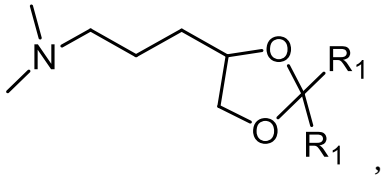
such as tuberculosis, HIV/AIDS, malaria, and COVID-19 represent significant challenges to human health. Mycobacteria, for example, is a genus of bacteria responsible for tuberculosis (TB). According to the World Health Organization, worldwide, TB is one of the top 10 causes of death and the leading cause of death from a single infectious agent. Despite current best efforts, there have been significant challenges in the development of effective vaccines for the prevention of many infectious diseases. New efforts in the identification of individual or combinations of antigenic peptides has helped improved the efficiency of vaccines. Nonetheless, significant opportunities remain in the engineering of adjuvants to help efficiently deliver and present these antigenic sequences to professional antigen presenting cells, like dendritic cells. mRNA coding for antigenic peptides or proteins combined with ionizable cationic lipid nanoparticles represent a particularly promising strategy in the development of a vaccine. There is a need for safe and effective therapies comprising LNP pharmaceutical compositions for delivery of mRNA for treatment and prevention of various diseases, including vaccine compositions.

SUMMARY

The disclosure relates to a lipid nanoparticle (LNP) vaccine composition comprising: (a) a nucleic acid; (b) an ionizable cationic lipid at a N/P ratio of 3 to 8 relative to the nucleic acid wherein the ionizable lipid has the chemical structure:



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wherein R_1 is alkyl group of C_{15}-C_{19} containing one or two olefins, andwherein the ionizable cationic lipid is present in the LNP vaccine composition in a total amount of 40-65 mol% of the total lipid content of the LNP composition, and the ionizable cationic lipid is optionally selected from the group DLin-KC3-DMA, KC3-01, KC3-OA, KC3-PA, KC3-C17 (8:1), KC3-C15 (C8:1) Compound 3 (Table 1A), Compound 8 (Table 1A); (c) a sterol in a total amount of 25-45 mol% of the total lipid content of the LNP composition; (d) one or more phospholipids in a total amount of phospholipids of 5-25 mol% of the total lipid content of the LNP composition; and (e) a conjugated lipid in a total amount of 0.5 – 2.5 mol% of the total lipid content of the LNP composition.

In one aspect, the present disclosure relates to a nucleic acid lipid nanoparticle (LNP) composition comprising:

a. a nucleic acid;

b. an ionizable cationic lipid at a N/P ratio of 3 to 8 relative to the nucleic acid in a total amount of 40-65 mol% of the total lipid content of the LNP composition;

c. a sterol in a total amount of 25-45 mol% of the total lipid content of the LNP composition;

d. one or more phospholipids in a total amount of phospholipids of 5-25 mol% of the total lipid content of the LNP composition, and comprising an anionic targeting ligand for targeting of the LNP to dendritic cells, selected from the group consisting of: (i) a phosphatidylserine (PS) lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition, or (ii) a phosphatidylglycerol (PG) in a total amount of 1.0 – 10 mol% of the total lipid content of the LNP composition; and

e. a conjugated lipid in a total amount of 0.5 – 2.5 mol% of the total lipid content of the LNP composition.

In some examples, the one or more phospholipids comprises a phosphatidylserine (PS) lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition. In some examples, the PS-lipid is DSPS or DPPS.

In some examples, the second phospholipid is selected from the group consisting of: DSPC, HSPC, DPPC, and sphingomyelin.

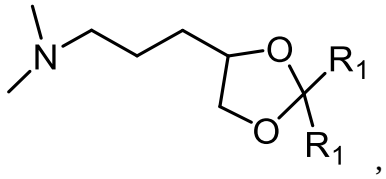
In some examples, the ionizable cationic lipid comprises either monounsaturated alkyl chains or di-unsaturated alkyl chains, wherein the olefins are separated by at least two methylene groups.

In some examples, the the ionizable cationic lipid is selected from the group consisting of: KC3-01, KC3-OA, KC3-PA, KC3-C17 (8:1), KC3-C15 (C8:1), and Compound 8 (Table 1A).

In some examples, the composition has an N/P ratio of 5-6 relative to the nucleic acid.

In some examples, the nucleic acid is an RNA. In some examples, the nucleic acid is mRNA. In some examples, the nucleic acid is a chemically modified RNA. In some examples, the nucleic acid is modified with N-methylpseudouridine.

