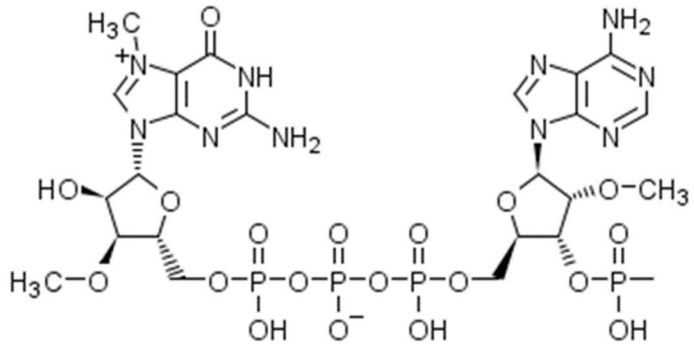
CLAIMS

1. An immunogenic composition comprising: (i) a first ribonucleic acid (RNA) polynucleotide comprising an open reading frame encoding a first antigen, said antigen comprising at least one influenza virus antigenic polypeptide or an immunogenic fragment thereof, and (ii) a second RNA polynucleotide comprising an open reading frame encoding a second antigen, said second antigen comprising at least one influenza virus antigenic polypeptide or an immunogenic fragment thereof, wherein the first and second RNA polynucleotides are formulated in a lipid nanoparticle (LNP).
2. The immunogenic composition of claim 1, wherein the first and second antigens comprise hemagglutinin (HA), or an immunogenic fragment or variant thereof.
3. The immunogenic composition of claim 1 or 2 wherein the first and second antigens each comprise an HA, or an immunogenic fragment thereof, that are from different subtypes of influenza virus.
4. The immunogenic composition of any one of claims 1-6, further comprising: (iii) a third RNA polynucleotide comprising an open reading frame encoding an antigen comprising at least one influenza virus antigenic polypeptide or an immunogenic fragment thereof, wherein the third antigen is from an influenza virus different from the strain of influenza virus of both the first and second antigens.
5. The immunogenic composition of claim 4, wherein the first, second and third RNA polynucleotides are formulated in a lipid nanoparticle.
6. The immunogenic composition of claim 5, further comprising: (iv) a fourth RNA polynucleotide comprising an open reading frame encoding a fourth antigen, said antigen comprising at least one influenza virus antigenic polypeptide or an immunogenic fragment thereof, wherein the fourth antigen is from influenza virus but is from a different strain of influenza virus to the first, second and third antigens.
7. The immunogenic composition of claim 6, wherein the first, second, third, and fourth RNA polynucleotides are formulated in a lipid nanoparticle.
8. The immunogenic composition of any one of claims 1-7, wherein the RNA polynucleotides are present in about equal ratios.
9. The immunogenic composition of any one of claims 1-8, wherein each RNA polynucleotide comprises a modified nucleotide.
10. The immunogenic composition of claim 9, wherein the modified nucleotide is selected from the group consisting of pseudouridine, 1-methylpseudouridine, 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.

11. The immunogenic composition of any one of claims 1-11, wherein each RNA polynucleotide comprises a 5′ terminal cap, a 5' UTR, a 3'UTR, and a 3′ polyadenylation tail.

12. The immunogenic composition of claim 11, wherein the 5′ terminal cap comprises:



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13. The immunogenic composition of claim 11, wherein the 5' UTR comprises SEQ ID NO: 1.

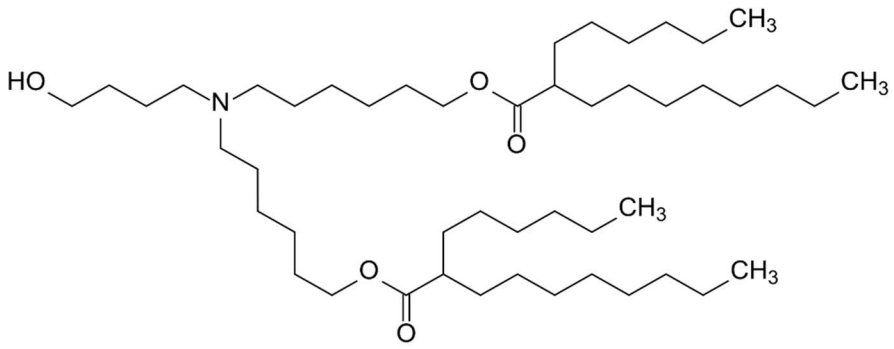
14. The immunogenic composition of claim 11, wherein the 3' UTR comprises SEQ ID NO: 2.

15. The immunogenic composition of claim 11, wherein the 3′ polyadenylation tail comprises SEQ ID NO: 3.

16. The immunogenic composition of any one of claims 1-15, wherein the RNA polynucleotide has an integrity greater than 85%.

