MESSENGER RNA VACCINES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims benefit of U.S. Provisional Patent Application No. 62/678,225, filed on May 30, 2018, which is hereby incorporated by reference in its entirety.

BACKGROUND

[0002] Vaccines are among the most important medicines for treating or preventing various diseases. A vaccine is a biological composition that induces active acquired immunity to a particular disease. Traditionally, a vaccine typically contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of a virus or other microbes, or its surface proteins. The agent stimulates the body's immune system to produce antibodies or cytokines to recognize and destroy any of the microorganisms associated with that agent that it may encounter in the future. Vaccines can be prophylactic or therapeutic.

SUMMARY

[0003] The present disclosure provides an improved and unconventional form of vaccines based on messenger RNA delivery that promises safer, more efficacious and cost-effective vaccines for treating and preventing various diseases.

[0003a] In one aspect, the present disclosure refers to use of a composition in the manufacture of a medicament for inducing an immune response *in vivo*, wherein the composition comprises a messenger RNA (mRNA) encoding an antigen, encapsulated in a lipid nanoparticle, at a dosing regimen sufficient to induce an antigen specific T cell response and/or an antigen specific antibody response.

[0003b] In one aspect, the present disclosure refers to use of a vaccine composition in the manufacture of a pharmaceutical composition for delivering a vaccine *in vivo*, wherein the vaccine composition comprises a messenger RNA (mRNA) encoding an antigen, encapsulated in a lipid nanoparticle, wherein the vaccine composition is to be administered intramuscularly or subcutaneously.

[0004] In one example, the present disclosure provides a method for inducing an immune response *in vivo* by administering to the subject a messenger RNA (mRNA) composition comprising an mRNA encoding an antigen, at a dosing regimen sufficient to induce an antigen specific T cell response and/or an antigen specific antibody response. In some examples, the mRNA is encapsulated in lipid nanoparticles (LNPs). In some examples, the composition is a vaccine composition.

[0005] In some examples, the composition is administered subcutaneously. In some examples, the composition is administered intradermally. In some examples, the composition is administered intramuscularly. In some examples, the composition is administered intravenously. In some examples, the composition is not administered intravenously.

[0006] In another example, the present disclosure provides a method of delivering a vaccine *in vivo* comprising administering to a subject a vaccine composition comprising an mRNA encoding an antigen, encapsulated in a LNP, and wherein the vaccine composition is administered intramuscularly or subcutaneously. In various examples, the vaccine composition is administered at a dosing regimen sufficient to induce an antigen specific T cell response and/or an antigen specific antibody response.

[0007] In some examples, an antigen encoded by the mRNA is a protein or a peptide.

[0008] In some examples, a composition comprising one or more than one mRNAs encoding one or more polypeptides is delivered.

[0009] In some examples, a suitable dosing regimen comprises injecting a single dose. In some examples, a suitable dosing regimen comprises administering multiple doses periodically.

[0010] In some embodimenst, the single dose or multiple doses (individually or collectively) range from about 0.1 μg – 100 mg mRNA. In some examples, the single dose or multiple doses (individually or collectively) range from about 0.1 μg – 50 mg mRNA, from about 0.1 μg – 25 mg mRNA, from about 0.1 μg – 10 mg mRNA, from about 0.1 μg – 5 mg mRNA, from about 0.1 μg – 1 mg mRNA, or from about 0.1 μg – 100 μg mRNA. In some examples, the single dose is or the multiple doses (individually or collectively) are selected from 0.1μg. 0.3 μg, 0.5 μg, 1 μg, 5 μg, 10 μg, 25 μg, 50 μg, or 100 μg.

[0011] In some examples, the single dose or the multiple doses (individually or collectively) range from about 0.01 μg/kg – 10 mg /kg (mRNA/body weight). In some examples, the single dose or the multiple doses (individually or collectively) range from about 0.01 μg/kg – 8 mg/kg, from about 0.01 μg/kg – 6 mg/kg, from about 0.01 μg/kg – 5 mg/kg, from about 0.1 μg/kg – 5 mg/kg, from about 0.1 μg/kg – 1 mg/kg, or from about 0.1 μg/kg – 0.5 mg/kg (mRNA/body weight).

[0012] In some examples, the multiple doses comprise the same individual dosage amount of mRNA. In some examples, the multiple doses comprise different individual dosage amount of mRNA.

[0013] In some examples, each of the multiple doses are injected 1-3 weeks apart. In some examples, each of the multiple doses is injected weekly. In some examples, at least two of the multiple doses are injected within 3 weeks. In some examples, the multiple doses are injected at the following schedule: Dose 1 on week 0, dose 2 on week 3, and dose 3 on week 5.

