

CLAIMS

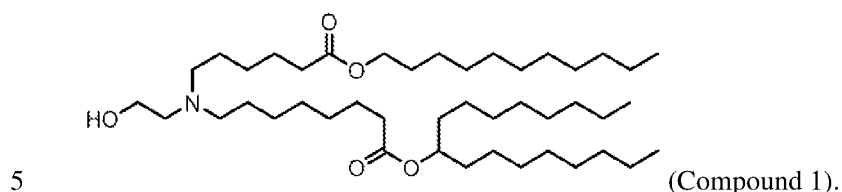
What is claimed is:

1. A composition comprising a human respiratory syncytial virus (hRSV) ribonucleic acid
5 (RNA) encoding a stabilized prefusion form of an hRSV F glycoprotein variant that lacks a cytoplasmic tail and has at least 90% identity to a wild-type hRSV F glycoprotein.
2. The composition of claim 1, wherein the cytoplasmic tail comprises the C-terminal 20-
30, 20-25, 15-30, 15-25, 15-20, 10-30, 10-25, 10-20, 10-15, 5-30, 5-25, 5-20, or 5-15 amino
10 acids of the of the hRSV F glycoprotein variant.
3. The composition of claim 2, wherein the cytoplasmic tail comprises the C-terminal 25
amino acids, 20 amino acids, 15 amino acids, or 10 amino acids of the hRSV F glycoprotein
variant.
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4. The composition of claim 3, wherein the cytoplasmic tail comprises of the following C-
terminal amino acids of the hRSV F glycoprotein variant:
CKARSTPVTLSKDQLSGINNIAFSN (SEQ ID NO: 25); TPVTLTKDQLSGINNIAFSN (SEQ
ID NO: 26); SKDQLSGINNIAFSN (SEQ ID NO: 27); or SGINNIAFSN (SEQ ID NO: 28).
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5. The composition of any one of claims 1-4, wherein the hRSV F glycoprotein variant
comprises a modification, relative to a wild-type hRSV F glycoprotein, selected from the group
consisting of: a P102X substitution, a substitution of amino acids 104-144 with a linker
molecule, an A149X substitution, an S155X substitution, an S190X substitution, a V207X
25 substitution, an S290X substitution, a L373X substitution, an I379X substitution, an M447X
substitution, and a Y458X substitution, wherein X is any amino acid.
6. The composition of claim 5, wherein the hRSV F glycoprotein variant comprises a
modification, relative to a wild-type hRSV F glycoprotein, selected from the group consisting of:
30 a P102A substitution, a substitution of amino acids 104-144 with a linker molecule, an A149C
substitution, an S155C substitution, an S190F substitution, a V207L substitution, an S290C
substitution, a L373R substitution, an I379V substitution, an M447V substitution, and a Y458C
substitution.

7. The composition of claim 6, wherein the hRSV F glycoprotein variant comprises the following modifications, relative to a wild-type hRSV F glycoprotein: a P102A substitution, a substitution of amino acids 104-144 with a linker molecule, an A149C substitution, an S155C substitution, an S190F substitution, a V207L substitution, an S290C substitution, a L373R substitution, an I379V substitution, an M447V substitution, and a Y458C substitution.
8. The composition of any one of the preceding claims, wherein the wild-type hRSV F glycoprotein comprises the sequence of SEQ ID NO: 1.
9. The composition of any one of the preceding claims, wherein the hRSV F glycoprotein variant comprises a sequence that has at least 95% or at least 98% identity to the sequence of SEQ ID NO: 8.
10. The composition of claim 9, wherein the hRSV F glycoprotein variant comprises the sequence of SEQ ID NO: 8.
11. The composition of any one of the preceding claims, wherein the hRSV RNA comprises an open reading frame (ORF) that comprises a sequence that has at least 90%, at least 95%, or at least 98% identity to the sequence of SEQ ID NO: 7.
12. The composition of claim 11, wherein the hRSV RNA comprises an ORF that comprises the sequence of SEQ ID NO: 7.
13. The composition of claim 11 or 12, wherein the hRSV RNA comprises a 5' untranslated region (UTR) that comprises the sequence of SEQ ID NO: 2.
14. The composition of any one of claims 11-13, wherein the hRSV RNA comprises a 3' UTR that comprises the sequence of SEQ ID NO: 4.
15. The composition of any one of claims 11-14, wherein the hRSV RNA comprises a sequence that has at least 90%, at least 95%, or at least 98% identity to the sequence of SEQ ID NO: 15.

