CLAIMS

1. A pharmaceutical composition comprising a nucleic acid molecule encapsulated in a lipid nanoparticle (LNP), wherein the LNP comprises:

a cationic lipid at a molar ratio between 35% and 45%,

a polyethylene glycol (PEG) conjugated (PEGylated) lipid at a molar ratio between 0.25% and 2.75%,

a cholesterol-based lipid at a molar ratio between 20% and 35%, and

a helper lipid at a molar ratio of between 25% and 35%,

wherein all the molar ratios are relative to the total lipid content of the LNP.

2. The composition of claim 1, wherein the LNP comprises:

a cationic lipid at a molar ratio of 40%,

a PEGylated lipid at a molar ratio of 1.5%,

a cholesterol-based lipid at a molar ratio of 28.5%, and

a helper lipid at a molar ratio of 30%.

3. The composition of claim 1 or 2, wherein:

the cationic lipid is OF-02, cKK-E10, GL-HEPES-E3-E10-DS-3-E18-1, GL-HEPES-E3-E12-DS-4-E10, or GL-HEPES-E3-E12-DS-3-E14; the PEGylated lipid is dimyristoyl-PEG2000 (DMG-PEG2000); the cholesterol-based lipid is cholesterol; and/or the helper lipid is 1,2-dioleoyl-SN-glycero-3-phosphoethanolamine (DOPE).

4. The composition of claim 1, wherein the LNP comprises:

OF-02 at a molar ratio of 40%,

DMG-PEG2000 at a molar ratio of 1.5%,

cholesterol at a molar ratio of 28.5%, and

DOPE at a molar ratio of 30%; or

cKK-E10 at a molar ratio of 40%,

DMG-PEG2000 at a molar ratio of 1.5%,

cholesterol at a molar ratio of 28.5%, and
DOPE at a molar ratio of 30%; or

GL-HEPES-E3-E10-DS-3-E18-1 at a molar ratio of 40%,
DMG-PEG2000 at a molar ratio of 1.5%,
cholesterol at a molar ratio of 28.5%, and
DOPE at a molar ratio of 30%; or

GL-HEPES-E3-E12-DS-4-E10 at a molar ratio of 40%,
DMG-PEG2000 at a molar ratio of 1.5%,
cholesterol at a molar ratio of 28.5%, and
DOPE at a molar ratio of 30%; or

GL-HEPES-E3-E12-DS-3-E14 at a molar ratio of 40%,
DMG-PEG2000 at a molar ratio of 1.5%,
cholesterol at a molar ratio of 28.5%, and
DOPE at a molar ratio of 30%.

5. The composition of any one of the preceding claims, wherein:
the LNP has an average diameter of 30-200 nm, optionally wherein the LNP has an average diameter of 80-150 nm;
the composition comprises 1-10, optionally 1 mg/mL of the LNP; and/or
the LNP comprises 1-20, optionally 5-10 or 6-8 nucleic acid molecules.

6. The composition of any one of the preceding claims, wherein the nucleic acid molecule(s) is an mRNA molecule comprising an open reading frame (ORF).

7. The composition of claim 6, wherein the mRNA molecule encodes an antigen, optionally a viral antigen or a bacterial antigen.

8. The composition of claim 7, wherein the antigen is derived from influenza virus.

9. The composition of claim 7 or 8, wherein the LNP comprises two or more mRNA molecules, wherein each mRNA molecule encodes a different antigen, optionally wherein the different antigens are from the same pathogen or from different pathogens.

10. The composition of claim 7 or 8, wherein the composition comprises two or more LNPs, wherein each LNP comprises an mRNA encoding a different antigen, optionally wherein the different antigens are from the same pathogen or from different pathogens.

11. The composition of claim 6, wherein the mRNA molecule comprises an open reading frame (ORF) encoding a respiratory syncytial virus (RSV) F protein antigen, optionally wherein:

the RSV F protein antigen comprises an amino acid sequence with at least 98% identity to SEQ ID NO: 16 or consists of an amino acid sequence of SEQ ID NO: 16; and/or

the RSV F protein antigen is a pre-fusion protein.