[0014] In some examples, the mRNA has a length of or greater than about 0.5 kb, 1 kb, 1.5 kb, 2 kb, 2.5 kb, 3 kb, 3.5 kb, 4 kb, 4.5 kb, 5 kb, 6kb, 7 kb, 8 kb, 9 kb, 10 kb, 11 kb, 12 kb, 13 kb, 14 kb, or 15 kb. In some examples, the mRNA comprises unmodified nucleotides. In some examples, the mRNA comprises one or more modified nucleotides. In some examples, the one or more modified nucleotides comprise pseudouridine, N-1-methyl-pseudouridine, 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine,

C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, 4'thiouridine, 4'-thiocytidine, and/or 2-thiocytidine.

[0015] In some examples, the mRNA comprises a 5' untranslated region (5' UTR) and/or a 3' untranslated region (3' UTR).

[0016] In some examples, the nanoparticle is a lipid-based or polymer-based nanoparticle. In some examples, the lipid-based nanoparticle is a liposome. In some examples, the lipid nanoparticle comprises one or more cationic lipids, one or more helper lipids, and/or one or more PEGylated lipids. In some examples, the lipid nanoparticle comprises one or more cationic lipids, one or more helper lipids, and one or more PEGylated lipids. In some examples, the lipid nanoparticle comprises one or more cationic lipids, one or more helper lipids, one or more cholesterol-based lipids, and one or more PEGylated lipids. As used herein, the terms "PEGylated lipids" and "PEG modified lipids" are used inter-changeably.

[0017] In some examples, the lipid nanoparticle comprises one or more PEGylated lipids. In some examples, the PEGylated lipids constitute at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, or at least 10% of the total lipids in the lipid nanoparticle. In some examples, the PEGylated lipids constitute at least 5% of the total lipids in the lipid nanoparticle. In some examples, the PEGylated lipids constitute about 5% of the total lipids in the lipid nanoparticle. In some examples, the PEGylated lipids constitute 10% or less, 9% or less, 8% or less, 7% or less, 6% or less, 5% or less, 4% or less, or 3% or less of the total lipids in the lipid nanoparticle. In some examples, the PEGylated lipids constitute 5% or less of the total lipids in the lipid nanoparticle.

[0018] In some examples, the lipid nanoparticle comprises one or more cationic lipids. In some examples, the one or more cationic lipids are selected from the group consisting of cKK-E12, OF-02, C12-200, MC3, DLinDMA, DLinkC2DMA, ICE (Imidazol-based), HGT5000, HGT5001, HGT4003, DODAC, DDAB, DMRIE, DOSPA, DOGS, DODAP, DODMA and DMDMA, DODAC, DLenDMA, DMRIE, CLinDMA, CpLinDMA, DMOBA, DOcarbDAP, DLinDAP, DLincarbDAP, DLinCDAP, KLin-K-DMA, DLin-K-

XTC2-DMA, 3-(4-(bis(2-hydroxydodecyl)amino)butyl)-6-(4-((2-hydroxydodecyl)(2-hydroxyundecyl)amino)butyl)-1,4-dioxane-2,5-dione (Target 23), 3-(5-(bis(2-hydroxydodecyl)amino)pentan-2-yl)-6-(5-((2-hydroxydodecyl)(2-hydroxyundecyl)amino)pentan-2-yl)-1,4-dioxane-2,5-dione (Target 24), and combinations thereof. In some examples, the at least one cationic lipid is selected from CCBene, ICE, ML-2, C12-200 or DLin-SS-DMA. In some examples, the one or more cationic lipids comprise ICE. In some examples, the one or more cationic lipids comprise ML-2. In some examples, the one or more cationic lipids comprise CCBene.

[0019] In some examples, the lipid nanoparticle comprises one or more helper lipids. In some examples, the helper lipids are selected from DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DOPE (1,2-dioleyl-sn-glycero-3-phosphoethanolamine), DOPC (1,2-dioleyl-sn-glycero-3-phosphotidylcholine) DPPE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine), DMPE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine), DOPG (1,2-dioleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol)).

[0020] In some examples, the lipid nanoparticle comprises one or more cholesterol-based lipids. I some examples, the cholesterol-based lipids are selected from DC-Choi (N,N-dimethyl-N-ethylcarboxamidocholesterol), 1,4-bis(3-N-oleylamino-propyl)piperazine (Gao, et al. Biochem. Biophys. Res. Comm. 179, 280 (1991); Wolf et al. BioTechniques 23, 139 (1997); U.S. Pat. No. 5,744,335), or ICE.