16. The composition of claim 15, wherein the hRSV RNA comprises the sequence of SEQ ID NO: 15.
17. The composition of any one of the preceding claims, wherein the hRSV RNA further comprises a 7mG(5')ppp(5')NlmpNp cap.
18. The composition of any one of the preceding claims, wherein the hRSV RNA further comprises a poly(A) tail, optionally having a length of 50 to 150 nucleotides.
19. The composition of any one of the preceding claims, wherein the hRSV RNA comprises a chemical modification.
20. The composition of claim 19, wherein the hRSV RNA is fully modified.
21. The composition of claim 19 or claim 20, wherein the chemical modification is 1-methylpseudouridine.
22. The composition of any one of the preceding claims, comprising 25 µg – 200 µg of the hRSV RNA.
23. The composition of any one of the preceding claims, further comprising a mixture of lipids that comprises a PEG-modified lipid, a non-cationic lipid, a sterol, an ionizable cationic lipid, or any combination thereof.
24. The composition of claim 23, wherein the mixture of lipids comprises 0.5-15 mol% PEG-modified lipid; 5-25 mol% non-cationic lipid; 25-55 mol% sterol; and 20-60 mol% ionizable cationic lipid.
25. The composition of claim 24, wherein the mixture of lipids comprises 1-5 mol% PEG-modified lipid; 10-20 mol% non-cationic lipid; 35-45 mol% sterol; and 40-50 mol% ionizable cationic lipid.

26. The composition of claim 24 or claim 25, wherein the PEG-modified lipid is 1,2 dimyristoyl-sn-glycerol, methoxypolyethyleneglycol (PEG2000 DMG), the non-cationic lipid is 1,2 distearoyl-sn-glycero-3-phosphocholine (DSPC), the sterol is cholesterol; and the ionizable cationic lipid has the structure of Compound 1:



27. The composition of any one of claims 23-26, wherein the mixture of lipids forms lipid nanoparticles.

10 28. The composition of claim 27, wherein the hRSV RNA is formulated in the lipid nanoparticles.

29. The composition of any one of the preceding claims, wherein at least 24 hours post-delivery of the composition to mammalian cells in vitro results in cell surface expression of the prefusion form of hRSV F glycoprotein at a level that is at least 5-fold higher than a control level.

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30. The composition of any one of the preceding claims, wherein at least 48 hours post-delivery of the composition to mammalian cells in vitro results in cell surface expression of the stabilized prefusion form of hRSV F glycoprotein at a level that is at least 50-fold higher than a control level.

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31. A method comprising administering to a subject the composition of any one of claims 1-30 in an amount effective to induce a neutralizing antibody response against hRSV in the subject.

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32. The method of claim 31, wherein the subject is immunocompromised.

33. The method of claim 31 or 32, wherein the subject has a pulmonary disease.

30 34. The method of any one of claims 31-33, wherein the subject is 5 years of age or younger.

35. The method of any one of claims 31-33, wherein the subject is 65 years of age or older.
36. The method of any one of claims 31-35, comprising administering to the subject at least two doses of the composition.
- 5 37. The method of any one of claims 31-36, wherein the hRSV F glycoprotein variant folds into a stabilized prefusion conformation.
38. The method of any one of claims 31-37, wherein at least 24 hours post-administration the
10 hRSV F glycoprotein variant is expressed in the subject at a level that is at least 5-fold higher, relative to a control.
39. The method of any one of claims 31-38, wherein at least 48 hours post-administration the RSV F glycoprotein variant is expressed in the subject at a level that is at least 50-fold higher,
15 relative to a control.
40. The method of any one of claims 31-39, wherein the RSV F glycoprotein variant is expressed in the subject for at least 24 hours longer, relative to a control.
- 20 41. The method of any one of claims 31-40, wherein a neutralizing antibody response is induced in the subject using a dose of the composition that is at least 5-fold lower, relative to a control.
42. The method of any one of claims 31-42, wherein the control is baseline, administration of
25 an RSV RNA encoding a wild-type RSV F glycoprotein, or administration of an RSV RNA encoding a stabilized prefusion form of an RSV F glycoprotein with a cytoplasmic tail.
43. A messenger ribonucleic acid (mRNA) comprising an open reading frame that comprises a sequence having at least 85%, at least 90%, at least 95%, or at least 98% identity to the
30 sequence of SEQ ID NO: 7.
44. A messenger ribonucleic acid (mRNA) comprising a sequence having at least 85%, at least 90%, at least 95%, or at least 98% identity to the sequence of SEQ ID NO: 15.