12. The composition of any one of claims 6-11, wherein:

the ORF is codon optimized;

the mRNA molecule comprises at least one 5' untranslated region (5' UTR), at least one 3' untranslated region (3' UTR), and at least one polyadenylation (poly(A)) sequence; and/or

the mRNA comprises at least one chemical modification;

at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% of the uracil nucleotides in the mRNA are chemically modified; and/or

at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% of the uracil nucleotides in the ORF are chemically modified, optionally wherein the chemical modification is selected from the group consisting of pseudouridine, N1-methylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 2-thio-l-methyl-1-deaza-pseudouridine, 2-thio-l-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-l-methyl-pseudouridine, 4-thio-

pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methyluridine, 5-methyluridine, 5-methoxyuridine, and 2'-O-methyl uridine.

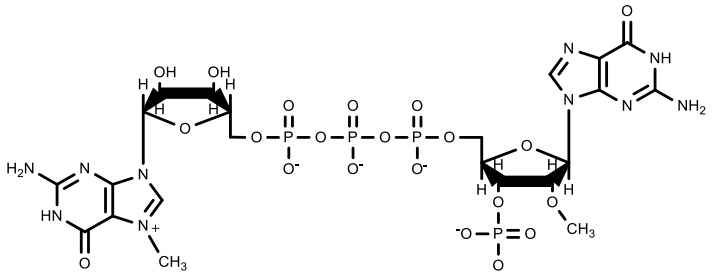
13. The composition of any one of claims 11-12, wherein:

the mRNA comprises a nucleic acid sequence with at least 80% identity to a nucleic acid sequence set forth in SEQ ID NO: 17; or

the mRNA comprises a nucleic acid sequence with at least 80% identity to a nucleic acid sequence set forth in SEQ ID NO: 21.

14. The composition of any one of claims 11-13, wherein the mRNA comprises of the following structural elements:

(i) a 5' cap with the following structure:


OH
OH
H
H
H
H
O
O
O

providing an amphiphilic solution comprising the cationic lipid, the PEGylated lipid, the cholesterol-based lipid, and the helper lipid, and

mixing the aqueous buffered solution and the amphiphilic solution at a ratio of 5:1 to 3:1, optionally 4:1, optionally wherein:

the aqueous buffered solution is an acidic buffered solution, optionally comprising 1 mM citrate and 150 mM sodium chloride with a pH of about 4.5; and/or

the amphiphilic solution is an ethanol solution.

18. A composition for use of any one of claims 1-16 for use in treating a subject in need thereof.

19. Use of a prophylactically effective amount of the composition of any one of claims 1-16 in the manufacture of a medicament for eliciting an immune response in a subject in need thereof, optionally comprising the medicament is to be administered to the subject intramuscularly, intranasally, intravenously, subcutaneously, or intradermally,

20. Use of a prophylactically effective amount of the composition of any one of claims 8-11 in the manufacture of a medicament for preventing influenza infections or reducing one or more symptoms of influenza infections, optionally comprising the medicament is to be administered the subject intramuscularly, intranasally, intravenously, subcutaneously, or intradermally, optionally wherein the composition elicits an immune response against one or more seasonal and/or pandemic influenza strains.

21. The composition for use or the use of any one of claims 18 or 20, comprising the subject to be administered one or more doses of the composition, each dose comprising 1-250, optionally 2.5, 5, 15, 45, or 135, μg of mRNA.

22. The composition for use or the use of any one of claims 18-21, comprising the subject to be administered two doses of the composition with an interval of 2-6, optionally 4, weeks.

23. A kit comprising a container comprising a single-use or multi-use dosage of the composition of any one of claims 1-16, optionally wherein the container is a vial or a pre-filled syringe or injector.