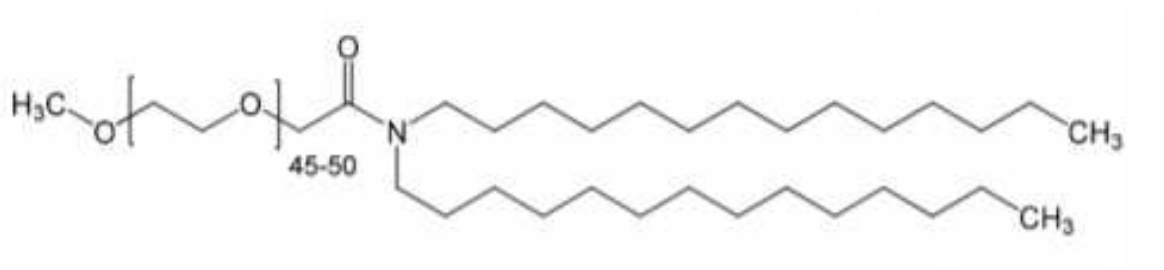
17. The immunogenic composition of any one of claims 1-16, wherein the RNA polynucleotide has a purity of greater than 85%.

18. The immunogenic composition of any one of claims 1-17, wherein the lipid nanoparticle comprises 20-60 mol % ionizable cationic lipid, 5-25 mol % neutral lipid, 25-55 mol % cholesterol, and 0.5-5 mol % PEG-modified lipid.

19. The immunogenic composition of any one of claims 1-18, wherein the cationic lipid comprises: 

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20. The immunogenic composition of any one of claims 1-19, wherein the PEG-modified lipid comprises:



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21. The immunogenic composition of any one of claims1-20, wherein the first antigen is HA from influenza A subtype H1 or an immunogenic fragment or variant thereof and the second antigen is HA from a different H1 strain to the first antigen or an immunogenic fragment or variant thereof.

22. The immunogenic composition of any one of claims1-21, wherein the first and second antigens are HA from influenza A subtype H3 or an immunogenic fragment or variant thereof and wherein both antigens are derived from different strains of H3 influenza virus.

23. The immunogenic composition of any one of claims6-22, wherein the first and second antigens are HA from influenza A subtype H1 or an immunogenic fragment or variant thereof and the third and fourth antigens are from influenza A subtype H3 or an immunogenic fragment or variant thereof and wherein the first and second antigens are derived from different strains of H1 virus and the third and fourth antigens are from different strains of H3 influenza virus.

24. The immunogenic composition of any one of claims 1-23, wherein at least the first and second RNA polynucleotides are formulated in a single lipid nanoparticle.

25. The immunogenic composition of any one of claims 1-24, wherein the first and second RNA polynucleotides are formulated in a single lipid nanoparticle.

26. The immunogenic composition of any one of claims 4-25, wherein the first, second, and third RNA polynucleotides are formulated in a single lipid nanoparticle.

27. The immunogenic composition of any preceding claim, wherein the first, second, third, and fourth RNA polynucleotides are formulated in a single LNP.

28. The immunogenic composition of any one of claims 1-23, wherein each of the RNA polynucleotides is formulated in a single LNP, wherein each single LNP encapsulates the RNA polynucleotide encoding one antigen.

29. The immunogenic composition of claim 28, wherein the first RNA polynucleotide is formulated in a first LNP; and the second RNA polynucleotide is formulated in a second LNP.

30. The immunogenic composition of claim 28, wherein the first RNA polynucleotide is formulated in a first LNP; the second RNA polynucleotide is formulated in a second LNP; and the third RNA polynucleotide is formulated in a third LNP.

31. The immunogenic composition of claim 28, wherein the first RNA polynucleotide is formulated in a first LNP; the second RNA polynucleotide is formulated in a second LNP; the third RNA polynucleotide is formulated in a third LNP; and the fourth RNA polynucleotide is formulated in a fourth LNP.

32. The immunogenic composition of any one of claims 1-31, for use in the eliciting an immune response against influenza in a subject.

33. Use of the immunogenic composition according to any one of claims 1-31, in the manufacture of a medicament for eliciting an immune response against influenza disease in a subject.

34. A method of purifying an RNA polynucleotide comprising an open reading frame encoding a first antigen, said antigen comprising at least one influenza virus antigenic polypeptide or an immunogenic fragment thereof, synthesized by in vitro transcription, the method comprising ultrafiltration and diafiltration.

35. The method according to claim 34, wherein the method does not comprise a chromatography step.

36. The method according to claim 34, wherein the purified RNA polynucleotide is substantially free of contaminants comprising short abortive RNA species, long abortive RNA species, double- stranded RNA (dsRNA), residual plasmid DNA, residual in vitro transcription enzymes, residual solvent and/or residual salt.