45. The mRNA of claim 43 or 44, wherein the mRNA encodes a stabilized prefusion form of an human respiratory syncytial virus (hRSV) ribonucleic F glycoprotein variant that lacks a cytoplasmic tail, wherein the RSV F glycoprotein variant has at least 85%, at least 90%, at least 95%, or at least 98% identity to a full-length wild-type RSV F glycoprotein.

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46. The mRNA of any one of claims 43-45, wherein the open reading from comprises the sequence of SEQ ID NO: 7.

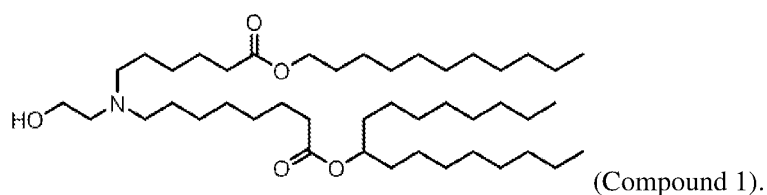
47. The mRNA of any one of claims 43-46, wherein the mRNA comprises the sequence of
10 SEQ ID NO: 15.

48. The mRNA of any one of claims 43-47 formulated in a lipid nanoparticle.

49. The mRNA of any one of claims 43-48, wherein the lipid nanoparticle comprises 0.5-
15 15% PEG-modified lipid; 5-25% non-cationic lipid; 25-55% sterol; and 20-60% ionizable cationic lipid.

50. The mRNA of claim 49, wherein the lipid nanoparticle comprises 1-5 mol% PEG-
20 modified lipid; 10-20 mol% non-cationic lipid; 35-45 mol% sterol; and 40-50 mol% ionizable cationic lipid.

51. The mRNA of any one of claims 43-50, wherein the PEG-modified lipid is 1,2 dimyristoyl-sn-glycerol, methoxypolyethyleneglycol (PEG2000 DMG), the non-cationic lipid is 1,2 distearoyl-sn-glycero-3-phosphocholine (DSPC), the sterol is cholesterol; and the ionizable
25 cationic lipid has the structure of Compound 1:



52. A composition comprising:
a human respiratory syncytial virus (hRSV) ribonucleic acid (RNA) encoding a stabilized
30 prefusion form of an RSV F glycoprotein variant that lacks a cytoplasmic tail, wherein the RSV F glycoprotein variant has at least 85% identity to a full-length wild-type RSV F glycoprotein;

a human metapneumovirus (hMPV) RNA encoding an hMPV F glycoprotein; and
a human parainfluenza virus 3 (hPIV3) RNA encoding an hPIV3 F glycoprotein.

53. The composition of claim 52, wherein the cytoplasmic tail comprises the C-terminal 20-
5 30, 20-25, 15-30, 15-25, 15-20, 10-30, 10-25, 10-20, 10-15, 5-30, 5-25, 5-20, or 5-15 amino
acids of the of the hRSV F glycoprotein variant.

54. The composition of claim 53, wherein the cytoplasmic tail comprises the C-terminal 25
amino acids, 20 amino acids, 15 amino acids, or 10 amino acids of the hRSV F glycoprotein
10 variant.

55. The composition of claim 54, wherein the cytoplasmic tail comprises of the following C-
terminal amino acids of the hRSV F glycoprotein variant:
CKARSTPVTLSKDQLSGINNIAFSN (SEQ ID NO: 25); TPVTLSKDQLSGINNIAFSN (SEQ
15 ID NO: 26); SKDQLSGINNIAFSN (SEQ ID NO: 27); or SGINNIAFSN (SEQ ID NO: 28).

