACAAACAGAGCTGCTCAATCTCCAATATAGAGACGGTGATAGAGTTCCAGCAAAAAA
20 ATAATCGGCTCCTTGAGATCACCCGCGAATTCTCAGTTAATGCCGGCGTCACAACTCCGGTGTCTA
CATACATGCTGACCAACTCGGAGCTGTTATCCTTAATAAATGACATGCCCATCACCAATGATCAAA
AAAACTGATGTCAAATAACGTCCAGATAGTAAGACAGCAGAGCTACAGCATCATGTGATTATC
AAAGAGGAGGTGCTGGCGTACGTGGTGCAGCTGCCCTGTATGGGGTGATTGACACCCCTTGTGG
AAGCTGCACACCTCCCCACTATGTACTACCAATACCAAGAAGGATCCAACATCTGCCTTACCCGC
ACCGATAGGGGATGGTATTGCGACAACGCCGGATCCGTCAGCTTCTTTCCACTTGCCGAAACTTGC
25 AAGGTTCAAGTCAAACCGGGTGTTCTGCGATACAATGAATTCCTTACCTTGCCAGCGAAGTTAAT
CTCTGTAATATTGACATCTTTAACCCCAAATACGATTGCAAAATTATGACGTCAAAAACCGATGTC
AGTTCAAGCGTTATCACCAGCTTGGGTGCTATCGTTTCATGCTATGGCAAAACCAAGTGACGGCT
AGTAACAAAAACCGCGGAATAATTAAGACATTCAGCAATGGTTGCGACTACGTATCAATAAGGG
TGTCGACACCGTTTCCGTGGGCAATACGCTGTACTATGTTAATAAACAGGAAGGCAAGTCACTGTA
30 TGTTAAAGGTGAACCCATCATCAACTTCTACGACCCCTGGTTTTCCCTCCGACGAGTTTGATGCC
AGCATATCACAGGTTAATGAAAAAATAAACGGCACATTGGCGTTTATCAGAAAGTCTGACGAGAA
ACTTCATAACGTGGAAGACAAGATAGAAGAGATATTGAGCAAAATCTATCATATTGAGAACGAGA
TCGCCAGGATCAAAAAGCTTATTGGGGAG (SEQ ID NO:15)

35 The underlined region represents a region coding for GCN4. The underlined region can be substituted with alternative sequences which achieve a same or similar function.

MELLILKTNAITAILAAVTLCFASSQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKCNG
TDAKVLIKQELDKYKSAVTELQLLMQSTPATNNKFLGFLQGVGSAIASGIAVSKVLHLEGEVNIKLSA
40 LLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPVINKQSCSISNIETVIEFQQKNNRLLLEITREFSVNA
GVTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYVVLPLYGVIDT
PCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPLAETCKVQSNRVFCDTMNSLTLPSEV
NLCNIDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFSNGCDYVSNKGVD
VTVSVGNTLYYVKNQEGKSLYVKGEPIINFYDPLVFPSEDFDASISQVNEKINGTLAFIRKSDEKLHNVEDK
50 IEEILSKIYHIENEIARIKKLIGE (SEQ ID NO:16)

45 The first underlined region represents a signal peptide sequence. The underlined region can be substituted with alternative sequences that achieve the same or similar functions, or it can be deleted, as shown below. The second underlined region represents GCN4. The underlined region can be substituted with alternative sequences which achieve a same or similar function, or can be deleted.

50 FASSQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVLIKQELDKYKSAV
TELQLLMQSTPATNNKFLGFLQGVGSAIASGIAVSKVLHLEGEVNIKLSALLSTNKAVVSLSNGVSVLTS
KVLDLKNYIDKQLLPVINKQSCSISNIETVIEFQQKNNRLLLEITREFSVNAGVTPVSTYMLTNSSELLSLIN
DMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYVVLPLYGVIDTPCWKLHTSPLCTTNTKEGSNI
55 CLTRTDRGWYCDNAGSVSFFPLAETCKVQSNRVFCDTMNSLTLPSEVNLCNIDIFNPKYDCKIMTSKTD
VSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFSNGCDYVSNKGVDTVSVGNTLYYVKNQEGKSLYVK
GEPIINFYDPLVFPSEDFDASISQVNEKINGTLAFIRKSDEKLHN (SEQ ID NO: 295)

MRK-9 membrane-bound RSV G protein:

ATGTCTAAAAACAAGGACCAGCGCACTGCTAAGACGCTGGAACGCACATGGGATACCCTGAACCA
 TCTGTTATTCATTTCCAGCTGCCTCTACAAGCTAAACCTTAAAAGTGTTGCACAAATCACACTCAGC
 5 ATCCTGGCAATGATTATTTCAACATCCCTGATCATAGCCGCAATCATATTTATCGCCTCAGCAAATC
ACAAAGTTACCCCGACCACAGCCATTATCCAGGACGCTACATCCCAAATCAAAAACACCACACCT
 ACATATCTCACTCAGAACCCGACGCTGGGCATTTACCATCCAACCCTTCCGAGATCACCTCTCAAAAT
 CACCACCATTTCTCGCTCTACTACCCCGGGAGTAAAGAGCACTCTTCAGAGCACAACCGTTAAAAC
 TAAAAATACCACCACCCTCAGACTCAGCCTTCGAAACCAACGACTAAACAGCGGCAAAATAAGC
 10 CTCCATCCAAACCGAATAACGACTTTTCAATTTTGAAGTCTTTAACTTTGTGCCATGCAGTATTTGCTC
 CAATAATCTACTTGCTGGGCTATCTGCAAGAGAATCCCTAACAAGAAGCCTGGAAAGAAGACAA
 CGACAAAGCCAACTAAGAAGCCGACACTTAAGACTACCAAAAAAGACCCTAAGCCGCAGACTACC
 AAGAGCAAGGAGGTTCCCAACAACCAAGCCTACAGAGGAGCCGACTATTAACACAACAAAGACCA
 ACATCATCACCACCCTGCTTACTTCTAATACTACCGGAAACCCAGAGCTGACGTCCAGATGGAGA
 15 CGTTCCATTCCACATCTTCCGAAGGGAATCCTAGTCCAGCCAGGTGAGCACAACCTCAGAATACC
 CGTCCAGCCCTCATCACCTCCTAATACCCCCCGGCAG (SEQ ID NO:17)

The underlined region represents a region coding for transmembrane domain. The underlined region can be substituted with alternative sequences which achieve a same or similar function, or can be deleted.

MSKNKDQRTAKTLERTWDTLNLHLLFISSCLYKLNLSVAQITLSILAMIISTSLIIAIIIFIASANHKVTP
 TAIHQDATSQIKNTTPTYLTQNPQLGISPSNPSEITSQITTLASTTPGVKSTLQSTTVKTKNTTTTQTQPSK
 PTTKQRQNKPPSKPNDFHFEVFNFPVPCISNNPTCWAICKRIPNKKPGKKTITTKPTKPTLKTTKKDP
 20 KPQTTKSKEVPTTKPTEPTINTTKTNIITLLTSNTTGNPELTSQMETFHSTSSSEGNPSPSQVSTTSEYPS
 25 QPSSPNTPRQ (SEQ ID NO:18)

The underlined region represents a transmembrane domain. The underlined region can be substituted with alternative sequences which achieve a same or similar function.

MRK-11 truncated RSV F protein (ectodomain only); construct modified to include an Ig secretion peptide signal sequence:

ATGGAGACGCCTGCCAGCTGCTGTTCTGCTGTTGTTGTGGCTGCCAGATACTACTGGGTTTGCA
AGCGGACAAAACATTACCGAAGAGTTCTATCAATCCACATGCTCTGCAGTGTCTAAGGGCTACCTT
 AGTGCATTACGAACCGGGTGGTATACGAGTGTAATCACCATTGAGCTGTCCAACATCAAGAAGAA
 35 CAAGTGCAATGGGACTGATGCCAAGGTGAACTTATCAACAAGAGCTCGACAAGTATAAGAAGC
 CCGTGACCGAACTACAACCTCTGATGCAATCGACTCAGGCTACTAACAACAGAGCTCGGAGGGAG
 CTGCCCAGATTTCATGAATTATACCTTAAACAACGCTAAAAAAACAAATGTGACCCTGAGTAAGAA
 GCGGAAACGAAGGTTCTTGGGCTTCTGCTCGGTGTGGGGTCTGCAATAGCAAGCGGCGTCTGCTGT
 GTCCAAGGTCCTTCACTTAGAAGGTGAGGTCAATAAGATCAAGTCCGCTCTCCTCTCTACCAACAA
 40 GGCAGTGGTGAGCCTGTCTAACGGTGTGTCCGTGCTGACATCGAAGGTACTGGACCTGAAAACT
 ACATCGACAAGCAGCTGCTGCCTATTGTGAATAAGCAATCCTGCAGTATCTCCAACATTGAGACAG
 TGATTGAATTCAGCAAAAGAACAATCGTTTGTGGAGATAACAAGAGAATTCAGTGTTAATGCCG
 GCGTTACCACTCCCGTGTCGACATACATGCTAACAATAAGCGAGCTGCTATCTCTCATTAAATGATA
 TGCCTATCACCAATGACCAGAAAAAATATGTCCAATAACGTGCAGATAGTCAGGCAGCAGTCC
 45 TACAGCATTATGAGCATAATTAAAGAGGAAGTGTTGGCTTACGTCTGTCAGCTTCCACTGTATGGC
 GTGATCGATAACCCCTTGTGGAAGCTGCATACTTCCCCCTTTGTACAACATAACCAAAGAAGGG
 AGTAATATATGCCTCACAAGGACTGACAGAGGCTGGTACTGCGACAACGCCGGGAGCGTCAGCTT
 TTTCCCGCAGGCCGAGACATGTAAGGTGCAGAGCAACCGTGTCTTTTGCGACACCATGAATAGCCT
 GACTTTGCCAAGTGAGGTCAACCTTTGCAACGTGGATATTTTTAACCTAAGTACGATTGTAAGAT
 50 AATGACATCCAAAACCGATGTTAGTAGCTCCGTGATCACTTCGCTGGGTGCGATAGTTAGCTGCTA
 TGGAAAGACAAAGTGTAACCGAAGTAACAAGAACCGCGGGATTATTAACACATTTAGCAATGGGT
 GCGACTACGTATCAAAACAAGGGGGTGGATACAGTCAGCGTGGGAAACACACTTTACTACGTTAAC
 AAGACGAAAGGAAATCCCTTTATGTGAAGGAGAACCAATTATCAACTTTTATGATCCCCCTCGTG
 TTTCCAAGTGATGAATTCGACGCAAGCATCTCGCAGGTGAACGAGAAAATCAATCAGAGTCTAGC
 55 TTTTATAAGGAAGTCTGATGAACTGCTTAGTGCCATTGGCGGGTACATACCGGAAGCCCCACGCGA
CGGTCAGGCTTACGTGAGGAAGGACGGCGAGTGGGTTCTGCTGTCCACTTTCCTT (SEQ ID NO:19)

The first underlined region represents region coding for human Igk signal peptide, second underlined region represents region coding for foldon. The underlined regions can be

substituted with alternative sequences which achieves same or similar functions, or can be deleted.

METPAQLLFLLLLWLPDTTGFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKNCNG
 TDAKVLIKQELDKYKNAVTELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFL
 GFLLGVSASIASGVA VSKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPV
 NKQSCSISNIETVIEFQQKNRRLLEITREFSVNAGVTTTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNN
 VQIVRQQSYSIMSIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA
 GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNVDFNPKYDCKIMTSKTDVSSSVITSLGAIVSC
 YGKTKCTASNKNRGIKTFNSGCDYVSNKGVDTVSVGNTLYYVVKQEGKSLYVKGEPIINFYDPLVFPS
 DEFDAISQVNEKINQSLAFIRKSDELLSAIGGYIPEAPRDGOAYVRKDGEWVLLSTFL (SEQ ID NO:20)

The first underlined region represents human Igk signal peptide, second underlined region represents foldon. The underlined regions can be substituted with alternative sequences which achieves same or similar functions, or can be deleted, as shown below.

FASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKNCNGTDAKVLIKQELDKYKNAV
 TELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFLGFLLGVSASIASGVA VSKV
 LHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPVKNKQSCSISNIETVIEFQQKN
 NRLLEITREFSVNAGVTTTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVL
 AYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVF
 CDTMNSLTLPSEVNLCNVDFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFNS
 GCDYVSNKGVDTVSVGNTLYYVVKQEGKSLYVKGEPIINFYDPLVFPSDEFDAISQVNEKINQSLAFI
 RKSDDELL (SEQ ID NO: 296)

MRK-12 DS-CAV1 (non-membrane bound form); modified to include an Ig secretion peptide signal sequence:

ATGGAGACTCCCCGCTCAGCTGCTGTTTTTGGCTCCTCCTATGGCTGCCGGATACACCGGCTTTGCCT
CTGGACAGAACATTACCGAGGAATTCTATCAGTCGACTTGTTCCGCAGTCTCGAAGGGGTACCTGA
 GTGCCCTGCGCACCGGGTGGTACACCAGTGTTATCACTATTGAGCTGTCCAACATTAAAGAAAATA
 AGTGTAATGGAAGTACGCGAAGGTGAAGTTGATAAAACAGGAGCTGGATAAATAACAAGAAATGC
 AGTGACCGAAGTGCAGCTCCTGATGCAGTCCACTCCAGCAACAAATAATCGCGCGAGACGCGAAC
 TCCCCCGCTTTATGAACTACACTCTGAATAATGCGAAGAAAACGAATGTGACACTAAGTAAGAAA
 AGAAAACGGCGATTCTTGGGTTCTGCTCGGGGTGGGATCTGCCATAGCAAGCGGGGTGGCGGT
 ATGTAAAGTCTTCACCTAGAAGGGGAGGTGAACAAAATTAAGAGTGCCCTGCTGAGACCAACA
 AGGCTGTGGTTTCACTGTCAAACGAGTAAGCGTGCTAACATTTAAAGTCTTGGACCTGAAGAATT
 ATATTGACAAGCAGCTCCTGCCCATTCTCAACAAACAGTCATGTTCCATTAGCAACATCGAAACAG
 TCATTGAGTTTCAGCAAAAAAACAACCGCCTCCTTGAGATTACGCGTGAGTTTTCCGTCAATGCTG
 GAGTCACGACACCGGTGTCCACTTACATGCTGACTAACAGCGAACTCCTGAGCCTAATCAATGACA
 TGCCCATTAATAACGACCAGAAAAAATTGATGTCCAATAACGTGCAGATAGTGCGCCAGCAATCTT
 ACTCCATAATGTGCATTATCAAGGAGGAAGTCCTGGCGTACGTTGTTTCAGCTGCCGCTGTATGGTG
 TGATAGATACGCCATGCTGGAACTGCACACATCCCCCTTTGCACAACGAATACTAAAGAGGGA
 AGTAACATTTGCTTGACCAGAACAGATCGGGGCTGGTACTGCGACAACGCTGGTAGTGTGTCATTT
 TTCCCCCAGGCAGAAACGTGTAAAGTCCAGAGCAATCGCGTGTCTGCGACACAATGAACTCACTT
 ACTTTGCCCTCAGAGGTCAATTTGTGTAATGTGGATATCTTCAACCCGAAATACGATTGTAAGATT
 ATGACGAGCAAAACAGACGTGTCTTCATCAGTGATAACAAGTCTGGGCGCAATAGTGTGTCATGCTA
 TGGTAAGACTAAGTGCAGTGCCTCCAATAAAAAACCGCGGCATCATCAAGACATTTTCAAATGGAT
 GCGACTACGTGTCAAACAAGGGCGTCGACACAGTAAGCGTTGGGAACACCCATATACTACGTCAAC
 AAGCAGGAGGGGAAAAGCCTATACGTGAAAGGCGAGCCAATCATCAATTTCTACGATCCACTGGT
 CTTTCCAAGTGACGAATTTGATGCCAGCATATCGCAGGTGAACGAGAAAAATAAATCAGTCACTCG
 CCTTCATCAGGAAGTCAGATGAGCTGCTGTCCGCCATCGGAGGATACATTCCAGAAGCCCCACGC
GACGGCCAGGCATACGTGCGGAAGGACGGCGAATGGGTCCTTTTGAGCACTTTTCTA (SEQ ID
 NO:21)

The first underlined region represents a region coding for human Igk signal peptide, the second underlined region represents a region coding for a foldon. The underlined regions can be substituted with alternative sequences which achieves same or similar functions, or can be deleted.

METPAQLLFLLLLWLPDTTGFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKCNG
TDAKVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKKTNTVLSKKRKRRL
GFLLVGSAIASGVAVCKVLHLEGEVNIKSALLSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPIL
NKQSCSISNIETVIEFQQKNRRLLEITREFSVNAGVTTTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNN
5 VQIVRQSSYSIMCIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA
GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSC
YGKTKCTASNKNRGIKTFSNGCDYVSNKGVDTVSVGNTLYYVVKQEGKSLYVKGEPIINFYDPLVFPS
DEFDASISQVNEKINQSLAFIRKSDELLSAIGGYIPEAPRDGOAYVRKDGEWVLLSTFL (SEQ ID NO:22)

The first underlined region represents human Igk signal peptide, the second underlined region
10 represents foldon. The underlined regions can be substituted with alternative sequences which
achieves same or similar functions, or can be deleted, as shown below.

FASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAV
15 TELQLLMQSTPATNNRARRELPRFMNYTLNNAKKTNTVLSKKRKRRLGFLLVGSAIASGVAVCKVL
HLEGEVNIKSALLSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNR
LLEITREFSVNAGVTTTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNNVQIVRQSSYSIMCIIKEEVLAY
VVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVF
DTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYKTKCTASNKNRGIKTFSN
20 GCDYVSNKGVDTVSVGNTLYYVVKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIR
KSDELL (SEQ ID NO: 297)

MRK-13 MRK-5 construct modified to include an Ig secretion peptide signal sequence:

ATGGAGACTCCAGCCCAATTACTGTTCTGCTACTCCTTTGGCTGCCCCGATACTACTGGATTTCGCTT
25 CGGGTCAGAATATTACAGAGGAGTTCTACCAAAGTACTTGCTCTGCAGTCTCCAAGGGATACCTGT
CCGCTCTGCGGACGGGATGGTATACCAAGTGTATAACGATCGAGTTGAGCAACATCAAGAAGAAC
AAATGTAATGGAACAGATGCCAAGGTGAACTGATCAAACAGGAGTTGGATAAATATAAGAATGC
TGTCACCGAACTGCAGCTATTGATGCAGTCCACCCAGGCTACCAACAACCGGGCCAGGCAGCAAC
AACAGAGATTTTTGGGTTTCTTGCTGGGCGTGGGGTCTGCCATCGCTTCAGGGGTGGCCGTGAGTA
30 AAGTCCTGCACCTGGAAGGCGAAGTCAACAAGATCAAGTCTGCATTACTAAGTACCAATAAAGCT
GTAGTTAGCCTGTCCAATGGCGTGAGTGTGCTTACTTCTAAGGTACTGGACCTGAAGAATAACATC
GACAAGCAACTACTACCCATTGTAAATAAGCAGTCATGTAGCATATCAAACATCGAGACAGTGAT
CGAATTTCAACAGAAGAATAACCGGCTGTTGGAGATAACACGGGAGTTCTCTGTAAATGCCGGCG
TGACGACCCCTGTCAGCACCTACATGCTCACGAATAGCGAGTTGCTTTCCCTGATTAATGATATGC
35 CGATTACAAATGACCAGAAGAAGCTGATGAGTAATAATGTCCAAATTGTCCGTCAGCAGAGCTAT
TCGATTATGTCCATCATCAAGGAGGAAGTCTTAGCCTATGTGGTGCAGCTCCCCCTCTACGGAGTGA
TTGACACACCGTGCTGGAAGCTGCACACCTCCCCTTTGTGTACAACCAATACCAAGGAGGGCTCCA
ACATCTGCCTTACTAGGACCGACAGGGGATGGTATTGCGACAACGCCGGGTCCGTCTCATTTTTTC
CTCAGGCGGAAACCTGTAAAGTACAGTCGAATCGAGTGTTTTGTGACACTATGAACAGCCTGACCT
40 TGCCTAGCGAGGTGAATCTGTGTAACGTTGATATCTTCAACCCTAAGTATGACTGTAAGATCATGA
CTTCAAAAATGATGTCTCCTCAAGCGTGATCACCTCTTTGGGCGCCATCGTGTGTCATGCTACGGAA
AGACGAAGTGCACCGCCTCTAACAAGAACCGAGGGATCATCAAAACATTCTCCAATGGCTGTGAT
TACGTCAAGTAACAAGGTGTGGACACAGTCTCCGTGGGCAATACGTTATATTATGTGAATAAGCA
GGAGGGAAAAAGTCTCTATGTGAAGGTGAACCGATAATCAATTTCTACGATCCCTTGGTGTTC
45 AAGCGACGAGTTCGACGCCTCGATCAGCCAGGTGAACGAGAAAAATCAACCAGTCTTTGGCATTCA
TCCGCAAGAGCGACGAGCTACTGCATAACGTGAACGCAGGCAAGAGTACTACCAAT (SEQ ID
NO:23)

The underlined region represents a region coding for human Igk signal peptide. The
underlined region can be substituted with alternative sequences which achieve a same or
50 similar function, or can be deleted.

METPAQLLFLLLLWLPDTTGFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKCNG
TDAKVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKKTNTVLSKKRKRRL
GFLLVGSAIASGVAVCKVLHLEGEVNIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNRRLLE
55 ITREFSVNAGVTTTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNNVQIVRQSSYSIMCIIKEEVLAYVVQ
LPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTM
NSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYKTKCTASNKNRGIKTFSNGCDY
VSNKGVDTVSVGNTLYYVVKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDE
LLHNVNAGKSTTN (SEQ ID NO:24)

The underlined region represents human Igk signal peptide. The underlined region can be substituted with alternative sequences which achieve a same or similar function, or can be deleted, as shown below.

5 FASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKNCNGTDAKVKLIKQELDKYKNAV
TELQLLMQSTQATNNRARQQQRFLGFLGVGSAIASGVAVSKVLHLEGEVNIKSALLSTNKAVVSL
SNGVSVLTSKVLDLKNYIDKQLLPVINKQSCSISNIETVIEFQQKNRLLLEITREFSVNAGVTPVSTYML
TNSSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLC
TTNTKEGSNICLTRDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNVDIFNPK
10 YDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFSNGCDYVSNKGVDTVSVGNTLYYV
NKQEGKSLYVKGEPIINFYDPLVFPSEDFDASISQVNEKINQSLAFIRKSDELLHNVNAGKSTTN (SEQ ID
NO: 298)

MRK-14 MRK-6 construct modified to include an Ig secretion peptide signal sequence:

15 ATGGAGACTCCCGCTCAGTTGTTGTTCTGCTACTGCTGTGGCTGCCTGATACAACCGGATTTGCTA
GTGGGCAGAATATCACCGAAGAATTCTATCAGAGCACTTGCAGTGCAGTGTCCAAAGGATATTTG
AGCGCCCTGCGCACTGGGTGGTACACAAGTGTCATCACAAATCGAGCTAAGTAACATTAATAAGAACG
CAAATGCAACGGGACTGACGCAAAGGTCAAACCTATTAAGCAAGAACTTGACAAATATAAGAACG
CTGTTACAGAGTTGCAGCTGCTAATGCAAAGCACTCAGGCTACCAATAACCGAGCGAGACAGCAG
20 CAGCAACGTTTCCTGGGTTTCCTGTTAGGTGTGGGTAGCGCAATTGCCAGTGGTGTAGCCGTGTCC
AAGGTGCTGCACCTGGAAGGGGAAGTGAATAAGATCAAGTCTGCACTGCTGTCCACCAATAAGGC
GGTCGTTTCGCTGTCTAACGGCGTCTCGTCTCAACAAGTAAAGTTCTGGATTTAAAGAACTATAT
TGATAAGCAATTGCTGCCTATCGTAAATAAGCAGAGTTGCAGCATTAGCAATATCGAGACAGTGA
TAGAATTTTCAGCAAAAGAACAATCGATTACTCGAAATCACACGCGAATTCAGTGTCAATGCCGGG
25 GTTACAACCCCTGTGTGACCTACATGCTTACCAATTCGAGCTTCTGTCTCTTATTAACGATATGC
CCATACGAACGATCAGAAGAACTGATGTCAAATAACGTCCAATTTGTGCGGCAGCAAAGCTAC
AGTATCATGAGCATCATCAAAGAGGAGGTGCTCGCCTATGTGGTCCAATTGCCGCTATACGGGGTCT
ATTGATACACCCTGTTGGAAGCTCCATACATCCCCACTTTGTACAACGAATACCAAGGAGGGGTCT
AACATTTGTCTGACCCGGACCGACAGAGGCTGGTATTGCGATAATGCTGGAAGCGTTAGTTTCTTT
30 CCTCAGGCAGAAACATGCAAGGTGCAGTCAAACAGAGTTTTCTGTGACACCATGAATTCCTTGACG
CTGCCTTCAGAAGTGAATCTGTGTAACGTGGATATCTTTAATCCGAAGTACGATTGTAAAATTATG
ACTAGCAAGACAGATGTCTCGTCCTCTGTGATCACTAGCCTGGGAGCGATTGTGAGCTGTTATGGT
AAAACAAAGTGTACTGCTAGCAATAAGAACAGGGGGATTATCAAAACGTTACGTAACGGCTGTGA
TTACGTATCCAACAAGGGGGTGGACACCGTGTGAGTCGGGAACACGCTCTACTACGTGAACAAGC
35 AGGAAGGTAAGTCGCTATACGTGAAGGGGGAACCCATAATCAATTTCTACGATCCGCTCGTGTTC
CTAGCGACGAATTCGACGCATCTATCAGCCAGGTGAACGAGAAGATCAATCAGAGTCTGGCCTTC
ATCCGCAAGTCCGACGAGCTGCTTAGTGCTATCGGAGGTTATATCCCTGAGGCCCGAGGGACGG
CCAAGCGTATGTGAGAAAGGACGGGGAATGGGTACTGTTGTCAACTTTCCTA (SEQ ID NO:25)

40 The first underlined region represents a region coding for human Igk signal peptide, the
second underlined region represents a region coding for a foldon. The underlined regions can
be substituted with alternative sequences which achieves same or similar functions, or can be
deleted.

45 METPAQLLFLLLLWLPDITTFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKNCNG
TDAKVKLIKQELDKYKNAVTELQLLMQSTQATNNRARQQQRFLGFLGVGSAIASGVAVSKVLHLE
GEVNIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPVINKQSCSISNIETVIEFQQKNRLLLE
ITREFSVNAGVTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYVVQ
LPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTM
NSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFSNGCDY
50 VSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFYDPLVFPSEDFDASISQVNEKINQSLAFIRKSDE
LLSAIGGYIPEAPRDGQAYVRKDGEWVLLSTFL (SEQ ID NO:26)

The first underlined region represents human Igk signal peptide, second underlined region
represents a foldon. The underlined regions can be substituted with alternative sequences
which achieves same or similar functions, or can be deleted, as shown below.

55 FASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKNCNGTDAKVKLIKQELDKYKNAV
TELQLLMQSTQATNNRARQQQRFLGFLGVGSAIASGVAVSKVLHLEGEVNIKSALLSTNKAVVSL
SNGVSVLTSKVLDLKNYIDKQLLPVINKQSCSISNIETVIEFQQKNRLLLEITREFSVNAGVTPVSTYML

TNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLC
TTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNVDIFNPK
YDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFNSNGCDYVSNKGVDTVSVGNTLYYV
NKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELL (SEQ ID NO: 299)

5

MRK-16 MRK-8 construct modified to include an Ig secretion peptide signal sequence:

ATGGAGACACCTGCCCAACTTCTGTTCCCTTCTTTTGCTCTGGCTGCCTGACACAACCGGCTTCGCAT
CTTCACAAAACATCACGGAAGAGTTTTACCAGAGCACATGCTCCGCGGTCTCTAAAGGCTATCTTT
10 CTGCCCTGCGGACTGGCTGGTATACCAGCGTCATCACCATAGAGCTGTCAAACATCAAGGAGAAC
AAGTGTAAACGGCACTGACGCCAAGGTCAAGCTTATAAAGCAGGAAGTGGACAAGTATAAAGAGTGC
TGTTACCGAGCTCCAGTTGCTTATGCAGTCCACCCCGCAACAACAATAAATTTCTGGGCTTTCT
ACAGGGCGTCGGAAGCGCCATCGCAAGCGGCATCGCTGTGAGCAAGGTGTTGCATCTGGAGGGAG
AGGTGAATAAGATAAAGAGTGCTCTGCTTTCCACTAACAAGCCGTGGTGAGCCTGAGCAATGGC
15 GTATCTGTTCTGACTTCTAAAGTCCTGGATCTCAAGAACTATATCGACAAGCAGCTCTTGCCCATTG
TCAACAAACAGTCCTGCTCCATTTCCAATATTGAGACCGTCATTGAGTTCCAACAGAAGAATAACC
GTTTGCTGGAAATTACAAGGGAATTCAAGTGTTAATGCCGGTGTAACCACCCCTGTGAGCACCTATA
TGCTCACCAACTCTGAAGTCTGAGTCTGATTAACGATATGCCCATTAATAATGATCAGAAGAAAC
TAATGAGTAACAATGTCCAGATAGTTCCGCGAGCAGTCATATTCCATTATGAGTATAATCAAGGAGG
20 AAGTGCTAGCCTACGTAGTTCAGTCTCCCCCTCTACGGCGTTATAGACACGCCATGTTGGAAGCTGCA
TACGAGTCTCTGTGCACTACAAATACCAAGGAGGGCAGTAACATATGCTTGACTAGAAGTATA
GAGGCTGGTACTGCGACAATGCAGGCTCCGTGTCATTCTTTCTCTCGCCGAGACGTGTAAAGTGC
AGAGTAACAGAGTGTTTTGTGACACAATGAAGTCAATTGACCTGCCTAGCGAAGTGAAGTATGCA
ACATCGACATTTTAAACCAAAATACGATTGCAAGATTATGACCTCTAAGACTGACGTATCTTCAT
25 CCGTCATAACTTCTCTAGGAGCGATCGTGAGCTGCTACGGTAAGACTAAATGCACGGCTAGTAATA
AAAATAGAGGTATCATTAAAGACTTTTAGTAACGGTTGCGATTATGTGTCAAACAAGGGAGTCGAC
ACTGTTTCAGTGGGCAATACTCTCTACTACGTTAACAACAGGAGGGTAAATCCCTTTATGTGAAA
GGGGAACCCATCATTAATTTTTATGACCCACTTGTGTTTCCTAGTGACGAGTTTGACGCTTCAATCA
GTCAAGTGAACGAAAAAATTAATGGCAGCTCGCGTTTATCAGGAAAAGCGACGAGAAGCTGCAT
30 AACGTGGAAGATAAGATCGAGGAGATTCTCTCGAAAATTTATCATATAGAGAATGAAATCGCAAG
AATCAAAAAGCTTATTGGGGAG (SEQ ID NO:27)

The first underlined region represents a region coding for human Igk signal peptide, the
second underlined region represents a region coding for GCN4. The underlined regions can
be substituted with alternative sequences which achieves same or similar functions, or can be
deleted.

35

METPAQLFLLLLWLPDTTGFASSQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKCNG
TDKVKLIKQELDKYKSAVTELQLLMQSTPATNNKFLGFLQGVGSAIASGIAVSKVLHLEGEVNKIKSA
40 LLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNBKQSCSISNIETVIEFQKNNRLLLEITREFSVNA
GVTPPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYVVQLPLYGVIDT
PCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPLAETCKVQSNRVFCDTMNSLTLPSEV
NLCNIDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFNSNGCDYVSNKGVD
VSVGNTLYYVKNQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINGTLAFIRKSDEKLHN
45 VEDK
IEEILSKIYHIENEIARIKKLIGE (SEQ ID NO:28)

The first underlined region represents human Igk signal peptide, second underlined region
represents GCN4. The underlined regions can be substituted with alternative sequences which
achieves same or similar functions, or can be deleted, as shown below.

45

FASSQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKCNGTDKVKLIKQELDKYKSAV
TELQLLMQSTPATNNKFLGFLQGVGSAIASGIAVSKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTS
50 KVLDLKNYIDKQLLPIVNBKQSCSISNIETVIEFQKNNRLLLEITREFSVNAGVTPPVSTYMLTNSELLSLIN
DMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNI
CLTRTDRGWYCDNAGSVSFFPLAETCKVQSNRVFCDTMNSLTLPSEVNLCNIDIFNPKYDCKIMTSKD
VSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFNSNGCDYVSNKGVDTVSVGNTLYYVKNQEGKSLYVK
55 GEPIINFYDPLVFPSDEFDASISQVNEKINGTLAFIRKSDEKLHN (SEQ ID NO: 300).

The protein vaccine evaluated in this study was DS-CAV1 stabilized prefusion F protein (1 mg/mL), as described in McLellan *et al. Science* 342, 592 (2013). The protein was buffered in 50 mM Hepes, 300 mM NaCl and was formulated with Adju-phos.

Briefly, groups of 10 mice were immunized intramuscularly with the following

5 vaccines:

Group	N	Vaccine	Concentration (μ g/ml)	Total dose /mouse (μ g)
1	10	mF (MRK-1)	100	10
3	"	mDS-CAV1 (MRK-4)	100	10
4	"	MRK-5	100	10
5	"	MRK-6	100	10
6	"	MRK-7	100	10
7	"	MRK-8	100	10
8	"	mG (MRK-9)	100	10
9	"	IgSP_sF (MRK-11)	100	10
10	"	IgSP_sDS-CAV1 (MRK-12)	100	10
11	"	MRK-13	100	10
12	"	MRK-14	100	10
14	"	MRK-16	100	10
15	"	DS-CAV1 protein/adju phos	100	10
16	10	mF (MRK-1)	20	2
18	"	mDS-CAV1 (MRK-4)	20	2
19	"	MRK-5	20	2
20	"	MRK-6	20	2
21	"	MRK-7	20	2
22	"	MRK-8	20	2
23	"	mG (MRK-9)	20	2
24	"	IgSP_sF (MRK-11)	20	2
25	"	IgSP_sDS-CAV1 (MRK-12)	20	2
26	"	MRK-13	20	2
27	"	MRK-14	20	2
29	"	MRK-16	20	2
30	"	DS-CAV1 protein/adju phos	20	2
31	"	naïve		

The animals were immunized on day 0 and day 21 of the experiment. On days 14 and 35, blood was drawn from each animal and used for serological assays. On days 42 and 49, a subset of the animals were sacrificed and spleens were harvested to support ELISPOT and intracellular cytokine staining studies.

10

The serum neutralizing antibody titers for the mouse immunogenicity study measured post dose 1 (PD1) and post dose 2 (PD2) are shown in Fig. 1. The PD2 serum neutralizing antibody titers are also provided in tabular form below:

Description	10 µg dose	2 µg dose
mF (MRK-1)	4075	1391
mDS-CAV1 (MRK-4)	3160	846
MRK-5	600	331
MRK-6	465	178
MRK-7	2259	2168
MRK-8	2318	656
mG (MRK-9)	86	39
IgSP_sF (MRK-11)	4559	3597
IgSP_sDS-CAV1 (MRK-12)	3458	2007
MRK-13	750	269
MRK-14	471	116
MRK-16	1077	1088
DS-CAV1 protein/adju phos	692	1166
Naive		<4

The results indicated that the neutralizing antibody titers are robust and several of the mRNA vaccines, including the RSV mF vaccine and the RSVmDS-CAV1 mRNA vaccine elicited neutralizing antibody titers higher than DS-CAV1 protein/adjuv-phos vaccine.

5

B. Assays for Cellular Immune Response:

Mouse IFN- γ ELISPOT Assay Procedures

I. Preparation of Splenocytes:

10 Spleens were placed in a 60-mm tissue culture dish and palpated up and down with a syringe handle to remove the cells. Minced spleens were then transferred to 15-mL tubes, centrifuged at 1200 rpm for 10 min, resuspended in an Ammonium-Chloride-Potassium (ACK) Lysing Buffer and incubated at room temperature for 5 minutes. R10 media was added to the tubes and cells were centrifuged at 1200 rpm for 10 minutes, and then washed
15 once more with R10 media. Following a second centrifugation, the cells were resuspended in 10 mL of R10 media and filtered through a 70 µm nylon cell strainer into a 50 mL centrifuge tube. The strainer was rinsed with an additional 10 mL of media and this was added to the cells. The cells were counted on a hemocytometer and the cell concentration was normalized across the groups.

20

II. ELISPOT ASSAY:

- 1) 96-well MultiScreen-IP sterile white filtration plates were coated with MABTECH purified anti-mouse IFN- γ , clone AN18 at 10 μ g/ml PBS in Bio-Hood (1:100 dilution) and incubated at 4 °C overnight
- 2) The following morning, the plates were washed with sterile PBS and blocked with R10 medium at 37 °C for 4 hrs.
- 3) Splenocytes were added to the plate at 4 x 10⁵ cells/well, and the cells were stimulated with peptide pools for RSV-F and RSV-G. The peptide pools were as follows.