The disclosure relates to a lipid nanoparticle (LNP) composition comprising: (a) a nucleic acid; (b) an ionizable cationic lipid at a N/P ratio of 3 to 8 relative to the nucleic acid wherein the ionizable lipid has the chemical structure:



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wherein R_1 is alkyl group of C_{15}-C_{19} containing one or two olefins, and wherein the ionizable cationic lipid is present in the LNP composition in a total amount of 40-65 mol% of the total lipid content of the LNP composition, and the ionizable cationic lipid is optionally selected from the group Compound 3 (Table 1A), Compound 8 (Table 1A), DLin-KC3-DMA, KC3-01, KC3-OA, KC3-PA, KC3-C17 (8:1), and KC3-C15 (C8:1); (c) a sterol in a total amount of 25-45 mol% of the total lipid content of the LNP composition; (d) one or more phospholipids in a total amount of phospholipids of 5-25 mol% of the total lipid content of the LNP composition, and comprising a dipalmitoylphosphatidylserine (DPPS) lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition; and (e) a conjugated lipid in a total amount of 0.5 – 2.5 mol% of the total lipid content of the LNP composition.

In some examples, a second phospholipid is selected from the group consisting of: DSPC, HSPC, and sphingomyelin.

In some examples, the ionizable cationic lipid is at an N/P ratio of 5-6 relative to the nucleic acid.

In some examples, the ionizable cationic lipid contains either two monounsaturated alkyl chains or two di-unsaturated alkyl chains, and wherein the olefins are separated by at least two methylene groups.

In some examples, the ionizable cationic lipid is selected from the group KC3-01, KC3-OA, KC3-PA, KC3-C17 (8:1), KC3-C15 (C8:1), and Compound 8 (Table 1A).

In some examples, the ionizable cationic lipid is 3-((*S*)-2,2-di((*Z*)-octadec-9-en-1-yl)-1,3-dioxolan-4-yl)-*N*,*N*-dimethylpropan-1-amine **(**KC3-OA).

In some examples, the nucleic acid is an mRNA. In some examples, the mRNA is chemically modified with N-methylpseudouridine.

The disclosure relates to a nucleic acid lipid nanoparticle (LNP) composition comprising: (a) a nucleic acid; (b) an ionizable cationic lipid at a N/P ratio of 3 to 8 relative to the nucleic acid in a total amount of 40-65 mol% of the total lipid content of the LNP composition; (c) a sterol in a total amount of 25-45 mol% of the total lipid content of the LNP composition; (d) one or more phospholipids in a total amount of phospholipids of 5-25 mol% of the total lipid content of the LNP composition; and (e) a conjugated lipid in a total amount of 0.5 – 2.5 mol% of the total lipid content of the LNP composition comprising a PEG-lipid having a poly(ethylene glycol) chain terminally attached to a linking moiety and two hydrocarbon chains terminally attached to the same linking moiety, wherein the hydrocarbon chains are saturated C_{12}-chains independently selected from n-dodecyl (lauryl) group and n-dodecanoyl (lauroyl) group.

In some examples, the linking moiety is glyceryl group, N-oxycarbonyl glycerophoshoryl ethanolaminocarbonyl group, oxycarbonylamide group, or oxyacetamide group. In some examples, the poly(ethyene glycol) chain is methoxy-poly(ethyene glycol) with an average molecular weight of 2000. In some examples, the PEG-lipid is mPEG-1,2-dilauroylglycerol (PEG-DLG), mPEG-1,2-dilaurylglycerol (PEG-DLG), PEG-1,2-dilaurylglycerol, PEG-DLPE, PEG-oxycarbonyl-N,N-didodecylamide, or mPEG-N,N-didodecylacetamide.

In some examples, the LNP has the z-average particle size of 60-150 nm and is freeze-thaw stable.

In some examples, the one or more phospholipids comprise a phosphatidylserine (PS) lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition, and wherein the LNP composition comprises a phosphatidylserine (PS) lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition.

The disclosure relates to a nucleic acid lipid nanoparticle (LNP) human vaccine composition comprising: (a) a nucleic acid; (b) an ionizable cationic lipid at a N/P ratio of 3 to 8 relative to the nucleic acid in a total amount of 40-65 mol% of the total lipid content of the LNP composition; (c) a sterol in a total amount of 25-45 mol% of the total lipid content of the LNP composition; (d) one or more phospholipids in a total amount of phospholipids of 5-25 mol% of the total lipid content of the LNP composition, and comprising a phosphatidylglycerol (PG) in a total amount of 1.0 – 10 mol% of the total lipid content of the LNP composition; and (e) a conjugated lipid in a total amount of 0.5 – 2.5 mol% of the total lipid content of the LNP composition.