[0021] In some examples, the administration of the composition results in expression of the antigen encoded by mRNA in the lymph nodes of the subject. In some examples, the administration of the composition results in expression of the antigen encoded by mRNA in the lymphocytes of the subject.

[0022] In some examples, the administration of the composition results in an antigen specific antibody response. In some examples, the antigen specific antibody response is measured by the presence of antigen-specific antibodies in serum.

[0023] In some examples, the administration of the composition results in an antigen specific T-cell response. In some examples, the antigen specific T-cell response is measured

by cytokine response. In some examples, the antigen specific T-cell response is measured by IFN-ɣ ELISPOT in splenocytes.

[0024] In some examples, the antigen specific antibody response and/or the antigen specific T-cell response is detectable at least 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 4 weeks, or 1 month post-administration.

[0025] An example of the disclosure provides a method of treating a disease, disorder or condition comprising delivering messenger RNA (mRNA) to a subject in need of treatment according to the methods described above.

[0026] In this application, the use of "or" means "and/or" unless stated otherwise. As used in this disclosure, the term "comprise" and variations of the term, such as "comprising" and "comprises," are not intended to exclude other additives, components, integers or steps. As used in this application, the terms "about" and "approximately" are used as equivalents. Both terms are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0027] Other features, objects, and advantages of the present disclosure are apparent in the detailed description, drawings and claims that follow. It should be understood, however, that the detailed description, the drawings, and the claims, while indicating examples of the present disclosure, are given by way of illustration only, not limitation. Various changes and modifications within the scope of the disclosure will become apparent to those skilled in the art.

BRIEF DESCRIPTION OF FIGURES

[0028] The drawings are for illustration purposes, not for limitation.

[0029] **Figure 1A and B** depicts comparison data on efficacy of three different lipid compositions in *in vivo* human erythropoietin (hEPO) mRNA delivery, as indicated by measuring hEPO protein in the serum.

[0030] **Figure 2** depicts comparison data on efficacy of three different routes of *in vivo* human erythropoietin (hEPO) mRNA delivery, as indicated by measuring hEPO protein in the serum.

[0031] **Figure 3** depicts images of FFL expression in different mouse tissues, showing comparison of delivery routes on tissue specific enrichment of FFL expression.

We claim:

1. Use of a composition in the manufacture of a medicament for inducing an immune response *in vivo*, wherein the composition comprises a messenger RNA (mRNA) encoding an antigen, encapsulated in a lipid nanoparticle, at a dosing regimen sufficient to induce an antigen specific T cell response and/or an antigen specific antibody response.

2. The use of claim 1, wherein the composition is to be administered subcutaneously.

3. The use of claim 1, wherein the composition is to be administered intradermally.

4. The use of claim 1, wherein the composition is to be administered intramuscularly.

5. The use of claim 1, wherein the composition is not to be administered intravenously.

6. The use of any one of the preceding claims, wherein the composition is a vaccine composition.

7. Use of a vaccine composition in the manufacture of a pharmaceutical composition for delivering a vaccine *in vivo*, wherein the vaccine composition comprises a messenger RNA (mRNA) encoding an antigen, encapsulated in a lipid nanoparticle, wherein the vaccine composition is to be administered intramuscularly or subcutaneously.

8. The use of claim 7, wherein the vaccine composition is to be administered at a dosing regimen sufficient to induce an antigen specific T cell response and/or an antigen specific antibody response.

9. The use of any one of the preceding claims, wherein the antigen is a protein.

10. The use of any one of claims 1-8, wherein the antigen is a peptide.

11. The use of any one of the preceding claims, wherein the composition comprises one or more mRNAs encoding one or more polypeptides.

12. The use of any one of the preceding claims, wherein the dosing regimen comprises that a single dose is to be injected.

13. The use of any one of claims 1-11, wherein the dosing regimen comprises that multiple doses are to be injected periodically.

14. The use of claim 12 or 13, wherein the single dose or the multiple doses range from 0.1 µg – 100 mg mRNA.

15. The use of claim 12 or 13, wherein the single dose or the multiple doses range from 0.1 µg – 50 mg, 0.1 µg – 25 mg, 0.1 µg – 10 mg, 1 µg – 1 mg, or 1 µg – 100 µg mRNA.

16. The use of claim 12 or 13, wherein the single dose is or the multiple doses are selected from 0.1µg. 0.3 µg, 0.5 µg, 1 µg, 5 µg, 10 µg, 25 µg, 50 µg, or 100 µg.

17. The use of claim 12 or 13, wherein the single dose or the multiple doses range from 0.01 µg/kg – 10 mg /kg (mRNA/body weight).