For RSV-F:

Sequence = sequence in FM	peptide ID	SEQ ID No:
MELPILKANAITTIL	RSV_F_1 – 15	29
ILKANAITTILTAVT	RSV_F_5 – 19	30
NAITTILTAVTFCFA	RSV_F_9 – 23	31
TILTAVTFCFASSQN	RSV_F_13 – 27	32
AVTFCFASSQNITEE	RSV_F_17 – 31	33
CFASSQNITEEFYQS	RSV_F_21 – 35	34
SQNITEEFYQSTCSA	RSV_F_25 – 39	35
TEEFYQSTCSAVSKG	RSV_F_29 – 43	36
YQSTCSAVSKGYLSA	RSV_F_33 – 47	37
CSAVSKGYLSALRTG	RSV_F_37 – 51	38
SKGYLSALRTGWYTS	RSV_F_41 – 55	39
LSALRTGWYTSVITI	RSV_F_45 – 59	40
RTGWYTSVITIELSN	RSV_F_49 – 63	41
YTSVITIELSNIKEN	RSV_F_53 – 67	42
ITIELSNIKENKCNG	RSV_F_57 – 71	43
LSNIKENKCNGTDAK	RSV_F_61 – 75	44
KENKCNGTDAKVCLI	RSV_F_65 – 79	45
CNGTDAKVCLIQEL	RSV_F_69 – 83	46
DAKVCLIQELDKYK	RSV_F_73 – 87	47
KLIQELDKYKNAVT	RSV_F_77 – 91	48
QELDKYKNAVTELQL	RSV_F_81 – 95	49
KYKNAVTELQLLMQS	RSV_F_85 – 99	50
AVTELQLLMQSTPAA	RSV_F_89 – 103	51
LQLLMQSTPAANNRA	RSV_F_93 – 107	52
MQSTPAANNRARREL	RSV_F_97 – 111	53
PAANNRARRELPRFM	RSV_F_101 – 115	54
NRARRELPRFMNYTL	RSV_F_105 – 119	55
RELPRFMNYTLNNAK	RSV_F_109 – 123	56
RFMNYTLNNAKKTNV	RSV_F_113 – 127	57
YTLNNAKKTNVTLISK	RSV_F_117 – 131	58
NAKKTNVTLISKRRKR	RSV_F_121 – 135	59
TNVTLISKRRKRRLG	RSV_F_125 – 139	60

Sequence = sequence in FM	peptide ID	SEQ ID No:
LSKKRKRRFLGFLLG	RSV_F_129 - 143	61
RKRRFLGFLLGVGSA	RSV_F_133 - 147	62
FLGFLLGVGSAIASG	RSV_F_137 - 151	63
LLGVGSAIASGIAVS	RSV_F_141 - 155	64
GSAIASGIAVSKVLH	RSV_F_145 - 159	65
ASGIAVSKVLHLEGE	RSV_F_149 - 163	66
AVSKVLHLEGEVNKI	RSV_F_153 - 167	67
VLHLEGEVNKIKSAL	RSV_F_157 - 171	68
EGEVNLIKSSALLSTN	RSV_F_161 - 175	69
NLIKSSALLSTNKAVV	RSV_F_165 - 179	70
SALLSTNKAVVSLSN	RSV_F_169 - 183	71
STNKAVVSLSNGVSV	RSV_F_173 - 187	72
AVVSLSNGVSVLTSK	RSV_F_177 - 191	73
LSNGVSVLTSKVLDL	RSV_F_181 - 195	74
VSVLTSKVLDLKNYI	RSV_F_185 - 199	75
TSKVLDLKNYIDKQL	RSV_F_189 - 203	76
LDLKNYIDKQLPIV	RSV_F_193 - 207	77
NYIDKQLPIV NKQS	RSV_F_197 - 211	78
KQLLPIV NKQSCSIS	RSV_F_201 - 215	79
PIV NKQSCSISNIET	RSV_F_205 - 219	80
KQSCSISNIETVIEF	RSV_F_209 - 223	81
SISNIETVIEFQQKN	RSV_F_213 - 227	82
IETVIEFQQKNRLL	RSV_F_217 - 231	83
IEFQQKNRLLLEITR	RSV_F_221 - 235	84
QKNRLLLEITREFSV	RSV_F_225 - 239	85
RLLEITREFSVNAGV	RSV_F_229 - 243	86
ITREFSVNAGVTPV	RSV_F_233 - 247	87
FSVNAGVTPVSTYM	RSV_F_237 - 251	88
AGVTPVSTYMLTNS	RSV_F_241 - 255	89
TPVSTYMLTNSSELLS	RSV_F_245 - 259	90
TYMLTNSSELLSLIND	RSV_F_249 - 263	91
TNSSELLSLINDMPIT	RSV_F_253 - 267	92
LLSLINDMPITNDQK	RSV_F_257 - 271	93
INDMPITNDQKKLMS	RSV_F_261 - 275	94
PITNDQKKLMSNNVQ	RSV_F_265 - 279	95
DQKKLMSNNVQIVRQ	RSV_F_269 - 283	96
LMSNNVQIVRQQSYS	RSV_F_273 - 287	97
NVQIVRQQSYSIMSI	RSV_F_277 - 291	98
VRQQSYSIMSIKKKE	RSV_F_281 - 295	99
SYSIMSIKKKEVLAY	RSV_F_285 - 299	100
MSIKKKEVLAYVVQL	RSV_F_289 - 303	101
KKEVLAYVVQLPLYG	RSV_F_293 - 307	102
LAYVVQLPLYGVIDT	RSV_F_297 - 311	103
VQLPLYGVIDTPCWK	RSV_F_301 - 315	104
LYGVIDTPCWKLHTS	RSV_F_305 - 319	105
IDTPCWKLHTSPLCT	RSV_F_309 - 323	106
CWKLHTSPLCTTNTK	RSV_F_313 - 327	107

Sequence = sequence in FM	peptide ID	SEQ ID No:
HTSPLCTTNTKEGSN	RSV_F_317 - 331	108
LCTTNTKEGSNICLT	RSV_F_321 - 335	109
NTKEGSNICLTRTDR	RSV_F_325 - 339	110
GSNICLTRTDRGWYC	RSV_F_329 - 343	111
CLTRTDRGWYCDNAG	RSV_F_333 - 347	112
TDRGWYCDNAGSVSF	RSV_F_337 - 351	113
WYCDNAGSVSFFPQA	RSV_F_341 - 355	114
NAGSVSFFPQAETCK	RSV_F_345 - 359	115
VSFFPQAETCKVQSN	RSV_F_349 - 363	116
PQAETCKVQSNRVFC	RSV_F_353 - 367	117
TCKVQSNRVFCDTMN	RSV_F_357 - 371	118
QSNRVFCDTMNSLTL	RSV_F_361 - 375	119
VFCDTMNSLTLPEV	RSV_F_365 - 379	120
TMNSLTLPEVNLCN	RSV_F_369 - 383	121
LTLPSEVNLCNVDIF	RSV_F_373 - 387	122
SEVNLCNVDIFNPKY	RSV_F_377 - 391	123
LCNVDIFNPKYDCKI	RSV_F_381 - 395	124
DIFNPKYDCKIMTSK	RSV_F_385 - 399	125
PKYDCKIMTSKTDVS	RSV_F_389 - 403	126
CKIMTSKTDVSSSVI	RSV_F_393 - 407	127
TSKTDVSSSVITSLG	RSV_F_397 - 411	128
DVSSSVITSLGAIVS	RSV_F_401 - 415	129
SVITSLGAIVSCYGK	RSV_F_405 - 419	130
SLGAIVSCYGKTKCT	RSV_F_409 - 423	131
IVSCYGKTKCTASNK	RSV_F_413 - 427	132
YGKTKCTASNKNRGI	RSV_F_417 - 431	133
KCTASNKNRGIKTF	RSV_F_421 - 435	134
SNKNRGIKTFSNCG	RSV_F_425 - 439	135
RGIKTFSNCGDYVS	RSV_F_429 - 443	136
KTFSNCGDYVSNKGV	RSV_F_433 - 447	137
NGCDYVSNKGVDTVS	RSV_F_437 - 451	138
YVSNKGVDTVSVGNT	RSV_F_441 - 455	139
KGVDTVSVGNTLYYV	RSV_F_445 - 459	140
TVSVGNTLYYVKNQE	RSV_F_449 - 463	141
GNTLYYVKNQEGKSL	RSV_F_453 - 467	142
YYVKNQEGKSLYVKG	RSV_F_457 - 471	143
KQEGKSLYVKGEPII	RSV_F_461 - 475	144
KSLYVKGEPIINFYD	RSV_F_465 - 479	145
VKGEPIINFYDPLVF	RSV_F_469 - 483	146
PIINFYDPLVFPSGE	RSV_F_473 - 487	147
FYDPLVFPSGEFDAS	RSV_F_477 - 491	148
LVFPSGEFDASISQV	RSV_F_481 - 495	149
SGEFDASISQVNEKI	RSV_F_485 - 499	150
DASISQVNEKINQSL	RSV_F_489 - 503	151
SQVNEKINQSLAFIR	RSV_F_493 - 507	152
EKINQSLAFIRKSDE	RSV_F_497 - 511	153
QSLAFIRKSDELLHN	RSV_F_501 - 515	154

Sequence = sequence in FM	peptide ID	SEQ ID No:
FIRKSDELLHNVNAG	RSV_F_505 - 519	155
SDELLHNVNAGKSTT	RSV_F_509 - 523	156
LHNVNAGKSTTNIMI	RSV_F_513 - 527	157
NAGKSTTNIMITAI	RSV_F_517 - 531	158
STTNIMITAIIVIV	RSV_F_521 - 535	159
IMITAIIVIVVILL	RSV_F_525 - 539	160
AIIVIVVILLSLIA	RSV_F_529 - 543	161
VIVVILLSLIAVGLL	RSV_F_533 - 547	162
ILLSLIAVGLLLYCK	RSV_F_537 - 551	163
LIAVGLLLYCKARST	RSV_F_541 - 555	164
GLLLYCKARSTPVT	RSV_F_545 - 559	165
YCKARSTPVTLSKDQ	RSV_G_549 - 563	166
RSTPVTLSKDQLSGI	RSV_F_553 - 567	167
VTLSKDQLSGINNIA	RSV_F_557 - 571	168
KDQLSGINNIAFSN	RSV_F_561 - 574	169

For RSV-G:

Sequence	peptide ID	SEQ ID No:
MSKNKDQRTAKTLER	RSV_G_1 - 15	170
KDQRTAKTLERTWDT	RSV_G_5 - 19	171
TAKTLERTWDTLNHL	RSV_G_9 - 23	172
LERTWDTLNHLFIS	RSV_G_13 - 27	173
WDTLNHLFISSCLY	RSV_G_17 - 31	174
NHLFISSCLYKLN	RSV_G_21 - 35	175
FISSCLYKLNLSVA	RSV_G_25 - 39	176
CLYKLNLSVAQITL	RSV_G_29 - 43	177
LNLSVAQITLSILA	RSV_G_33 - 47	178
SVAQITLSILAMIIS	RSV_G_37 - 51	179
ITLSILAMIISTSLI	RSV_G_41 - 55	180
ILAMIISTSLIIAAI	RSV_G_45 - 59	181
IISTSLIIAAIFIA	RSV_G_49 - 63	182
SLIIAAIFIASANH	RSV_G_53 - 67	183
AAIFIASANHKVTS	RSV_G_57 - 71	184
FIASANHKVTSTTTI	RSV_G_61 - 75	185
ANHKVTSTTTIIQDA	RSV_G_65 - 79	186
VTSTTTIIQDATSQI	RSV_G_69 - 83	187
TTIIQDATSQIKNTT	RSV_G_73 - 87	188
QDATSQIKNTTPTYL	RSV_G_77 - 91	189
SQIKNTTPTYLTQSP	RSV_G_81 - 95	190
NTTPTYLTQSPQLGI	RSV_G_85 - 99	191
TYLTQSPQLGISPSN	RSV_G_89 - 103	192
QSPQLGISPSNPSEI	RSV_G_93 - 107	193
LGISPSNPSEITSQI	RSV_G_97 - 111	194
PSNPSEITSQITLIL	RSV_G_101 - 115	195
SEITSQITLILASTT	RSV_G_105 - 119	196
SQITLILASTTPGVK	RSV_G_109 - 123	197

Sequence	peptide ID	SEQ ID No:
TILASTTPGVKSTLQ	RSV_G_113 - 127	198
STTPGVKSTLQSTTV	RSV_G_117 - 131	199
GVKSTLQSTTVGTKN	RSV_G_121 - 135	200
TLQSTTVGTKNTTTT	RSV_G_125 - 139	201
TTVGTKNTTTTQAQP	RSV_G_129 - 143	202
TKNTTTTQAQPSKPT	RSV_G_133 - 147	203
TTTQAQPSKPTTKQR	RSV_G_137 - 151	204
AQPSKPTTKQRQNKP	RSV_G_141 - 155	205
KPTTKQRQNKPPSKP	RSV_G_145 - 159	206
KQRQNKPPSKPNNDP	RSV_G_149 - 163	207
NKPPSKPNNDPHEFV	RSV_G_153 - 167	208
SKPNNDPHEFVFNFV	RSV_G_157 - 171	209
NDFHFEVFNFVPCSI	RSV_G_161 - 175	210
FEVFNFVPCSICSNN	RSV_G_165 - 179	211
NFVPCSICSNNPTCW	RSV_G_169 - 183	212
CSICSNNPTCWAICK	RSV_G_173 - 187	213
SNNPTCWAICKRIPN	RSV_G_177 - 191	214
TCWAICKRIPNKKPG	RSV_G_181 - 195	215
ICKRIPNKKPGKKT	RSV_G_185 - 199	216
IPNKKPGKKTTKPT	RSV_G_189 - 203	217
KPGKKTTKPTEEPT	RSV_G_193 - 207	218
KTTTKPTEEPTFKTA	RSV_G_197 - 211	219
KPTEEPTFKTAKEDP	RSV_G_201 - 215	220
EPTFKTAKEDPKPQT	RSV_G_205 - 219	221
KTAKEDPKPQTGSG	RSV_G_209 - 223	222
EDPKPQTGSGEVPT	RSV_G_213 - 227	223
PQTGSGEVPTTKPT	RSV_G_217 - 231	224
GSGEVPTTKPTGEPT	RSV_G_221 - 235	225
VPTTKPTGEPTINTT	RSV_G_225 - 239	226
KPTGEPTINTTKTNI	RSV_G_229 - 243	227
EPTINTTKTNITTL	RSV_G_233 - 247	228
NTTKTNITTLTNSN	RSV_G_237 - 251	229
TNITTLTNSNTRN	RSV_G_241 - 255	230
TLLTNSNTRNPELT	RSV_G_245 - 259	231
TSNTRNPELTSQME	RSV_G_249 - 263	232
TRNPELTSQMETFHS	RSV_G_253 - 267	233
ELTSQMETFHSTSSE	RSV_G_257 - 271	234
QMETFHSTSSEGNPS	RSV_G_261 - 275	235
FHSTSSEGNPSQV	RSV_G_265 - 279	236
SSEGNPSQVSITS	RSV_G_269 - 283	237
NPSQVSITSEYLS	RSV_G_273 - 287	238
SQVSITSEYLSQPSS	RSV_G_277 - 291	239
ITSEYLSQPSSPNT	RSV_G_281 - 295	240
YLSQPSSPNTPR	RSV_G_285 - 297	241

4) Plates were incubated at 37°C, 5% CO₂ for 20-24 hrs.

- 5) The following day, the plates were thoroughly washed and 100 μ L/well MABTECH detection antibody, clone R4-6A2 was added to 0.25 μ g/ml in PBS/1%FBS (1:4000 dilution) in each well. Plates were incubated for 2 hrs and then washed thoroughly with PBS/0.05% Tween 20
- 6) Streptavidin-AP was diluted 1:3000 in PBS/1% FBS and 100 μ L was added to each well.
- 7) Plates were incubated for 60 min at room temperature and washed thoroughly with PBS/Tween 20 (0.05%).
- 8) 100 μ L of 1-STEP NBT/BCIP was added to each well, plates were held at room temperature for several minutes, washed with tap water, and allowed to dry overnight.
- 9) Plates were imaged using AID imager system and data were processed to calculate the number of IFN- γ secreting cells per million splenocytes.

The data showed that RNA/LNP vaccines gave much higher cellular immune responses than the protein antigen formulated with alum, which elicited little to no detectable cellular immune responses. See Fig. 2, where columns with a * indicate that the number of spots of interferon gamma were too high to count accurately.

III. Intracellular cytokine staining:

Splenocytes were harvested as described above. Freshly harvested splenocytes were rested overnight in R10 media at 1×10^7 cells per mL. The following morning, 100 μ L of cells were added to each well according to plate template for a final number of 1×10^6 cells/ well. Pooled RSV-F or RSV-G peptides were used to stimulate the cells. The RSV-F peptide pools were as described above. The RSV-G peptide pools were either as described above or purchased from JPT (catalog PM-RSV-MSG). Cells were incubated for 1 hr at 37 $^{\circ}$ C, and BFA and monensin were added to each well to a final concentration of 5 μ g each.

To stain the cells, 20 μ L of 20 mM EDTA was added to each cell well, and the cells were incubated for 15 minutes at Room Temperature (RT). The plates were centrifuged at 500xg for 5 minutes and the supernatant was aspirated. The plates were then washed with PBS and centrifuged again. ViVidye was reconstituted with DMSO and diluted in PBS. 125 μ L diluted ViVidye was added to each well and incubated at room temperature for 15 minutes. The plates were centrifuged, the supernatant was removed and the plates were washed again with 175 μ L FACSWash. A BD cytofix/cytoperm solution was added to each

well, and the plates were incubated for 20-25 minutes at 2-8 °C. The plates were then centrifuged and washed twice with a BD perm wash buffer. Finally, FC block was added to a final concentration of 0.01 mg/mL in a volume of 125 mL per well in the BD perm wash buffer. The cells were stained with an intracellular antibody cocktail made as follows:

- 5 a) IL-10 FITC:
- b) IL-17A PE:
- c) IL-2 PCF594:
- d) CD4 PerCPcy5.5:
- e) TNF PE Cy7:
- 10 f) IFN γ APC:
- g) CD8a BV510:
- h) CD3 APC Cy7:
- i) Perm Wash:

The cells were incubated with the antibody cocktail (20 μ L per test well) at 2-8 °C for 15 35 minutes, washed twice with the BD perm wash buffer, and resuspended in 200 μ L per well of BD stabilizing fixative. Samples were acquired on an LSRII and data were analyzed using Flojo software. The percentage of CD4⁺ splenocytes that respond to the peptide pools and produced IFN- γ , IL-2, or TNF α are shown in FIGs. 3A, 3B, and 3C and the percentage of CD8⁺ splenocytes that respond to the peptide pools and produce Ifn- γ , IL-2 or TNF α are 20 shown in FIGs. 4A, 4B, and 4C. The data were a that RSV-F mRNA/LNP vaccines and RSV-G mRNA/LNP vaccines but not DS-CAV1 protein antigens elicit robust Th1 biased CD4⁺ immune responses in mice. In addition, RSV-F mRNA/LNP vaccines but not RSV-G mRNA/LNP vaccines or DS-CAV1 protein antigens elicit robust Th1 biased CD8⁺ immune responses in mice.

25 *Example 13: Mouse immunogenicity*

In this example, additional assays were carried out to evaluate the immune response to RSV vaccine antigens delivered using an mRNA/LNP platform in comparison to protein antigens.

30 Again, female Balb/c (CRL) mice (6-8 weeks old; N= 10 mice per group) were administered mRNA vaccines or protein vaccines. The mRNA vaccines were generated and formulated in MC3 lipid nanoparticles. The mRNA vaccines evaluated in this study included the followings:

MRK-1 membrane-bound RSV F protein

MRK-2 secreted RSV F protein

MRK-3 secreted DS-CAV1

MRK-4 membrane-bound DS-CAV1 (stabilized prefusion F protein)

MRK-5 RSV F construct

5 MRK-7 RSV F construct

MRK-8 RSV F construct

MRK-9 membrane-bound RSV G protein

Influenza M1

10 Listed below are the DNA sequences encoding the mRNA sequences for MRK-2, MRK-3 and Influenza M1. Also shown are the corresponding amino acid sequences. All other sequences are provided elsewhere herein.

MRK-2 non-membrane bound form RSV F protein/MRK_02_F (soluble, Merck A2 strain)/

15

ATGGAGCTGTTGATCCTTAAGGCCAACGCCATCACTACTATTCTCACCGCGGTAA
CATTCTGCTTCGCCTCCGGGCAGAACATCACCGAGGAGTTCTACCAGTCTACGTG
CTCCGCCGTCTCCAAAGGTTACCTGTCCGCATTAAGGACGGGGTGGTACACTTCC
20 GTCATAACTATTGAACTGAGTAACATAAAAAAGAACAAAGTGTAATGGGACGGAT
GCCAAGGTGAAGCTCATCAAGCAAGAGCTTGACAAATACAAGAATGCAGTGACA
GAGCTCCAACCTTCTCATGCAGTCTACACAGGCCACGAATAACCGTGCCCGAAGA
GAACTGCCTAGATTTATGAATTACACTTTGAACAACGCCAAAAAGACCAACGTG
ACTCTAAGCAAAAAAAGGAAACGGCGTTTTTCTGGGCTTTCTGCTGGGGGTTGGTA
25 GCGCCATCGCATCTGGCGTGGCAGTCAGTAAAGTTTTGCACCTTGAGGGGGAGGT
CAACAAAATCAAGAGCGCGCTGTTATCAACAAACAAGGCAGTCGTGTCCCTCTC
CAATGGCGTGTCTGTCTGACCTCTAAAGTACTGGATCTCAAGAACTATATCGAC
AAACAACCTGCTACCAATCGTCAATAAGCAGAGTTGCTCTATTTCCAATATTGAGA
CCGTGATCGAGTTTCAACAGAAGAATAACAGATTGTTGGAGATCACCGAGGAAT
TCAGCGTCAATGCAGGGGTGACCACACCCGTATCTACCTACATGCTGACCAACTC
30 GGAACCTCCTCTCCTTAATAAACGACATGCCTATTACTAACGACCAAAAAAAGTTG
ATGTCCAACAATGTCCAGATCGTGCGACAGCAATCTTATTCAATTATGTCCATTA
TAAAAGAGGAGGTGCTGGCGTACGTAGTGCAGCTGCCCCCTTTACGGAGTGATCG
ACACCCCATGCTGGAAGCTCCACACCTCCCCCTGTGCACCACTAATACCAAAGA
AGGCAGCAACATCTGTCTGACCCGTACCGACCGCGGATGGTACTGCGATAATGC
35 AGGTAGCGTCTCTTTTTTTCCCGAGGCTGAAACTTGCAAGGTTCAAGTCCAACCGG
GTATTCTGTGACACGATGAACAGTCTCACCCCTACCATCAGAGGTGAACCTGTGCA
ATGTGGACATATTTAACCCTAAATATGACTGTAAGATCATGACCTCCAAAACCTGA
CGTTTCCAGCAGTGTGATAACCTCACTGGGCGCAATAGTTTCATGCTATGGAAAG
ACTAAGTGCACTGCCTCTAACAAAAATCGAGGTATTATTAAGACCTTTAGCAATG
40 GCTGCGATTATGTCAGTAACAAAGGTGTTGATACAGTGAGTGTGGGCAACACATT
ATACTATGTTAACAAGCAAGAAGGCAAGAGCCTCTATGTGAAGGGAGAACCAAT
CATTAATTTTTACGATCCGCTGGTCTTTCCAGCGATGAGTTCGATGCATCCATCT
CTCAGGTGAATGAAAAAATTAACCAATCACTGGCTTTCATACGGAAGAGCGATG
AACTGCTGAGCGCCATCGGGGGATACATCCCTGAAGCTCCGAGGGACGGCCAAG

CTTATGTCCGCAAAGACGGAGAGTGGGTGTTGCTCAGTACCTTCCTC (SEQ ID NO: 242) The underlined region represents a region coding for a foldon. The underlined region can be substituted with alternative sequences which achieve a same or similar function.

5 MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIE
LSNIKKNCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTQATNNRARRELPRFMN
YTLNNAKKTNTVLSKKRRRFLGFLGVSASGVAVSKVLHLEGEVNIKISALLS
TNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPVINKQSCSISNIETVIEFQQKNNRLLE
ITREFSVNAGVTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSI
10 IKEEVLAYVVLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRDRGWYCDNAGS
VSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSV
ITSLGAIVSCYGKTKCTASNKNRGIKTFNNGCDYVSNKGVDTVSVGNTLYYVVKQE
GKSLYVKGEPIINFYDPLVFPSEDFDASISQVNEKINQSLAFIRKSDELLSAIGGYIPEAP
RDGOAYVRKDGWVLLSTFL(SEQ ID NO: 243)

15 The first underlined region represents a signal peptide sequence. The first underlined regions can be substituted with alternative sequences that achieve the same or similar functions, or it can be deleted. The second underlined region represents a foldon. The second underlined region can be substituted with alternative sequences which achieve a same or similar function.

20 MRK-3 non-membrane bound form DS-CAV1 (stabilized prefusion F protein)//MRK_03_
DS-CAV1 (soluble, S155C/S290C/S190F/V207L)/SQ-030271:

ATGGAAGTCTGATTCTTAAGGCGAATGCCATAACCACTATCTTGACCGCAGTTA
25 CTTTTTGCTTCGCCTCTGGGCAGAATATTACCGAAGAGTTCTACCAGTCCACGTG
CAGTGCCGTGTCTAAGGGCTACCTTTCCGCGCTTCGCACTGGCTGGTACACGTCA
GTCATAACGATCGAACTCTCTAATATAAAGGAAAATAAGTGTAACGGAACAGAC
GCTAAGGTCAAGTTAATCAAGCAGGAGCTGGACAAATATAAGAATGCCGTAACG
GAGCTCCAGCTGCTCATGCAGAGCACGCCAGCTACAAACAACAGGGGCACGCCGT
30 GAGCTCCCCCGATTATGAAGTACACATTGAACAACGCCAAGAAAATAACGTG
ACTTTGTCCAAGAAGAGGAAGCGGCGATTCTTAGGGTTCCTTTTGGGGGTAGGCT
CGGCGATTGCCAGTGGGGTTGCCGTATGCAAGGTGCTCCACCTGGAAGGGGAGG
TGAACAAGATTAAGTCGGCTCTGCTCAGTACAAACAAGCTGTCGTCTCATTGTC
AAACGGAGTCAGTGTATTGACATTTAAAGTCCTCGACCTGAAGAAGTATATAGAT
35 AAACAGTTACTCCCAATCTTGAATAAGCAGTCCTGTAGCATCAGCAACATTGAGA
CAGTGATCGAGTTCCAGCAGAAGAATAATCGCCTACTCGAGATCACCAGAGAAT
TCTCAGTCAATGCCGGAGTAACCACTCCTGTCAGCACATACATGCTCACAACTC
TGAAGTCTTAAGCCTGATTAATGATATGCCTATCACAAATGATCAGAAGAACTC
ATGAGCAATAATGTGCAGATTGTAAGACAGCAGAGTTATTCTATAATGTGTATTA
40 TTAAGGAGGAGGTACTGGCCTATGTGGTTCAACTTCCTCTGTATGGGGTGATAGA
TACACCATGCTGGAAGCTGCACACCAGCCCACTGTGTACGACCAATAACAAGGA
GGGCTCCAATATTTGCTTAACACGGACTGACCGGGGGTGGTATTGCGACAATGCC
GGATCAGTCTCCTTCTTCCCCCAAGCAGAGACCTGCAAGGTGCAGTCCAATAGAG
TTTTCTGCGACACAATGAAGTCTGACCTACCTAGCGAAGTTAACTTATGCAA
45 CGTGGATATTTTTAATCCGAAGTATGATTGTAAAATCATGACTAGCAAAACGGAT
GTTAGCTCCAGCGTAATCACCTCCCTAGGCGCTATCGTGAGCTGTTATGGCAAGA
CGAAGTGCAGTGCATCTAATAAAAAATAGGGGTATTATTAACCTTCAGCAATG
GCTGCGACTATGTGAGCAATAAGGGCGTGGACACCGTGTGAGTGGGAAACACCC
TCTATTATGTGAACAAGCAGGAGGGAAAATCCCTTTATGTAAAGGGCGAACCCA
50 TTATCAATTTCTATGACCCCTGGTTTTCCCAAGCGACGAGTTCGACGCATCTATC
TCTCAAGTGAACGAGAAAATCAATCAGAGTCTTGCCCTTATCAGAAAATCCGATG

AGCTGCTTTCCGCCATCGGTGGCTATATCCCAGAAGCCCCAAGAGACGGACAAG
CGTACGTCCGGAAGATGGTGAGTGGGTCCTCCTCTACCTTTCTT (SEQ ID NO:
 244)

The underlined region represents a region coding for a foldon. The underlined region can be substituted with alternative sequences which achieve a same or similar function.

MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIE
 LSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMN
 YTLNNAKKTNTLSKKRKRRLGFLGVSASGVA VCKVLHLEGEVNIKSALLS
 TNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLE
 ITREFSVNAGVTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMC
 IIEEVLAYVYVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAG
 SVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNL CNVDIFNPKYDCKIMTSKTDVSS
 VITSLGAIVSCYGKTKCTASNKNRGIKTF SNGCDYVSNKGVDTVSVGNTRYVYVKNQ
 EGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLSAIGGYIPEA
PRDGQAYVRKDG EWVLLSTFL (SEQ ID NO: 245)

The first underlined region represents a signal peptide sequence. The first underlined regions can be substituted with alternative sequences that achieve the same or similar functions, or it can be deleted. The second underlined region represents a foldon. The second underlined region can be substituted with alternative sequences which achieve a same or similar function.

Influenza M-1 (A/California/04/2009(H1N1), ACP44152)+hIgκ

ATGGAGACTCCTGCACAGCTGCTGTTTCTGCTATTGTTGTGGCTTCCGGACACTAC
TGGGTCCCTCCTCACCGAGGTGGAACATACGTGCTGTCCATCATACCATCCGGG
 CCCTTGAAAGCCGAGATCGCCCAGAGACTCGAATCTGTATTTCGCAGGAAAGAAC
 ACGGATTTGGAGGCACTAATGGAATGGCTGAAGACCCGTCCGATCCTGTCTCCTC
 TCACAAAGGGGATTCTTGGATTTGTCTTTACCCTCACCGTCCCGAGCGAGCGCGG
 TCTCCAGCGCAGACGTTTTGTACAGAAATGCACTGAATGGCAACGGCGATCCCAAT
 AACATGGATCGTGCGGTAAAGCTTTATAAAAAGCTGAAGAGAGAAATCACTTTC
 CATGGGGCTAAAGAGGTGAGTCTCTCCTATTCAACCGGGGCATTGGCCTCTTGCA
 TGGGTCTTATATACAATCGAATGGGCACCGTTACCACCGAGGCCGCATTTGGTCT
 GGTTTGTGCTACGTGCGAGCAAATCGCAGATAGCCAGCATCGGTCCCATCGGCA
 GATGGCCACCACTACGAACCCTCTAATTCGACATGAAAATCGCATGGTCCTGGCT
 AGCACCACCGCAAAGGCAATGGAGCAGATGGCGGGCTCTAGTGAACAGGCAGC
 CGAGGCAATGGAAGTGGCCAATCAGACCAGGCAGATGGTCCATGCTATGCGGAC
 TATTGGTACCCACCCGTCCAGCAGTGCTGGACTGAAGGATGACCTCCTTGAGAAC
 CTGCAGGCATACCAGAAACGAATGGGGGTGCAAATGCAGAGATTCAAG (SEQ ID
 NO: 246)

The underlined region represents a region coding for human Igκ signal peptide. The underlined region can be substituted with alternative sequences which achieve a same or similar function.

METPAQLLFLLLLWLPD TTGSLLTEVETYVLSIIPSGPLKAEIAQRLESVFAGKNTDLE
 ALMEWLKTRPILSPLTKGILGFVFTLTVPSEGLQRRRFVQNALNGNGDPNNMDRAV
 KLYKKLKREITFHGAKEVSLSYSTGALASCMGLIYNRMGTVTTEAAFGLVCA TCEQI
 ADSQHRSHRQMATTNTNPLIRHENRMVLASTTAKAMEQMAGSSEQAAEAMEVANQT
 RQMVHAMRTIGTHPSSSAGLKDDLLENLQAYQKRMGVQMQRFK (SEQ ID NO: 247)

The underlined region represents human Igκ signal peptide. The underlined region can be substituted with alternative sequences which achieve a same or similar function.

The influenza M1 mRNA was combined with MRK-1, MRK-4 or MRK-9 in an effort to increase the immune response by having the cells that take up the mRNAs make virus like particles (VLPs).

Protein vaccine evaluated in this study was DS-CAV1 stabilized prefusion F protein as described in McLellan et al. *Science* 342, 592 (2013); 1 mg/mL. The protein was buffered in 50 mM Hepes, 300 mM NaCl and was formulated with Adju-phos.

Groups of 10 mice were immunized intramuscularly with 100 groups of 10 mice were immunized intramuscularly with 100 μ L of vaccine, delivered with 50 μ L injections into each quadriceps. The groups were vaccinated with the following vaccines:

Table 1. Vaccines

Group	Vaccine	Concentration (ug/ml)	Total dose /mouse (ug)
1	mF (MRK-1)	100	10
2	sF (MRK-2)	100	10
3	mDS-CAV1 (MRK-4)	100	10
4	sDS-CAV1 (MRK-3)	100	10
5	mG (MRK-9)	100	10
6	mF (MRK-1) + Influenza M1 (1:1 mixture)	100	10
7	mDS-CAV1 (MRK-4) + Influenza M1 (1:1 mixture)	100	10
8	mG (MRK-9) + Influenza M1 (1:1 mixture)	100	10
9	MRK-5	100	10
10	MRK-7	100	10
11	MRK-8	100	10
12	DS-CAV1 protein/adju phos	100	10
13	mF (MRK-1)	20	2
14	sF (MRK-2)	20	2
15	mDS-CAV1 (MRK-4)	20	2
16	sDS-CAV1 (MRK-3)	20	2
17	mG (MRK-9)	20	2
18	VLP/mF (MRK-1)	20	2
19	VLP/mDS-CAV1 (MRK-4)	20	2
20	VLP/G (MRK-9)	20	2
21	MRK-5	20	2
22	MRK-7	20	2
23	MRK-8	20	2
24	DS-CAV1 protein/adju phos	20	2
25	naïve	N/A	N/A

The animals were immunized on day 0 and day 21 of the experiment. On days 14 and 35, blood was drawn from each animal and used for serological assays. On day 42, a subset of the animals were sacrificed and spleens were harvested to support ELISPOT and intracellular cytokine staining studies.

On day 27, the mice were challenged intranasally with 1×10^6 PFU RSV A2. Four days post inoculation, the animals were sacrificed by CO₂ inhalation and lung and nasal turbinates were removed and homogenized in 10 volumes of Hanks Balanced Salt Solution (Lonza) containing SPG on wet ice. The samples were clarified by centrifugation at 2000 rpm for 10 minutes, aliquotted, flash frozen, and immediately stored frozen at -70°C.

A. RSV Neutralization Assay:

Neutralizing antibody titers were determined as described above. The titers are shown in Fig. 5 (PD1= samples taken post-dose 1, PD2= samples taken post-dose2). The results showed that mRNA/LNP vaccines were strongly immunogenic and elicited high neutralizing antibody titers, as was demonstrated in the previous experiment. Attempts to generate a significantly higher neutralizing antibody by co-delivering mRNAs expressing influenza M1 with mRNAs expressing membrane-bound protein antigen were not successful.

B. Intracellular Cytokine Staining.

Intracellular cytokine staining was conducted in the same manner described above in Examples 13. The CD4 ICS responses to RSV-F and G peptide pools are shown in Figs. 6A, 6B, and 6C. As in the previous study, the ICS results showed that mRNA vaccines expressing RSV-F and RSV-G elicited robust Th1-biased CD4 immune responses.

The CD8 ICS responses are shown in Figs. 7A, 7B, and 7C. The data confirm the previous observation that mRNAs expressing RSV-F antigens but not mRNAs expressing RSV-G or DS-CAV1 protein/adju phos elicited robust Th1 biased CD8 responses.

C. Mouse Challenge Results

The procedure for measuring viral titers is outlined below. Briefly, samples were diluted and added in duplicate to 24- well plates containing confluent HEp-2 cell monolayers. The plates were incubated at 37°C for one hour. Following the one hour incubation, sample inoculum was aspirated and 1ml of overlay containing 0.75% methylcellulose was added. The plates were incubated at 37°C for 5 days. Following the 5 day incubation, the cells were fixed and stained with crystal violet/ glutaraldehyde solution. Plaques were counted and titers were expressed as pfu/gram of tissue. As shown in Fig. 8, no virus was recovered from the lungs of any of the mice immunized with the mRNA vaccines formulated with MC3 LNP

and only one animal at the lower dose of DS-CAV1 protein /adju phos vaccine had any virus detectable in the nose.

Example 14: Cotton Rat Immunogenicity and Efficacy

In this example, assays were carried out to test the immunogenicity and efficacy of mRNA/LNP vaccines in the cotton rat RSV challenge model.

More specifically, female cotton rats (SAGE) were used and immunizations began at 3-7 weeks of age. The mRNA vaccines used were generated and formulated in MC3 lipid nanoparticles. The mRNA vaccines evaluated in this study included:

- MRK-1 membrane-bound RSV F protein
- MRK-2 secreted RSV F protein (truncated ectodomain)
- MRK-3 secreted DS-CAV1 (trimeric ectodomain)
- MRK-4 membrane-bound DS-CAV1 (stabilized prefusion F protein)
- MRK-9 membrane-bound RSV G protein
- Influenza M1 protein

Protein vaccine evaluated in this study was DS-CAV1 stabilized prefusion F protein as described in McLellan et al. *Science* 342, 592 (2013); 1 mg/mL. The protein was buffered in 50 mM Hepes, 300 mM NaCl and was formulated with Adju-phos.

Groups of 10 cotton rats were immunized intramuscularly with 120 μ L of vaccine, delivered with 60 μ L injections into each quadricep. The groups were vaccinated with the following vaccines as set out in Table 2:

Table 2. Vaccine Formulations Tested for Immunogenicity in Cotton Rats

Group	Vaccine	Conc (μ g/ml)	Dose (μ g)
1	mF (MRK-1), I.M.	250	30
2	sF (MRK-2) I.M.	250	30
3	mDS-CAV1 (MRK-4), I.M.	250	30
4	sDS-CAV1 (MRK-3), I.M.	250	30
5	mG (MRK-9), I.M.	250	30
6	VLP/mF (MRK10 + MRK-1), I.M.	250	30
7	VLP/mG (MRK10 + MRK-9), I.M.	250	30
8	VLP/mDS-CAV1 (MRK10 + MRK-4), I.M.	250	30
9	DS-CAV1 protein/adju phos, I.M.	250	30
10	RSV A2 5.5log10pfu, I.N.	NA	NA
11	None	NA	NA

The animals were immunized on day 0 and day 28 of the experiment. On days 28 and 56, blood was drawn from each animal and used for serological assays. On day 56, the cotton rats were challenged intranasally with $1 \times 10^{5.5}$ PFU RSV A2. Four days post inoculation, animals were sacrificed by CO₂ inhalation and lung (left lobes) and nasal turbinates were removed and homogenized in 10 volumes of Hanks Balanced Salt Solution (Lonza) containing SPG on wet ice. The samples were clarified by centrifugation at 2000 rpm for 10 minutes, aliquoted, flash frozen, and immediately stored frozen at -70 °C.

