

CLAIMS

What is claimed is:

1. An immunogenic composition comprising:
 - (i) one or more influenza virus proteins selected from one or more influenza virus hemagglutinin (HA) proteins, one or more influenza virus neuraminidase (NA) proteins, or a combination thereof; and
 - (ii) one or more ribonucleic acid molecules, wherein the one or more ribonucleic acid molecules encode one or more influenza virus proteins selected from one or more influenza virus HA proteins, one or more influenza virus NA proteins, or a combination thereof.
2. The immunogenic composition of claim 1, wherein the one or more influenza virus proteins in (i) are recombinant influenza virus proteins, optionally wherein the one or more influenza virus proteins in (i) are present in an inactivated influenza virus (IIV).
3. The immunogenic composition of any of the preceding claims, wherein the one or more ribonucleic acid molecules are mRNA molecules.
4. The immunogenic composition of any of the preceding claims, wherein the immunogenic composition comprises no more than eight influenza virus proteins in (i) and ribonucleic acid molecules in (ii) that encode no more than eight influenza virus proteins.
5. The immunogenic composition of any of the preceding claims,
 - wherein the one or more influenza virus proteins of (i) comprise 1-8 influenza virus proteins selected from an influenza virus H1 HA, an influenza virus H3 HA, an influenza virus HA from a B/Victoria lineage, an influenza virus HA from a B/Yamagata lineage, an influenza virus N1 NA, an influenza virus N2 NA, an influenza virus NA from a B/Victoria lineage, or an influenza virus NA from a B/Yamagata lineage; and
 - wherein the one or more ribonucleic acid molecules of (ii) encode 1-8 influenza virus proteins selected from an influenza virus H1 HA, an influenza virus H3 HA, an influenza virus

HA from a B/Victoria lineage, an influenza virus HA from a B/Yamagata lineage, an influenza virus N1 NA, an influenza virus N2 NA, an influenza virus NA from a B/Victoria lineage, or an influenza virus NA from a B/Yamagata lineage.

6. The immunogenic composition of any of the preceding claims, wherein the immunogenic composition comprises no more than four influenza virus proteins in (i) and ribonucleic acid molecules in (ii) that encode no more than four influenza virus proteins.

7. The immunogenic composition of any one of the preceding claims, wherein the immunogenic composition is an octavalent, a hexavalent, or a hexadecavalent immunogenic composition.

8. The immunogenic composition of any one of the preceding claims,
wherein the one or more influenza virus proteins in (i) comprise four recombinant influenza virus HA proteins; and
wherein the one or more ribonucleic acid molecules encode four influenza virus NA proteins.

9. The immunogenic composition of any one of claims 1-7,
wherein the one or more influenza virus proteins in (i) comprise four recombinant influenza virus NA proteins; and
wherein the one or more ribonucleic acid molecules encode four influenza virus HA proteins.

10. The immunogenic composition of any one of claims 1-5,
(a) wherein the one or more influenza virus proteins comprise:
a first influenza virus HA protein, wherein the first influenza virus HA protein is an H1 HA;
a second influenza virus HA protein, wherein the second influenza virus HA protein is an H3 HA; and
a third influenza virus HA protein from a B/Victoria influenza virus lineage; and

(b) wherein the one or more ribonucleic acid molecules encode:

a first influenza virus NA protein, wherein the first influenza virus NA protein is an N1 NA;

a second influenza virus NA protein wherein the second influenza virus NA protein is an N2 NA; and

a third influenza virus NA protein from a B/Victoria influenza virus lineage.

11. The immunogenic composition of claim 10, wherein the H1 HA is from an H1N1 influenza virus strain, the H3 HA is from an H3N2 influenza virus strain, the N1 NA is from an H1N1 influenza virus strain, and/or the N2 NA is from an H3N2 influenza virus strain.

12. The immunogenic composition of claim 11, wherein the H1 HA and the N1 NA are from the same H1N1 influenza virus strain and/or the H3 HA and N2 NA are from the same H3N2 influenza virus strain.

13. The immunogenic composition of any one of claims 10-12, wherein each of the first, second, and third influenza virus HA proteins is a recombinant influenza virus HA.

14. The immunogenic composition of any one of claims 1-13, wherein the one or more ribonucleic acid molecules encode four full-length influenza virus NA proteins.

15. The immunogenic composition of any one of claims 1-5,

(a) wherein the one or more influenza virus proteins comprise:

a first influenza virus NA protein, wherein the first influenza virus NA protein is an N1 NA;

a second influenza virus NA protein, wherein the second influenza virus NA protein is an N2 NA; and

a third influenza virus NA protein from a B/Victoria influenza virus lineage; and

(b) wherein the one or more ribonucleic acid molecules encode:

a first influenza virus HA protein, wherein the first influenza virus HA protein is an H1 HA;

a second influenza virus HA protein, wherein the second influenza virus HA protein is an H3 HA; and

a third influenza virus HA protein from a B/Victoria influenza virus lineage.