56. The composition of any one of claims 52-55, wherein the hRSV F glycoprotein variant
further comprises a modification, relative to the wild-type hRSV F glycoprotein, selected from
the group consisting of: a P102X substitution, a substitution of amino acids 104-144 with a
20 linker molecule, an A149X substitution, an S155X substitution, an S190X substitution, a V207X
substitution, an S290X substitution, a L373X substitution, an I379X substitution, an M447X
substitution, and a Y458X substitution, wherein X is any amino acid.

57. The composition of claim 56, wherein the hRSV F glycoprotein variant further comprises
25 a modification, relative to the wild-type hRSV F glycoprotein, selected from the group consisting
of: a P102A substitution, a substitution of amino acids 104-144 with a linker molecule, an
A149C substitution, an S155C substitution, an S190F substitution, a V207L substitution, an
S290C substitution, a L373R substitution, an I379V substitution, an M447V substitution, and a
Y458C substitution.

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58. The composition of claim 57, wherein the hRSV F glycoprotein variant further comprises
the following modifications, relative to the wild-type hRSV F glycoprotein: a P102A
substitution, a substitution of amino acids 104-144 with a linker molecule, an A149C
substitution, an S155C substitution, an S190F substitution, a V207L substitution, an S290C

substitution, a L373R substitution, an I379V substitution, an M447V substitution, and a Y458C substitution.

59. The composition of any one of claims 52-58, wherein the wild-type hRSV F glycoprotein
5 comprises the sequence of SEQ ID NO: 1.

60. The composition of any one of claims 52-59, wherein the hRSV F glycoprotein variant
comprises a sequence that has at least 95% or at least 98% identity to the sequence of SEQ ID
NO: 8.
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61. The composition of claim 60, wherein the hRSV F glycoprotein variant comprises the
sequence of SEQ ID NO: 8.

62. The composition of any one of claims 52-61, wherein the hRSV RNA comprises an open
15 reading frame (ORF) that comprises a sequence that has at least 90%, at least 95%, or at least
98% identity to the sequence of SEQ ID NO: 7.

63. The composition of claim 62, wherein the hRSV RNA comprises an ORF that comprises
the sequence of SEQ ID NO: 7.
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64. The composition of claim 62 or 63, wherein the hRSV RNA comprises a 5' untranslated
region (UTR) that comprises the sequence of SEQ ID NO: 2.

65. The composition of any one of claims claim 62-64, wherein the hRSV RNA comprises a
25 3' UTR that comprises the sequence of SEQ ID NO: 4.

66. The composition of any one of claims 62-65, wherein the hRSV RNA comprises a
sequence that has at least 90%, at least 95%, or at least 98% identity to the sequence of SEQ ID
NO: 15.
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67. The composition of claim 66, wherein the hRSV RNA comprises the sequence of SEQ
ID NO: 15.

68. The composition of any one of claims 52-67, wherein the hMPV F glycoprotein comprises a sequence that has at least 90%, at least 95%, or at least 98% identity to the sequence of SEQ ID NO: 11.
- 5 69. The composition of claim 68, wherein the hMPV F glycoprotein comprises the sequence of SEQ ID NO: 11.
70. The composition of any one of claims 52-69, wherein the hMPV RNA comprises an ORF that comprises a sequence that has at least 90%, at least 95%, or at least 98% identity to the
10 sequence of SEQ ID NO: 10.
71. The composition of claim 70, wherein the hMPV RNA comprises an ORF that comprises the sequence of SEQ ID NO: 10.
- 15 72. The composition of claim 70 or 71, wherein the hMPV RNA comprises a 5' UTR that comprises the sequence of SEQ ID NO: 2.
73. The composition of any one of claims 70-72, wherein the hMPV RNA comprises a 3' UTR that comprises the sequence of SEQ ID NO: 4.
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74. The composition of any one of claims claim 70-73, wherein the hMPV RNA comprises a sequence that has at least 90%, at least 95%, or at least 98% identity to the sequence of SEQ ID NO: 16.
- 25 75. The composition of claim 74, wherein the hMPV RNA comprises the sequence of SEQ ID NO: 16.
76. The composition of any one of claims 52-75, wherein the hPIV3 F glycoprotein comprises a sequence that has at least 90%, at least 95%, or at least 98% identity to the sequence
30 of SEQ ID NO: 14.
77. The composition of claim 76, wherein the hPIV3 F glycoprotein comprises the sequence of SEQ ID NO: 14.