A. RSV Neutralization Assay

Neutralizing antibody titers were determined as described above.

The titers determined post dose 1 and post dose 2 are shown in Fig. 9. The neutralizing titers were robust in cotton rats following a single immunization and overall were several fold higher than those elicited by the DS-CAV1 protein antigen formulated with adju-phos or with infection with RSV A2 virus. The highest neutralizing antibody titers were elicited by RNA vaccines expressing full length RSV-F protein, truncated F-protein (ectodomain), mDS-CAV1 (stabilized prefusion F protein containing the RSV F transmembrane domain), and sDS-CAV1 (a truncated form of the stabilized prefusion F protein) as well as mRNA combination, including full length F protein and influenza M1 (termed “VLP/mF” in the graph above).

Titers determined post-dose two indicate that overall, neutralizing antibody titers were quite high for both mRNA vaccines and for the DS-CAV1 protein comparator. Surprisingly, in this study, as in the two mouse immunogenicity studies, relatively high neutralizing antibody titers were observed for the mG and mG+influenza M1 mRNA vaccine groups after the second dose of vaccine. With other vaccine modalities used to delivery RSV-G antigens, it was reported that neutralizing antibody activity is not observed *in vitro* unless complement is included in the assay.

B. Competition ELISA

The immune response to specific epitopes on RSV F-protein for neutralizing antibodies was characterized. The antigenic site II is the binding site for palivizumab, a monoclonal antibody developed for the prevention of lower respiratory infection with RSV in at risk infants and toddlers. Antigenic site Ø is a binding site for more potent neutralizing antibodies that are elicited by natural infection with RSV. A competition ELISA was developed to characterize the antigenic site Ø and antigenic site II response to the various mRNA-based vaccines.

Methods

ELISA plates were coated with either prefusion F protein or postfusion F protein (McLellan *et al.*, 2013). After coating, the plates were washed and blocked with blocking buffer (PBST/3% nonfat dried milk). Test sera from the cotton rat challenge study was then diluted with blocking buffer and titrated in the ELISA plate. Biotinylated D25 (a monoclonal antibody that binds to antigenic site Ø) or biotinylated palivizumab (a monoclonal antibody that binds to antigenic site II) were diluted in blocking buffer and added to each well of the ELISA plate (biotinylated D25 is only used with plates coated with prefusion F protein; biotinylated palivizumab may be used with plates coated with prefusion or postfusion F protein as antigenic site II is present on both forms of the antigen). Following incubation, plates were washed and streptavidin-tagged horse radish peroxidase was added to each well of the ELISA plate. Plates were incubated at room temperature for 1 hr, washed, and incubated with TMB substrate (ThermoScientific). The color was allowed to develop for 10 minutes and then quenched with 100 µL of 2N sulfuric acid and the plates were read at 450 nM on a microplate reader. The results are shown in Fig. 10. Fig. 10 illustrates the ability of cotton rat sera to compete with either D25 binding to prefusion F protein or palivizumab binding to postfusion F protein.

Background binding titers were seen in both the naïve mice and in those immunized with mG or with VLP/mG (neither of which will express the epitopes bound by D25 or palivizumab). The unlabeled monoclonal antibodies were included in the experiment as positive controls and those data are shown in the right-hand column of Fig. 10. No D25 competing titers were evident in cotton rats immunized with MRK-1, MRK-2, MRK-9, MRK10+MRK-1, or MRK10+MRK-9. Only immunization with a mRNA encoding the DS-CAV1 sequence (MRK-4, MRK-3, and MRK10+MRK-4) elicited D25-competing antibody titers, illustrating that these mRNAs produce a form of RSV F protein that is primarily in the prefusion conformation. In contrast, palivizumab competing titers were far higher in animals immunized with MRK-1 or MKR02 mRNAs, illustrating that these mRNAs were produced as postfusion RSV F protein in cotton rats.

C. Cotton Rat Challenge Results

Procedures for measuring RSV titers in the cotton rat nose were followed as described above for mice. Nasal titers are shown in Fig. 11. In this assay, the limit of detection was 40 pfu/g of tissue. It was found that only one vaccinated animal (one mouse vaccinated with mDS-CAV1 (MRK4) mRNA encapsulated with MC3 LNP) had any detectable virus presence in the nose. In contrast, the geometric mean titer of RSV A2 virus in animals that were not vaccinated but were challenged in the same study was >10,000 pfu/g tissue.

Example 15: African Green Monkey Immunogenicity and Efficacy

In this example, assays were carried out to test the immunogenicity and efficacy of mRNA/LNP vaccines in the African Green Monkey RSV challenge model.

More specifically, male and female adult African Green Monkeys with body weights ranging from 1.3 to 3.75 kg, which were confirmed to be RSV-negative by neutralizing antibody titer, were used. The mRNA vaccines used were generated and formulated in MC3 lipid nanoparticles. The mRNA vaccines evaluated in this study included:

MRK-1 membrane-bound RSV F protein

MRK-4 membrane-bound DS-Cav1 (stabilized prefusion F protein)

Groups of four African Green Monkeys were immunized intramuscularly with 1000 μ L of vaccine, delivered with 500 μ L injections into each deltoid. The groups were vaccinated with the following vaccines as set out in Table 3.

Table 3. Vaccine Formulations Tested for Immunogenicity in African Green Monkeys

Group	Vaccine	Conc (μ g/ml)	Dose (μ g)
1	mF (MRK-1), I.M.	125	125
2	mDS-Cav1 (MRK-4), I.M.	125	125
3	mF (MRK-1) + mDS-Cav1 (MRK-4), I.M.	125	125 (62.5 μ g each mRNA)
4	RSV A2 5.5log10pfu, I.N.	NA	NA
5	None	NA	NA

The animals were immunized on day 0, day 28, and day 56 of the experiment. On days 0, 14, 28, 42, 56 and 70, blood was drawn from each animal and used for serological assays. On day 70, the African Green Monkeys were challenged intranasally with $1 \times 10^{5.5}$ PFU RSV A2. Nasopharyngeal swabs were collected on days 1-12, 14, and on day 18 post challenge, and lung lavage samples were collected on days 3, 5, 7, 9, 12, 14, and 18 post challenge to test for viral replication.

A. RSV Neutralization Assay

Neutralizing antibody titers (NT_{50}) were determined as described above.

The NT_{50} titers determined post dose 1 and post dose 2 are shown in Fig. 12. Titers were seen to increase after each dose for both groups receiving mRNA vaccines as well as the group receiving RSV A2. The GMTs obtained with mRNA vaccines at week 10 (2 weeks post-dose 3) were more than 2 orders of magnitude higher than in the animals that received RSV A2.

B. Competition ELISA

The immune response to specific epitopes on RSV F-protein for neutralizing antibodies was characterized using the competition assays described above.

The palivizumab and D25 competing antibody titers measured at week 10 (2 weeks PD3) are presented in Figs. 13A-13B. The GMT palivizumab competing titers were 5 fold higher in the groups that received mF or the combination of mF+mDS-Cav1 compared to the group that received mDS-Cav1. While the GMT D25 competing antibody titers were 2 fold higher in the groups that received mDS-Cav1 or the combination of mF+ mDS-Cav1 than in the group that received mF mRNA. The prefusion F stabilized antigen (mDS-Cav1), was able to elicit prefusion specific responses.

C. African Green Monkey Challenge Results

As mentioned above, in order to evaluate vaccine efficacy African Green Monkeys were challenged intranasally with $1 \times 10^{5.5}$ PFU RSV A2 on day 70 post vaccination and nasopharyngeal swabs and lung lavage samples were collected post challenge to test for the presence of virus.

In order to measure RSV titers in the African Green Monkey nasopharyngeal swabs and lung lavage samples an RSV RT-qPCR assay to detect RSV A was carried out as follows:

1) Equipment and Materials:

A. Equipment

1. Stratagene Mx3005P Real Time PCR system and MxPro Software
2. Jouan GR422 centrifuge or equivalent
3. Jouan Plate carriers or equivalent

B. Reagents

1. Quantitect® Probe RT-PCR kit (1000) catalog # 204445
2. Water, Molecular Biology Grade DNAase-free and Protease free, 5 Prime, catalog # 2900136
3. TE buffer, 10mM Tris 1mM EDTA ph 8.0, Fisher Bioreagents, catalog # BP2473-100
4. Viral primers: RSV A Forward and Reverse primers, Sigma custom, HPLC purified. Primer stocks are reconstituted to 100 μ M in Molecular grade water and stored at -20 °C.
5. RSV dual labeled probe, Sigma custom, HPLC purified. Probe stocks are reconstituted to 100 μ M in TE buffer and stored at -20 °C protected from light.

6. RSV A standard were generated in-house and stored at -20 °C. Standards for the assay were generated by designing primer pairs to the N gene of RSV A. The product length for the RSV A standard is 885 bp. QIAGEN OneStep RT-PCR was used to generate this standard.

5

Table 4. Primers

Primers	Sequences
RSV A F N gene	5' CTC AAT TTC CTC ACT TCT CCA GTG T (SEQ ID NO: 248)
RSV A R N gene	5' CTT GAT TCC TCG GTG TAC CTC TGT (SEQ ID NO: 249)
RSV A FAM N gene	5' FAM-TCC CAT TAT GCC TAG GCC AGC A (BHQ1) (SEQ ID NO: 250)

7. Promega, Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Product #AS1150)

10 C. Supplies

1. Stratagene Optical cap 8x strip, catalog # 401425
2. Stratagene Mx3000P 96 well plates, skirted, catalog # 401334
3. ART filtered pipet tips

15 2) RT-PCR Reactions and set up

A. Preparation of Complete Master Mix

1. Prepare complete Master Mix following the set up below for a final reaction volume of 50 µL. The following table is volume per well. Final primer concentration is 300 nM and final probe concentration is 200 nM.

20

Table 5. Reagents

Reagent	mL
2X Master Mix	25
RSV A F 100uM	0.2
RSV A R 100uM	0.2
RSV A FAM 100uM	0.1
RT enzyme mix	0.5
Water	19

2. Add 45 µL of complete master mix to each well. Cover plate with plate cover and wrap in aluminum foil to protect from light.

B. Preparation of Standard curve

1. Remove standard from -20 °C.
2. Dilute standards to final concentrations of 1×10^6 copy/5 μ L to 1 copy/5 μ L using 10-fold dilutions.

C. Sample preparation

1. Nasopharyngeal swab and lung lavage samples are prepared for the RT-PCR reaction using the Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Promega, product #AS1150)
2. 200 μ L of sample is extracted following the manufactures protocol and eluted into 50 μ L to be used in PCR reactions.

D. Additions of samples

1. Add 5 μ L of extracted samples to appropriate wells. After addition of samples, carefully cap sample wells before adding standard curves.
2. Add 5 μ L of diluted standard to appropriate wells and cap.
3. Add 5 μ L of molecular grade water to No Template Control (NTC) wells.
4. Wrap plates in aluminum foil and transfer plates to centrifuge.
5. Spin plates for 2 mins at 100 rpm to pull down any samples or master mix that may be on the sides of well.
6. Wrap plates in aluminum foil and transfer to Stratagene instrument.

E. Thermo cycler: Stratagene MX 3005P

1. Place plates in Stratagene Mx3005P and set thermal profile conditions to:

Table 6. Thermocycler Steps

Step	Time	Temperature
Reverse Transcription	30 min	50
PCR initial activation step	15 min	95
2-step cycling:		
Denaturation	16 sec	94
Combined annealing/extension	60 sec	62
Number of cycles	40	

2. Analyze results using the Stratagene Mx3005p software

The mean RNA copy number detected in the lung and nose samples are presented in Figs. 14A-14B. The animals that received mRNA encoding mF, mDS-Cav1 or mF + mDS-Cav1 formulated in MC3 showed complete protection (no virus detected) in lungs similar to

the control group immunized with RSV A2. The animals that received mRNA vaccines also showed a greater than 2 log reduction in virus detected in the nose on the majority of the assay days compared to the no vaccine control group.

5 *Example 16: Immunogenicity in RSV-Experienced African Green Monkeys*

The immunogenicity of mRNA vaccines formulated in MC3 LNP was tested in RSV-experienced African Green Monkeys.

Healthy adult, African Green Monkeys of either sex (n=5/group), weighing more than 1.3 kg, that were confirmed to be RSV seropositive by ELISA and neutralizing antibody
10 titers, were selected for the study. The pool of animals selected for this study had been experimentally infected with RSV in previous vaccine studies and were distributed across study groups based on their pre-study RSV neutralization titers so that all groups would have similar group GMTs at study start. RSV-experienced animals provide a model of immune
15 memory recall response to vaccination that may reflect the responses that can be anticipated in seropositive human adults.

A single vaccine dose was administered to each animal at week 0 by the intramuscular (IM) route. A control group receiving only the MC3 LNP was also included in the study design. Vaccines were administered as described in Table 7. After vaccination, the animals
20 were observed daily for any changes at the inoculation site or other changes in activity or feeding habits that might indicate an adverse reaction to the vaccine, but none were noted. Serum samples were collected for assessment of RSV neutralizing antibody titers, as well as palivizumab (site II) and D25 (site Ø) competing antibody titers. PBMC samples were collected to assess cell-mediated immune responses.

25 Table 7. Vaccine Formulations Tested for Immunogenicity in RSV Seropositive African Green Monkeys

Group	Vaccine	Conc (µg/ml)	Dose (µg)
1	mF (MRK-1), I.M.	125	125
2	mDS-Cav1 (MRK-4), I.M.	125	125
3	mF (MRK-1) + mDS-Cav1 (MRK-4), I.M.	125	125 (62.5 µg each mRNA)
4	RSV A2 5.5 _{log10} pfu, I.N.	NA	NA
5	None	NA	NA

Individual animal NT₅₀ titers were measured in serum samples collected at baseline
30 and 2 weeks post vaccination using methods described above, and the results are shown in

Fig. 15. Vaccination with the mRNA vaccines resulted in, on average, a 150-fold increase in serum neutralization titers. The fold increase was comparable for all mRNA vaccines. No increase in titers was observed in the LNP only vaccine control group. The durability of the serum neutralization titers was assessed by measuring the titers every 2 to 4 weeks post vaccination. The GMTs for each group measured out to week 24 post vaccination are presented in Fig. 16. The titers remain about 50 fold higher than baseline at week 24.

To evaluate the quality of the boosted responses in the vaccinated animals, both palivizumab (site II) and D25 (site Ø) competing antibody titers were determined. As described above, antigenic site II is a neutralization epitope found on both the prefusion and the postfusion conformation of the F protein, while site Ø is a prefusion specific neutralization epitope. The palivizumab (site II) and D25 (site Ø) competing antibody titers measured 4 weeks post vaccination using the methods described above are summarized in Figs. 17A-17B. All of the mRNA vaccines resulted in a boost in palivizumab competing titers of approximately 7 fold from baseline. Although D25 competing antibody titers were below the limit of detection of the assay before immunization in all but one animal in the MC3 LNP only control group, D25 competing antibody titers were elicited in all animals receiving an mRNA based vaccine. The GMTs were highest in the groups receiving mDS-Cav1 or the combination of mF + mDS-Cav1. No increase in palivizumab or D25 (site Ø) competing antibody titers were seen in the LNP only control group.

The mRNA vaccines were also found to boost T cell responses in the RSV-experienced African green monkeys as determined by ICS assay at week 6 post vaccination (Figs. 18A-18B).

ICS assays for African Green Monkeys were conducted as follows:

A. Day 1: Thawing PBMCs

1. PBMC vials were removed from liquid nitrogen and placed on dry ice until ready to thaw.
2. Cells were thawed quickly with gentle agitation in 37 °C set point water bath.
3. For each subject, cell suspension was transferred to an appropriately labeled 15 mL or 50 mL tube, using a serologic pipette.
4. Approximately 0.5 mL R10 medium was slowly added to the cells, which were then swirled gently to mix the media and cell suspension.
5. Three times the frozen cell volume of R10 media was then added drop wise to each tube, swirling each after 0.5 mL to 1.0 mL of R10 media were added.

6. R10 Media was then added at a rate of 1.0 mL to 2.0 mL at a time until approximately 10 to 15 mL was added to each tube.
7. The tubes were swirled to mix the media and cell suspension, and then centrifuged at 250xg (setpoint) for 8 to 10 minutes at room temperature.
8. The supernatant was removed and the cells were gently resuspended in 5 mL of R10 medium.
9. The cell suspensions were then transferred into a 12 well tissue culture plate.
10. The tissue culture plates were placed in a 37 °C +/- 2 °C, 4% to 6% CO₂ incubator overnight.

10 B. Day 2: Counting and Stimulation Procedure for PBMC

PBMC counting

1. PBMCs from each well of the 12-well tissue culture plate were placed into labeled 50mL conical tubes.
2. Cells were then counted by trypan blue exclusion on a hemacytometer or by Guava PC and resuspended to 1×10^7 cells per mL.

Stimulation Set-up

1. 100 μ L of the resuspended PBMCs were then added to each well of a 96-well sterile U bottom tissue culture plate for a final number of 1×10^6 cells/ well.
2. Peptide pools corresponding to the RSV F protein sequence were generated as follows. For optimal results the peptides were combined into two pools, RSV F1 and RSV F2. RSVF1 includes the first 71 peptides in the following list, and RSV F2 includes the following 70 peptides:

Table 8. Peptides

First aa number	15-mer	aa #		SEQ ID NO:
1	MELPILKANAITTIL	1 - 15	start F protein pool 1	29
5	ILKANAITTILTAVT	5 - 19		30
9	NAITTILTAVTFCFA	9 - 23		31
13	TILTAVTFCFASSQN	13 - 27		32
17	AVTFCFASSQNITEE	17 - 31		33
21	CFASSQNITEEFYQS	21 - 35		34
25	SQNITEEFYQSTCSA	25 - 39		35
29	TEEFYQSTCSAVSKG	29 - 43		36
33	YQSTCSAVSKGYLSA	33 - 47		37
37	CSAVSKGYLSALRTG	37 - 51		38
41	SKGYLSALRTGWYTS	41 - 55		39
45	LSALRTGWYTSVITI	45 - 59		40

First aa number	15-mer	aa #		SEQ ID NO:
49	RTGWYTSVITIELSN	49 - 63		41
53	YTSVITIELSNIKEN	53 - 67		42
57	ITIELSNIKENKCNG	57 - 71		43
61	LSNIKENKCNGTDAK	61 - 75		44
65	KENKCNGTDAKVCLI	65 - 79		45
69	CNGTDAKVCLIKQEL	69 - 83		46
73	DAKVCLIKQELDKYK	73 - 87		47
77	KLIKQELDKYKNAV	77 - 91		48
81	QELDKYKNAVTELQL	81 - 95		49
85	KYKNAVTELQLMQS	85 - 99		50
89	AVTELQLMQSTPAA	89 - 103		51
93	LQLMQSTPAANNRA	93 - 107		52
97	MQSTPAANNRARREL	97 - 111		53
101	PAANNRARRELPRFM	101 - 115		54
105	NRARRELPRFMNYTL	105 - 119		55
109	RELPRFMNYTLNNAK	109 - 123		56
113	RFMNYTLNNAKKTNV	113 - 127		57
117	YTLNNAKKTNVTLISK	117 - 131		58
121	NAKKTNVTLISKRRKR	121 - 135		59
125	TNVTLISKRRKRRLG	125 - 139		60
129	LSKKRRKRRLGFLG	129 - 143		61
133	RKKRRKRRLGFGVSA	133 - 147		62
137	FLGFLGFGVSAIASG	137 - 151		63
141	LLGVGSAIASGIAVS	141 - 155		64
145	GSAIASGIAVSKVLH	145 - 159		65
149	ASGIAVSKVLHLEGE	149 - 163		66
153	AVSKVLHLEGEVNKI	153 - 167		67
157	VLHLEGEVNKIKSAL	157 - 171		68
161	EGEVNLIKSSALLSTN	161 - 175		69
165	NKIKSALLSTNKAVV	165 - 179		70
169	SALLSTNKAVVSLSN	169 - 183		71
173	STNKAVVSLSNGVSV	173 - 187		72
177	AVVSLSNGVSVLTSK	177 - 191		73
181	LSNGVSVLTSKVLDL	181 - 195		74
185	VSVLTSKVLDLKNYI	185 - 199		75
189	TSKVLDLKNYIDKQL	189 - 203		76
193	LDLKNYIDKQLLPV	193 - 207		77
197	NYIDKQLLPVKNQS	197 - 211		78
201	KQLLPVKNQSCSIS	201 - 215		79
205	PIVKNQSCSISNIET	205 - 219		80
209	KQSCSISNIETVIEF	209 - 223		81
213	SISNIETVIEFQQKN	213 - 227		82
217	IETVIEFQQKNRLL	217 - 231		83
221	IEFQQKNRLLLEITR	221 - 235		84
225	QKNRLLLEITREFSV	225 - 239		85
229	RLLEITREFSVNAGV	229 - 243		86
233	ITREFSVNAGVTPV	233 - 247		87
237	FSVNAGVTPVSTYM	237 - 251		88
241	AGVTPVSTYMLTNS	241 - 255		89

First aa number	15-mer	aa #		SEQ ID NO:
245	TPVSTYMLTNSSELLS	245 - 259		90
249	TYMLTNSSELLSLIND	249 - 263		91
253	TNSSELLSLINDMPIT	253 - 267		92
257	LLSLINDMPITNDQK	257 - 271		93
261	INDMPITNDQKKLMS	261 - 275		94
265	PITNDQKKLMSNNVQ	265 - 279		95
269	DQKKLMSNNVQIVRQ	269 - 283		96
273	LMSNNVQIVRQQSYS	273 - 287		97
277	NVQIVRQQSYSIMSI	277 - 291		98
281	VRQQSYSIMSIKKE	281 - 295		99
285	SYSIMSIKKEVLAY	285 - 299	start F protein pool 2	100
289	MSIKKEVLAYVVQL	289 - 303		101
293	KKEVLAYVVQLPLYG	293 - 307		102
297	LAYVVQLPLYGVIDT	297 - 311		103
301	VQLPLYGVIDTPCWK	301 - 315		104
305	LYGVIDTPCWKLHTS	305 - 319		105
309	IDTPCWKLHTSPLCT	309 - 323		106
313	CWKLHTSPLCTTNTK	313 - 327		107
317	HTSPLCTTNTKEGSN	317 - 331		108
321	LCTTNTKEGSNICLT	321 - 335		109
325	NTKEGSNICLTRTDR	325 - 339		110
329	GSNICLTRTDRGWYC	329 - 343		111
333	CLTRTDRGWYCDNAG	333 - 347		112
337	TDRGWYCDNAGSVSF	337 - 351		113
341	WYCDNAGSVSFFPQA	341 - 355		114
345	NAGSVSFFPQAETCK	345 - 359		115
349	VSFFPQAETCKVQSN	349 - 363		116
353	PQAETCKVQSNRVFC	353 - 367		117
357	TCKVQSNRVFCDTMN	357 - 371		118
361	QSNRVFCDTMNSLTL	361 - 375		119
365	VFCDTMNSLTLPEV	365 - 379		120
369	TMNSLTLPEVNLCN	369 - 383		121
373	LTLPSEVNLCNVDIF	373 - 387		122
377	SEVNLCNVDIFNPKY	377 - 391		123
381	LCNVDIFNPKYDCKI	381 - 395		124
385	DIFNPKYDCKIMTSK	385 - 399		125
389	PKYDCKIMTSKTDVS	389 - 403		126
393	CKIMTSKTDVSSSVI	393 - 407		127
397	TSKTDVSSSVITSLG	397 - 411		128
401	DVSSSVITSLGAIVS	401 - 415		129
405	SVITSLGAIVSCYGK	405 - 419		130
409	SLGAIVSCYGKTKCT	409 - 423		131
413	IVSCYGKTKCTASNK	413 - 427		132
417	YGKTKCTASNKNRGI	417 - 431		133
421	KCTASNKNRGIKTF	421 - 435		134
425	SNKNRGIKTFSGNC	425 - 439		135
429	RGIKTFSGNCDYVS	429 - 443		136
433	KTFSNGCDYVSNKGV	433 - 447		137

First aa number	15-mer	aa #		SEQ ID NO:
437	NGCDYVSNKGVDTVS	437 - 451		138
441	YVSNKGVDTVSVGNT	441 - 455		139
445	KGVDTVSVGNTLYYV	445 - 459		140
449	TVSVGNTLYYV NKQE	449 - 463		141
453	GNTLYYV NKQEGKSL	453 - 467		142
457	YYV NKQEGKSLYVKG	457 - 471		143
461	KQEGKSLYVKGEPII	461 - 475		144
465	KSLYVKGEPIINFYD	465 - 479		145
469	VKGEPIINFYDPLVF	469 - 483		146
473	PIINFYDPLVFPSGE	473 - 487		147
477	FYDPLVFPSGEFDAS	477 - 491		148
481	LVFPSGEFDASISQV	481 - 495		149
485	SGEFDASISQVNEKI	485 - 499		150
489	DASISQVNEKINQSL	489 - 503		151
493	SQVNEKINQSLAFIR	493 - 507		152
497	EKINQSLAFIRKSDE	497 - 511		153
501	QSLAFIRKSDELLHN	501 - 515		154
505	FIRKSDELLHNVNAG	505 - 519		155
509	SDELLHNVNAGKSTT	509 - 523		156
513	LHNVNAGKSTTNIMI	513 - 527		157
517	NAGKSTTNIMITAI	517 - 531		158
521	STTNIMITAIIVIV	521 - 535		159
525	IMITAIIVIVVILL	525 - 539		160
529	AIIIVIVVILLSLIA	529 - 543		161
533	VIVVILLSLIAVGLL	533 - 547		162
537	ILLSLIAVGLLLYCK	537 - 551		163
541	LIAVGLLLYCKARST	541 - 555		164
545	GLLLYCKARSTPVTL	545 - 559		165
549	YCKARSTPVTLSKDQ	549 - 563		166
553	RSTPVTLSKDQLSGI	553 - 567		167
557	VTL SKDQLSGINNIA	557 - 571		168
561	KDQLSGINNIAFSN	561 - 575		169
		561-574	14mer	-

3. Peptide pools (either RSV F1 or RSV F2 pool) were added to the cells to a final concentration of 2.5 µg/mL.
4. One mock well was prepared for each subject. The volume of DMSO corresponding to the volume of the peptide pool was added to the mock well.
5. Positive control wells were stimulated with a solution of PMA (20 ng/mL)/Ionomycin (1.25 µg/mL).
6. CD28/ CD49d cocktail was added to each well at a final concentration of 2 µg/mL.
7. Following the addition of peptides and the CD28/CD49d cocktail, the plates were incubated 30-60 minutes in 37 degree incubator.

8. 5 mL of Brefeldin A (0.5 mg/mL) was then added to each well, and the plates were then incubated for an additional 4-5 hours in 37 °C 5%CO₂ incubator.
9. Plates were then removed and 20 µL of 20 mM EDTA (dissolved in 1X PBS) was added to each cell well.
- 5 10. The plates were then held at 4 °C overnight.

C. Day 3: Staining

1. Plates were centrifuged at 500xg for 5 min, and the supernatant was removed.
2. Each well was washed with 175 mL of FACS Wash, and the plate was centrifuged
10 again at 500xg for 5 min, and the supernatant was removed.
3. The PBMCs were stained with the extracellular antibodies as follows according to manufacturer recommended volume:
 - i. CD8 APCH7 : 5 µL per test
 - ii. CD3 PE: 20 µL per test
 - 15 iii. CD4 PCF594: 5 µL per test
 - iv. ViViDye: 3 µL per test
4. After the cocktail was added to all wells, 120 µL of FACS wash was added to each well and mixed. The plates were incubated in the dark at room temperature for 25-30 minutes.
- 20 5. Plates were then centrifuged plate at 500xg for 5 minutes and washed with 175 µL per well of FACS wash.
6. 200 µL of BD Cytofix/cytoperm solution was added to each well and the plates were incubated 20 to 25 minutes 4 °C.
7. Plates were then centrifuged plate at 500xg for 5 minutes and washed twice with 175
25 µL per well of PD perm wash buffer.
8. The PBMCs were then stained with the intracellular antibodies as follows:
 - i. IFN-g FITC 20 µL per test
 - ii. TNF PEcy7 5 µL per test
 - iii. IL-2 APC 20 µL per test
- 30 9. After the cocktail was added to all wells, 120 µL of BD PermWash was added to each well, and the plates were incubated in the dark at room temperature for 25 minutes.
10. Following the incubation, the plates were centrifuged at 500xg for 5 minutes, washed with 175 µL BD perm wash buffer and the cells were then resuspended in 200 µL per

well of BD stabilizing fixative. Samples were then stored overnight at 4°C and acquired on an LSRII within 24 hrs of fixing.

As shown in Figs. 18A-18B, mRNA vaccines (mF, mDS-Cav1 or mF+ mDS-Cav1) resulted in increases in RSV F specific CD4⁺ and CD8⁺ T cell responses that were positive for IFN- γ , IL-2, and TNF- α . Overall the responses were comparable across all mRNA vaccine groups. T cell responses were not boosted in the MC3 LNP only control group.

Example 17: Immunogenicity and Efficacy Against RSV-B in Cotton Rat; Effectiveness of mRNA vaccine encapsulated with MC3

The immunogenicity and efficacy of experimental mRNA RSV vaccine formulations against challenge with RSV-B was tested in cotton rats. The study compared mRNAs encoding different forms of RSV-F protein encapsulated in MC3 lipid nanoparticle.

More specifically, female cotton rats (SAGE) were used and immunizations began at 3-7 weeks of age. The mRNA vaccines evaluated in this study included:

MRK-1 membrane-bound RSV F protein

MRK-4 membrane-bound DS-Cav1 (stabilized prefusion F protein)

The groups included in the study are as summarized in Table 9. The study evaluated all mRNA vaccines at a single dose of 25 mg. Control groups included in the study received either RSV A2 ($1 \times 10^{5.5}$ pfu) or no vaccine. Two doses of vaccine were administered to each animal (at week 0 and 4) except for the group receiving RSV A2 which received a single intranasal inoculum at week 0. Serum samples were collected for assessment of RSV neutralizing antibody titers. At week 8 cotton rats were challenged intranasally with RSV B strain RSV 18537. Four days post challenge the animals were euthanized and nose and lung tissue were collected to assess vaccine efficacy by measuring RSV levels in the tissue.

Table 9. Vaccine Formulations Tested for Immunogenicity and Efficacy in Cotton Rats

Group	No. of Cotton Rats	Vaccine Formulation (mRNA/ LNP)	Concentration (μ g/mL)	Final mRNA Dose (μ g)
1	6	mF (MRK-1) mRNA/ MC3, I.M.	250	25
2	6	mDS-Cav1 (MRK-4) mRNA/ MC3, I.M.	250	25
3	6	RSV A2 (intranasal)	NA	5.5 log 10 pfu
4	6	No Vaccine	NA	NA

Individual animal neutralizing antibody (NT₅₀) titers were measured in serum samples collected at week 4 (4 weeks post-dose 1) and week 8 (4 weeks post-dose 2; day of challenge). At week 4 all of the animals responded to vaccination with mRNA vaccines as well as with the RSV A2 challenge. Titers increased in both mRNA vaccine groups following the second immunization. Both the mRNA vaccines and the RSV A2 infection resulted in roughly equivalent neutralizing antibody titers against RSV A and RSV B. The individual animal and group geometric mean NT₅₀ titers measured at weeks 4 and 8 (4 weeks post-dose 1 (PD1) and 4 weeks post-dose 2 (PD2; day of challenge)) are presented in Fig. 19.

The *in vivo* efficacy of the various vaccine formulations was evaluated by measuring inhibition of viral replication in the lungs and nasal passages of the immunized cotton rats after challenge with RSV B strain 18537 using the methods described above. The data are shown in Fig. 20. Complete inhibition of virus replication was observed in the lungs and the nose of cotton rats immunized with wt RSV A2. Both mF and mDS-Cav1 mRNAs completely protected both the lung and the nose from challenge with RSV B 18537, despite being designed based on sequences from RSV A. Both mF and mDS-Cav1 mRNA vaccines were equally effective against RSV B challenge when formulated with MC3 lipid nanoparticles.

Each of the sequences described herein encompasses a chemically modified sequence or an unmodified sequence which includes no nucleotide modifications.

Example 18: Mouse immunogenicity

In this example, assays are carried out to evaluate the immune response to RSV vaccine antigens delivered using an chemically unmodified mRNA/LNP platform in comparison to protein antigens.

Female Balb/c (CRL) mice (6-8 weeks old; N= 10 mice per group) are administered RSV mRNA vaccines or protein vaccines. The mRNA vaccines are generated and formulated in MC3 lipid nanoparticles. The mRNA vaccines to be evaluated in this study include (each in a chemically unmodified form):

MRK-1 membrane-bound RSV F protein

MRK-4 membrane-bound DS-CAV1 (stabilized prefusion F protein)

MRK-5 RSV F construct

MRK-6 RSV F construct

MRK-7 RSV F construct

MRK-8 RSV F construct

MRK-9 membrane-bound RSV G protein

MRK-11 truncated RSV F protein (ectodomain only); construct modified to include an Ig secretion peptide signal sequence

MRK-12 DS-CAV1 (non-membrane bound form); modified to include an Ig secretion peptide signal sequence

MRK-13: MRK-5 construct modified to include an Ig secretion peptide signal sequence

MRK-14: MRK-6 construct modified to include an Ig secretion peptide signal sequence

MRK-16: MRK-8 construct modified to include an Ig secretion peptide signal sequence

The animals are immunized on day 0 and day 21 of the experiment. On days 14 and 35, blood is drawn from each animal and used for serological assays. On days 42 and 49, a subset of the animals are sacrificed and spleens are harvested to support ELISPOT and intracellular cytokine staining studies.

A. RSV Neutralization Assay:

Mouse sera from each group are pooled and evaluated for neutralization of RSV-A (Long strain) using the following procedures:

11. All sera samples are heat inactivated by placing in dry bath incubator set at 56°C for 30 minutes. Samples and control sera are then diluted 1:3 in virus diluent (2% FBS in EMEM) and duplicate samples are added to an assay plate and serially diluted.

12. RSV-Long stock virus is removed from the freezer and quickly thawed in 37°C water bath. Viruses are diluted to 2000 pfu/mL in virus diluent

13. Diluted virus is added to each well of the 96-well plate, with the exception of one column of cells.

14. HEp-2 cells are trypsinized, washed, resuspended at 1.5×10^5 cells/ml in virus diluent, and 100 mL of the suspended cells are added to each well of the 96-well plate. The plates are then incubated for 72 hours at 37°C, 5% CO₂.

15. Following the 72 hour incubation, the cells are washed with PBS, and fixed using 80% acetone dissolved in PBS for 10-20 minutes at 16-24°C. The fixative is removed and the plates are allowed to air-dry.

16. Plates are then washed thoroughly with PBS + 0.05% Tween. The detections monoclonal antibodies, 143-F3-1B8 and 34C9 are diluted to 2.5 plates are then

washed thoroughly with PBS + 0.05%. 50 plates are then washed thoroughly with PBS + 0.1% of the 96-well plate. The plates are then incubated in a humid chamber at 16-24°C for 60-75 minutes on rocker

17. Following the incubation, the plates are thoroughly washed.

18. Biotinylated horse anti-mouse IgG is diluted 1:200 in assay diluent and added to each well of the 96-well plate. Plates are incubated as above and washed.

19. A cocktail of IRDye 800CW Streptavidin (1:1000 final dilution), Sapphire 700 (1:1000 dilution) and 5mM DRAQ5 solution (1:10,000 dilution) is prepared in assay diluent and 50 mL of the cocktail is added to each well of the 96-well plate.

Plates are incubated as above in the dark, washed, and allowed to air dry.

20. Plates are then read using an Aeries Imager. Serum neutralizing titers are then calculated using a 4 parameter curve fit in Graphpad Prism.

The serum neutralizing antibody titers for the mouse immunogenicity study are measured post dose 1 (PD1) and post dose 2 (PD2).