16. The immunogenic composition of claim 15, wherein the N1 NA is from an H1N1 influenza virus strain, the N2 NA is from an H3N2 influenza virus strain, the H1 HA is from an H1N1 influenza virus strain, and/or the H3 HA is from an H3N2 influenza virus strain.

17. The immunogenic composition of claim 16, wherein the H1 HA and the N1 NA are from the same H1N1 influenza virus strain and/or the H3 HA and the N2 NA are from the same H3N2 influenza virus strain.

18. The immunogenic composition of any one of claims 15-17, wherein each of the first, second, and third influenza virus NA proteins is a recombinant influenza virus NA, optionally wherein each of the first, second, and third influenza virus NA proteins is a modified recombinant tetrameric influenza virus NA comprising four modified recombinant monomeric NA molecules, wherein each of the modified recombinant monomeric NA molecules comprises a head region of the influenza virus NA and a heterologous tetramerization domain, but lacking a cytoplasmic tail, a transmembrane region, and all or substantially all of a stalk region of the influenza virus NA, wherein the four modified recombinant monomeric NA molecules form modified recombinant tetrameric NA when expressed in a host cell.

19. The immunogenic composition according to claim 18, wherein the heterologous tetramerization domain is a *Staphylothermus marinus* tetrabrachion tetramerization domain, a GCN4 leucine zipper tetramerization domain, a tetramerization domain from a paramyxovirus phosphoprotein, or a human vasodilator stimulated phosphoprotein (VASP) tetramerization domain.

20. The immunogenic composition according to claim 18, wherein the second influenza virus NA protein is a modified recombinant tetrameric N2 NA comprising four modified recombinant monomeric influenza virus N2, wherein each of the modified monomeric influenza virus N2 comprises:

a head region of an influenza virus N2, and
wherein the modified recombinant monomeric influenza virus N2 does not contain a cytoplasmic tail, a transmembrane region and all or substantially all of a stalk region of the influenza virus N2, and wherein the modified recombinant monomeric influenza virus N2 does not include a heterologous oligomerization domain,
optionally wherein the modified recombinant monomeric influenza virus N2 lacks amino acids 1-70, 1-71, 1-72, 1-73, 1-74, 1-75, 1-76, 1-77, 1-78, 1-79, 1-80, 1-81, 1-82, 1-83, or 1-84 of the influenza virus N2.

21. The immunogenic composition according to any of the preceding claims, wherein at least one of the one or more influenza virus proteins comprises an influenza virus HA protein and/or an influenza virus NA protein having a molecular sequence identified or designed from a machine learning model and/or wherein at least one of the one or more ribonucleic acid molecules encode one or more influenza virus proteins having a molecular sequence identified or designed from a machine learning model.
22. The immunogenic composition according to any of the preceding claims, wherein the immunogenic composition further comprises an adjuvant, optionally wherein the adjuvant comprises a squalene-in-water adjuvant or a liposome-based adjuvant.
23. The immunogenic composition according to any of the preceding claims, wherein the one or more ribonucleic acid molecules comprise at least one chemically modified nucleotide, optionally wherein the at least one chemically modified nucleotide comprises a pseudouridine, optionally N1-methylpseudouridine, a 2'-fluoro ribonucleotide, a 2'-methoxy ribonucleotide, and/or a phosphorothioate bond.
24. The immunogenic composition according to any of the preceding claims, wherein the one or more influenza virus HA proteins are recombinant influenza virus HA proteins produced by a baculovirus expression system in cultured insect cells and/or wherein one or more of the influenza virus NA proteins are recombinant influenza virus NA produced in Chinese Hamster Ovary (CHO) cells.

25. The immunogenic composition according to any of the preceding claims, wherein the one or more ribonucleic acid molecules are encapsulated in a lipid nanoparticle (LNP), optionally wherein the composition does not further comprise an adjuvant.
26. The immunogenic composition according to any of the preceding claims, wherein the composition comprises at least two ribonucleic acid molecules encapsulated in the same LNP.
27. The immunogenic composition according to any of the preceding claims, wherein the influenza virus proteins in (i) and/or the ribonucleic acid molecules in (ii) are from standard of care influenza strains.
28. The immunogenic composition according to any one of claims 25-27, wherein the LNP comprises a cationic lipid, a polyethylene glycol conjugated (PEGylated) lipid, a cholesterol-based lipid, and a helper lipid.
29. The immunogenic composition according to claim 28, wherein the LNP comprises:
a cationic lipid at a molar ratio between 35% and 45%, such as 40%,
a PEGylated lipid at a molar ratio between 0.25% and 2.75%, such as 1.5%,
a cholesterol-based lipid at a molar ratio between 25% and 35%, such as 28.5%, and
a helper lipid at a molar ratio between 25% and 35%, such as 30%.
30. The immunogenic composition according to any one of claims 28-29, wherein the cationic lipid is selected from the group comprising OF-02, cKK-E10, GL-HEPES-E3-E10-DS-3-E18-1, GL-HEPES-E3-E12-DS-4-E10, and GL-HEPES-E3-E12-DS-3-E14, preferably wherein the cationic lipid is cKK-E10.
31. The immunogenic composition according to any one of claims 28-30, wherein the PEGylated lipid is dimyristoyl-PEG2000, wherein the cholesterol-based lipid is cholesterol, and/or wherein the helper lipid is dioleoyl-SN-glycero-3-phosphoethanolamine.
32. The immunogenic composition according to any one of claims 28-31, wherein the LNP comprises:

cKK-E10 at a molar ratio of 40%;
dimyristoyl-PEG2000 at a molar ratio of 1.5%;
cholesterol at a molar ratio of 28.5%; and
dioleoyl-SN-glycero-3-phosphoethanolamine at a molar ratio of 30%.

33. The immunogenic composition according to any one of claims 25-28, wherein the LNP comprises (i) ALC-0315 as the cationic lipid, (ii) N,N ditetradecylacetamide-polyethylene glycol as the PEGylated lipid, (iii) DSPC as the helper lipid, and (iv) cholesterol.

34. The immunogenic composition according to any one of claims 25-28 or 33, wherein the LNP comprises (i) ALC-0315 as the cationic lipid at a molar ratio of about 25% to about 65%, (ii) N,N ditetradecylacetamide-polyethylene glycol as the PEGylated lipid at a molar ratio of about 0.5% to about 2.6%, (iii) DSPC as the helper lipid at a molar ratio of about 5% to about 15%, and (iv) cholesterol at a molar ratio of about 20% to about 60%, such as i) ALC-0315 as the cationic lipid at a molar ratio of about 46.3%, (ii) ALC-0159 as the PEGylated lipid at a molar ratio of about 1.6%, (iii) DSPC as the helper lipid at a molar ratio of about 9.4%, and (iv) cholesterol at a molar ratio of about 42.7%.

35. The immunogenic composition according to any of the preceding claims, wherein each of the influenza virus proteins in (i) is present in the immunogenic composition in an amount ranging from about 0.1 µg to about 90 µg, optionally about 1 µg to about 60 µg or about 5 µg to about 45 µg and/or wherein each of the ribonucleic acid molecules is present in the immunogenic composition in an amount ranging from about 0.1 µg to about 150 µg, optionally from about 1 µg to about 60 µg or about 5 µg to about 45 µg.

36. The immunogenic composition according to any of the preceding claims, wherein the immunogenic composition is formulated for intramuscular injection.

37. A vaccine comprising the immunogenic composition according to any of the preceding claims and a pharmaceutical carrier.

38. Use of a vaccine according to claim 37 for the manufacture of a medicament for immunizing a subject against influenza virus.

39. The use of claim 38, wherein the vaccine prevents influenza virus infection in the subject.
40. The use of claim 38 or 39, wherein the vaccine raises a protective immune response in the subject.
41. The use of claim 40, wherein the protective immune response comprises an HA antibody response and/or an NA antibody response.
42. The use of any one of claims 38-41, wherein the subject is human.
43. The use of any one of claims 38-42, wherein the vaccine is formulated to be administered intramuscularly, intradermally, subcutaneously, intravenously, intranasally, by inhalation, or intraperitoneally.
44. The use of any one of claims 38-43, wherein the vaccine treats or prevents disease caused by either or both a seasonal and a pandemic influenza strain.
45. The use of any one of claims 38-44, wherein the subject is human and the human is 6 months of age or older, less than 18 years of age, at least 6 months of age and less than 18 years of age, at least 18 years of age and less than 65 years of age, at least 6 months of age and less than 5 years of age, at least 5 years of age and less than 65 years of age, at least 60 years of age, or at least 65 years of age.
46. Use of a vaccine according to claim 37 for the manufacture of a medicament for reducing one or more symptoms of influenza virus infection in a subject.
47. Use of a vaccine according to claim 37 for the manufacture of a medicament for enhancing or broadening a protective immune response in a subject, wherein the vaccine increases the vaccine efficacy of a standard of care influenza virus vaccine composition by an amount ranging from about 5% to about 100%, such as at least about 20%.
48. The use according to claim 57, wherein the standard of care influenza virus vaccine composition is an inactivated influenza virus composition comprising inactivated influenza virus from an H1N1 strain, an H3N2 strain, and a B/Victoria lineage or wherein the standard of care

influenza virus vaccine composition comprises recombinant influenza virus HA from an H1N1 strain, an H3N2 strain, and a B/Victoria lineage.

49. The use of any one of claims 37-48, wherein the vaccine is formulated for administration to the subject in two doses of the vaccine with an interval of 2-6 weeks, optionally 4 weeks, between doses.