78. The composition of any one of claims 52-77, wherein the hPIV3 RNA comprises an ORF that comprises a sequence that has at least 90%, at least 95%, or at least 98% identity to the sequence of SEQ ID NO: 13.
- 5 79. The composition of claim 78, wherein the hPIV3 RNA comprises an ORF that comprises the sequence of SEQ ID NO: 13.
80. The composition of claim 78 or 79, wherein the hPIV3 RNA comprises a 5' UTR that comprises the sequence of SEQ ID NO: 2.
- 10 81. The composition of any one of claims 78-80, wherein the hPIV3 RNA comprises a 3' UTR that comprises the sequence of SEQ ID NO: 4.
82. The composition of any one of claims 78-81, wherein the hPIV3 RNA comprises a
15 sequence that has at least 90%, at least 95%, or at least 98% identity to the sequence of SEQ ID NO: 17.
83. The composition of claim 82, wherein the hPIV3 RNA comprises the sequence of SEQ ID NO: 17.
- 20 84. The composition of any one of claims 52-83, wherein the hRSV RNA, the hMPV RNA, and/or the hPIV3 RNA further comprises a 7mG(5')ppp(5')NlmpNp cap.
85. The composition of any one of claims 52-84, wherein the hRSV RNA, the hMPV RNA,
25 and/or the hPIV3 RNA further comprises a poly(A) tail, optionally having a length of 50 to 150 nucleotides.
86. The composition of any one of claims 52-83, wherein the hRSV RNA, the hMPV RNA, and/or the hPIV3 RNA comprises a chemical modification.
- 30 87. The composition of claim 86, wherein the hRSV RNA, the hMPV RNA, and/or the hPIV3 RNA is fully modified.

88. The composition of claim 86 or claim 87, wherein the chemical modification is 1-methylpseudouridine.

89. The composition of any one of claims 52-88, comprising 25 µg – 200 µg of the hRSV RNA, the hMPV RNA, and/or the hPIV3 RNA.

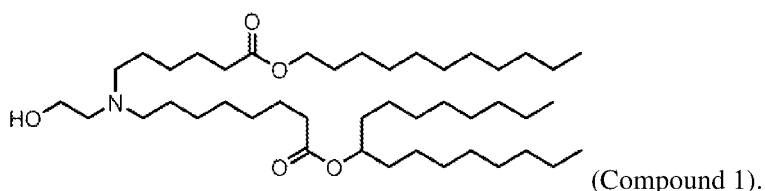
90. The composition of any one of claims 52-89, further comprising a mixture of lipids that comprises a PEG-modified lipid, a non-cationic lipid, a sterol, an ionizable cationic lipid, or any combination thereof.

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91. The composition of claim 90, wherein the mixture of lipids comprises 0.5-15% PEG-modified lipid; 5-25% non-cationic lipid; 25-55% sterol; and 20-60% ionizable cationic lipid.

92. The composition of claim 91, wherein the lipid nanoparticle comprises 1-5 mol% PEG-modified lipid; 10-20 mol% non-cationic lipid; 35-45 mol% sterol; and 40-50 mol% ionizable cationic lipid.

93. The composition of claim 91 or claim 92, wherein the PEG-modified lipid is 1,2 dimyristoyl-sn-glycerol, methoxypolyethyleneglycol (PEG2000 DMG), the non-cationic lipid is 1,2 distearoyl-sn-glycero-3-phosphocholine (DSPC), the sterol is cholesterol; and the ionizable cationic lipid has the structure of Compound 1:



94. The composition of any one of claims 90-93, wherein the mixture of lipids forms lipid nanoparticles.

95. The composition of claim 94, wherein the hRSV RNA, the hMPV RNA, and the hPIV3 RNA are formulated in the lipid nanoparticles.

96. A method comprising administering to a subject the composition of any one of claims 52-95 in an amount effective to induce a neutralizing antibody response against hRSV, hMPV, and/or hPIV3 in the subject.

5 97. The method of claim 96, wherein the subject is immunocompromised.

98. The method of claim 96 or 97, wherein the subject has a pulmonary disease.

99. The method of any one of claims 96-98, wherein the subject is 5 years of age or younger.

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100. The method of any one of claims 96-98, wherein the subject is 65 years of age or older.

101. The method of any one of claims 96-100, comprising administering to the subject at least two doses of the composition.

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