Table 10. Flagellin Nucleic Acid Sequences

Name	Sequence	SEQ ID NO:
NT (5' UTR, ORF, 3' UTR)	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTAT AGGGAAATAAGAGAGAAAAAGAGTAAGAAGAAATATAAG AGCCACCATGGCACAAGTCATTAATACAAACAGCCTGTCGCTG TTGACCCAGAATAACCTGAACAAATCCCAGTCCGCACTGGGCA CTGCTATCGAGCGTTTGTCTTCCGGTCTGCGTATCAACAGCGCG AAAGACGATGCGGCAGGACAGGCGATTGCTAACCGTTTTACCG CGAACATCAAAGGTCTGACTCAGGCTTCCCGTAACGCTAACGA CGGTATCTCCATTGCGCAGACCACTGAAGGCGCGCTGAACGAA ATCAACAACAACCTGCAGCGTGTGCGTGAAGTGGCGGTTTCACT CTGCGAATGGTACTAACTCCCAGTCTGACCTCGACTCCATCCAG GCTGAAATCACCCAGCGCCTGAACGAAATCGACCGTGTATCCG GCCAGACTCAGTTCAACGCGGTGAAAGTCCTGGCGCAGGACAA CACCTTGACCATCCAGGTTGGTGCCAACGACGGTGAAACTATC GATATTGATTTAAAAGAAATCAGCTCTAAAACACTGGGACTTG ATAAGCTTAATGTCCAAGATGCCTACACCCCGAAAGAACTGC TGTAACCGTTGATAAAACTACCTATAAAAATGGTACAGATCCT ATTACAGCCCAGAGCAATACTGATATCCAAACTGCAATTGGCG GTGGTGCAACGGGGGTTACTGGGGCTGATATCAAATTTAAAGA TGGTCAATACTATTTAGATGTTAAAGGCGGTGCTTCTGCTGGTG TTTATAAAGCCACTTATGATGAAACTACAAAGAAAGTTAATAT TGATACGACTGATAAACTCCGTTGGCAACTGCGGAAGCTACA GCTATTCGGGGAACGGCCACTATAACCCACAACCAAATTGCTG AAGTAACAAAAGAGGGTGTGATACGACCACAGTTGCGGCTCA ACTTGCTGCAGCAGGGGTTACTGGCGCCGATAAGGACAATACT AGCCTTGTA AAACTATCGTTTGAGGATAAAAACGGTAAGGTTA TTGATGGTGGCTATGCAGTGAAAATGGGCGACGATTTCTATGC CGCTACATATGATGAGAAAACAGGTGCAATTACTGCTAAAACC ACTACTTATACAGATGGTACTGGCGTTGCTCAAACTGGAGCTGT GAAATTTGGTGGCGCAAAATGGTAAATCTGAAGTTGTTACTGCT ACCGATGGTAAGACTTACTTAGCAAGCGACCTTGACAAACATA	251

Name	Sequence	SEQ ID NO:
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ORF Sequence, NT	ATGGCACAAAGTCATTAATACAAACAGCCTGTCGCTGTTGACCC AGAATAACCTGAACAAATCCCAGTCCGCACTGGGCACTGCTAT CGAGCGTTTGTCTTCCGGTCTGCGTATCAACAGCGCGAAAGAC GATGCGGCAGGACAGGCGATTGCTAACCGTTTTACCGCGAACA TCAAAGGTCTGACTCAGGCTTCCCGTAACGCTAACGACGGTAT CTCCATTGCGCAGACCACTGAAGGCGCGCTGAACGAAATCAAC AACAACCTGCAGCGTGTGCGTGAAGTGGCGGTTTCACTGCGA ATGGTACTAACTCCCAGTCTGACCTCGACTCCATCCAGGCTGAA ATCACCCAGCGCCTGAACGAAATCGACCGTGTATCCGGCCAGA CTCAGTTCAACGGCGTGAAAGTCTTGGCGCAGGACAACACCCCT GACCATCCAGGTTGGTGCCAACGACGGTGAAACTATCGATATT GATTTAAAGAAATCAGCTCTAAAACACTGGGACTTGATAAGC TTAATGTCCAAGATGCCTACACCCCGAAAGAACTGCTGTAAC CGTTGATAAAACTACCTATAAAAATGGTACAGATCCTATTACA GCCCAGAGCAATACTGATATCCAAACTGCAATTGGCGGTGGTG CAACGGGGGTTACTGGGGCTGATATCAAATTTAAAGATGGTCA ATACTATTTAGATGTTAAAGGCGGTGCTTCTGCTGGTGTTTATA AAGCCACTTATGATGAAACTACAAAGAAAGTTAATATTGATAC GACTGATAAAACTCCGTTGGCAACTGCGGAAGCTACAGCTATT CGGGGAACGGCCACTATAACCCACAACCAAATTGCTGAAGTAA CAAAAGAGGGTGTTGATACGACCACAGTTGCGGCTCAACTTGC TGCAGCAGGGGTTACTGGCGCCGATAAGGACAATACTAGCCTT GTAAAACTATCGTTTGAGGATAAAAACGGTAAGGTTATTGATG GTGGCTATGCAGTGAAGTGGGCGACGATTTCTATGCCGCTAC ATATGATGAGAAAACAGGTGCAATTACTGCTAAAACCACTACT TATACAGATGGTACTGGCGTTGCTCAAACCTGGAGCTGTGAAAT TTGGTGGCGCAAATGGTAAATCTGAAGTTGTTACTGCTACCGAT GGTAAGACTTACTTAGCAAGCGACCTTGACAAACATAACTTCA GAACAGGCGGTGAGCTTAAAGAGGTAAATACAGATAAGACTG AAAACCCACTGCAGAAAATTGATGCTGCCTTGGCACAGGTTGA TACACTTCGTTCTGACCTGGGTGCGGTTTCAAGAACCGTTTCACT CCGCTATCACCAACCTGGGCAATACCGTAAATAACCTGTCTTCT GCCCGTAGCCGTATCGAAGATTCCGACTACGCAACCGAAGTCT CCAACATGTCTCGCGCGCAGATTCTGCAGCAGGCCGGTACCTC CGTTCTGGCGCAGGCGAACCAGGTTCCGCAAAACGTCCTCTCTT TACTGCGT	252
mRNA Sequence (assumes T100 tail)	G*GGGAAAU AAGAGAGAAAAGAAGAGUAAGAAGAAAUUAA GAGCCACCAUGGCACAAGUCAUUAUACAAACAGCCUGUCGC UGUUGACCCAGAAUAACCUGAACAAAUCCAGUCCGCACUGG GCACUGCUAUCGAGCGUUUGUCUUCGCGUCUGCGUAUCAACA GCGCGAAAGACGAU GCGGCAGGACAGGCGAUUGCUAACCGUU UUACCGCGAACAUCAAAGGUCUGACUCAGGCUUCCCGUAACG CUAACGACGGUAUCUCCAUUGCGCAGACCACUGAAGGCGCGC UGAACGAAAUCAACAACAACCGCAGCGUGUGCGUGAACUGG CGGUUCAGUCUGCGAAUGGUACUAAUCCAGUCUGACCUCG ACUCCAUCAGGCUGAAAUACCCAGCGCCUGAACGAAAUUCG ACCGUGUAUCCGGCCAGACUCAGUUAACGGCGUGAAAGUCC UGGCGCAGGACAACACCCUGACCAUCCAGGUUGGUGCCAACG	253

Name	Sequence	SEQ ID NO:
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Table 11. Flagellin Amino Acid Sequences

Name	Sequence	SEQ ID NO:
ORF Sequence, AA	MAQVINTNSLSLLTQNNLNKSQSALGTAIERLSSGLRINSAKDDAA GQAIANRFTANIKGLTQASRNANDGISIAQTTEGALNEINNNLQRV RELAVQSANGTNSQSDLDSIQAEITQRLNEIDRVSGQTQFNGVKVL AQDNLTITQVGANDGETIDIDLKEISSKTLGLDKLNVQDAYTPKET AVTVDKTTYKNGTDPITAQSNTDIQTAIGGGATGVTGADIKFKDG QYYLDVKGGASAGVYKATYDETTKKVNIDTTDKTPLATAEATAI RGTATITHNQIAEVTKEGVDTTVAQAALAAAGVTGADKDNTSLV KLSFEDKNGKVIDGGYAVKMGDDFYAATYDEKTGAITAKTTTYT DGTGVAQTGAVKFGGANGKSEVVTATDGKTYLASDLDKHNFRT GGELKEVNTDKTENPLQKIDAALAQVDTLRSDLGAVQNRFNSAIT NLGNTVNNLSSARSRIEDSDYATEVSNMSRAQILQQAGTSVLAQA NQVPQNVLSLLR	254
Flagellin- GS linker- circumspor ozoite protein (CSP)	MAQVINTNSLSLLTQNNLNKSQSALGTAIERLSSGLRINSAKDDAA GQAIANRFTANIKGLTQASRNANDGISIAQTTEGALNEINNNLQRV RELAVQSANSTNSQSDLDSIQAEITQRLNEIDRVSGQTQFNGVKVL AQDNLTITQVGANDGETIDIDLKQINSQTLGLDTLNVQKQYKVS TAATVTGYADTTIALDNSTFKASATGLGGTDQKIDGDLKFDDTTG KYYAKVTVTGGTGKDGYYEVSVDKTNGEVTLAGGATSPLTGGLP ATATEDVKNVQVANADLTEAKAALTAAGVTGTASVVKMSYTDN NGKTIDGGLAVKVGDDYYSATQNKDGSISINTTKYTADDGTSKTA LNKLGGADGKTEVVSIGGKTYAASKAEGHNFKAQPDLEAAATT TENPLQKIDAALAQVDTLRSDLGAVQNRFNSAITNLGNTVNNLTS	255

Name	Sequence	SEQ ID NO:
	<u>ARSRIEDSDYATEVSNMSRAQILQQAGTSVLAQANQVPQNVLSLL</u> <u>RGGGSGGGGSMAPDPNANPNANPNANPNANPNANPNANPNANPN</u> <u>NPANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPN</u> <u>ANPNANPNKNNQGNQGNMPNDPNRNVDENANANNAVKNNN</u> <u>NEEPSDKHIEQYLKKIKNSISTEWSPCSVTCGNGIQVRIKPGSANKP</u> <u>KDEL DYENDIEKKICKMEKCSSVFNVVNS</u>	
Flagellin- RPVT linker- circumspor ozoite protein (CSP)	MMAPDPNANPNANPNANPNANPNANPNANPNANPNANPNANPN ANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNKNN QGNGQGNMPNDPNRNVDENANANNAVKNNNNEEPSDKHIEQY LKKIKNSISTEWSPCSVTCGNGIQVRIKPGSANKPKDEL DYENDIEK KICKMEKCSSVFNVVNSRPVTMAQVINTNSLSLLTQNNLNKSQSA LGTAIERLSSGLRINSADDAAGQAIANRFTANIKGLTQASRNAND GISIAQTTEGALNEINNNLQVRRELAVQSANSTNSQSDLDSIQAEIT QRLNEIDRVSGQTQFNGVKVLAQDNTLTIQVGANDGETIDIDLKQI NSQTLGLDTLNVQOKYKVSDDTAATVTGYADTTIALDNSTFKASAT GLGGTDQKIDGDLKFDDTTGKYAKVTVTGGTGKDGYYEVSVD KTNGEVTLAGGATSPLTGGPLPATATEDVKNVQVANADLTEAKAA LTAAGVTGTASVVKMSYTDNNGKTIDGGLAVKVGDDYYSATON KDGSISINTTKYTADDGTSKTALNKLGGADGKTEVVSIGGKTYAA SKAEGHNFKAQPDLEAAAATTENPLOKIDAALAOVDTLRSDLG AVQNRFNSAITNLGNTVNNLTSARSRIEDSDYATEVSNMSRAQILQ QAGTSVLAQANQVPQNVLSLLR	256

Additional mRNA Vaccines

MRK_04

SQ-030271

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30 ATCACTACCATCATTATTGTGATTATCGTAATTCTGCTATCCTTGATTGCTGTCGGGCTGCTTCTGT
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MRK_04_no AAALys**SQ-038059**

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MRK_04_no4A**SQ-038058**

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MRK_04_nopolyA_3mut**SQ-038057**

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 CTGAGTGCGCTCCGCACAGGATGGTACACCTCCGTGATCACCATCGAGCTCAGCAATATTAAAGA
 GAACAAGTGCAATGGTACCGACGCTAAAGTCAAACCTTATCAAGCAGGAACCTCGACAAATATAAGA
 ACGTGTGACCGAGCTGCAGTTATTGATGCAGAGTACACCTGCCACCAATAACAGAGCTAGGAGG
 GAGTTGCCTAGGTTTATGAAGTACACTCTCAACAACGCGAAGAAAACCAATGTGACGCTATCCAA
 10 GAAACGGAAGAGGAGGTTCTCTGGGGTTTCTTTTAGGGGTGGGCTCTGCCATTGCTTCCGGCGTGGC
 TGTATGTAAAGTTCTCCACCTCGAGGGAGAGGTTAATAAGATTAAGTCGGCCCTGCTGAGTACTAA
 CAAAGCAGTGGTGTGCTGAGTAACGGAGTAAGTGTGTAAACATTTAAGGTGCTGGACCTCAAGA
 ATTATATTGACAAACAGTTGCTTCTTATTCTAAACAAACAGAGCTGTTCAATAAGTAATATTGAAA
 CTGTTATTGAGTTTCAGCAGAAGAACAACAGGCTTCTTGAGATTACACGCGAGTTCAGTGTCAATG
 CCGGCGTTACAACACCCGTGTCTACCTACATGCTGACGAATTCTGAGCTTCTCTCTCTATAAACG
 15 ACATGCCCCATTACGAATGACCAAAAAGAACTTATGTCCAACAACGTGCAGATTGTGCGACAGCAA
 TCCTATAGCATTATGTGTATCATCAAGGAAGAGGTACTCGCTTATGTTGTGCAGCTACCACTCTAT
 GGTGTGATTGACACCCCTGTTGGAAGCTGCATACCAGTCCACTCTGCACCACTAACACAAAGGAA
 GGGAGCAATATTTGCCTCACTCGAACCGACAGGGGGTGGTATTGCGATAATGCGGGCTCCGTGTCC
 TTCTTTCCACAGGCTGAAACTTGTAAAGGTACAGTCAAACCGCGTGTCTGTGATACTATGAATTCTC
 20 TGACTCTTCCCAGCGAGGTTAATCTCTGCAACGTGACATTTTCAATCCTAAATATGACTGCAAGA
 TCATGACCAGCAAGACCGACGTCTCCAGCTCAGTAATCACTAGCCTAGGGGGCCATTGTAAGCTGCT
 ATGGCAAAACCAAGTGTACTGCCTCTAATAAGAACAGAGGCATAATTAACCTTTTCAATGGC
 TGTGACTATGTGTGCAATAAGGGCGTCGACACGGTCTCAGTAGGGAATACCCTCTACTACGTAAAC
 AAACAGGAAGGCAAATCCCTTTATGTAAAGGGCGAGCCATCATAAATTTCTACGACCCACTTGTG
 25 TTCCCCAGTGATGAATTGATGCATCAATCTCCAGGTGAACGAAAAGATCAATCAATCCCTTGCT
 TTTATACGAAAGTCAGATGAACTCTGCATAACGTGAATGCTGGGAAATCTACAACCAACATCATG
 ATCACTACCATCATTATTGTGATTATCGTAATTCTGCTATCCTTGATTGCTGTGCGGGCTGCTTCTGT
 ACTGTAAGGCCAGATCGACGCCTGTGACCCTTTCAAAGACCAACTTAGCGGTATCAATAATATTG
 CCTTTAGCAAT (SEQ ID NO: 259)

Table 12. RSV mRNA Sequences

Name	mRNA Sequence	SEQ ID NO:
RSV #1	AUGGAGCUGCUCUAUCCUCAAAAGCAAUAUGCCAUCACCACUAUCCU GACCGCCGUCACUUUCUGCUUCGCCUCCGGCCAAAUAUACCGGA AGAGUUCUAUACAGUCCACCUGCUCUGCCGUUUCUAAAGGUUACC UGUCAGCCCUUAGAACAGGGUGGUUAUACCUCUGUUAUUACCAUU GAGUUGUCCAACAUUAAGAAGAACAAAGUGCAAUUGGCACAGACGC UAAGGUUAAGCUCUAUAAAGCAGGAGCUCGACAAAUAUAAAAAUG CCGUCACGGAGCUGCAGUUAUUGAUGCAGAGCACCCAGGCGACA AACAAACCGUGCACGACGCGAGCUACCCCGAUUCAUGAACUACAC CCUCAAUAAUGCAAAGAAGACAAUUGUGACGCUCUCUAAGAAGC GCAAGCGUCGCUUUCUGGGCUUUCUUCUCGGGGUUGGGAGCGCG AUCGCAAGCGGCGUGGCUGUAUCAAAGUGCUUCAUUAAGAGGG AGAAGUGAAUAAAAUCAAAAGUGCUCUGCUAUCUACAACAAAGG CCGUUGUAUCACUGUCCAACGGAGUGUCCGUGCUCACGUCCAAA GUGCUAGAUUUUGAAGAAUUAUCAUGAUAAAGCAGCUGCUCCCUAU UGUGAACAAACAAUCAUGUCCAUCAGUAACAUAUUGAAACAGUCA UCGAGUUUCAACAGAAAAACAAUAGACUGCUGGAGAUUACCAGA GAAUUUUCGGUUAACGCCGCGUGACUACCCUGUAAGCACCUA CAUGUUGACAAACUCCGAACUUUUGUCACUGAUAAACGAUAUGC CUAUUACUAAUGAUCAGAAAAAAUUGAUGUCCAUAUUAUUAAGGA AUCGUCAGGCAACAGUCCUACAGUAUCAUGUCUAUUUAUUAAGGA GGAGGUCCUUGCAUACGUGGUGCAACUGCCAUUAUACGGAGUCA UUGAUACUCCUGUUGGAAACUCCAUAACAAGCCCCUGUGCACU ACUAAACACUAAAGAGGGAUCAAUAUUUGUCUCACUCGGACAGA UAGAGGUUGGUACUGUGAUAAUGCUGGCUCAGUGCAUUCUUUC CACAGGCUGAAACCUGCAAGGUUCAGUCAACAGGGUGUUUUGC GAUACCAUGAAUUCUCUAACCCUCCCCAGUGAGGUGAACCUUGUG	260

Name	mRNA Sequence	SEQ ID NO:
	UAAUGUGGAUAUAUUAACCCCAAGUAUGAUUGUAAGAUAUGA CCUCCAAGACGGACGUGAGUAGCAGUGUUAUACCCUCCUGGGG GCCAUUGUAUCCUGCUACGGAAAAACGAAUAGUACUGCCUCGAA CAAAAAUAGGGGAUAUCAAAAACUUUAGUAAUGGAUGCGACU ACGUUAUCUAAUAAAGGUGUUGACACAGUGUCAGUCGGCAACACA CUGUAUUACGUGAAUAAGCAAGAAGGGAAGUCGCUGUAUGUCA AGGGGAGCCUAUCAUUAUUUUUUAUGACCCACUGGUUUUCCCCA GCGAUGAGUUCGACGCCAGCAUUAAGUCAGGUUAAUGAGAAAAUC AACCAGUCCUUGGCAUUUAUUCGUAAGAGUGAUGAAUUGCUGCA UAAUGUGAACGCUGGUAAAUCCACUACCAACAUAUGAUAAUA CCAUCAUCAUAGUAAUAAUAGUAAUUUUACUGUCUCUGAUCGCU GUGGGCCUGUUAUGUAUUGCAAAGCCCGCAGUACUCCUGUCAC CUUAUCAAAAGGACCAGCUGUCUGGGAUAAACAACAUCGCGUUCU CCAAU	
RSV # 2	AUGGAACUGCUCAUUUUGAAGGCAAACGCUAUCACGACAAUACU CACUGCAGUGACCUUCUGUUUUGCCUCAGGCCAGAACAUAACCG AGGAGUUUUUAUCAUUCUACAUGCAGCGCUGUAUCUAAAGGCUAC CUGAGUGCGCUCCGCACAGGAUGGUACACCUCCGUGAUCACCAU CGAGCUCAGCAAUAUUAAGAGAAACAAGUGCAAUGGUACCGACG CUAAAGUCAAAACUUAUCAAGCAGGAACUCGACAAAUUAAAAAC GCUGUGACCGAGCUGCAGUUAUUGAUGCAGAGUACACCGGCCAC CAUAACAGAGCUAGGAGGGAGUUGCCUAGGUUUUUGAACUACA CUCUCAACAACGCGAAAAAAACCAUUGUGACGCUAUCCAAGAAA CGGAAGAGGAGGUUCCUGGGGUUUUUUAGGGGUGGGCUCUGC CAUUGCUUCCGGCGUGGCUGUAUGUAAAGUUCUCCACCUCGAGG GAGAGGUUAUAAGAUUAAGUCGGCCUUGCUGAGUACUAAACAA GCAGUGGUGUCGUGAGUAACGGAGUAAGUGUGUUAACAUUUAA GGUGCUGGACCUCAGAAUUAUUAUGACAAACAGUUGCUUCCUA UUCUAAACAAACAGAGCUGUUAUUAAGUAAUUAUGAAACUGUU AUUGAGUUUCAGCAGAAGAACAACAGGCUUCUUGAGAUUACACG CGAGUUCAGUGUCAAUGCCGGCGUUAACAACACCCGUGUCUACCU ACAUGCUGACGAAUUCUGAGCUUCUCUCUCAUAAACGACAUG CCCAUUAACGAAUGACCAAAAAAAACUUAUGUCCAACAACGUGCA GAUUGUGCGACAGCAAUCCUAUAGCAUUAUGUGUAUCAUCAAGG AAGAGGUACUCGCUUAUGUUGUGCAGCUACCACUCUAUGGUGUG AUUGACACCCCUUGUUGGAAGCUGCAUACCAGUCCACUCUGCAC CACUAACACAAAGGAAGGGAGCAUAUUUGCCUCACUCGAACCG ACAGGGGGUGGUUUUGCGAUAAUGCGGGCUCGUGUCCUUCUUU CCACAGGCUGAAACUUGUAAGGUACAGUCAAAACCGCGUGUUCUG UGAUACUAUGAAUUCUCUGACUCUCCCAGCGAGGUUAAUCUCU GCAACGUCGACAUUUCAAUCCUAAAUUGACUGCAAGAUAUG ACCAGCAAGACCGACGUCUCCAGCUCAGUAAUCACUAGCCUAGG GGCCAUUGUAAGCUGCUAUGGCAAAACCAAGUGUACUGCCUCUA AUAAGAACAGAGGCAUAAUUAACCUUUUCAAUUGGCUGUGAC UAUGUGUCGAAUAAGGGCGUCGACACGGUCUCAGUAGGGAUAC CCUCUACUACGUUAACAAACAGGAAGGCAAAUCCUUUAUGUAA AGGGCGAGCCCAUCAUAAAUUUUACGACCCACUUGUGUCCCC AGUGAUGAAUUCGAUGCAUAAUCUCCAGGUGAACGAAAAGAU CAAUCAAUCCCUUGCUUUUAACGAAAGUCAGAUGAACUCCUGC AUAACGUGAAUGCUGGGAAAUCAACAACCAACAUAUGAUCACU ACCAUCAUUAUUGUGAUUAUCGUAAUUCUGCUAUCCUUGAUUGC UGUCGGGCUGCUUCUGUACUGUAAGGCCAGAUACGACCCUGUGA CCCUUCAAAGACCAACUUAAGCGGUAUCAAUAAUUAUGCCUUU AGCAAU	261
MRK-1 membrane-bound RSV F protein/MRK_01 _F (full length,	AUGGAGCUGCUCAUCCUCAAAGCAAAUGCCAUCACCACUAUCCUG ACCGCCGUCACUUUCUGCUUCGCCUCCGGCCAAAUAUACCCGAA GAGUUCUAUCAGUCCACCUGCUCUGCCGUUUUAAGGUUACCUG UCAGCCCUUAGAACAGGGUGGUUAUACCUCUGUUAUUAACAUUGAG UUGUCCAACAUUAAGAAGAACAAGUGCAAUGGCACAGACGCUAAG	262

Name	mRNA Sequence	SEQ ID NO:
Merck A2 strain)/SQ-030268	GUUAAGCUCAUCAAGCAGGAGCUCGACAAAUUAAAAAUGCCGUC ACGGAGCUGCAGUUUUUGAUGCAGAGCAGGAGGCGACAAACAAC CGUGCAGCAGCGAGCUACCCCGAUUCAUGAACUACACCCUCAU AAUGCAAAGAAGACAAAUGUGACGCUCUCUUAAGAAGCGCAAGCGU CGCUUUCUGGGCUUUCUUCUCGGGGUUGGGAGCGCGAUCGCAAGC GGCGUGGCUGUAUCAAAGUGCUUCAUCUUGAGGGGAGAAGUGAAU AAAAUCAAAAGUGCUCUGCUAUCUACAAACAAAGCCGUUGUAUCA CUGUCCAACGGAGUGUCCGUGCUCACGUCCAAGUGCUAGAUUUUG AAGAAUUACAUCGAUAAGCAGCUGCUCCCUAUUUGUGAACAAACAA UCAUGUUCCAUCAGUAACAUAUGAAACAGUCAUCGAGUUUCAACAG AAAAACAUAAGACUGCUGGAGAUUACCAGAGAAUUUUCGGUUUAC GCCGGCGUGACUACCCUGUAAGCACCACAUUGUUGACAAACUCC GAACUUUUGUCACUGAUAAACGAUAUGCCUAUUACUAAUGAUCAG AAAAAAUUGAUGUCCAUAUUGUCCAUAUCGUCAGGCAACAGUCC UACAGUAUCAUGUCUAUUUAUUAAGGAGGAGGUCCUUGCAUACGUG GUGCAACUGCCAUUAUACGGAGUCAUUGAUACUCCUGUUGGAAA CUCCAUAACAAGCCCCUGUGCACUACUAAACUAAAGAGGGGAUCA AAUAUUUGUCUCACUCGGACAGAUAGAGGUUGGUACUGUGAUAAU GCUGGCUCAGUGCAUUCUUUCCACAGGCUGAAACCUGCAAGGUU CAGUCAAAACAGGGUGUUUUGCGAUACCAUGAAUUCUCUAACCCUC CCCAGUGAGGUGAACCUGUGUAUUGUGGAUAUAUUCACCCCAAG UAUGAUUGUAAGAUAUGACCUCCAAGACGGACGUGAGUAGCAGU GUUAUCACCUCUCCUGGGGGCCAUUGUAUCCUGCUACGAAAAACG AAAUUGUACUGCCUCGAACAAAAAUAGGGGAUAUCAAAAACUUUU AGUAAUGGAUGCGACUACGUUAUUAUUAAGGUGUUGACACAGUG UCAGUCGGCAACACACUGUAUUACGUGAAUAAGCAAGAAGGGAAG UCGCGUAUGUCAAAAGGGGAGCCUAUCAUUAUUUUUAUGACCCA CUGGUUUUCCCCAGCGAUGAGUUCGACGCCAGCAUUAAGUCAGGUU AAUGAGAAAAUCAACCAGUCCUUGGCAUUUAUUCGUAAGAGUGAU GAAUUGCUCCAUAUUGUGAACGCUGGUAAAUCCACUACCAACAUU AUGAUAAUACCAUCAUCAUAGUAAUAUAGUAAUUUUACUGUCU CUGAUCGCUUGGGGCCUGUUAUGUAUUGCAAAGCCCGCAGUACU CCUGUCACCUUAUCAAAAGGACCAGCUGUCUGGGAUAAACAACU GCGUUCUCCAUA	
MRK-4 membrane-bound DS-CAV1 (stabilized prefusion F protein)/MRK_04_Prefusion F/DS-CAV1 (Full length, S155C/S290C/S190F/V207L)/SQ-030271	AUGGAACUGCUCAUUUUUGAAGGCAAACGCUAUCACGACAAUACU CACUGCAGUGACCUUCUGUUUUGCCUCAGGCCAGAACAUAAACCG AGGAGUUUUUAUCAAUUCAUGCAGCGCUGUAUCUAAAGGCUAC CUGAGUGCGCUCCGCACAGGAUGGUACACCUCUGAUCACCAU CGAGCUCAGCAAUUUAAAAGAGAACAAGUGCAAUGGUACCGACG CUAAAGUCAAAACUUAUCAAGCAGGAACUCGACAAAUUAAAAAC GCUGUGACCGAGCUGCAGUUAUUGAUGCAGAGUACACCUGCCAC CAAUAACAGAGCUAGGAGGGAGUUGCCUAGGUUUUGAACUACA CUCUCAACAACGCGAAAAAAACCAAUGUGACGCUAUCCAAGAAA CGGAAGAGGAGGUUCCUGGGGUUUUUUAGGGGUGGGCUCUGC CAUUGCUUCCGGCGUGGCUGUAUGUAAAGUUCUCCACCUCGAGG GAGAGGUUAAUAAGAUUAAGUCGGCCUUGCUGAGUAUAACAAA GCAGUGGUGUCGCUGAGUAACGGAGUAAGUGUGUUAACAUAUUAA GGUGCUGGACCUCAGAUAUUAUUGACAAACAGUUGCUUCCUA UUCUAAACAAACAGAGCUGUUAUAUAGUAAUAUUGAAACUGUU AUUGAGUUUCAGCAGAAGAACAACAGGCUUCUUGAGAUUACACG CGAGUUCAGUGUCAUUGCCGGCGUUAACAACCCCGUGUCUACCU ACAUGCUGACGAAUUCUGAGCUUCUCUCUCUCAAUAAACGACAUG CCCAUUAACGAUUGACCAAAAAAAACUUAUGUCCAACAACGUGCA GAUUGUGCGACAGCAAUCCUAUAGCAUUAUGUGUAUCAUCAAGG AAGAGGUACUCGCUUAUGUUGUGCAGCUACCACUCUUAUGGUGUG AUUGACACCCUUGUUGGAAGCUGCAUACCAGUCCACUCUGCAC CACUAACACAAAGGAAGGGAGCAUAUUUGCCUCACUCGAACCG ACAGGGGGUGGUUAUUGCGAUAAUGCGGGCUCCGUGUCCUUCUUU CCACAGGCUGAAACUUGUAAGGUACAGUCAAAACCGCGUGUUCUG	263

Name	mRNA Sequence	SEQ ID NO:
	UGAUACUAUGAAUUCUCUGACUCUUCCCCAGCGAGGUUAAUCUCU GCAACGUCGACAUUUUCAAUCCUAAAU AUGACUGCAAGAUAUG ACCAGCAAGACCGACGUCUCCAGCUCAGUAAUCACUAGCCUAGG GGCCAUUGUAAGCUGCUAUGGCAAAACCAAGUGUACUGCCUCUA AUAAGAACAGAGGCAUAAUUAAAACCUUUUCAAUUGGCUGUGAC UAUGUGUCGAAUAAGGGCGUCGACACGGUCUCAGUAGGGAUAUAC CCUCUACUACGUUAACAAACAGGAAGGCAAAUCCCUUU AUGUAA AGGGCGAGCCCAUCAUAAAUUUUCUACGACCCACUUGUGUCCCC AGUGAUGAAUUCGAUGCAUCAAUUCCCAGGUGAACGAAAAGAU CAAUCAAUCCCUUGCUUUUAUACGAAAGUCAGAUGAACUCCUGC AUAACGUGAAUUGCUGGGAAUUCUACAACCAACAUCAUGAUCACU ACCAUCAUUAUUGUGAUUAUCGUAAUUCUGCUAUCCUUGAUUGC UGUCGGGCUGCUUCUGUACUGUAAGGCCAGAUUCGACGCCUGUGA CCCUUUCAAAAGACCAACUUAAGCGGUAUCAAUAAUAUUGCCUUU AGCAAU	
MRK-5 RSV F Construct	AUGGAACUGCUCAUCCUUAAGCCAACGCGAUAAACGACCAUUCU GACCGCCGUGACCUUCUGCUUCGCCAGCGGCCAGAAAUUACCG AAGAGUUUUACCAGAGCAGCUGCUCUGCCGUGAGCAAAGGUUAU CUGAGCGCUUUAGAACUGGCUGGUACACCAGUGUU AUUACU AU AGAGCUGUCAAAU AUUAAAAAGAAUAAAUGCAACGGGACCGAUG CCAAAGUAAAAUUAUUAAGCAGGAUUGGACAAGUAUAAGAAU GCAGUGACAGAGUUGCAGCUCUGAUGCAGAGCACACAAGCUAC AAACAAUCGCGCUCGCCAGCAGCAACAGCGUUUUUAGGGUUC UGCUAGGGGUGGGGUCAGCCAUUGCCUCUGGAGUGGCAGUGUCC AAAGUGCUGCAUCUGGAAGGGGAAGUUAACAAGAUAAAAUCCGC ACUCCUCAGCACCAAUAAAGCCGUGGUCUCCCUUGUCCAUUGGAG UAUCAGUUUUGACAAGCAAGGUGCUGGACCUGAAGAAUUAUAUA GAUAAGCAGUUAUCUGCCAUAUGUGAAUAAACAGUCAUGCUCAAU UAGCAACAUAUGAGACAGUUAUCGAAUUCAGCAGAAAAAUAAUA GGCUUCUGGAAUAACUCGCGAAUUCUCAGUAAAUGCCGGAGUG ACCACACCCGU AUCGACUUAU AUGCUUACAAACUCUGAACUGUU GUCCUUGAUUAACGAUAUGCCAUAACAAAUGACCAGAAGAAGC UAAUGAGCAACAAUGUGCAGAUUGUAAGACAGCAGUCUUAUCUA AUAUUGUCUAUAUAUAAAAGAGGAGGUGUUGGCAUAUGUGGUGC AACUGCCUCUCUAUGGCGUGAUUCGAUACUCCUUGCUGGAAGUUA CAUACAUCUCCACUGUGUACAACUAAUACUAAGGAGGGUAGCAA UAUUUGUCUGACACGCACAGAUUCGGGGUUGGU AUUGCGACAACG CGGGCAGUGUGAGCUUUUUCUCCUAGGCCGAAACCUGUAAGGUU CAAUCUAAUCGGGU AUUUUGCGACACAAUGAACAGCCUGACCCU UCCGUCCGAAGUUA AUUUUGUGCAACGUCGACAUCUCAAUCCUA AAUAUGACUGCAAAAUCAUGACUUCUAAAACCGACGU AUCCAGC UCAGUGAUAAACAAGCCUUGGGGCAAUUGUAAGCUGCUAUGGCAA GACGAAGUGCACCGCUAGUAACAAGAACCGGGGGAUUAUUAAGA CUUUUUCGAACGGAUGCGAUUACGUCUCCAACAAAGGCGUCGAU ACUGUGUCCGUGGGAAACACCCUCUACU AUGUGAACAAAGCAGGA AGGCAAAAGCCUCUACGUCAAAGGAGAGCCUAUCAUCAAUUUCU ACGACCCUCUAGUAUUCUCCUUCAGACGAAUUGACGCAUCAAUU UCCCAGGUGAACGAGAAAAUAAAUCAAAGCUUAGCCUUUAUCCG CAAGAGUGAUGAGUUGCUUCACAACGUC AACGCCGGCAAUAUCAA CCACUAAU	264
MRK-6 RSV F Construct	AUGGAACUCUUGAUCCUGAAGGCUAAUGCAAUAACAACAAUUCU GACAGCAGUCACCUUUUGCUUCGCCAGCGGACAGAAU AUUACGG AGGAGUUUU AUCAAUCUACCUGUAGUGCCGUGAGCAAGGGGUAC CUGUCUGCCCUGAGGACGGGAUGGUACACAUCGUGAUACCAU CGAGUUGUCUAACAUAUAAAAGAAACAAGUGCAACGGAACUGACG CCAAGGUGAAGCUCAUUAAGCAAGAGCUCGACAAAUUAAGAAU GCGGUUACAGAACUACAGCUACUAAUGCAGUCCACACAGGCAAC CAAUAACCGAGCACGUCAGCAGCAGCAACGCUUCCUUGGCUUCC UGCUCGGGGUUGGCUCGGCAAUUGCAUCCGGAGUGGCUGUUUCC	265

Name	mRNA Sequence	SEQ ID NO:
	AAGGUUUUUGCACCUUGAGGGAGAGGUCAAUAAGAUAAGAGCGC CCUCCUGUCAACUAAUAAGGCCGUGGUCAGCCUUUCCAACGGUG UUUCUGUGUUAACCUCAAAAAGUGCUCGACCUUAAAAACUUAUC GAUAAGCAGCUGCUGGCCAUAGUGAACAAACAGUCCUGUUCUUAU CAGUAAUAUCGAGACAGUGAUCGAAUCCAGCAGAAGAACAUC GUCUGCUGGAAAUAACAAGGGAGUUCAGCGUAAACGCUGGAGUC ACAACCCCCGUGUCCACUUAACUAGCUGACCAAUUCCGAGCUGCU GAGUUUGAUUAAUGAUUAGCCCAUACGAACGAUCAGAAGAAAC UGAUGUCGAAUAAUGUUCAGAUUCGUUAGGCAGCAGUCUUAUAGC AUCUAGAGUAUUAUCAAAAGAGGAGGUCCUCGCCUAUGUGGUUCA GCUGCCUCUCUACGGCGUUAUAGACACCCCAUGCUGGAAGCUUC ACACCUCUCCUCUGUGUACGACCAAUACAAAGGAGGGCUCAAAC AUUUGCCUUACCCGCACAGAUAGAGGAUGGUACUGCGAUAAUAGC UGGCUCUGUGUCUUUCUUUCCUCAGGCCGAAACAUGUAAGGUAC AGUCCAAUAGGGUAUUUUGCGACACCAUGAACUCCCUAACCUUA CCAAGUGAAGUGAACCUCUGCAAUGUGGACAUCUUUAACCCGAA GUAUGACUGCAAAAUCAUGACUCCAAGACAGACGUGUCCAGUA GUGUGAUUACCUCACUGGGCGCAAUCGUUUAUGCUAUGGGAAG ACAAAGUGCACCGCAAGCAACAAGAAUCGGGGCAUCAUCAAAC CUUCAGUAACGGUUGUGACU AUGUUUCAAACAAGGGAGUCGAUA CCGUGUCGGUGGGCAAUACUCUUUACUACGUGAAUAAACAGGAG GGGAAAUCACUGUAUGUGAAAGGUGAGCCGAUCAUUAACUUUA CGACCCUCUCGUGUUUCCUCCGAUGAGUUCGACGCAUCCAUA GUCAGGUCAAUGAGAAAAUCAACCAUUCUCUGCCUUAUUAAGA AAAUCUGACGAAUACUGAGUGCCAUUGGAGGAUUAUUAUCCGGA GGCUCCAGGGACGGGCAGGCUUACGUCCGAAAGGAUGGAGAAU GGGUCCUACUGAGCACAUUUCUA (The underlined region represents a sequence coding for foldon. The underlined region can be substituted with alternative sequences which achieve a same or similar function.)	
MRK-7 RSV F Construct	AUGGAGCUCCUGAUUCUUGAAGGCGAAUGCCAUAACCAUCCU CACCGCAGUAACUUCUGUUUCGCAAGUGGCCAGAAUAUAACAG AAGAGUUCUAUCAGUCAACCUGUAGCGCAGUCUCAAAGGGGUUAU UUAUCAGCACUGAGAACCGGUUGGUUAUACCAGUGUUUAUACAUA AGAGCUGAGUAACAUAAGGAGAAUAAGUGCAACGGCACUGACG CCAAGGUCAAGCUCAUCAAACAGGAACUCGAUAAUACAAGAAC GCUGUCACUGAACUGCAGCUGCUGAUGCAAAGCACCCCCGCCACC AACAAUAGGGCCCCGAGAGAGCUUCCUAGAUUUUAUGAACUACAC UCUGAACAAACGCCAAAAAGACCAUGUAACACUGUCAAGAAAC AGAAACAGCAGGCUAUUGCAAGCGGUGUGGCUGUGUCUAAAGUG CUGCAUCUCGAGGGGGAGGUCAAACAAGAUCAAUCCGCAUUGCU CAGCACCAACAAGGCUGUGGUGAGCCUGUCCAUGGUGUCUCAG UGCUCACCAGCAAAGUGCUGGACCUGAAGAAUUAUUAUGAUAA CAGCUGCUACCCAUAGUCAACAAACAGUCAUGCUCCAUAUCUAA UAUUGAGACUGUCAUCGAGUUCACAGAAGAACAUCGCCUGC UGGAGAUUACCAGGGAGUUCUAGUCAUAGCCGGGGCAGCAGACA CCCGUUAGUACUUAUUAUGCUUACCAACUCCGAGCUUCUCUUU GAUCAUAGACAUGCCAAUUAUUAACGACCAGAAGAAGUUGAUGU CUAACAAUGUACAGAUUGUUCGCCAGCAGUCCUAUUAUUAUG UCGAUUAUUAAGAGGAGGUUCUUGCAUACGUCGUGCAGUUGCC AUUAUUAUGGAGUCAUCGACACCCCCUGCUGGAAACUGCAUACGU CACCAUUAUGCACCACGAAUACAAAGGAGGGCAGUAAUUAUUGU CUUACACGGACUGAUCGAGGCUGGUUAUUGUGAUAAACGCAGGCUC GGUGUCAUUCUUUCCACAGGCUGAAACCUGUAAGGUGCAAUCUA AUAGGGUGUUUUGCGAUACCAUGAAUUCUCUGACUCUGCCCAGU GAGGUCAAUUUGUGUAACGUGGACAUCUUAACCCAAAGUACGA CUGCAAGAUAUGACAUCUAAGACAGAUGUGUCAUCCAGCGUUA UCACGAGCCUCGGCGCUAUGUCUCCUGUACGGCAAGACCAAG UGCACCGCUAGCAACAAGAAUCGGGGAAUCAUAAAACCUUUUC UAACGGUUGUGACUACGUGAGCAACAAGGGGGUGGAUACCGUCU	266