18. The use of claim 12 or 13, wherein the single dose or the multiple doses range from 0.01 µg/kg – 8 mg /kg, 0.01 µg/kg – 6 mg /kg, 0.01 µg/kg – 5 mg /kg, 0.1 µg/kg – 5 mg /kg, 0.1 µg/kg – 1 mg /kg, or 0.1 µg/kg – 0.5 mg /kg (mRNA/body weight).

19. The use of any one of claims 13-18, wherein each of the multiple doses comprise the same dosage amount of mRNA.

20. The use of any one of claims 13-18, wherein each of the multiple doses comprise a different dosage amount of mRNA.

21. The use of any one of claims 13-20, wherein each of the multiple doses are to be injected 1-3 weeks apart.

22. The use of any one of claims 13-20, wherein each of the multiple doses are to be injected weekly.

23. The use of any one of claims 13-20, wherein at least two of the multiple doses are to be injected within 3 weeks.

24. The use of any one of claims 13-20, wherein the multiple doses are to be injected at the following schedule: Dose 1 week 0, dose 2 in week 3, and dose 3 in week 5.

25. The use of any one of the preceding claims, wherein the mRNA comprises one or more modified nucleotides.

26. The use of any one of the preceding claims, wherein the mRNA comprises a 5' untranslated region (5' UTR) and/or a 3' untranslated region (3' UTR).

27. The use of any one of the preceding claims, wherein the lipid nanoparticle comprises one or more cationic lipids, one or more helper lipids, and one or more PEG lipids.

28. The use of any one of the preceding claims, wherein the lipid nanoparticle comprises at least one cationic lipid selected from the group consisting of cKK-E12, OF-02,

C12-200, MC3, DLinDMA, DLinkC2DMA, ICE (Imidazol-based), HGT5000, HGT5001, HGT4003, DODAC, DDAB, DMRIE, DOSPA, DOGS, DODAP, DODMA and DMDMA, DODAC, DLenDMA, DMRIE, CLinDMA, CpLinDMA, DMOBA, DOcarbDAP, DLinDAP, DLincarbDAP, DLinCDAP, KLin-K-DMA, DLin-K-XTC2-DMA, 3-(4-(bis(2-hydroxydodecyl)amino)butyl)-6-(4-((2-hydroxydodecyl)(2-hydroxyundecyl)amino)butyl)-1,4-dioxane-2,5-dione (Target 23), 3-(5-(bis(2-hydroxydodecyl)amino)pentan-2-yl)-6-(5-((2-hydroxydodecyl)(2-hydroxyundecyl)amino)pentan-2-yl)-1,4-dioxane-2,5-dione (Target 24), and combinations thereof.

29. The use of claim 28, wherein the at least one cationic lipid is selected from CCBene, ICE, ML-2, C12-200 or DLin-SS-DMA.

30. The use of claim 29, wherein the at least one cationic lipid is ICE.

31. The use of claim 29, wherein the at least one cationic lipid is ML-2.

32. The use of claim 29, wherein the at least one cationic lipid is CCBene.

33. The use of any one of the preceding claims, wherein the one or more helper lipids are selected from DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DOPE (1,2-dioleyl-sn-glycero-3-phosphoethanolamine), DOPC (1,2-dioleyl-sn-glycero-3-phosphotidylcholine) DPPE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine), DMPE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine), DOPG (1,2-dioleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol)).

34. The use of any one of the preceding claims, wherein the lipid nanoparticle comprises one or more PEG lipids selected from derivatized ceramides such as N-Octanoyl-Sphingosine-l-[Succinyl(Methoxy Polyethylene Glycol)-2000] (C8 PEG-2000

ceramide); PEG-modified lipids having a polyethylene glycol chain of up to 5 kDa in length covalently attached to a lipid with alkyl chain(s) of C_6-C_{20} length, a PEGylated cholesterol and PEG-2K

35. The use of any one of the preceding claims, wherein the PEG-modified lipid comprises at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, or at least 10% of the total lipids in the liposome.

36. The use of any one of the preceding claims, wherein the administration of the composition results in expression of the antigen encoded by mRNA in the lymphocytes of the subject.

37. The use of any one of the preceding claims, wherein the administration of the composition results in an antigen specific antibody response.

38. The use of claim 36, wherein the antigen specific antibody response is measured by the presence of antigen-specific antibodies in serum.

39. The use d of any one of the preceding claims, wherein the administration of the composition results in an antigen specific T-cell response.

40. The use of claim 38, wherein the antigen specific T-cell response is measured by IFN-ɤ ELISPOT in splenocytes.