Name	mRNA Sequence	SEQ ID NO:
	CAGUCGGUAAACACCCUGUACUACGUGAAUAAACAGGAGGGGAAG UCAUUGUACGUGAAGGGUGAACCUAUCAUCAACUUUUUUGACCC CCUCGUCUCCCAUCAGACGAGUUUGACGCGUCCAUCUCUCAGG UGAAUGAGAAGAUUAACCAGAGCCUGGCUUUUAUCCGCAAAUCA GACGAACUACUGCACAAGUGCAACGCGUGGCAAGAGCACAACAA UAUAAUGAUACAACCAUCAUCAUCGUCAUUAUUGUGAUCUUGU UAUACUGAUCGCGUGGGGCUCCUCCUUUAUUGCAAGGCUCGU AGCACCCUGUCACCCUCAGUAAAGAUACAGCUGUCAGGGAUCAA UAAUAUCGCGUUUAGCAAC	
MRK-8 RSV F Construct	AUGGAAUUAUUAAUUUUUGAAGACAAAUGCUAUAACCGCGAUACUA GCGGCUGUGACUCUUUGUUUCGCAUCAAGCCAGAAUUAUUACAGAA GAAUUUUUAUCAAUCCACCUGCAGCGCUGUAUCGAAAGGUUACCUC AGCGCGCUUAGGACAGGAUGGUUAUACCUCCGUUAUCACGAUUGAA CUGAGUAAUAUCAAGGAAAACAAGUGUAACGGAACAGACGCCAAG GUCAAACUUAUUAAACAAGAACUGGACAAGUAUAAGUCUGCAGUG ACCGAAUUGCAGCUCCUGAUGCAGAGUACCCUGCAACUAACAAC AAGUUUUUGGGCUUUCUGCAAGGCGUGGGUAGCGCGAUCGCCUCC GGAAUCGCGGUCUCCAAAGUGUUGCACCUGGAGGGAGAAGUUAAC AAGAUCAAAUCGGCUCUGUUGAGUACCAACAAGGCAGUGGUGUCA CUGAGCAACGGUGUAAGCGUGUUAACAAGCAAGGUUAUUGGACUUA AAGAACUAUAUUGACAAACAGCUGCUCCCCAUCGUGAACAACAG AGCUGCUCAAUCUCCAUAUAGAGACGGUGAUAGAGUUCAGCAA AAAAUAAUCGGCUCUUGAGAUACCCGCGAAUUCUGAGUAAU GCCGGCGUCACAACUCCGGUGUCUACAUACAUGCUGACCAACUCG GAGCUGUUAUCCUUAUAUAAUUGACAUGCCCAUCACCAAUGAUCAA AAAAAACUGAUGUCAAAUAACGUCCAGAUAGUAAGACAGCAGAGC UACAGCAUCAUGUCGAUUAUCAAAAGAGGAGGUGCUGGCGUACGUG GUGCAGCUGCCCCUGUAUGGGGUGAUUGACACCCUUGUUGGAAG CUGCACACCUCUCCACUAUGUACUACCAAUACCAAGAAGGAUCC AACAUUCGCCUUAACCCGCACCGAUAGGGGAUGGUUAUUGCGACAAC GCCGGAUCCGUCAGCUUCUUUCCACUUGCCGAAACUUGCAAGGUU CAGUCAAAACGGGUGUUCUGCGAUACAAUGAAUCCCUUACCUUG CCCAGCGAAGUUAUUCUCUGUAAUAUUGACAUCUUUAACCCCAA UACGAUUGCAAAAUUAUGACGUCAAAAACCGAUGUCAGUUCAAGC GUUAUACACCAGCUUGGGUGCUAUCGUUUAUGCUAUGGCAAAACC AAGUGUACGGCUAGUAACAAAAACCGCGGAUAAUUAAGACAUUC AGCAAUGGUUGCGACUACGUUAUCAAUAAGGGUGUCGACACCGUU UCCGUGGGCAAUACGCUGUACUAUGUUAUAAACAGGAAGGCAAG UCACUGUAUGUUAAGGUGAACCCAUCAUCAACUUCUACGACCCC CUGGUUUUCCCCUCCGACGAGUUUGAUGCCAGCAUAUCACAGGUU AAUGAAAAAAUAAACGGCACAUUGGCGUUUAUCAGAAAGUCUGAC GAGAAACUUCAUAACGUGGAAGACAAGAUAGAAGAGAUAAUUGAG <u>CAAAAUUCUAUCAUAUUGAGAACGAGAUCGCCAGGAUCAAAAAGCU</u> <u>UAUUGGGGAG</u> (The underlined region represents a region coding for GCN4. The underlined region can be substituted with alternative sequences which achieve a same or similar function.)	267
MRK-9 membrane-bound RSV G protein	AUGUCUAAAAACAAGGACCAGCGCACUGCUAAGACGCGUGGAACG CACAUGGGAUACCCUGAACCAUCUGUUAUUCAUUUCCAGCUGCC UCUACAAGCUAAACCUUAAAAGUGUUGCACAAAUCACACUCAGC <u>AUCCUGGCAAUGAUUAUUUCAACAUCCCUGAUCAUAGCCGCAAU</u> <u>CAUAUUUAUCGCCUCAGCAAAUCACAAAGUUACCCCGACCACAG</u> CCAUUAUCCAGGACGCUACAUCCCAAUCAAACACACACCU ACAUAUCUCACUCAGAACCCGCAGCUGGGCAUUUCACCAUCCAA CCCUCCGAGAUACCCUCUCAAUACACCAUUCUCGCCUCUACU ACCCCGGGAGUAAAGAGCACUCUUCAGAGCACAAACCGUUAAC UAAAAAUACCACCACACUCAGACUCAGCCUUCGAAACCAACGA CUAACAGCGGCAAAAUAAAGCCUCCAUCCAAACCGAAUAACGAC UUUCAUUUCGAAGUCUUUAACUUUGUGCCAUGCAGUAUUUGCUC CAAUAAUCCUACUUGCUGGGCUAUCUGCAAGAGAAUCCCUAACA	268

Name	mRNA Sequence	SEQ ID NO:
	AGAAGCCUGGAAAGAAGACAACGACAAAGCCAACUAAGAAGCCG ACACUUAAGACUACCAAAAAAGACCCUAAGCCGAGACUACCAA GAGCAAGGAGGUUCCCACAACCAAGCCUACAGAGGAGCCGACUA UUAACACAACAAAGACCAACAUCAUACCACCCUGCUUACUUCU AAUACUACCGGAAACCCAGAGCUGACGUCCCAGAUGGAGACGUU CCAUUCCACAUCUUCGGAAGGGAAUCCUAGUCCCAGCCAGGUGA GCACAACCUCAGAAUACCCGUCCCAGCCCUCAUACCUCUAAUA CCCCCGGCAG (The underlined region represents a region coding for transmembrane domain. The underlined region can be substituted with alternative sequences which achieve a same or similar function.)	
MRK-11 truncated RSV F protein (ectodomain only); construct modified to include an Ig secretion peptide signal sequence	<u>AUGGAGACGCCUGCCCAGCUGCUGU</u> <u>UCCUGCUGUUGUUGUGGCU</u> <u>GCCAGAUACUACUGGGUUGCAAGCGGACAAAACAUUACCGAAG</u> <u>AGUUCUAUCAAUCCACAUGCUCUGCAGUGUCUAAGGGCUACCUU</u> <u>AGUGCAUUAACGAACCGGGUGGUUAUACGAGUGUAAUCACCAUUGA</u> <u>GCUGUCCAACAUCAAGAAGAACAAGUGCAAUGGGACUGAUGCCA</u> <u>AGGUGAAACUUAUCAAAACAAGAGCUCGACAAGUAUAAGAACGCC</u> <u>GUGACCGAACUACAACUCCUGAUGCAAUCGACUCAGGCUACUAA</u> <u>CAACAGAGCUCGGAGGGAGCUGCCCAGAUUCAUGAAUUAUACCU</u> <u>UAAACAACGCUAAAAAAACAAUUGUGACCCUGAGUAAGAAGCGG</u> <u>AAACGAAGGUUCCUGGGCUUCCUGCUCGGUGUGGGGUCUGCAAU</u> <u>AGCAAGCGGCGUCGUGUGUCCAAGGUCCUUCACUUAAGAAGGUG</u> <u>AGGUCAAUAAGAUAAGUCCGCUCUCCUCUCUACCAACAAGGCA</u> <u>GUGGUGAGCCUGUCUAACGGUGUGUCCGUGCUGACAUCAAGGU</u> <u>ACUGGACCUGAAAAACUACAUCGACAAGCAGCUGCUGCCUAUUG</u> <u>UGAAUAAGCAAUCCUGCAGUAUCUCCAACAUUGAGACAGUGAUU</u> <u>GAAUUUCAGCAAAAGAACAUCGUUUGUUGGAGAUAAACAAGAGA</u> <u>AUUCAGUGUUAUUGCCGGCGUUAACACUCCCGUGUCGACAUAACA</u> <u>UGCUAACAAAUAGCGAGCUGCUAUCUCUCAUUAUUGAUUAGCCU</u> <u>AUCACCAAUGACCAGAAAAACUUAUGUCCAUAUACGUGCAGAU</u> <u>AGUCAGGCAGCAGUCCUACAGCAUUAUGAGCAUAAUUAAGAGG</u> <u>AAGUGUUGGCUUACGUCGUCCAGCUUCCACUGUAUGGCGUGAUC</u> <u>GAUACCCCUUGUUGGAAGCUGCAUACUUCUUUUUUUGUACAAC</u> <u>UAAUACCAAAGAAGGGAGUAAUUAUUGCCUCACAAGGACUGACA</u> <u>GAGGCUGGUACUGCGACAACGCCGGGAGCGUCAGCUUUUUUCCCG</u> <u>CAGGCCGAGACAUGUAAGGUGCAGAGCAACCGUGUCUUUUUGCGA</u> <u>CACCAUGAAUAGCCUGACUUUGCCAAGUGAGGUCAACCUUUGCA</u> <u>ACGUGGAUUAUUUUUAACCCUAAGUACGAUUGUAAGAUAAUGACA</u> <u>UCCAAAACCGAUGUUAUGUAGCUCGUGAUCACUUCGUGGGUGC</u> <u>GAUAGUUAGCUGCUAUGGAAAGACAAAGUGUACCGCAAGUAACA</u> <u>AGAACCGCGGGAUUAUUAACAACAUUUAAGCAAUGGGUGCGACUAC</u> <u>GUACUCAAACAAGGGGGUGGAUACAGUCAGCGUGGGAAACACACU</u> <u>UUACUACGUUAACAAGCAGGAAGGGAAAUCCCUUUAUGUGAAGG</u> <u>GAGAACCAAUUAUCAACUUUUUAUGAUCCCCUCGUGUUUCCAAGU</u> <u>GAUGAAUUCGACGCAAGCAUCUCGCAGGUGAACGAGAAAAUCAU</u> <u>UCAGAGUCUAGCUUUAUUAAGGAAGUCUGAUGAACUGCUUAGUG</u> <u>CCAUUGGCGGGUACAUAACCGGAAGCCCCACGCGACGUCAGGCU</u> <u>UACGUGAGGAAGGACGGCGAGUGGGUUCUGCUGUCCACUUUCCU</u> <u>U</u> (The first underlined region represents region coding for human Igk signal peptide, second underlined region represents region coding for foldon. The underlined regions can be substituted with alternative sequences which achieves same or similar functions.)	269
MRK-12 DS- CAV1 (non- membrane bound form); modified to include an Ig secretion peptide signal sequence	<u>AUGGAGACUCCCGCUCAGCUGCUGU</u> <u>UUUUGCUCCUCCUAUGGCUG</u> <u>CCGGAUACCACCGGCUUUGCCUCUGGACAGAACAUAUACCGAGGAA</u> <u>UUCUAUCAGUCGACUUGUCCCGCAGUCUCGAAGGGGUACCUGAGU</u> <u>GCCCUGCGCACCGGGUGGUACACCAGUGUUAUCACUAUUGAGCUG</u> <u>UCCAACAUAUAAAGAAAAUAAGUGUAAUGGAACUGACGCGAAGGUG</u> <u>AAGUUGAUAAAACAGGAGCUGGAUAAAUAACAAGAAUGCAGUGACC</u> <u>GAACUGCAGCUCCUGAUGCAGUCCACUCCAGCAACAAUAAUACGC</u> <u>GCGAGACGCGAACUCCCCCGCUUAUGAACUACACUCUGAAUAAU</u>	270

Name	mRNA Sequence	SEQ ID NO:
	GCGAAGAAAACGAAUGUGACACUAAGUAAGAAAAGAAAACGGCGA UUUCUUGGGUUCUGUCUGGGGUGGGAUCUGCCAUAAGCAAGCGGG GUGGCGGUAUGUAAAGUCCUUCACCUAGAAGGGGAGGUGAACAAA AUUAAGAGUGCCUGCUGAGCACCAACAAGGCUGUGGUUUCACUG UCAACCGGAGUAAGCGUGCUAACAUUUAAAGUCUUGGACCUGAAG AAUUAUUAUGACAAGCAGCUCCUGCCCAUUCUCAACAAACAGUCA UGUCCAUAUAGCAACAUCAAGAACAGUCAUUGAGUUUCAGCAAAAA AACAACCGCCUCCUUGAGAUUACGCGUGAGUUUCCGUCAAUGCU GGAGUCACGACACCGGUGUCCACUACAUGCUGACUAAACAGCGAA CUCCUGAGCCUAAUCAUAGACAUGCCCAUACUAACGACCAGAAA AAAUUGAUGUCCAUAACGUGCAGAUAGUGCGCCAGCAAUCUAC UCCAUAUUGUGCAUUAUCAAGGAGGAAGUCCUGGCGUACGUUGU CAGCUGCCCGCUGUAUGGUGUGAUAGAUAACGCCAUGCUGGAAACUG CACACAUCUUUUUUGCACAACGAAUACUAAAAGAGGGAAGUAAC AUUUGCUUGACCAGAACAGAUCCGGGGCUGGUACUGCGACAACGCU GGUAGUGUGUCAUUUUUCCCGCAGGCAGAAACGUGUAAAAGUCCAG AGCAAUCGCGUGUUCUGCGACACAAUGAACUCACUUAUUUGCCC UCAGAGGUCAAUUUGUGUAAUGUGGAUAUCUUAACCCGAAAUAC GAUUGUAAGAUUAUGACGAGCAAAACAGACGUGUCUUAUCAGUG AUAACAAGUCUGGGCGCAAUAGUGUCAUGCUAUGGUAAGACUAG UGCACUGCCUCCAUAUAAAACCGCGCAUCAUAAGACAUUUUA AAUGGAUGCGACUACGUGUCAAAACAAGGGCGUCGACACAGUAAGC GUUGGGAACACCCUAUACUACGUCAACAAGCAGGAGGGGAAAAGC CUAUACGUGAAAGGCGAGCCAAUCAUAUUUCUACGAUCCACUG GUCUUUCCAAGUGACGAAUUUGAUGCCAGCAUAUCGCAGGUGAAC GAGAAAAUAAUACAGUCACUCGCCUUAUCAGGAAGUCAGAUGAG CUGCUGUCCGCCAUCGGAGGAUACAUCCAGAAGCCCCACGCGAC <u>GGCCAGGCAUACGUGCGGAAGGACGGCGAAUGGGUCCUUUUGAGC</u> <u>ACUUUUUCUA</u> (The first underlined region represents a region coding for human Igk signal peptide, The second underlined region represents a region coding for a foldon. The underlined regions can be substituted with alternative sequences which achieves same or similar functions.)	
MRK-13 MRK-5 construct modified to include an Ig secretion peptide signal sequence	<u>AUGGAGACUCCAGCCCAAUACUGUUCUGCUACUCCUUUGGCU</u> <u>GCCCGAUACUACUGGAUUCGCUUCGGGUCAGAAUUAUACAGAGG</u> AGUUCUACCAAAGUACUUGCUUGCAGUCUCCAAGGGAUACCUG UCCGCUUGCGGACGGGAUGGUUAUACCAGUGUUAUAAACGAUCGA GUUGAGCAACAUCAAGAAGAACAUAUGUAAUGGAACAGAUGCCA AGGUGAAACUGAUCAAAACAGGAGUUGGAUAAAUAUAGAAGUCU GUCACCGAACUGCAGCUAUUGAUGCAGUCCACCCAGGCUACCAA CAACCGGGCCAGGCAGCAACAACAGAGAUUUUUUGGGUUUCUUGC UGGGCGUGGGGUCUGCCAUCGCUUCAGGGGUGGCCGUGAGUAAA GUCCUGCACCUGGAAGGCGAAGUCAACAAGAUCAAGUCUGCAUU ACUAAAGUACCAAUAAGGCUAGUUAAGCCUGUCCAAGGCGUGA GUGUGCUUACUUCUAAGGUACUGGACCUGAAGAACUACAUCGAC AAGCAACUACUACCCAUUGUAAAUAAGCAGUCAUGUAGCAUAC AAACAUCGAGACAGUGAUCGAAUUUCAACAGAAGAAUAACCGGC UGUUGGAGAUAAACACGGGAGUUCUCUGUAAAUGCCGGCGUGACG ACCCUGUCAGCACCUAACUGCUCACGAAUAGCGAGUUGCUUUC CCUGAUUAAUGAUUAGCCGAUUACAAAUGACCAGAAGAAGCUGA UGAGUAAUAAUGUCCAAAUUGUCCGUCAGCAGAGCUAUUCGAUU AUGUCCAUAUCAAGGAGGAAGUCUUAAGCCUAUGUGGUGCAGCU <u>CCCCUCUACGGAGUGAUUGACACACCGUGCUGGAAGCUGCACA</u> CCUCCCCUUUGUGUACAACCAAUACCAAGGAGGGCUCCAACAUC UGCCUUAUCUAGGACCGACAGGGGAUGGUUAUUGCGACAACGCCGG GUCCGUCUUAUUUUUCCUCAGGCGGAAACCGUAAGGUACAGU CGAAUCGAGUGUUUUGUGACACUAUGAACAGCCUGACCUUGCCU AGCGAGGUGAAUCUGUGUAACGUUGAUUUCUUAACCCUAAGUA UGACUGUAAGAUAUGACUUCAAAAACUGAUGUCUCCUCAAGCG UGAUCACCUCUUUGGGCGCCAUCGUGUCAUGCUACGGAAGACG	271

Name	mRNA Sequence	SEQ ID NO:
	AAGUGCACCGCCUCUAACAAGAACCGAGGGAUCAUCAAACAUAU CUCCAAUGGCUGUGAUUACGUCAGUAACAAAGGUGUGGACACAG UCUCCGUGGGCAAUACGUUAUAUUAUGUGAAUAAGCAGGAGGGA AAAAGUCUCUAUGUGAAGGGUGAACCGAUAAUCAAUUUCUACGA UCCCUUGGUGUUUCCAAGCGACGAGUUCGACGCCUCGAUCAGCC AGGUGAACGAGAAAAUCAACCAGUCUUUGGCAUUCAUCCGCAAG AGCGACGAGCUACUGCAUAACGUGAACGCAGGCAAGAGUACUAC CAAU (The underlined region represents a region coding for human Igk signal peptide. The underlined region can be substituted with alternative sequences which achieve a same or similar function)	
MRK-14 MRK-6 construct modified to include an Ig secretion peptide signal sequence:	<u>AUGGAGACUCCCGCUCAGUUGUUGUCCUGCUACUGCUGUGGCUG</u> <u>CCUGAUACAACCGGAUUUGCUAGUGGGCAGAAU AUCACCGAAGAA</u> UUCUAUCAGAGCACUUGCAGUGCAGUGUCCAAAGGAUAUUUGAGC GCCCUGCGCACUGGGUGGUACACAAGUGUCAUCACAAUCGAGCUA AGUAACAUAUAAAAAAAAACAAUGCAACGGGACUGACGCAAAGGUC AAACUCAUUAAGCAAGAACUUGACAAAUUAAGAACCGUGUUAACA GAGUUGCAGCUGCUAAUGCAAAGCACUCAGGCUACCAUAUACCGA GCGAGACAGCAGCAGCAACGUUUCUGGGUUCUGUUAAGGUGUG GGUAGCGCAAUUGCCAGUGGUGUAGCCGUGUCCAAGGUGCUGCAC CUGGAAGGGGAAGUGAAUAAGAUCAGUCUGCACUGCUGUCCACC AAUAAGGCGGUCGUUUCGUGUCUAACGGCGUCUCGGUCCUAACA AGUAAAGUUCUGGAUUUAAAGAACUAUAUUGAUAAAGCAAUUGCU GCCUAUCGUAAAUAAGCAGAGUUGCAGCAUUAAGCAAUUCGAGAC AGGAUAGAAUUUCAGCAAAAGAACAAUCGAUUAUCGAAAUACAC ACGCGAAUUCAGUGUCAAUGCCGGGUUACAACCCCUGUGUCGAC CUACAUGCUUACCAAUUCCGAGCUUCUGUCUCUUAUUAACGAUUA GCCCCAUCACGAACGAUCAGAAGAAACUGAUGUCAAUAACGUCCA AAUUGUGCGGCAGCAAAGCUACAGUAUCAUGAGCAUCAUCAAAGA GGAGGUGCUCGCCU AUGUGGUCCA AUUGCCGCUAUACGGGGUCAU UGAUACACCCUGUUGGAAGCUCCAUAUACUCCCAUUGUACAAC GAAUACCAAGGAGGGGUCUAACAUAUUGUCUGACCCGGACCGACAG AGGCUGGUAAUUGCGAUAAUGCUGGAAGCGUUAUUAUUCUUAU GGCAGAAACAUGCAAGGUGCAGUCAACAGAGUUUUCUGUGACAC CAUGAAUUCUUGACGUGCCUUCAGAAGUGAAUCUGUGUAACGU GGAUAUCUUUAAUCCGAAGUACGAUUGUAAAAUUAUGACUAGCAA GACAGAUGUCUCGUCCUCUGUGAUCACUAGCCUGGGAGCGAUUGU GAGCUGUUAUGGUAAAACAAAGUGUACUGCUAGCAAUAAGAACAG GGGGAUUAUCAAACGUUCAGUAACGGCUGUGAUUACGUAUCCAA CAAGGGGGUGGACACCGUGUCAGUCGGGAACACGCUCUACUACGU GAACAAGCAGGAAGGUAAGUCGCUAUACGUGAAGGGGGAAACCAU AAUCAAUUUCUACGAUCCGCUUGUGUUUCCUAGCGACGAAUUCGA CGAUCUAUCAGCCAGGUGAACGAGAAGAUCAAUCAGAGUCUGGC CUUCAUCCGCAAGUCCGACGAGCUGCUU <u>AGUGCUAUCGGAGGUUA</u> <u>UAUCCUGAGGCCCCGAGGGACGGCCAAGCGUAUGUGAGAAAGGA</u> <u>CGGGGAAUGGGUACUGUUGUCAACUUUCCUA</u> (The first underlined region represents a region coding for human Igk signal peptide, The second underlined region represents a region coding for a foldon. The underlined regions can be substituted with alternative sequences which achieves same or similar functions.)	272
MRK-16 MRK-8 construct modified to include an Ig secretion peptide signal sequence:	<u>AUGGAGACACCUGCCCAACUUCUGUUCUUCUUUUGCUCUGGCU</u> <u>GCCUGACACAACCGGCUUCGCAUCUUCACAAAACAUCACGGAAG</u> AGUUUUACCAGAGCACAUGCUCGCGGUCUCUAAAGGCUAUCUU UCUGCCCUGCGGACUGGCUUGUAUACCAGCGUCAUCACCAUAGA GCUGUCAAAACAUAAGGAGAACAAGUGUAACGGCACUGACGCCA AGGUCAAGCUUAUAAAGCAGGAACUGGACAAGUAUAAGAGUGCU GUUACCGAGCUCCAGUUGCUUAUGCAGUCCACCCCCGCAACAAA CAAUAAAUUUCUGGGCUUUCUACAGGGCGUCGGAAGCGCCAUCG CAAGCGCAUCGCUUGAGCAAGGUGUUGCAUCUGGAGGGAGAG GUGAAUAAGAUAAAGAGUGCUCUGCUUCCACUAACAAAGCCGU	273

Name	mRNA Sequence	SEQ ID NO:
	GGUGAGCCUGAGCAAUGGCGUAUCUGUUCUGACUUCUAAAAGUCC UGGAUCUCAAGAACUUAUUCGACAAGCAGCUCUUGCCCAUUGUC AACAAACAGUCCUGCUCCAUUUCCAUAUUGAGACCGUCAUUGA GUUCCAACAGAAGAAUAACCGUUUGCUGGAAAUAACAAGGGAAU UCAGUGUUAUUGCCGGUGUAACCACCCCUGUGAGCACCUAUUG CUCACCAACUCUGAACUGCUGAGUCUGAUUAACGAUAUGCCCAU UACUAAUGAUCAGAAGAAACUAAUGAGUAACAAUGUCCAGAUAG UUCGGCAGCAGUCAUAUCCAUAUUGAGUAUAAUCAAGGAGGAA GUGCUAGCCUACGUAGUUCAGCUCUCCCCUCUACGGCGUUAUAGAC ACGCCAUGUUGGAAGCUGCAUACGAGUCCUCUGUGCACUACAAA UACCAAGGAGGGCAGUAACAU AUGCUUGACUAGAACUGAUAGAG GCUGGUACUGCGACAAUGCAGGCUCCGUGUCAUUCUUUCCUCUC GCCGAGACGUGUAAAAGUGCAGAGUAACAGAGUGUUUUGUGACAC AAUGAACUCAUUGACCCUGCCUAGCGAAGUGAACUUAUGCAACA UCGACAUUUUUAAACCCAAAAUACGAUUGCAAGAUUAUGACCUCU AAGACUGACGUAUUCUUAUCCGUCUAUACUUCUCUAGGAGCGAU CGUGAGCUGCUACGGUAAGACUAAAUGCACGGCUAGUAAUAAAA AUAGAGGUAUCAUUAAGACUUUUAGUAACGGUUGCGAUUAUGUG UCAACAAGGGAGUCGACACUGUUUCAGUGGGCAAUACUCUCUA CUACGUUAACAAACAGGAGGGUAAAUCCCUUUAUGUGAAAGGGG AACCAUCAUUAUUUUUAUGACCCACUUGUGUUUCCUAGUAGAC GAGUUUGACGCUUCAUACAGUCAAGUGAACGAAAAAAUUAUGG CAGCUCGCGUUUAUCAGGAAAAGCGACGAGAAGCUGCAUAACG UGGAAGAUAAAGAUUCGAGGAGAUUCUCUCGAAAAUUUAUCAUAUA GAGAAUGAAAUCGCAAGAAUCAAAAAGCUUAUUGGGGAG (The first underlined region represents a region coding for human Igk signal peptide, The second underlined region represents a region coding for GCN4. The underlined regions can be substituted with alternative sequences which achieves same or similar functions.)	
MRK-2 non-membrane bound form RSV F protein/MRK_02_F (soluble, Merck A2 strain)/	AUGGAGCUGUUGAUCCUUAAGGCCAACGCCAUCACUACUAAUUCU CACCGCGGUAACAUAUUCUGCUUCGCCUCCGGGCAGAACAUACCG AGGAGUUCUACCAGUCUACGUGCUCCGCCGUCUCCAAAGGUUAC CUGUCCGCAUUAAGGACGGGGUGGUACACUUCGGUCAUAACUUA UGAACUGAGUAACAUA AAAAAGAACAAGUGUAAUGGGACGGAUG CCAAGGUGAAGCUCAUAAGCAAGAGCUUGACAAUACAAGAAU GCAGUGACAGAGCUCCAACUUCUCAUGCAGUCUACACAGGCCAC GAAUAACCGUGCCCGAAGAGAACUGCCUAGAUUUUAUGAAUUAACA CUUUGAACAACGCCAAAAAGACCAACGUGACUCUAAGCAAAAAA AGGAAACGGCGUUUUCUGGGCUUUCUGCUGGGGGUUGGUAGCGC CAUCGCAUCUGGCGUGGCAGUCAGUAAAGUUUUGCACCUGAGG GGGAGGUCAACAAAUAAGAGCGCGCUGUUAUCAACAAACAAG GCAGUCGUGUCCCUUCUCAAUGGCGUGUCUGUCCUGACCUCUAA AGUACUGGAUCUCAAGAACUUAUUCGACAAACAACUGCUACCAA UCGUCAAUAAGCAGAGUUGCUCUAUUUCCAUAUUGAGACCGUG AUCGAGUUUACAACAGAAGAAUAACAGAUUGUUGGAGAUACCCAG GGAUUUCAGCGUCAUUGCAGGGGUGACCCACACCCGUAUUCACCU ACAUGCUGACCAACUCGGAACUCCUCUCCUUAUAUAAACGACAUG CCUAAUUAUAACGACCAAAAAAAGUUGAUGUCCAACAUAUGUCCA GAUCGUGCGACAGCAAUCUUAUUAUUAUUGUCCAUAUAAAAAG AGGAGGUGCUGGCGUACGUAGUGCAGCUGCCCCUUUACGGAGUG AUCGACACCCCAUGCUGGAAGCUCCACACCUCUCCCCCUGUGCACC ACUAAUAACAAAGAAGGCAGCAACAUCUGUCUGACCCGUACCGA CCGCGGAUGGUACUGCGAUAAUGCAGGUAGCGUCUCUUUUUUUC CCCAGGCUGAAACUUGCAAGGUUCAGUCCAACCGGGUUAUUCUGU GACACGAUGAACAGUCUCACCCUACCAUCAGAGGUGAACCUGUG CAAUGUGGACAUAUUUAACCCUAAAUAUGACUGUAAGAUAUGA CCUCCAAAACUGACGUUUCAGCAGUGUCAUAACCUCACUGGGC GCAAUAGUUUCAUGCUAUGGAAAGACUAAGUGCACUGCCUCUAA CAAAAUCGAGGUAAUUAUUAAGACCUUUAAGCAAUGGCUGCGAUU	274

Name	mRNA Sequence	SEQ ID NO:
	AUGUCAGUAAACAAAGGUGUUGAUACAGUGAGUGUGGGCAACACA UUAUACUAUGUUAACAAGCAAGAAGGCAAGAGCCUCUAUGUGAA GGGAGAACCAAUCAUUAUUUUUACGAUCCGCUUGUCUUUCCCA GCGAUGAGUUCGAUGCAUCCAUCUCUCAGGUGAAUGAAAAAAU AACCAAUACUGGCUUUAUACGGAAGAGCGAUGAACUGCUGAG <u>CGCAUCGGGGGAUACAUCCUGAAGCUCGAGGGACGGCCAAG</u> <u>CUUAUGUCCGCAAAGACGGAGAGUGGGUGUUGCUCAGUACCUUC</u> <u>CUC</u> (The underlined region represents a region coding for a foldon. The underlined region can be substituted with alternative sequences which achieve a same or similar function.)	
MRK-3 non- membrane bound form DS-CAV1 (stabilized prefusion F protein)//MRK_0 3_DS-CAV1 (soluble, S155C/S290C/S1 90F/V207L)/SQ- 030271	AUGGAACUGCUGAUUCUUAAGGCGAAUGCCAUAACCACUAUCUU GACCGCAGUUACUUUUUGCUUCGCCUCUGGGCAGAAUUAUACCG AAGAGUUCUACCAGUCCACGUGCAGUGCCGUGUCUAAGGGCUAC CUUUCGCGCUUCGCACUGGCUUGGUACACGUCAGUCAUAACGAU CGAACUCUCUAAUUAUAAAGGAAAAUAAAGUGUAACGGAACAGACG CUAAGGUCAAGUUAUUAAGCAGGAGCUGGACAAAUAUAAGAAU GCCGUAACGGAGCUCCAGCUGCUCAUGCAGAGCACGCCAGCUAC AAACAACAGGGCACGCCGUGAGCUCUCCCGAUUUAUGAACUACA CAUUGAACAAACGCCAAGAAAAACUAACGUGACUUUGUCCAAGAAG AGGAAGCGGCGAUUCUUAAGGUUCCUUUUGGGGGUAGGCUCGGC GAUUGCCAGUGGGGUUGCCGUAUGCAAGGUGCUCACCUGGAAG GGGAGGUGAACAAAGAUUAAGUCGGCUCUGCUAGUACAAACAAA GCUGUCGUCUUAUUGUCAAACGGAGUCAGUGUAUUGCAUUUAA AGUCCUCGACCUGAAGAACUAUAUAGAUAAACAGUUACUCCCAA UCUUGAAUAAGCAGUCCUGUAGCAUCAGCAACAUAUGAGACAGUG AUCGAGUUCAGCAGAAGAAUAAUCGCCUACUCGAGAUACCCAG AGAAUUCUCAGUCAUUGCCGGAGUAACCACUCCUGUCAGCACAU ACAUGCUCACAAACUCUGAACUCCUAAGCCUGAUUAAUGAUUG CCUAUCACAAUAGAUCAAGAAACUCAUGAGCAUAUAAUGUGCA GAUUGUAAGACAGCAGAGUUAUUCUAUAAUGUGUAUUUAUAAAG GAGGAGGUACUGGCCUAUGUGGUUCAAUCUCCUCUGUAUGGGGU GAUAGAUACACCAUGCUGGAAGCUGCACACCAGCCCACUGUGUA CGACCAUAACAAGGAGGGCUCCAUAUUUGCUUAACACGGACU GACCGGGGGUGGUUUUGCGACAAUGCCGGAUCAGUCUCCUUCUU CCCCCAAGCAGAGACCUGCAAGGUGCAGUCCAUAAGAGUUUUCU GCGACACAAUGAACUCGCUGACCCUACCUAGCGAAGUUAACUUA UGCAACGUGGAUUAUUUUUAAUCCGAAGUAUGAUUGUAAAAUCAU GACUAGCAAAACGGAUGUUAAGCUCCAGCGUAAUACCCUCCUAG GCGCUAUCGUGAGCUGUUAUGGCAAGACGAAGUGCACUGCAUCU AAUAAAAAUAGGGGUUAUUAUAAAACCUUCAGCAAUGGCUGCGA CUAUGUGAGCAAUAAGGGCGUGGACACCGUGUCAGUGGGAAACA CCCUCUAUUUGUGAACAAGCAGGAGGGAAAAUCCCUUUUAUGUA AAGGGCGAACC CAUUAUCAUUUCUUAUGACCCCCUGGUUUUCCC AAGCGACGAGUUCGACGCAUCUAUCUCUCAAGUGAACGAGAAAA UCAAUCAGAGUCUUGCCUUUAUCAGAAAAUCCGAUGAGCUGCUU <u>UCCGCCAUCGGUGGCUAAUCCAGAAAGCCCCAAGAGACGGACA</u> <u>AGCGUACGUCCGGAAAGAUGGUGAGUGGGUCCUCCUCUACCU</u> <u>UUCUU</u> (The underlined region represents a region coding for a foldon. The underlined region can be substituted with alternative sequences which achieve a same or similar function)	275
Influenza M-1 (A/California/04/ 2009(H1N1), ACP44152)+hIg κ	AUGGAGACUCCUGCACAGCUGCUGUUCUGCUAUUGUUGUGGCUU CCGGACACUACUGGGUCCCUCCUACCGAGGUGGAAACAUACGUG CUGUCCAUAUAACAUCCGGGCCCUUGAAAGCCGAGAUCCGCCAG AGACUCGAAUCUGUAUUCGCAGGAAAGAACACGGAUUUGGAGGCA CUAAUGGAAUGGCUGAAGACCCGUCCGAUCCUGUCUCCUCUCACA AAGGGGAUUCUUGGAUUUGUCUUUACCCUACCCGUCCCGAGCGAG CGCGGUCUCCAGCGCAGACGUUUUGUACAGAAUGCACUGAAUGGC AACGGCGAUCCCAAUAACAUGGAUCGUGCGGUAAAGCUUUUAUAAA AAGCUGAAGAGAGAAAUCACUUUCCAUGGGGCUAAAGAGGUGAGU	276

Name	mRNA Sequence	SEQ ID NO:
	CUCUCCUAUUC AACC GGGGCAUUGGCCUCUUGCAUGGGUCUUAUAU UACAAUCGAAUGGGCACC GUUACCACCGAGGCCGCAUUUGGUCUG GUUUGUGCUACGUGCGAGCAAUUCGAGAUAGCCAGCAUCGGUCC CAUCGGCAGAUUGGCCACCACUACGAACCCUCUAAUUCGACAUGAA AAUCGCAUGGUCCUGGCUAGCACCACCGCAAAGGCAUUGGAGCAG AUGGCGGGCUCUAGUGAACAGGCAGCCGAGGCAAUGGAAGUGGCC AAUCAGACCAGGCAGAUUGGUCCAUGCUAUGCGGACU AUUGGUACC CACCCGUCCAGCAGUGCUGGACUGAAGGAUGACCUCUUGAGAAC CUGCAGGCAUACCAGAAACGAAUGGGGGUGCAA AUGCAGAGAUUC AAG (The underlined region represents a region coding for human Igk signal peptide. The underlined region can be substituted with alternative sequences which achieve a same or similar function)	
MRK_04 SQ-030271	AUGGAACUGCUCAUUUUGAAGGCAAACGCUAUCACGACAAUACU CACUGCAGUGACCUUCUGUUUUGCCUCAGGCCAGAACAUAAACCG AGGAGUUUU AUCAAUCUACAUGCAGCGCUGUAUCUAAAGGCUAC CUGAGUGCGCUCCGCACAGGAUGGUACACCUC CGUGAUACCAU CGAGCUCAGCAAUAUUAAGAGAACAAGUGCAAUGGUACCGACG CUAAAGUCAACUUAUCAAGCAGGAACUCGACAAAU AUAAAAAC GCUGUGACCGAGCUGCAGUUAUUGAUGCAGAGUACACCUGCCAC CAAUAACAGAGCUAGGAGGGAGUUGCCUAGGUUU AUGAACUACA CUCUCAACAACGCGAAAAAAACCAAUGUGACGCUAUCCAAGAAA CGGAAGAGGAGGUUCCUGGGGUUUCUUUUAGGGGUGGGCUCUGC CAUUGCUUCCGGCGUGGCUGUAUGUAAAGUUCUCCACCUCGAGG GAGAGGUUAAUAAGAUUAAGUCGGCCCGCUGAGUACUAAACAAA GCAGUGGUGUCGUGAGUAACGGAGUAAGUGUGUUAACAUUUAA GGUGCUGGACCUC AAGAAUUAUUAUGACAAACAGUUGCUUCCUA UUCUAAACAAACAGAGCUGUUAUUAAGUAAUUAUGAAACUGUU AUUGAGUUUCAGCAGAAGAACAACAGGCUUCUUGAGAUUACACG CGAGUUCAGUGUCA AUGCCGGCGUUAACAACCCCGUGUCUACCU ACAUGCUGACGAAUUCUGAGCUUCUCUCUCUCAA AAACGACAUG CCCAUUAACGAAUGACCAAAAAAAACUUAUGUCCAACAACGUGCA GAUUGUGCGACAGCAAUCCUAUAGCAUUAUGUGUAUCAUCAAGG AAGAGGUACUCGCUUAUGUUGUGCAGCUACCACUCUAUGGUGUG AUUGACACCCCGUGUUGGAAGCUGCAUACCAGUCCACUCUGCAC CACUAACACAAAGGAAGGGAGCAAUAUUUGCCUCACUCGAACCG ACAGGGGGUGGU AUUGCGAUAAUGCGGGCUCCGUGUCCUUCUUU CCACAGGCUGAAACUUGUAAGGUACAGUCAAAACCGCGUGUUCUG UGAUACUAUGAAUUCUCUGACUCUUC CAGCGAGGUUAAUCUCU GCAACGUCGACAUUUUCAAUCCUAAAU AUGACUGCAAGAUAUG ACCAGCAAGACCGACGUCUCCAGCUCAGUAAUCACUAGCCUAGG GGCCAUUGUAAGCUGCUAUGGCAAACCAAGUGUACUGCCUCUA AUAAGAACAGAGGCAUAAUUA AAAACCUUUUCAA AUGGCUGUGAC UAUGUGUCGAAUAAGGGCGUCGACACGGUCUCAGUAGGGAUAC CCUCUACUACGUUAACAAACAGGAAGGCAA AUCCCUUUAUGUAA AGGGCGAGCCCAUCAUAAAUUUCUACGACCCACUUGUGUUC C AGUGAUGAAUUCGAUGCAUCAAUCUCCAGGUGAACGAAAAGAU CAAUCAAUCCCUUGCUUUUAUACGAAAGUCAGAUGAACUCCUGC AUAACGUGAAUGCUGGGAAAUCAACAACCAACAUAUGAUCACU ACCAUCAUUAUUGUGAUUAUCGUAAUUCUGCUAUCCUUGAUUGC UGUCGGGCUGCUUCUGUACUGUAAGGCCAGAU CGACGCCUGUGA CCCUUUCAAAAGACCAACUUAAGCGGUAUCAAUAAUAUUGCCUUU AGCAAU	277
MRK_04_no AAALys SQ-038059	AUGGAACUGCUCAUUUUGAAGGCAAACGCUAUCACGACAAUACU CACUGCAGUGACCUUCUGUUUUGCCUCAGGCCAGAACAUAAACCG AGGAGUUUU AUCAAUCUACAUGCAGCGCUGUAUCUAAAGGCUAC CUGAGUGCGCUCCGCACAGGAUGGUACACCUC CGUGAUACCAU CGAGCUCAGCAAUAUUAAGAGAACAAGUGCAAUGGUACCGACG CUAAAGUCAACUUAUCAAGCAGGAACUCGACAAAU AUAAAGAAC GCUGUGACCGAGCUGCAGUUAUUGAUGCAGAGUACACCUGCCAC	278

Name	mRNA Sequence	SEQ ID NO:
	CAAUAAACAGAGCUAGGAGGGAGUUGCCUAGGUUUUGAACUACA CUCUCAACAACGCGAAGAAGACCAAUGUGACGCUAUCCAAGAAA CGGAAGAGGAGGUUCCUGGGGUUUUUUUAGGGGUGGGCUCUGC CAUUGCUUCCGGCGUGGCUGUAUGUAAAGUUCUCCACCUCGAGG GAGAGGUUAAUAAGAUUAAGUCGGCCCGUCUGAGUACUAAACAAA GCAGUGGUGUCGUGAGUAACGGAGUAAGUGUGUUAACAUUUAA GGUGCUGGACCUCAGAAAUUAUUAUUGACAAACAGUUGCUUCCUA UUCUAAACAAACAGAGCUGUUAUUAAGUAAUUAUUGAAACUGUU AUUGAGUUUCAGCAGAAGAACAACAGGCUUCUUGAGAUUACACG CGAGUUCAGUGUCAUUGCCGGCGUUACAACACCCGUGUCUACCU ACAUGCUGACGAAUUCUGAGCUUCUCUCUCUCAUAAACGACAUG CCCAUUACGAAUGACCAAAAAGAAACUUAUGUCCAACAACGUGCA GAUUGUGCGACAGCAAUCCUAUAGCAUUAUGUGUAUCAUCAAGG AAGAGGUACUCGCUUAUGUUGUGCAGCUACCACUCUAUGGUGUG AUUGACACCCCGUGUUGGAAGCUGCAUACCAGUCCACUCUGCAC CACUAAACACAAAGGAAGGGAGCAAUUUUGCCUCACUCGAACCG ACAGGGGGUGGUAAUUGCGAUAAUGCGGGCUCGUGUCCUUCUUU CCACAGGCUGAAACUUGUAAGGUACAGUCAAAACCGCGUGUUCUG UGAUACUAUGAAUUCUCUGACUCUUCUCCAGCGAGGUUAAUCUCU GCAACGUCGACAUUUUCAAUCCUAAAUAUGACUGCAAGAUCUAG ACCAGCAAGACCGACGUCUCCAGCUCAGUAAUCACUAGCCUAGG GGCCAUUGUAAGCUGCUAUGGCAAGACCAAGUGUACUCCUCUA AUAAGAACAGAGGCAUAAUUAAGACCUUUUCAAUUGGCUGUGAC UAUGUGUCGAAUAAGGGCGUCGACACGGUCUCAGUAGGGAUAC CCUCUACUACGUUAAACAAACAGGAAGGCAAAUCCCUUUUAUGUAA AGGGCGAGCCCAUCAUAAAUUUUCUACGACCCACUUGUGUUCUCC AGUGAUGAAUUCGAUGCAUCAAUCUCCAGGUGAACGAAAAGAU CAAUCAAUCCCUUGCUUUUAUACGAAAGUCAGAUAACUCCUGC AUAACGUGAAUGCUGGGAAAUCAACAACCAACAUCAGUACACU ACCAUCAUUAUUGUGAUUAUCGUAAUUCUGCUAUCCUUGAUUGC UGUCGGGCUGCUUCUGUACUGUAAGGCCAGAUACGACCCUGUGA CCCUUCAAAGGACCAACUUAAGCGUAUCAAUAAUUAUUGCCUUU AGCAAU	
MRK_04_no4A SQ-038058	AUGGAACUGCUCAUUUUUGAAGGCAAACGCUAUCACGACAAUACU CACUGCAGUGACCUUCUGUUUUGCCUCAGGCCAGAACAUAAACCG AGGAGUUUUUAUCAAUUCUACAUGCAGCGCUGUAUCUAAAGGCUAC CUGAGUGCGCUCUCCGCACAGGAUGGUACACCUCUGUGAUACCAU CGAGCUCAGCAAUAUUAAGAGAAACAAGUGCAAUGGUACCGACG CUAAAGUCAAAACUUAUCAAGCAGGAACUCGACAAAUUAAGAAC GCUGUGACCGAGCUGCAGUUAUUGAUGCAGAGUACACCUGCCAC CAAUAAACAGAGCUAGGAGGGAGUUGCCUAGGUUUUGAACUACA CUCUCAACAACGCGAAGAAGACCAAUGUGACGCUAUCCAAGAAA CGGAAGAGGAGGUUCCUGGGGUUUUUUUAGGGGUGGGCUCUGC CAUUGCUUCCGGCGUGGCUGUAUGUAAAGUUCUCCACCUCGAGG GAGAGGUUAAUAAGAUUAAGUCGGCCCGUCUGAGUACUAAACAAA GCAGUGGUGUCGUGAGUAACGGAGUAAGUGUGUUAACAUUUAA GGUGCUGGACCUCAGAAAUUAUUAUUGACAAACAGUUGCUUCCUA UUCUAAACAAACAGAGCUGUUAUUAAGUAAUUAUUGAAACUGUU AUUGAGUUUCAGCAGAAGAACAACAGGCUUCUUGAGAUUACACG CGAGUUCAGUGUCAUUGCCGGCGUUACAACACCCGUGUCUACCU ACAUGCUGACGAAUUCUGAGCUUCUCUCUCUCAUAAACGACAUG CCCAUUACGAAUGACCAGAAGAAACUUAUGUCCAACAACGUGCA GAUUGUGCGACAGCAAUCCUAUAGCAUUAUGUGUAUCAUCAAGG AAGAGGUACUCGCUUAUGUUGUGCAGCUACCACUCUAUGGUGUG AUUGACACCCCGUGUUGGAAGCUGCAUACCAGUCCACUCUGCAC CACUAAACACAAAGGAAGGGAGCAAUUUUGCCUCACUCGAACCG ACAGGGGGUGGUAAUUGCGAUAAUGCGGGCUCGUGUCCUUCUUU CCACAGGCUGAAACUUGUAAGGUACAGUCAAAACCGCGUGUUCUG UGAUACUAUGAAUUCUCUGACUCUUCUCCAGCGAGGUUAAUCUCU	279

Name	mRNA Sequence	SEQ ID NO:
	GCAACGUCGACAUUUUCAAUCCUAAAUAUGACUGCAAGAUAUG ACCAGCAAGACCGACGUCUCCAGCUCAGUAAUCACUAGCCUAGG GGCCAUUGUAAGCUGCUAUGGCAAGACCAAGUGUACUGCCUCUA AUAAGAACAGAGGCAUAAUUAAGACCUUUUCAAUUGGCUGUGAC UAUGUGUCGAAUAAGGGCGUCGACACGGUCUCAGUAGGGAUAUAC CCUCUACUACGUUAACAAACAGGAAGGCAAAUCCCUUUUAUGUAA AGGGCGAGCCCAUCAUAAAUUUCUACGACCCACUUGUGUUCUCC AGUGAUGAAUUCGAUGCAUCAUUCUCCAGGUGAACGAGAAGAU CAAUCAAUCCCUUGCUUUUAUACGAAAGUCAGAUGAACUCCUGC AUAACGUGAAUUGCUGGGAAAUCUACAACCAACAUCAUGAUCACU ACCAUCAUUAUUGUGAUUAUCGUAAUUCUGCUAUCCUUGAUUGC UGUCGGGCUGCUUCUGUACUGUAAGGCCAGAUCGACGCCUGUGA CCCUUCAAAGGACCAACUAGCGGUAUCAAUAAUUAUUGCCUUU AGCAAU	
MRK_04_nopoly A_3mut SQ-038057	AUGGAACUGCUCAUUUUGAAGGCAAACGCUAUCACGACAAUACU CACUGCAGUGACCUUCUGUUUUGCCUCAGGCCAGAACAUAAACCG AGGAGUUUUUAUCAAUUCUACAUGCAGCGCUGUAUCUAAAGGCUAC CUGAGUGCGCUCCGCACAGGAUGGUACACCUCCGUGAUCACCAU CGAGCUCAGCAAUAUUAAAGAGAACAAGUGCAAUGGUACCGACG CUAAAGUCAAAACUUAUCAAGCAGGAACUCGACAAAUUAAGAAC GCUGUGACCGAGCUGCAGUUAUUGAUGCAGAGUACACCUGCCAC CAUAACAGAGCUAGGAGGGAGUUGCCUAGGUUUUAUGAACUACA CUCUCAACAACGCGAAGAAAACCAUUGUGACGCUAUCCAAGAAA CGGAAGAGGAGGUUCCUGGGGUUUUCUUUUAGGGGUGGGCUCUGC CAUUGCUUCCGGCGUGGCUGUAUGUAAAGUUCUCCACCUCGAGG GAGAGGUUAUAAGAUAUAGUCGGCCUUGCUGAGUACUAAACAA GCAGUGGUGUCGUGAGUAACGGAGUAAGUGUGUUAACAUUUAA GGUGCUGGACCUCAGAAUUAUAUUGACAAACAGUUGCUUCCUA UUCUAAACAAACAGAGCUGUUAUUAAGUAAUUAUGAAACUGUU AUUGAGUUUCAGCAGAAGAACAACAGGCUUCUUGAGAUUACACG CGAGUUCAGUGUCAUUGCCGGCGUUAACAACACCCGUGUCUACCU ACAUGCUGACGAAUUCUGAGCUUCUCUCUCUCAUAAACGACAUG CCCAUUAACGAAUGACCAAAAAGAAACUUAUGUCCAACAACGUGCA GAUUGUGCGACAGCAAUCCUAUAGCAUUAUGUGUAUCAUCAAGG AAGAGGUACUCGCUUAUGUUGUGCAGCUACCACUCUAUGGUGUG AUUGACACCCCUUGUUGGAAGCUGCAUACCAGUCCACUCUGCAC CACUAACACAAAGGAAGGGAGCAAUAUUUGCCUCACUCGAACCG ACAGGGGGUGGUAAUUGCGAUAAUGCGGGCUCGUGUCCUUCUUU CCACAGGCUGAAACUUGUAAGGUACAGUCAAAACCGCGUGUUCUG UGAUACUAUGAAUUCUCUGACUCUCCCAGCGAGGUAAUUCUCU GCAACGUCGACAUUUUCAAUCCUAAAUAUGACUGCAAGAUAUG ACCAGCAAGACCGACGUCUCCAGCUCAGUAAUCACUAGCCUAGG GGCCAUUGUAAGCUGCUAUGGCAAAACCAAGUGUACUGCCUCUA AUAAGAACAGAGGCAUAAUUAACCUUUUCAAUUGGCUGUGAC UAUGUGUCGAAUAAGGGCGUCGACACGGUCUCAGUAGGGAUAAC CCUCUACUACGUUAACAAACAGGAAGGCAAAUCCCUUUUAUGUAA AGGGCGAGCCCAUCAUAAAUUUCUACGACCCACUUGUGUUCUCC AGUGAUGAAUUCGAUGCAUCAUUCUCCAGGUGAACGAAAAGAU CAAUCAAUCCCUUGCUUUUAUACGAAAGUCAGAUGAACUCCUGC AUAACGUGAAUUGCUGGGAAAUCUACAACCAACAUCAUGAUCACU ACCAUCAUUAUUGUGAUUAUCGUAAUUCUGCUAUCCUUGAUUGC UGUCGGGCUGCUUCUGUACUGUAAGGCCAGAUCGACGCCUGUGA CCCUUCAAAGACCAACUAGCGGUAUCAAUAAUUAUUGCCUUU AGCAAU	280

It should be understood that each of the ORF sequences provided herein may be combined with a 5' and/or 3' UTR, such as those described herein. It should also be

understood that the 5' and/or 3' UTR for each construct may be omitted, modified or substituted for a different UTR sequences in any one of the vaccines as provided herein.

EQUIVALENTS

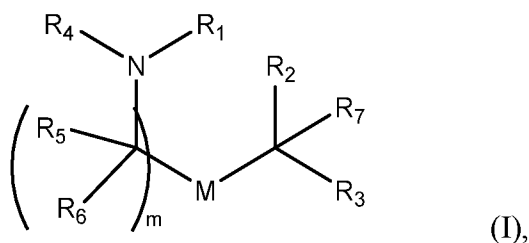
5 Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the disclosure described herein. Such equivalents are intended to be encompassed by the following claims.

 All references, including patent documents, disclosed herein are incorporated by reference in their entirety.

What is claimed is:

CLAIMS

1. A vaccine comprising at least one ribonucleic acid (RNA) polynucleotide having an
 5 open reading frame encoding at least one respiratory syncytial virus (RSV) antigenic
 polypeptide formulated in a lipid nanoparticle comprising at least one cationic lipid selected
 from compounds of Formula (I):



10 or a salt or isomer thereof, wherein:

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'',
 and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄
 alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are
 15 attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR,
 -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a carbocycle,
 heterocycle, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -N(R)₂,
 -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -N(R)R₈,
 20 -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR,
 -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂,
 -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R,
 -C(O)N(R)OR, and -C(R)N(R)₂C(O)OR, and each n is independently selected from 1, 2, 3,
 4, and 5;

25 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-,
 -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group,
 and a heteroaryl group;

30 R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R,

-S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

5 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl,

-R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

10 each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13.

2. The vaccine of claim 1, wherein a subset of compounds of Formula (I) includes those in which when R₄ is -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, or -CQ(R)₂, then (i) Q is not -N(R)₂ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

3. The vaccine of claim 1, wherein a subset of compounds of Formula (I) includes those in which

20 R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

25 R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈,
30 -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms selected from N, O, and S which is substituted with one or more substituents selected from

oxo (=O), OH, amino, mono- or di-alkylamino, and C₁₋₃ alkyl, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

5 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

10 R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

15 each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

20 or salts or isomers thereof.

4. The vaccine of claim 1, wherein a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and

25 -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR,

30 -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a

5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S,

-OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂,

-N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈,

-O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR,

-N(OR)C(O)R , $\text{-N(OR)S(O)}_2\text{R}$, -N(OR)C(O)OR , -N(OR)C(O)N(R)_2 , -N(OR)C(S)N(R)_2 ,
 $\text{-N(OR)C(=NR}_9\text{)N(R)}_2$, $\text{-N(OR)C(=CHR}_9\text{)N(R)}_2$, $\text{-C(=NR}_9\text{)R}$, -C(O)N(R)OR , and
 $\text{-C(=NR}_9\text{)N(R)}_2$, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a
5- to 14-membered heterocycle and (i) R_4 is $\text{-(CH}_2\text{)}_nQ$ in which n is 1 or 2, or (ii) R_4
5 is $\text{-(CH}_2\text{)}_n\text{CHQR}$ in which n is 1, or (iii) R_4 is -CHQR , and -CQ(R)_2 , then Q is either a 5- to
14-membered heteroaryl or 8- to 14-membered heterocycloalkyl;
each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 M and M' are independently selected from -C(O)O- , -OC(O)- , -C(O)N(R')- , -N(R')C(O)- ,
10 -C(O)- , -C(S)- , -C(S)S- , -SC(S)- , -CH(OH)- , -P(O)(OR')O- , $\text{-S(O)}_2\text{-}$, -S-S- , an aryl group,
and a heteroaryl group;
 R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle;
 R_9 is selected from the group consisting of H, CN, NO_2 , C_{1-6} alkyl, -OR , $\text{-S(O)}_2\text{R}$,
15 $\text{-S(O)}_2\text{N(R)}_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle;
each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl,
 -R*YR'' , -YR'' , and H;
each R'' is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
20 each R^* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;
each Y is independently a C_{3-6} carbocycle;
each X is independently selected from the group consisting of F, Cl, Br, and I; and
 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
or salts or isomers thereof.

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5. The vaccine of claim 1, wherein a subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR'' , -YR'' , and -R''M'R' ;

30 R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR'' , -YR'' , and -R*OR'' , or R_2 and R_3 , together with the atom to which they are attached, form a heterocycle or carbocycle;

- R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5;
- each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
- each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
- M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;
- R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
- R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;
- R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;
- each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
- each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;
- each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;
- each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;
- each Y is independently a C₃₋₆ carbocycle;
- each X is independently selected from the group consisting of F, Cl, Br, and I; and
- m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
- or salts or isomers thereof.

6. The vaccine of claim 1, wherein a subset of compounds of Formula (I) includes those in which
- R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₂₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is -(CH₂)_nQ or -(CH₂)_nCHQR, where Q is -N(R)₂, and n is selected from 3, 4, and 5;

- 5 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

- 10 R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;
 each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;
 15 each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₁₋₁₂ alkenyl;
 each Y is independently a C₃₋₆ carbocycle;
 each X is independently selected from the group consisting of F, Cl, Br, and I; and
 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
 or salts or isomers thereof.

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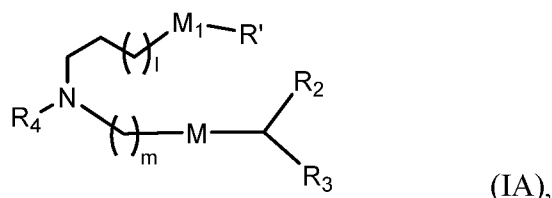
7. The vaccine of claim 1, wherein a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

- 25 R₂ and R₃ are independently selected from the group consisting of C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;
 R₄ is selected from the group consisting of -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, and -CQ(R)₂, where Q is -N(R)₂, and n is selected from 1, 2, 3, 4, and 5;
 30 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl,
 $-R^*YR''$, $-YR''$, and H;
 5 each R'' is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
 each R^* is independently selected from the group consisting of C_{1-12} alkyl and C_{1-12} alkenyl;
 each Y is independently a C_{3-6} carbocycle;
 each X is independently selected from the group consisting of F, Cl, Br, and I; and
 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
 10 or salts or isomers thereof.

8. The vaccine of claim 1, wherein a subset of compounds of Formula (I) includes those of Formula (IA):



15 or a salt or isomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6, 7, 8, and 9; M_1 is a bond or M' ; R_4 is unsubstituted C_{1-3} alkyl, or $-(CH_2)_nQ$, in which Q is OH, $-NHC(S)N(R)_2$, $-NHC(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)R_8$, $-NHC(=NR_9)N(R)_2$, $-NHC(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, heteroaryl or heterocycloalkyl; M and M' are independently selected from $-C(O)O-$, $-OC(O)-$, $-C(O)N(R')$, $-P(O)(OR')O-$, $-S-S-$, an aryl group, and a heteroaryl group; and R_2 and R_3 are independently
 20 selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl.

9. The vaccine of any one of claims 1-8, wherein the at least one antigenic polypeptide is glycoprotein G.

10. The vaccine of any one of claims 1-9, wherein the at least one antigenic polypeptide is glycoprotein F, optionally designed to maintain a prefusion conformation.

11. The vaccine of any one of claims 1-10, further comprising an adjuvant.

12. The vaccine of any one of claims 1-10, further comprising a pharmaceutically acceptable carrier.

13. The vaccine of any one of claims 1-12, wherein the at least one RNA polynucleotide is encoded by at least one nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, and 27, and/or wherein the at least one RNA polynucleotide comprises at least one nucleic acid sequence of any of SEQ ID NO: 260-280.

14. The vaccine of any one of claims 1-12, wherein the at least one RNA polynucleotide is encoded by at least one fragment of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, and 27, and/or wherein the at least one RNA polynucleotide comprises at least one fragment of a nucleic acid sequence of any of SEQ ID NO: 260-280.

15. The vaccine of any one of claims 1-12, wherein the amino acid sequence of the RSV antigenic polypeptide is an amino acid sequence selected from the group consisting of SEQ ID NO: 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 28.

16. The vaccine of any one of claims 1-15, wherein the open reading frame is codon-optimized.

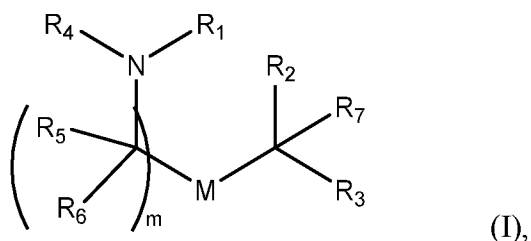
17. The vaccine of any one of claims 1-16, wherein the vaccine is multivalent.

18. The vaccine of any one of claims 1-17, wherein the at least one RNA polynucleotide encodes at least 2 antigenic polypeptides.

19. The vaccine of claim 18, wherein the at least one RNA polynucleotide encodes at least 10 antigenic polypeptides.

20. The vaccine of claim 19, wherein the at least one RNA polynucleotide encodes at least 100 antigenic polypeptides.

21. The vaccine of any one of claims 1-20, wherein the at least one RNA polynucleotide encodes 2-100 antigenic polypeptides.
22. The vaccine of any one of claims 1-21, wherein the at least one RNA polynucleotide comprises at least one chemical modification.
23. The vaccine of claim 22, wherein the chemical modification is selected from the group consisting of 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-methyl uridine.
24. The vaccine of any one of claims 1-23, wherein the nanoparticle has a mean diameter of 50-200 nm.
25. The vaccine of any one of claims 1-23, wherein the lipid nanoparticle further comprises a PEG-modified lipid, a sterol, and a non-cationic lipid.
26. The vaccine of claim 25, wherein the non-cationic lipid is a neutral lipid and the sterol is a cholesterol.
27. The vaccine of any one of claims 1-26, wherein the nanoparticle has a polydispersity value of less than 0.4.
28. The vaccine of any one of claims 1-27, wherein the nanoparticle has a net neutral charge at a neutral pH value.
29. A vaccine, comprising:
at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding at least one respiratory syncytial virus (RSV) antigenic polypeptide, at least one 5' terminal cap, and optionally at least one chemical modification, formulated within a lipid nanoparticle comprising a cationic lipid selected from compounds of Formula (I):



or a salt or isomer thereof, wherein:

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'',

5 and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR,

10 -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a carbocycle, heterocycle, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -N(R)₂, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(R)N(R)₂C(O)OR, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

20 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

25 R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

30 each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;
 each Y is independently a C₃₋₆ carbocycle;
 each X is independently selected from the group consisting of F, Cl, Br, and I; and
 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13.

5

30. The vaccine of claim 29, wherein a subset of compounds of Formula (I) includes those in which when R₄ is -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, or -CQ(R)₂, then (i) Q is not -N(R)₂ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

10

31. The vaccine of claim 29, wherein a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

15 R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a

20 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂,
 25 -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms selected from N, O, and S which is substituted with one or more substituents selected from oxo (=O), OH, amino, mono- or di-alkylamino, and C₁₋₃ alkyl, and each n is independently selected from 1, 2, 3, 4, and 5;

30 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

- 5 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

- 10 each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

- 15 32. The vaccine of claim 29, wherein a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄

- 20 alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S,

- 25 -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(=NR₉)

- 30 N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a 5- to 14-membered heterocycle and (i) R₄ is -(CH₂)_nQ in which n is 1 or 2, or (ii) R₄ is -(CH₂)_nCHQR in which n is 1, or (iii) R₄ is -CHQR, and -CQ(R)₂, then Q is either a 5- to 14-membered heteroaryl or 8- to 14-membered heterocycloalkyl;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

- 5 R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle;
 R_9 is selected from the group consisting of H, CN, NO₂, C_{1-6} alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle;
each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
10 each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR'', -YR'', and H;
each R'' is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;
each Y is independently a C_{3-6} carbocycle;
15 each X is independently selected from the group consisting of F, Cl, Br, and I; and
m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
or salts or isomers thereof.

33. The vaccine of claim 29, wherein a subset of compounds of Formula (I) includes
20 those in which

- R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR'', -YR'', and -R''M'R';
 R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR'', -YR'', and -R*OR'', or R_2 and R_3 , together with the atom to which they are
25 attached, form a heterocycle or carbocycle;
 R_4 is selected from the group consisting of a C_{3-6} carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a
5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S,
-OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂,
30 -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈,
-O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR,
-N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂,
-N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and
-C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-,
-C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group,
5 and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR-, -S(O)₂R,
-S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

10 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl,
-R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

15 each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

20 34. The vaccine of claim 29, wherein a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and
-R''M'R';

25 R₂ and R₃ are independently selected from the group consisting of H, C₂₋₁₄ alkyl, C₂₋₁₄
alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are
attached, form a heterocycle or carbocycle;

R₄ is -(CH₂)_nQ or -(CH₂)_nCHQR, where Q is -N(R)₂, and n is selected from 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

30 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-,
-C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group,
and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₁₋₁₂ alkenyl;

5 each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

10 35. The vaccine of claim 29, wherein a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

15 R₂ and R₃ are independently selected from the group consisting of C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, and -CQ(R)₂, where Q is -N(R)₂, and n is selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

20 each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

25 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₁₋₁₂ alkenyl;

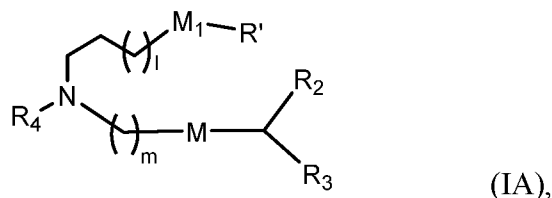
30 each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

36. The vaccine of claim 29, wherein a subset of compounds of Formula (I) includes those of Formula (IA):



or a salt or isomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6, 7, 8, and 9; M_1 is a bond or M' ; R_4 is unsubstituted C_{1-3} alkyl, or $-(CH_2)_nQ$, in which Q is OH, $-NHC(S)N(R)_2$, $-NHC(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)R_8$, $-NHC(=NR_9)N(R)_2$, $-NHC(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, heteroaryl or heterocycloalkyl; M and M' are independently selected from $-C(O)O-$, $-OC(O)-$, $-C(O)N(R')-$, $-P(O)(OR')O-$, $-S-S-$, an aryl group, and a heteroaryl group; and R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl.

37. The vaccine of any one of claims 29-36, wherein the 5' terminal cap is $7mG(5')ppp(5')NlmpNp$.

38. The vaccine of any one of claims 29-37, wherein the at least one chemical modification is selected from the group consisting of pseudouridine, N1-methylpseudouridine, N1-ethylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 5-methyluridine, 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-methyl uridine.

39. The vaccine of any one of claims 29-38, wherein the lipid nanoparticle further comprises a PEG-modified lipid, a sterol, and a non-cationic lipid.

40. The vaccine of claim 40, wherein the non-cationic lipid is a neutral lipid and the sterol is a cholesterol.

41. The vaccine of any one of claims 1-40 for use in the manufacture of a medicament for use in a method of inducing an antigen specific immune response in a subject, the method

comprising administering to the subject the RSV vaccine in an amount effective to produce an antigen specific immune response.

42. The vaccine of any one of claims 1-40 for use in a method of inducing an antigen specific immune response in a subject, the method comprising administering to the subject the RSV vaccine in an amount effective to produce an antigen specific immune response.

43. A method of inducing an antigen specific immune response in a subject, comprising administering to the subject the RSV vaccine of any one of claims 1-40 in an amount effective to produce an antigen specific immune response.

44. The method of claim 43, wherein the antigen specific immune response comprises a T cell response.

45. The method of claim 43, wherein the antigen specific immune response comprises a B cell response.

46. The method of any one of claims 43-45, wherein the method of inducing an antigen specific immune response involves a single administration of the RSV vaccine.

47. The method of any one of claims 43-46, further comprising administering a booster dose of the vaccine.

48. The method of any one of claims 43-47, wherein the vaccine is administered to the subject by intradermal or intramuscular injection.

49. The method of any one of claims 43-48, wherein an anti-RSV antigenic polypeptide antibody titer produced in the subject is increased by at least 1 log relative to a control.

50. The method of claim 49, wherein an anti-RSV antigenic polypeptide antibody titer produced in the subject is increased by 1-3 log relative to a control.

51. The method of any one of claims 43-48, wherein the anti-RSV antigenic polypeptide antibody titer produced in the subject is increased at least 2 times relative to a control.

52. The method of claim 51, wherein the anti-RSV antigenic polypeptide antibody titer produced in the subject is increased at least 5 times relative to a control.

5 53. The method of claim 52, wherein the anti-RSV antigenic polypeptide antibody titer produced in the subject is increased at least 10 times relative to a control.

54. The method of claim 51, wherein the anti-RSV antigenic polypeptide antibody titer produced in the subject is increased 2-10 times relative to a control.

10

55. The method of any one of claims 49-54, wherein the control is an anti-RSV antigenic polypeptide antibody titer produced in a subject who has not been administered RSV vaccine.

56. The method of any one of claims 49-54, wherein the control is an anti-RSV antigenic polypeptide antibody titer produced in a subject who has been administered a live attenuated or inactivated RSV vaccine.

15

57. The method of any one of claims 49-54 wherein the control is an anti-RSV antigenic polypeptide antibody titer produced in a subject who has been administered a recombinant or purified RSV protein vaccine.

20

58. The method of any one of claims 49-54, wherein the control is an anti-RSV antigenic polypeptide antibody titer produced in a subject who has been administered a RSV VLP vaccine.

25

59. The method of any one of claims 43-58, wherein the effective amount is a dose equivalent to an at least 2-fold reduction in the standard of care dose of a recombinant RSV protein vaccine, and wherein an anti-RSV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-RSV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant RSV protein vaccine, or a live attenuated RSV vaccine, or a RSV VLP vaccine.

30

60. The method of claim 59, wherein the effective amount is a dose equivalent to an at least 4-fold reduction in the standard of care dose of a recombinant RSV protein vaccine, and

wherein an anti-RSV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-RSV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified RSV protein vaccine, or a live attenuated or inactivated RSV vaccine, or a RSV VLP vaccine.

5

61. The method of claim 60, wherein the effective amount is a dose equivalent to an at least 10-fold reduction in the standard of care dose of a recombinant RSV protein vaccine, and wherein an anti-RSV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-RSV antigenic polypeptide antibody titer produced in a control subject
10 administered the standard of care dose of a recombinant or purified RSV protein vaccine, or a live attenuated or inactivated RSV vaccine, or a RSV VLP vaccine.

62. The method of claim 61, wherein the effective amount is a dose equivalent to an at least 100-fold reduction in the standard of care dose of a recombinant RSV protein vaccine, and wherein an anti-RSV antigenic polypeptide antibody titer produced in the subject is
15 equivalent to an anti-RSV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified RSV protein vaccine, or a live attenuated or inactivated RSV vaccine, or a RSV VLP vaccine.

20 63. The method of claim 62, wherein the effective amount is a dose equivalent to an at least 1000-fold reduction in the standard of care dose of a recombinant RSV protein vaccine, and wherein an anti-RSV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-RSV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified RSV protein vaccine, or a
25 live attenuated or inactivated RSV vaccine, or a RSV VLP vaccine.

64. The method of any one of claims 43-63, wherein the effective amount is a total dose of 50-1000 μg .

30 65. The method of claim 64, wherein the effective amount is a total dose of 100 μg .

66. The method of claim 64, wherein the effective amount is a dose of 25 μg administered to the subject a total of two times.

67. The method of claim 64, wherein the effective amount is a dose of 100 µg administered to the subject a total of two times.

68. The method of claim 64, wherein the effective amount is a dose of 400 µg administered to the subject a total of two times.

69. The method of claim 64, wherein the effective amount is a dose of 500 µg administered to the subject a total of two times.

70. The method of any one of claims 43-69, wherein the efficacy of the vaccine against RSV is greater than 60%.

71. The method of claim 70, wherein the efficacy of the vaccine against RSV is greater than 65%.

72. The method of claim 71, wherein the efficacy of the vaccine against RSV is greater than 70%.

73. The method of claim 72, wherein the efficacy of the vaccine against RSV is greater than 75%.

74. The method of claim 73, wherein the efficacy of the vaccine against RSV is greater than 80%.

75. The method of claim 74, wherein the efficacy of the vaccine against RSV is greater than 85%.

76. The method of claim 75, wherein the efficacy of the vaccine against RSV is greater than 90%.

77. The method of any one of claims 43-76, wherein the vaccine immunizes the subject against RSV for up to 1 year or up to 2 years.

78. The method of any one of claims 43-76, wherein the vaccine immunizes the subject against RSV for more than 2 years.

79. The method of claim 78, wherein the vaccine immunizes the subject against RSV for more than 3 years.

80. The method of claim 79, wherein the vaccine immunizes the subject against RSV for more than 4 years.

81. The method of claim 80, wherein the vaccine immunizes the subject against RSV for 5-10 years.

82. The method of any one of claims 43-81, wherein the subject is about 5 years old or younger, wherein subject is between the ages of about 1 year and about 5 years, wherein subject is between the ages of about 6 months and about 1 year, wherein the subject is about 6 months or younger, or wherein the subject is about 12 months or younger.

83. The method of any one of claims 43-81, wherein the subject is an elderly subject about 60 years old, about 70 years old, or older.

84. The method of any one of claims 43-81, wherein the subject is a young adult between the ages of about 20 years and about 50 years.

85. The method of any one of claims 43-81, wherein the subject was born full term.

86. The method of any one of claims 43-81, wherein the subject was born prematurely at about 36 weeks of gestation or earlier, wherein the subject was born prematurely at about 32 weeks of gestation or earlier, or wherein the subject was born prematurely between about 32 weeks and about 36 weeks of gestation.

87. The method of any one of claims 43-81, wherein the subject is pregnant.

88. The method of any one of claims 43-87, wherein the subject has a chronic pulmonary disease (*e.g.*, chronic obstructive pulmonary disease (COPD) or asthma).

89. The method of any one of claims 43-88, wherein the subject has been exposed to RSV, wherein the subject is infected with RSV, or wherein the subject is at risk of infection by RSV.

90. The method of any one of claims 43-89, wherein the subject is immunocompromised.

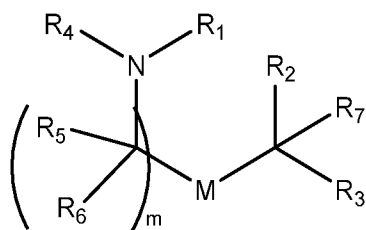
91. The method of any one of claims 43-90, further comprising administering a second (booster) dose, and optionally a third dose, of the RSV vaccine.

92. The method of any one of claims 43-91, wherein the effective amount of the RSV vaccine results in a 5-200 fold increase in serum neutralizing antibodies against RSV, relative to a control.

93. The method of claim 43-91, wherein a single dose of the RSV vaccine results in an about 2-10 fold increase in serum neutralizing antibodies against RSV, relative to a control.

94. A vaccine, comprising:

at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding membrane-bound respiratory syncytial virus (RSV) F protein, membrane-bound DS-Cav1 (stabilized prefusion RSV F protein), or a combination of membrane-bound RSV F protein and membrane-bound DS-Cav1 formulated in a lipid nanoparticle comprising a cationic lipid selected from compounds of Formula (I):



(I),

or a salt or isomer thereof, wherein:

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR,

- 5 -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a carbocycle, heterocycle, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -N(R)₂, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂,
10 -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(R)N(R)₂C(O)OR, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

- 15 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

- 20 R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

- 25 each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl; each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl; each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13.

30

95. The vaccine of claim 94, wherein a subset of compounds of Formula (I) includes those in which when R₄ is -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, or -CQ(R)₂, then (i) Q is not -N(R)₂ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

96. The vaccine of claim 94, wherein a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and
5 -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, a
10 -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, 15 -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms selected from N, O, and S which is substituted with one or more substituents selected from oxo (=O), OH, amino, mono- or di-alkylamino, and C₁₋₃ alkyl, and each n is independently
20 selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, 25 and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

30 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

5

97. The vaccine of claim 94, wherein a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

10 R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a

15 5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂,
20 -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a 5- to 14-membered heterocycle and (i) R₄ is -(CH₂)_nQ in which n is 1 or 2, or (ii) R₄ is -(CH₂)_nCHQR in which n is 1, or (iii) R₄ is -CHQR, and -CQ(R)₂, then Q is either a 5- to 14-membered heteroaryl or 8- to 14-membered heterocycloalkyl;

25 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

30 R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

5 each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

10 98. The vaccine of claim 94, wherein a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

15 R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S,

20 -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and
25 -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group,

30 and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl,
-R*YR'', -YR'', and H;
each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;
5 each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;
each Y is independently a C₃₋₆ carbocycle;
each X is independently selected from the group consisting of F, Cl, Br, and I; and
m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
or salts or isomers thereof.

10

99. The vaccine of claim 94, wherein subset of compounds of Formula (I) includes those
in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and
-R''M'R';

15 R₂ and R₃ are independently selected from the group consisting of H, C₂₋₁₄ alkyl, C₂₋₁₄
alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are
attached, form a heterocycle or carbocycle;

R₄ is -(CH₂)_nQ or -(CH₂)_nCHQR, where Q is -N(R)₂, and n is selected from 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

20 each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-,
-C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group,
and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

25 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl,
-R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₁₋₁₂ alkenyl;

30 each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

100. The vaccine of claim 94, wherein a subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, $-R^*YR''$, $-YR''$, and $-R''M'R'$;

- 5 R_2 and R_3 are independently selected from the group consisting of C_{1-14} alkyl, C_{2-14} alkenyl, $-R^*YR''$, $-YR''$, and $-R^*OR''$, or R_2 and R_3 , together with the atom to which they are attached, form a heterocycle or carbocycle;

R_4 is selected from the group consisting of $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, $-CHQR$, and $-CQ(R)_2$, where Q is $-N(R)_2$, and n is selected from 1, 2, 3, 4, and 5;

- 10 each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

M and M' are independently selected from $-C(O)O-$, $-OC(O)-$, $-C(O)N(R')-$, $-N(R')C(O)-$, $-C(O)-$, $-C(S)-$, $-C(S)S-$, $-SC(S)-$, $-CH(OH)-$, $-P(O)(OR')O-$, $-S(O)_2-$, $-S-S-$, an aryl group, and a heteroaryl group;

- 15 R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, $-R^*YR''$, $-YR''$, and H;

each R'' is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;

- 20 each R^* is independently selected from the group consisting of C_{1-12} alkyl and C_{1-12} alkenyl;

each Y is independently a C_{3-6} carbocycle;

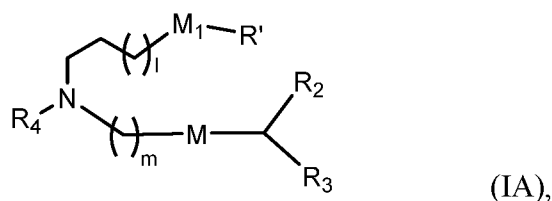
each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

25

101. The vaccine of claim 94, wherein a subset of compounds of Formula (I) includes those of Formula (IA):



or a salt or isomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6,

- 30 7, 8, and 9; M_1 is a bond or M' ; R_4 is unsubstituted C_{1-3} alkyl, or $-(CH_2)_nQ$, in which Q is

OH , $-NHC(S)N(R)_2$, $-NHC(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)R_8$,

-NHC(=NR₉)N(R)₂, -NHC(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, heteroaryl or heterocycloalkyl; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, -S-S-, an aryl group, and a heteroaryl group; and R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, and C₂₋₁₄ alkenyl.

5

102. The vaccine of any one of claims 94-101, wherein the at least one RNA polynucleotide is encoded by the sequence set forth as SEQ ID NO: 5, or wherein the at least one RNA polynucleotide comprises the sequence set forth as SEQ ID NO: 263.

10

103. The vaccine of any one of claims 94-102, wherein the at least one RNA polynucleotide is encoded by the sequence set forth as SEQ ID NO: 7, 257, 258, or 259, or wherein the at least one RNA polynucleotide comprises the sequence set forth as SEQ ID NO: 277, 278, 279, or 280.

15

104. The vaccine of any one of claims 94-103, wherein a single dose of the RSV vaccine results in a 2-10 fold increase in serum neutralizing antibodies against RSV, relative to a control.

20

105. The vaccine of claim 104, wherein a single dose of the RSV vaccine results in an about 5 fold increase in serum neutralizing antibodies against RSV, relative to a control.

106. The vaccine of claim 104 or 105, wherein the serum neutralizing antibodies are against RSV A and/or RSV B.

25

107. A pharmaceutical composition for use in vaccination of a subject comprising an effective dose of the RNA vaccine of any one of claims 1-42 or 94-106, wherein the effective dose is sufficient to produce detectable levels of antigen as measured in serum of the subject at 1-72 hours post administration.

30

108. The composition of claim 107, wherein the cut off index of the antigen is 1-2.

109. A pharmaceutical composition for use in vaccination of a subject comprising an effective dose of the RNA vaccine of any one of claims 1-42 or 94-106,

wherein the effective dose is sufficient to produce a 1,000-10,000 neutralization titer produced by neutralizing antibody against said antigen as measured in serum of the subject at 1-72 hours post administration.

Fig. 1

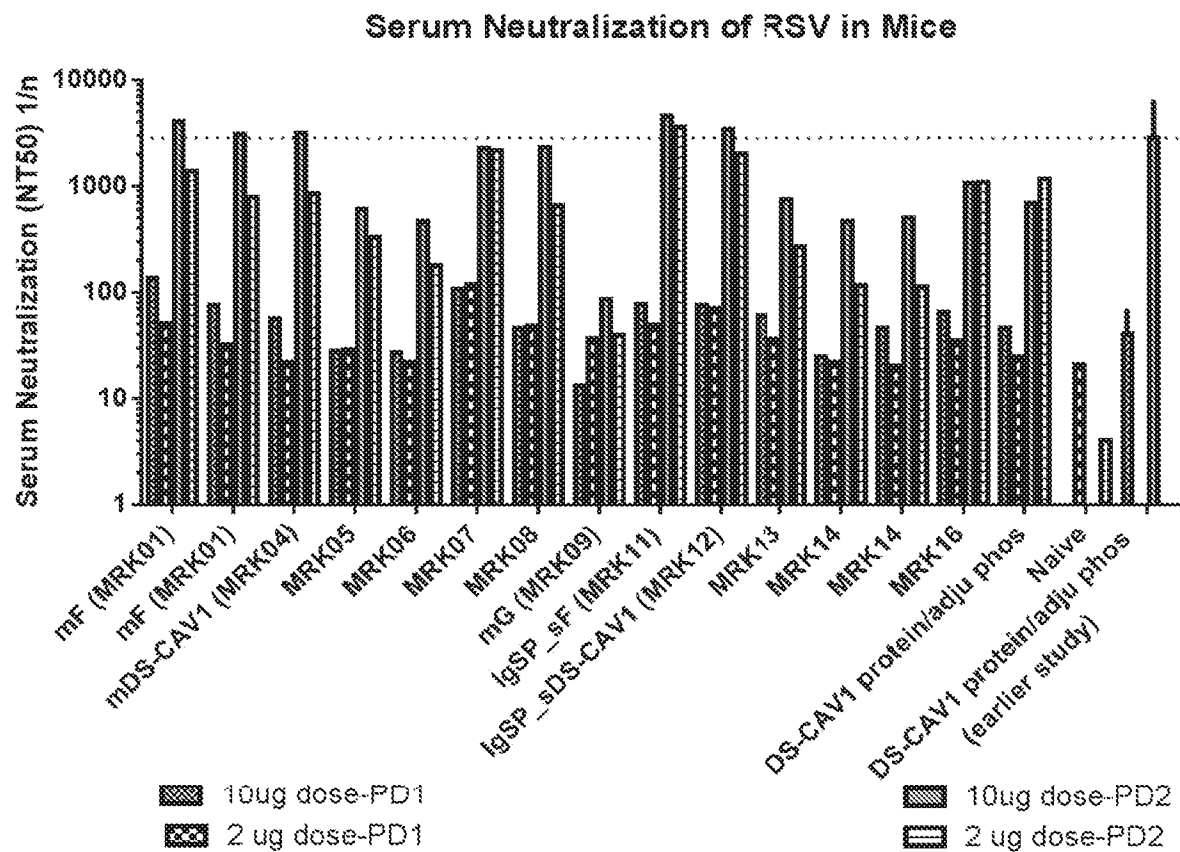


Fig. 2

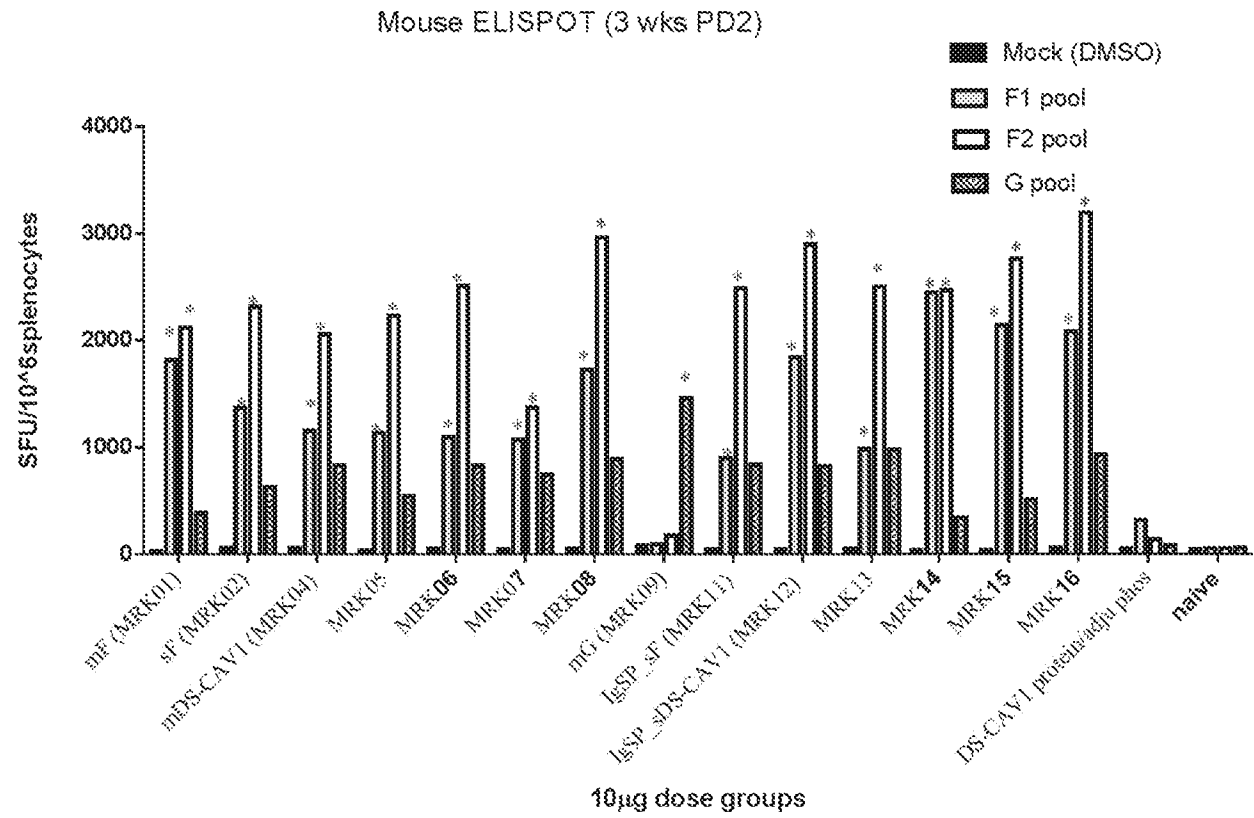


Fig. 3A

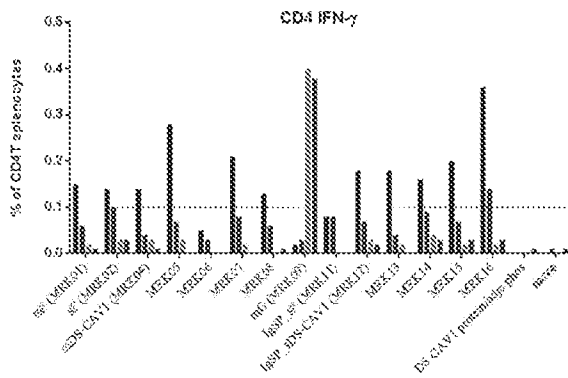


Fig. 3B

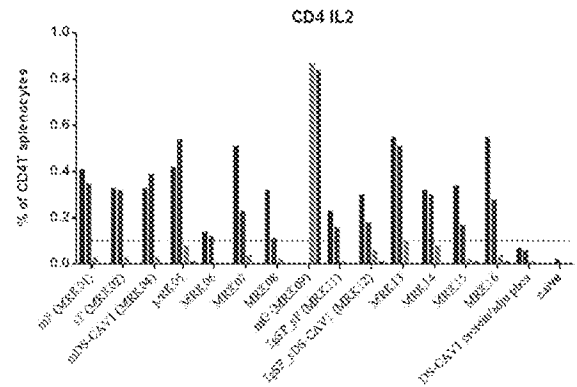
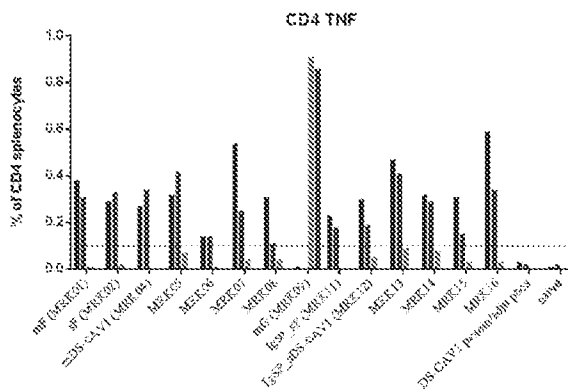


Fig. 3C



- RSV F pool 1
- RSV F pool 2
- RSV G custom
- RSV G new (catalog)

Fig. 4A

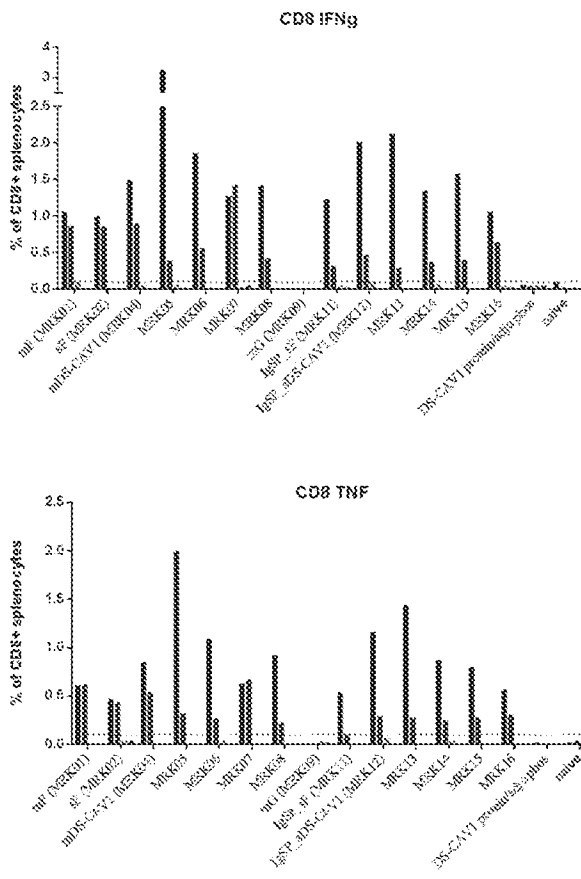


Fig. 4B

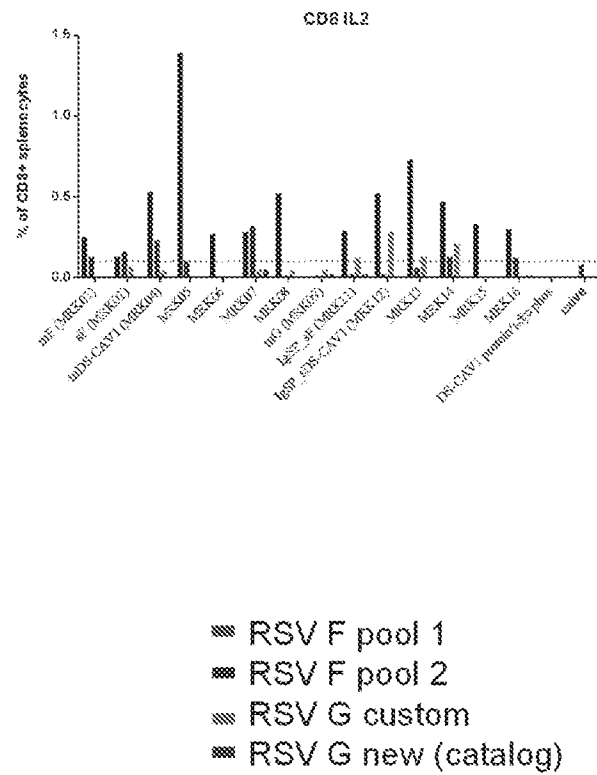


Fig. 4C

Fig. 5

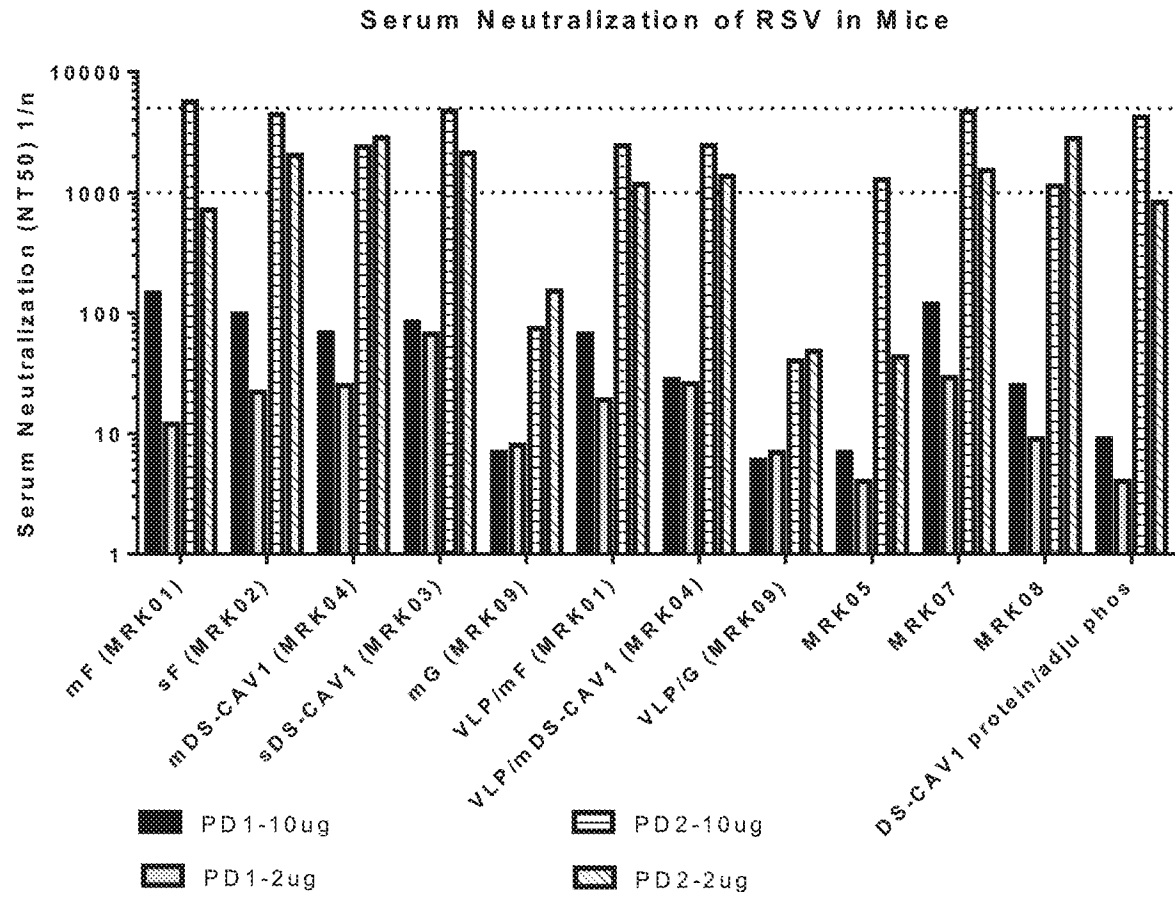


Fig. 6A

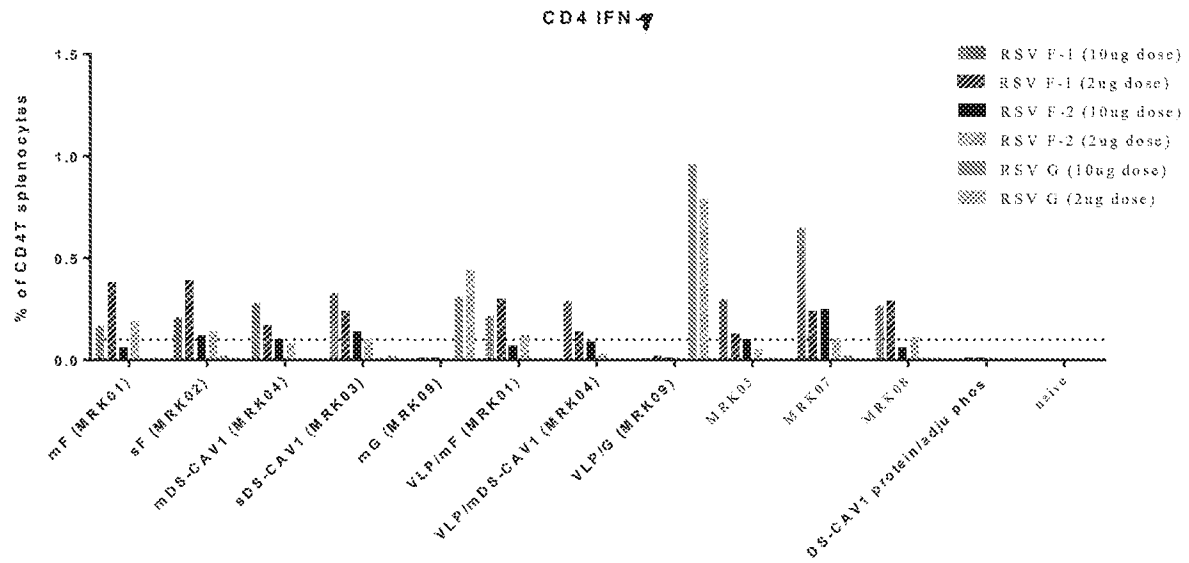


Fig. 6B

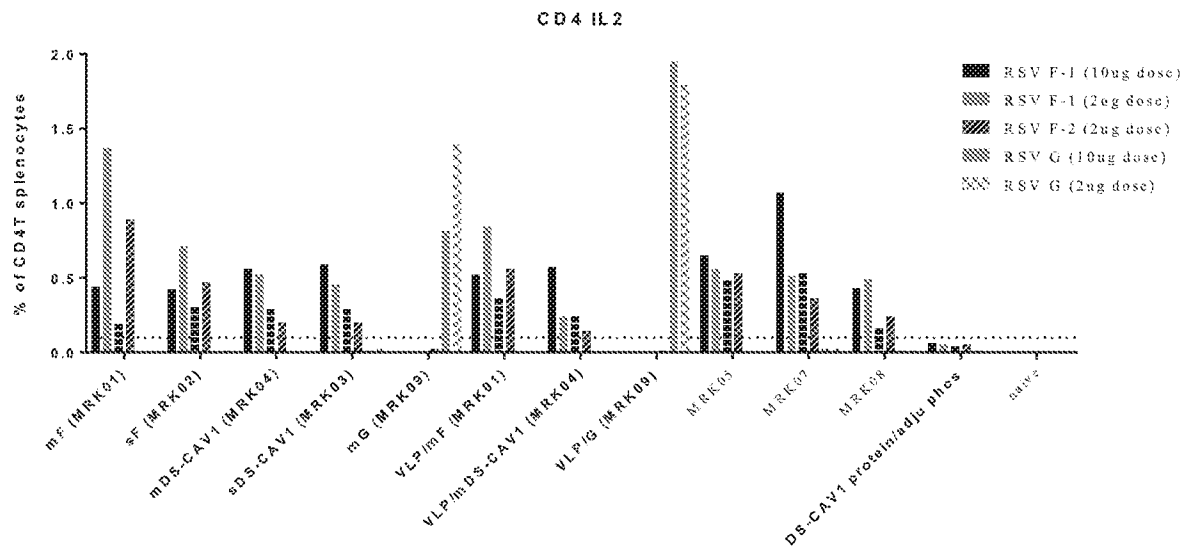


Fig. 6C

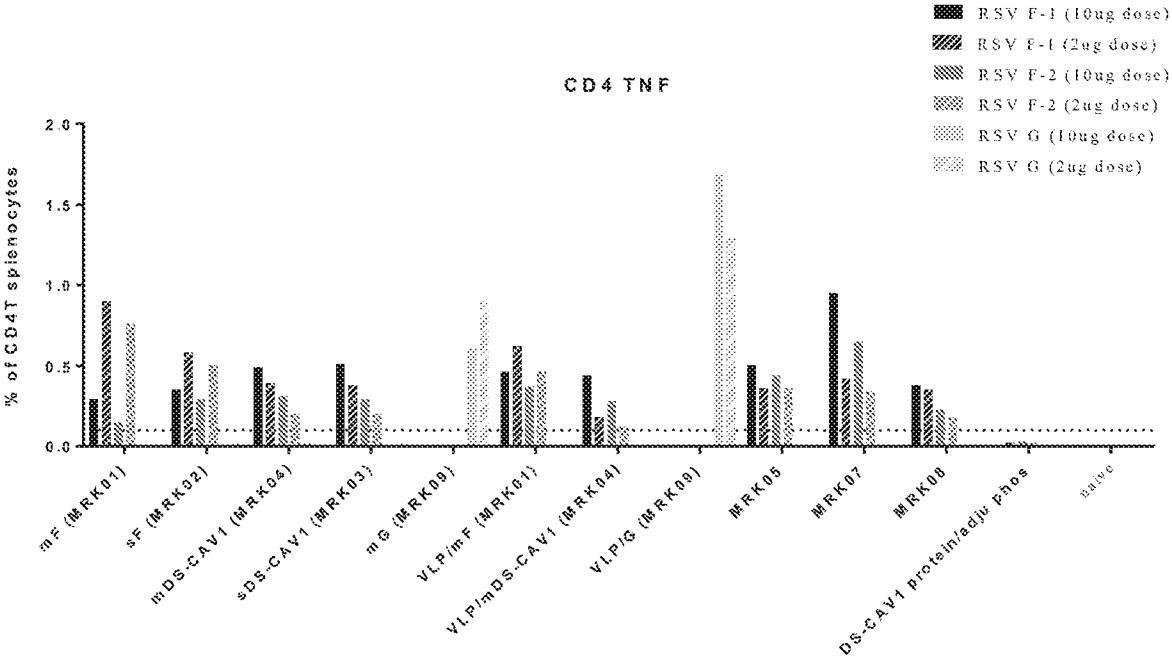


Fig. 7A

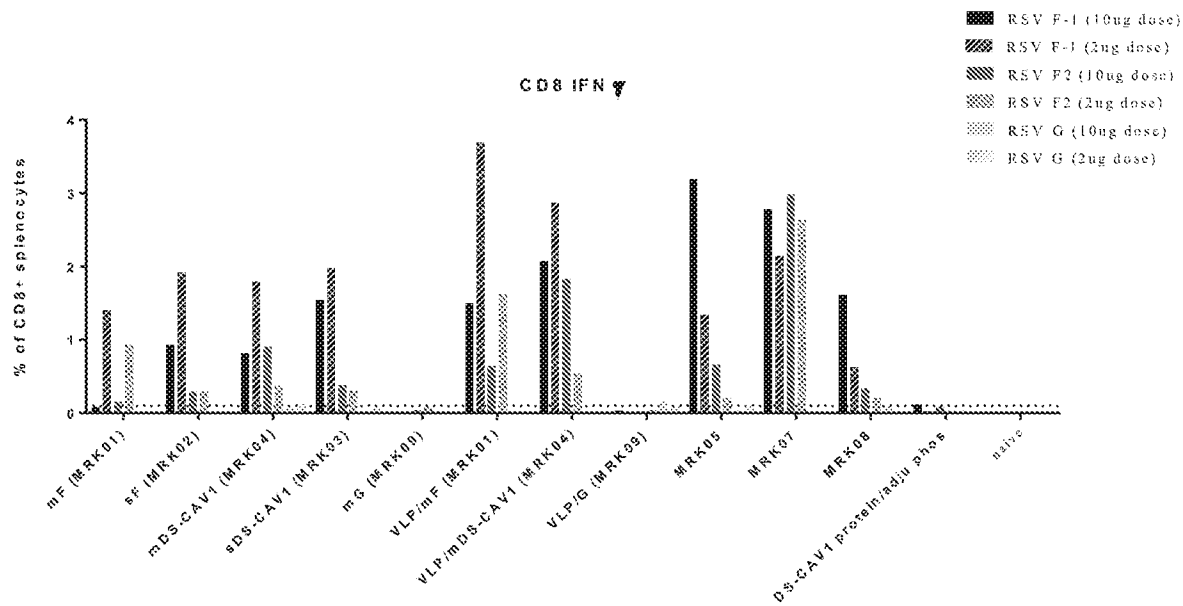


Fig. 7B

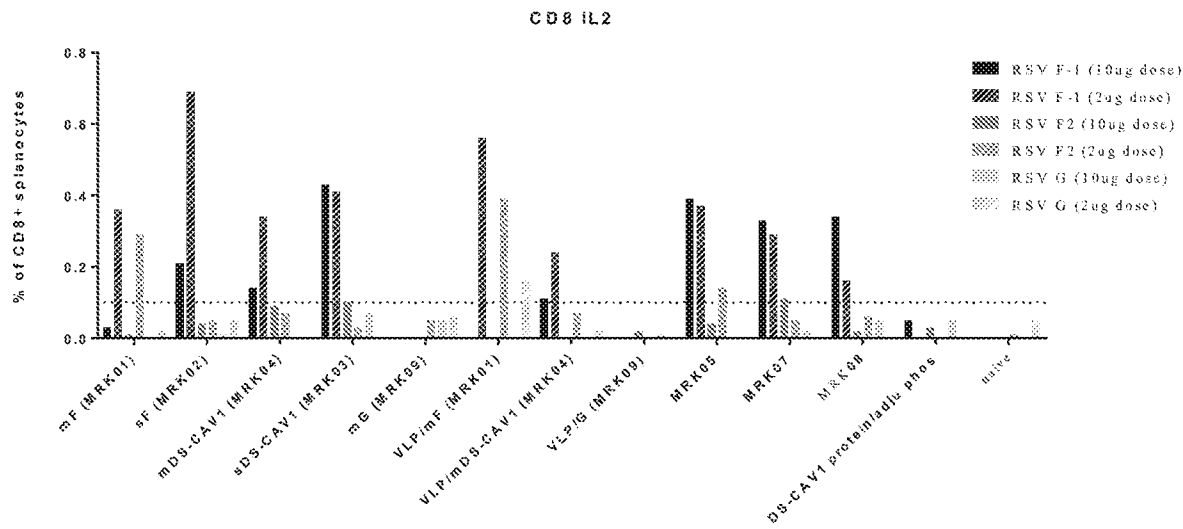


Fig. 7C

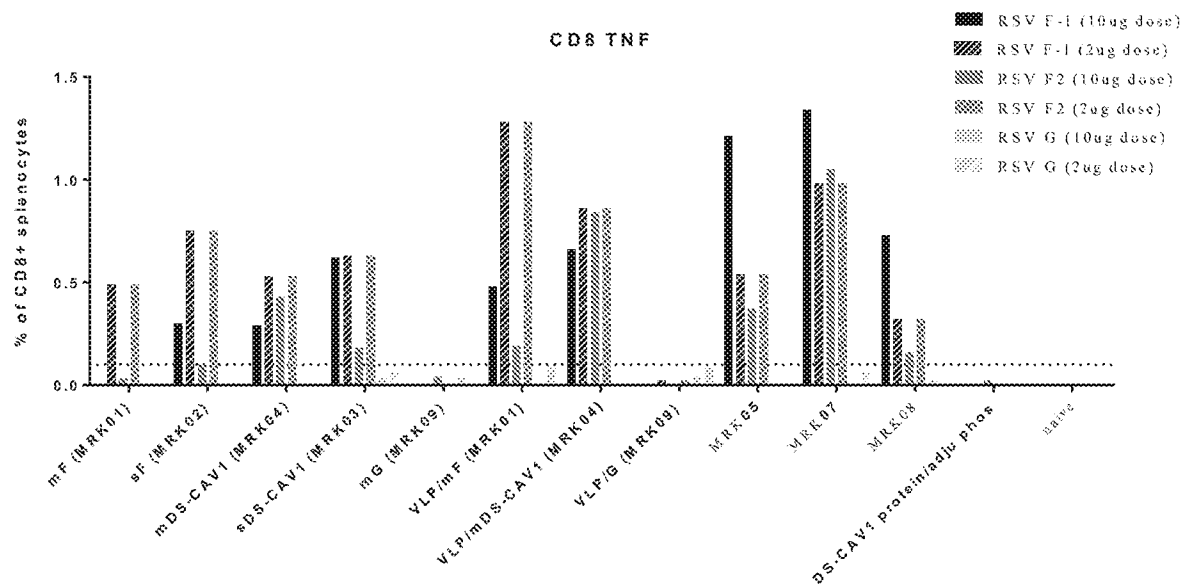


Fig. 8

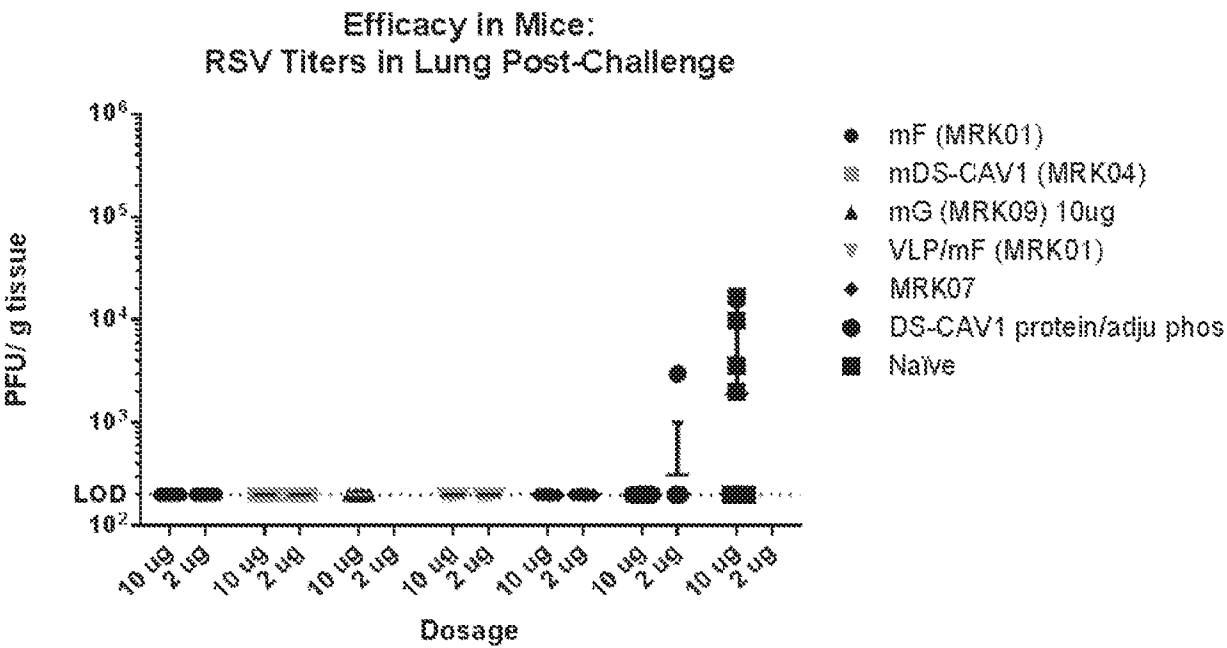


Fig. 9

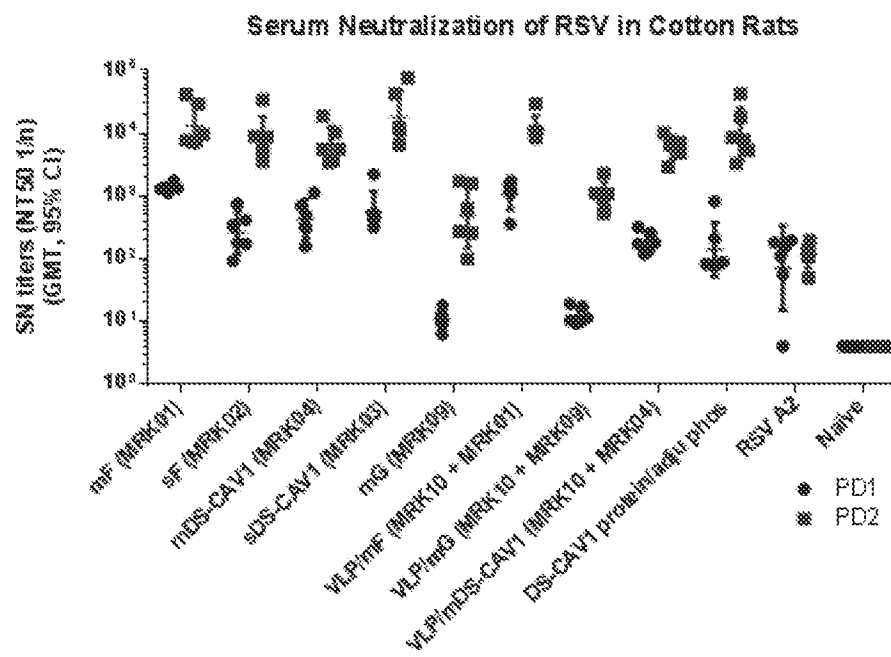


Fig 10. Competition ELISA from Cotton Rat Immunogenicity Study

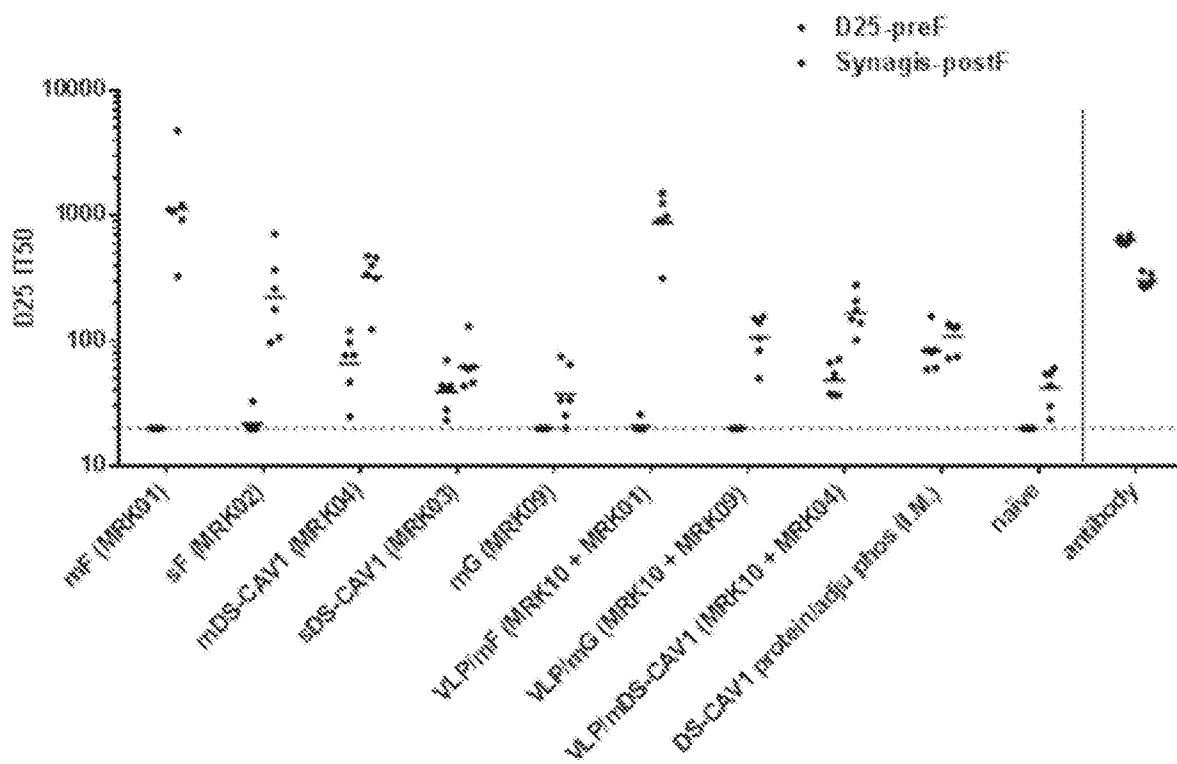


Fig. 11

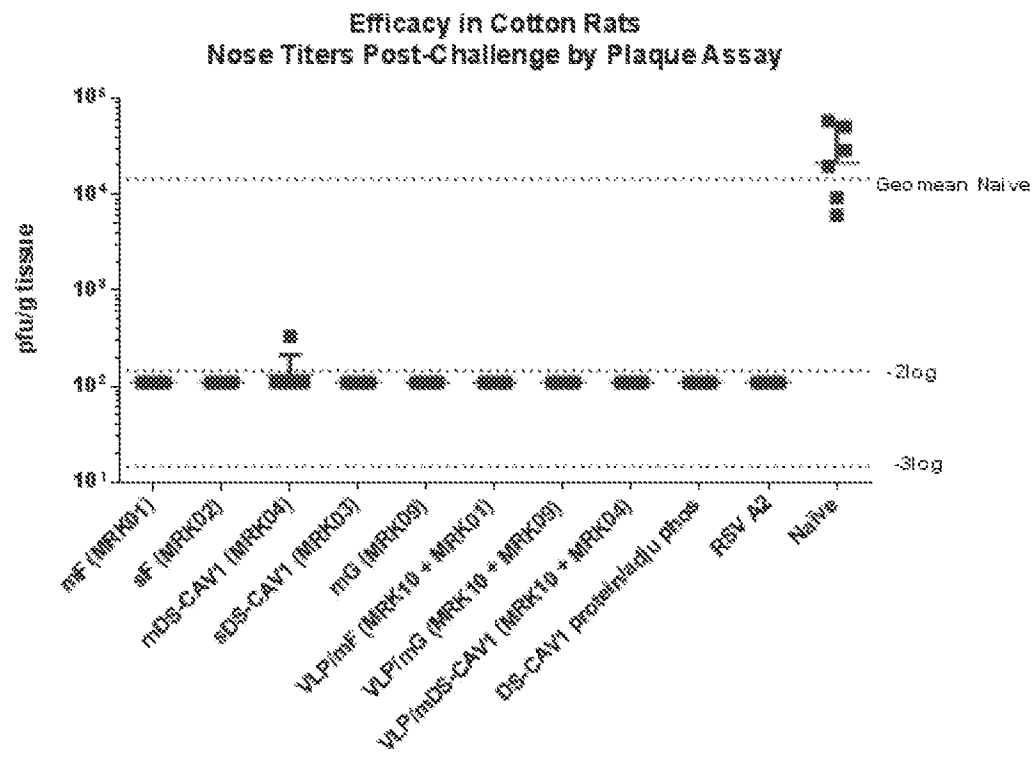


Fig. 12

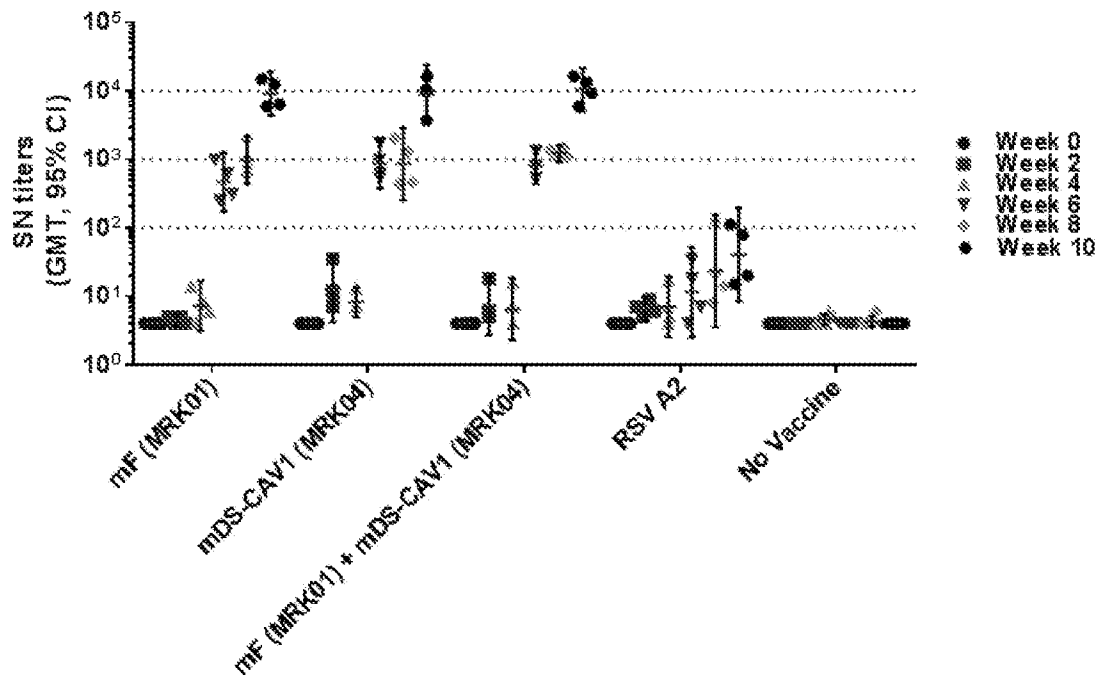


Fig. 13A

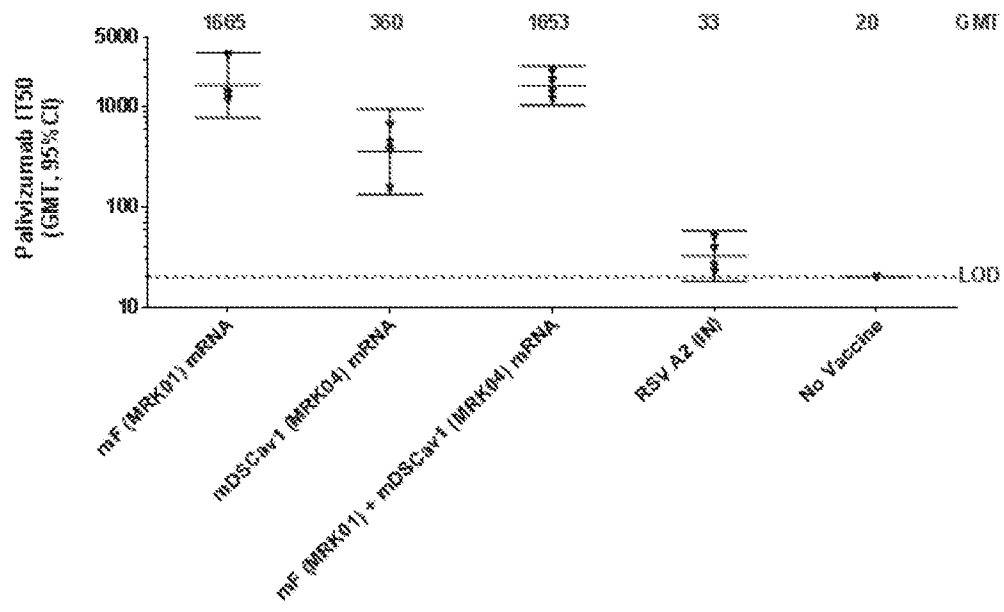


Fig. 13B

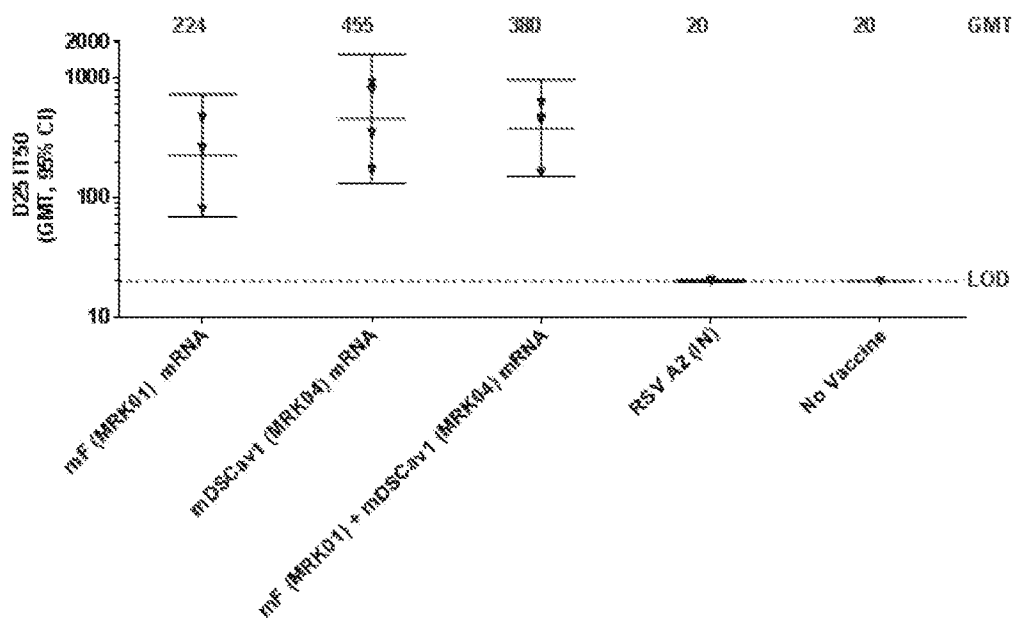


Fig. 14A

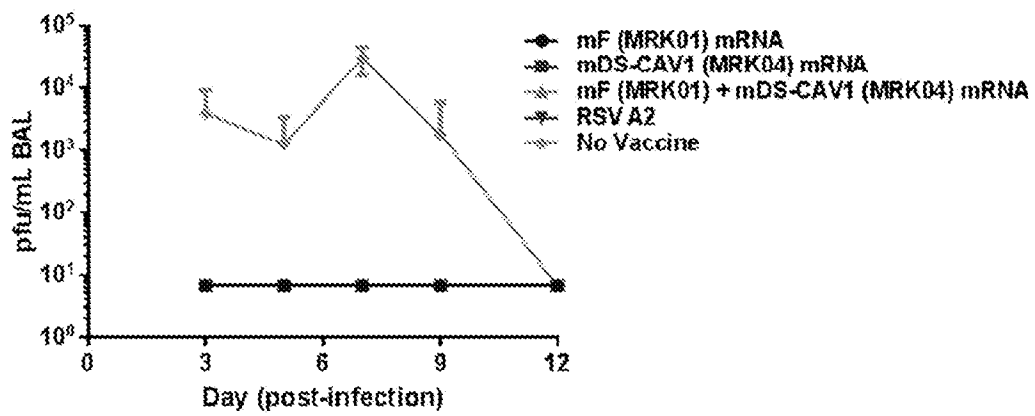


Fig. 14B

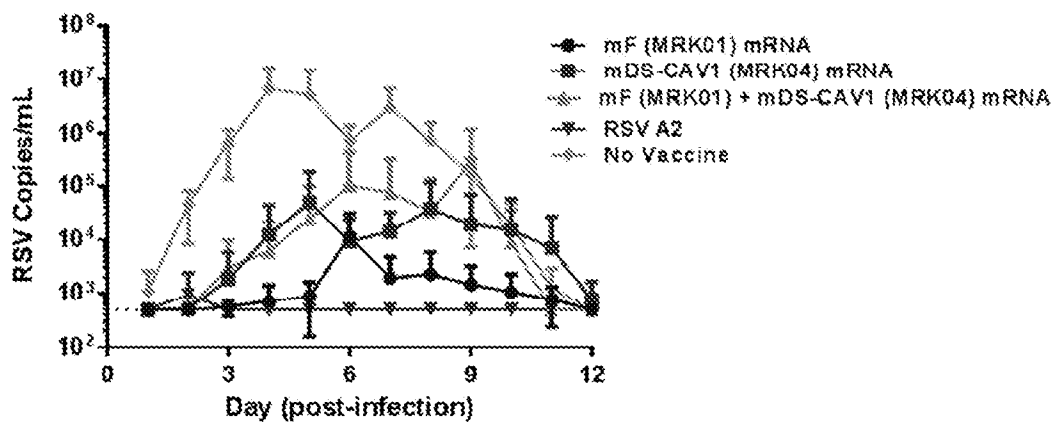


Fig. 15

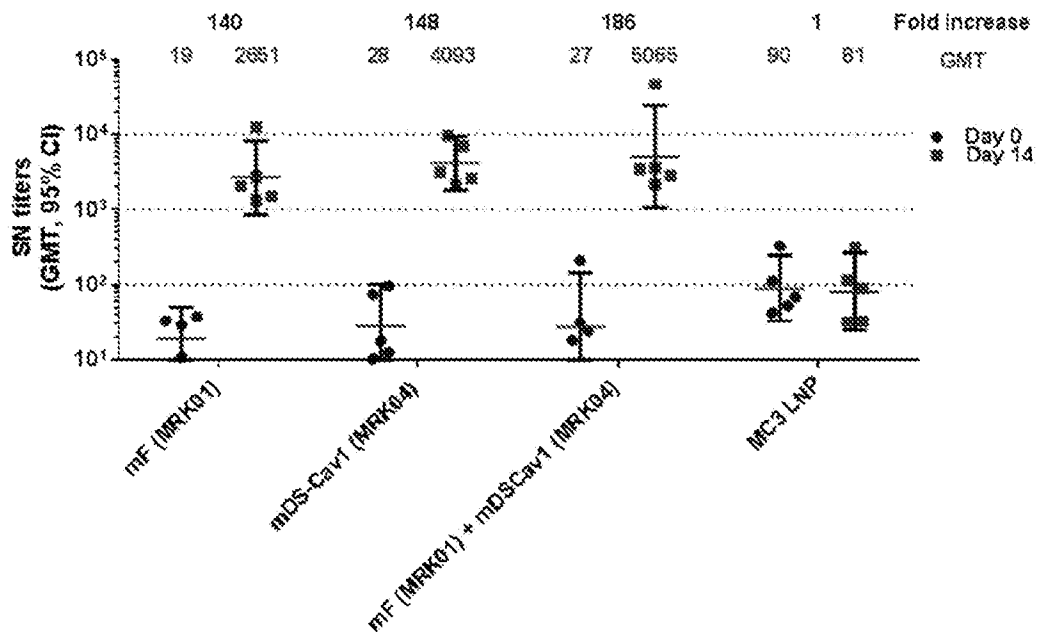


Fig. 16

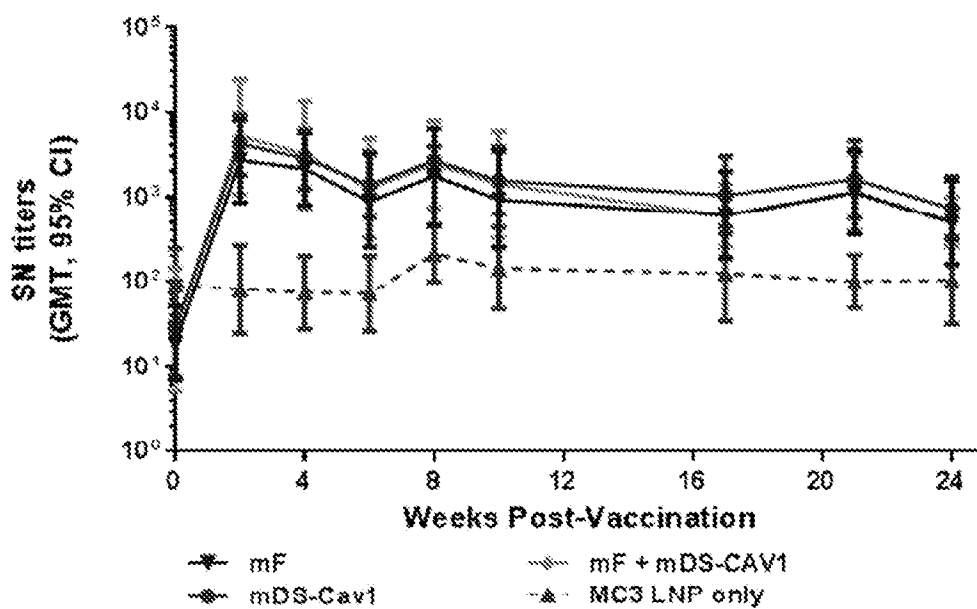


Fig. 17A

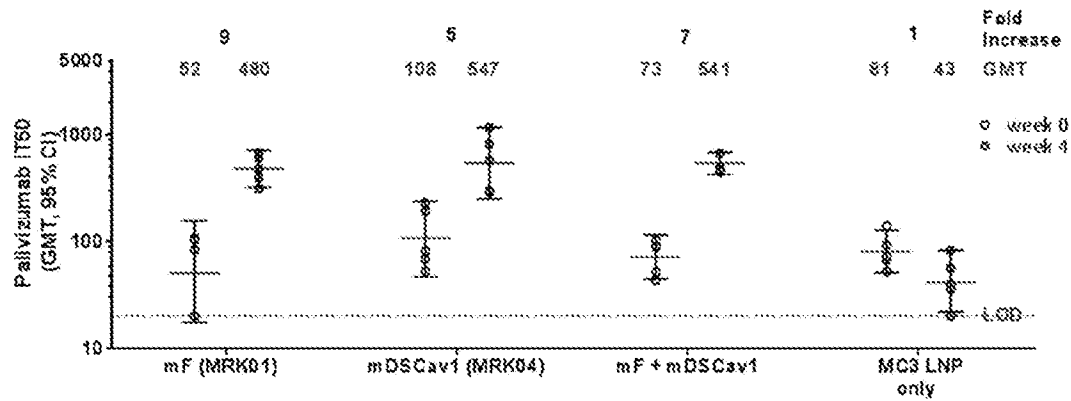


Fig. 17B

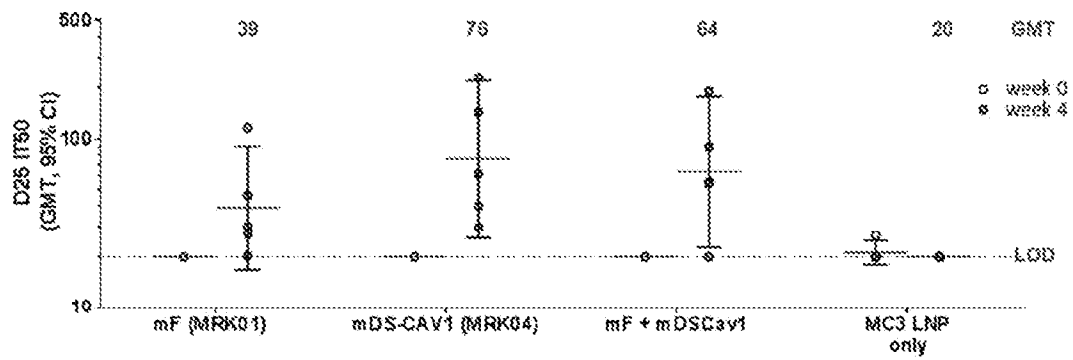


Fig. 18A

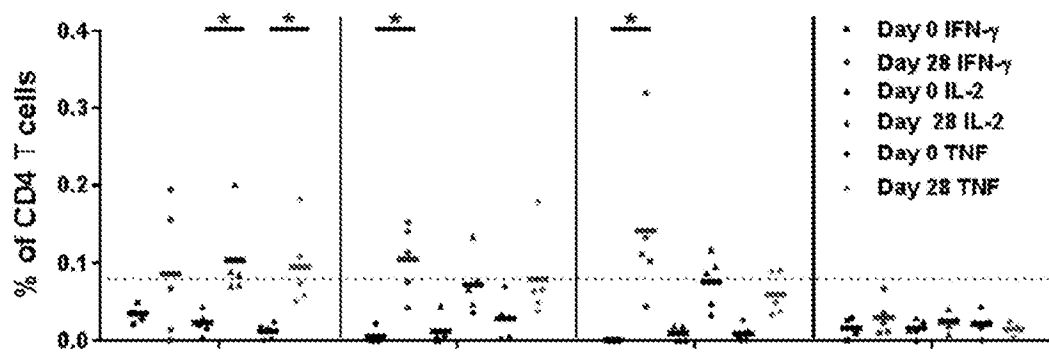


Fig. 18B

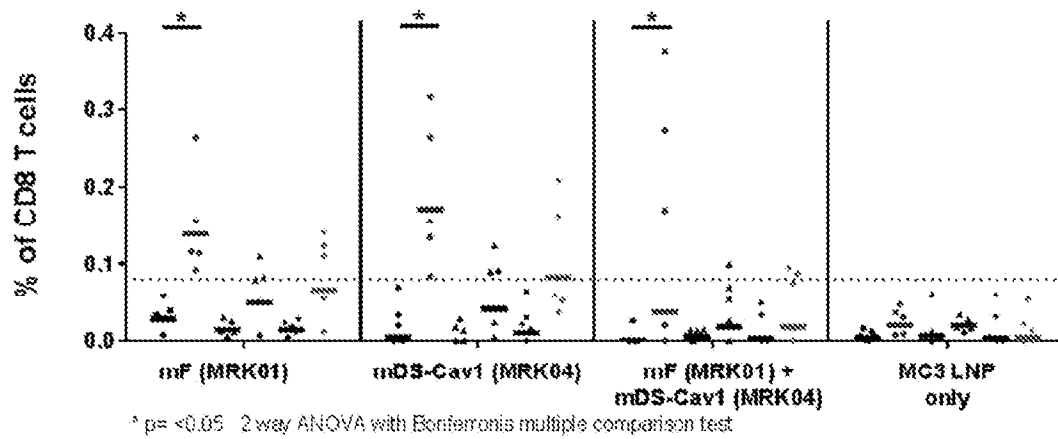


Fig. 19

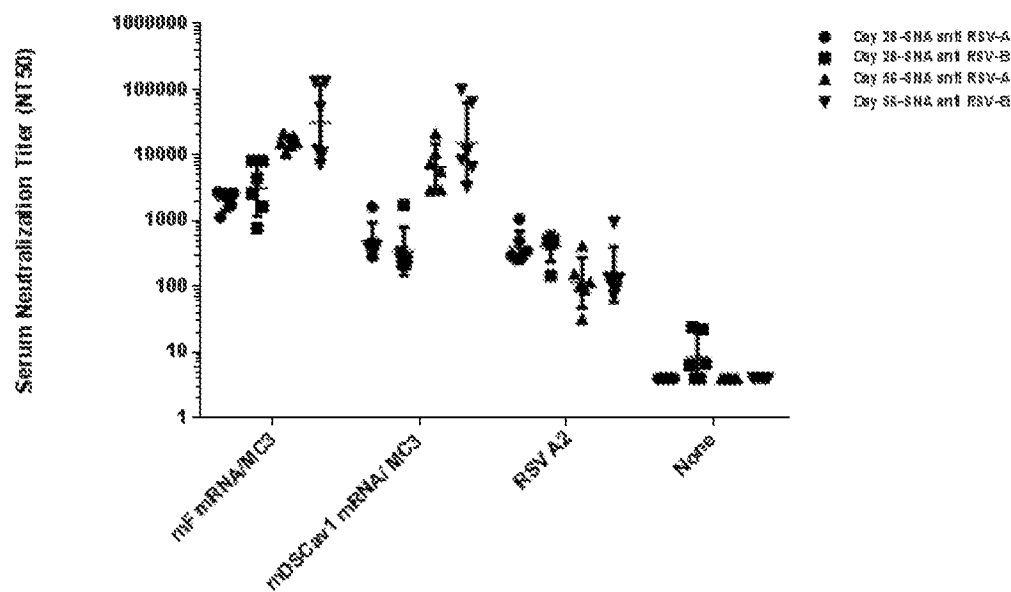
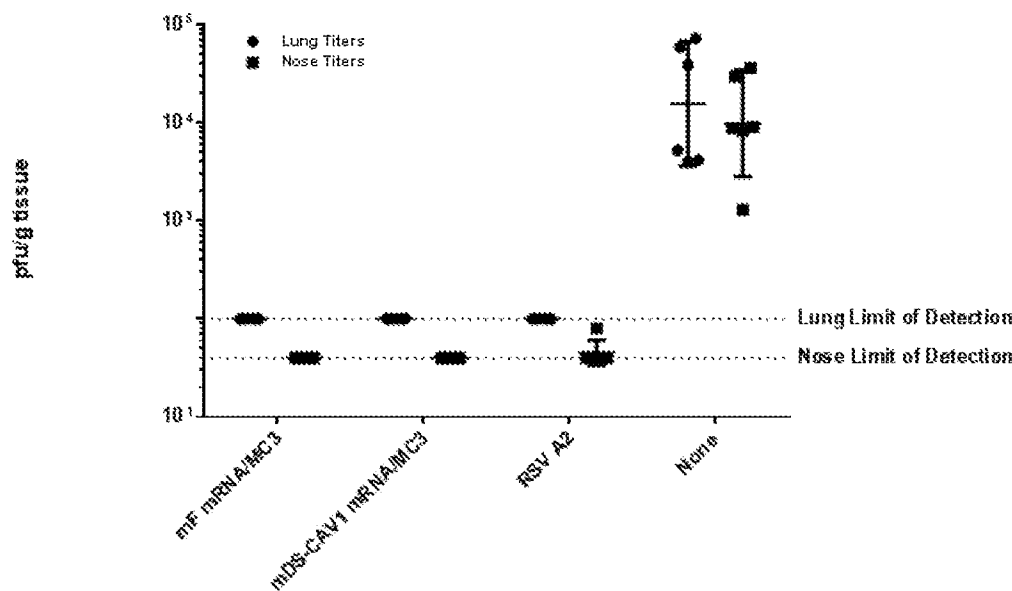


Fig. 20



Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

 International application No.
PCT/US2018/022630

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/155 (2006.01)**A61K 31/7105 (2006.01)****A61K 31/7115 (2006.01)****A61K 9/51 (2006.01)****A61P 31/14 (2006.01)**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Search conducted in databases WPIAP, EPODOC, MEDLINE, full text English language databases, CAPLUS, BIOSIS, EMBASE and the Internet (with the Google search engine) using the following CPC and IPC classification codes and keywords: A61K 2039/53, A61K 2039/55555, A61K 39/155, A61K 9/51, RSV, HRSV, Respiratory Syncytial Virus, DS-Cav1, F protein, ribonucleic acid, RNA, mRNA, open reading frame, vaccine, antigen, immunogen, immunise, cationic lipid, lipid nanoparticle and related terms.

Search of sequences SEQ ID NOs: 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, and 260-280 using GenomeQuest.

Search of applicant and inventor names using the databases Auspat, Patentscope, PubMed and IP Australia Internal databases.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 June 2018	Date of mailing of the international search report 26 June 2018
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au	Authorised officer Shawn Lyons AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262832081

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/US2018/022630
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/024668 A2 (CUREVAC GMBH) 26 February 2015 See the abstract; page 21, line 7 - page 32, line 6; Example 1; page 64, line 19 - page 67, line 4; Example 1; page 51, line 14 - page 52, line 2; page 62, lines 8-32; page 50, lines 1-17; Figures 8-11; Examples 4 and 5; page 20, lines 4-13; Figure 6 and page 78, lines 24-34; Figure 6; Tables 4-5 on pages 86, 88 and 90; Figure 14 and the paragraph bridging pages 80-81; page 3, lines 23-26; page 73, lines 10-22; Table 5 on page 88	1-109
X	GEALL A.J. et al., "Nonviral delivery of self-amplifying RNA vaccines", PNAS, 4 September 2012, Vol. 109, No. 36, pages 14604-14609 See page 14608 and the section entitled 'RNA Synthesis'; the abstract; page 14608 and the section entitled 'LNP/RNA Formulation'; page 14605 and the section entitled 'Vaccine Formulation Characterization'; the section entitled 'Immunogenicity of Candidate Vaccines Encoding Respiratory Syncytial Virus Fusion Glycoprotein (RSV-F)' on pages 14605-14606 and Figure 5; Figures 3-5	1-109
X	KALLEN K-J. et al., "A development that may evolve into a revolution in medicine: mRNA as the basis for novel, nucleotide-based vaccines and drugs", Therapeutic Advances in Vaccines, 2014, Vol. 2, No. 1, pages 10-31 See the paragraph bridging pages 18 and 19	1-109
X	WO 2012/006369 A2 (NOVARTIS AG) 12 January 2012 See page 32, 17-19; page 34, lines 11-18; the section entitled 'Large mammal study' on pages 40-43; page 15, lines 18-25; page 5, line 3; the table on page 36; Figure 13; page 25, lines 4-27; Figure 14; page 35, lines 7-12; page 42, lines 4-19; page 40, line 15; page 24, line 29 - page 25, line 3; page 25, lines 13-27; page 40, line 15	1-109
X	WO 2012/006378 A1 (NOVARTIS AG) 12 January 2012 See the abstract; page 1, lines 14-21; page 17, line 32 - page 18, line 30; page 37, line 20 - page 40, line 8; Figure 13; Figure 14; page 26, line 30 - page 27, line 11	1-109
X	WO 2016/037053 A1 (NOVARTIS AG) 10 March 2016 See paragraph bridging pages 43-44; page 50, lines 28-36; page 108, line 5 - page 111, line 15; page 51, lines 9-11; paragraph bridging pages 59-60; page 59, lines 13-32; page 58, lines 15-17	1-109
X	WO 2015/095340 A1 (NOVARTIS AG) 25 June 2015 See page 171, line 2 - page 173, line 4; page 54, lines 18-32; paragraph bridging pages 69-70; page 71, lines 7-21; page 69, lines 14-27	1-109
X	WO 2013/006842 A2 (NOVARTIS AG) 10 January 2013 See paragraphs [0096]-[00107]; paragraph [0031]; Examples I-V; paragraphs [00332]-[00345]; paragraph [00184]; paragraphs [00308]-[00310]	1-109
A	WO 2016/176330 A1 (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA; ACUITAS THERAPEUTICS INC.) 03 November 2016 See pages 92-96, Table 3 and page 25, lines 13-22	1-109
A	WO 2015/199952 A1 (ACUITAS THERAPEUTICS INC.) 30 December 2015 See Table 3 and page 5, lines 15-29	1-109
A	WO 2006/050280 A2 (CENTOCOR, INC.) 11 May 2006 See SEQ ID NO: 2	13-15
A	WO 2010/077717 A1 (NOVAVAX, INC.) 08 July 2010 See SEQ ID NO: 8 and SEQ ID NO: 26	13-15
Form PCT/ISA/210 (fifth sheet) (January 2015)		

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/US2018/022630
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2015/189425 A1 (GLAXOSMITHKLINE BIOLOGICALS SA) 17 December 2015 See SEQ ID NO: 2	13-15
P,X	WO 2017/070626 A2 (MODERNATX, INC.) 27 April 2017 See the abstract; page 6, line 15 - page 7, line 35; page 128, line 11 - page 168, line 11; page 11, lines 19-22; page 53, lines 3-12; page 14, line 10; page 63, line 18 - page 78 line 16; page 17, lines 8-24; page 18, lines 1-2; page 27, lines 30-33; page 87, lines 17-19; page 18, line 34 - page 19, line 18; page 19, line 19 - page 20, line 15; page 20, line 16 - page 21, line 18; page 21, line 19 - page 22, line 26	1-109
P,X	WO 2017/070622 A1 (MODERNATX, INC.) 27 April 2017 See claims 1-167, in particular claim 162; pages 120-132; Table 12; SEQ ID NOs: 1-28 and 260-280	1-109

Form PCT/ISA/210 (fifth sheet) (January 2015)

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Information on patent family members		PCT/US2018/022630	
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Patent Document/s Cited in Search Report		Patent Family Member/s	
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Patent Document/s Cited in Search Report		Patent Family Member/s	
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INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/US2018/022630	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(January 2015)			

INTERNATIONAL SEARCH REPORT		International application No.			
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.					

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Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.			
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Publication Number	Publication Date	Publication Number	Publication Date		
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This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.		
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