In some examples, the ionizable cationic lipid is selected from the group consisting of: KC3-01, KC3-OA, KC3-PA, KC3-C17 (8:1), KC3-C15 (C8:1), and Compound 8 (Table 1A).
In some examples, the sterol is cholesterol; and the phosphatidylglycerol (PG) is an anionic phospholipid selected from the group consisting of: distearoylphosphatidylglycerol (DSPG) and dipalmitoyphosphatidylglycerol (DPPG).

In some examples, the nucleic acid is mRNA and the ionizable cationic lipid is present at a N/P ratio of 4 to 7 relative to the nucleic acid.
In some examples, the conjugated lipid is selected from PEG-DMG, PEG-DLG, and PEG-DLPE.

In some examples, the one or more phospholipids comprise a phospholipid selected from the group consisting of: distearoylphosphatidylcholine (DSPC) and hydrogenated soy phosphatidylcholine (HSPC).

The disclosure relates to a method of making a nucleic acid delivery composition comprising lipids wherein the lipids comprise phosphatidylserine, the method comprising a step of dissolving the phosphatidylserine in ethanol wherein the phosphatidylserine is in the form of an ammonium salt of the phosphatidylserine.

In another aspect, the present disclosure relates to a method of making the composition as described herein, wherein the one or more phospholipids comprise phosphatidyl-(L-serine), the method comprising a step of dissolving the phosphatidyl-(L-serine) in ethanol wherein the phosphatidyl-(L-serine) is in the form of an ammonium salt of the phosphatidyl-(L-serine).

In some examples, the phosphatidylserine is DPPS. In some examples, the phosphatidylserine is dissolved in ethanol to the concentration of more than 0.2 mM. In some

examples, the ammonium salt is a salt comprising an ammonium selected from the group consisting of: ammonium, alkyammonium, dialkylammonium, trialkylammonium, and tetraalkylammonium. In some examples, the ammonium form selected from the group consisting of: ammonia, dimethylamine, diethylamine, triethylamine, trimethylamine, 2-(dimethyamino)ethanol, diethanolamine, 2-(diethyamino)ethanol, ethanolamine, ethylenediamine, N-methyl-glucamine, imidazole, histidine, lysine, arginine, 4-(2-hydroxyethyl)-morpholine, piperazine, 1-(2-hydroxyethyl)-pyrrolidine, triethanolamine, or tromethamine (tris(hydroxymethyl)aminomethane).

The disclosure relates to a method for delivery of a nucleic acid into cells comprising the step of: contacting the lipid nanoparticle (LNP) with the cells, wherein the cells are human dendritic cells, wherein the LNP composition was obtained by a process of the disclosure.

In some examples, the LNP comprises an ionizable cationic lipid of the disclosure.

In some examples, the cells are in a human or animal subject. In some examples, the LNP is administered to the subject intramuscularly, subcutaneously, intradermally, or topically.

The disclosure relates to an LNP composition comprising: (a) a nucleic acid, wherein the nucleic acid is an mRNA; (b) a cholesterol sterol in a total amount of 25-40 mol% of the total lipid content of the LNP composition; (c) an ionizable cationic lipid having a N/P ratio of 3 to 8 relative to the nucleic acid in a total amount of 40-65 mol% of the total lipid content of the LNP composition; (d) one or more phospholipids in a total amount of phospholipids of 5-25 mol% of the total lipid content of the LNP composition, the one or more phospholipids are selected from the group consisting of (i) an ammonium salt of dipalmitoylphosphatidyl-L-serine ((L-serine)DPPS) lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition; and (ii) a distearoylphosphatidylcholine (DSPC) phospholipid in a total amount of 5-25 mol% of the total lipid content of the LNP composition; and (e) a PEG-containing conjugated lipid in a total amount of 0.5 – 2.5 mol% of the total lipid content of the LNP composition.

The disclosure relates to a method for administering a nucleic acid to a subject in need thereof comprising administering a nucleic acid lipid nanoparticle (LNP) composition to a human subject, the nucleic acid LNP composition comprising: (a) a nucleic acid; (b) an ionizable cationic lipid at a N/P ratio of 3 to 8 relative to the nucleic acid in a total amount of 40-65 mol% of the total lipid content of the LNP composition; (c) a sterol in a total amount of 25-45 mol% of

the total lipid content of the LNP composition; (d) one or more phospholipids in a total amount of phospholipids of 5-25 mol% of the total lipid content of the LNP composition, and comprising a phosphatidylglycerol (PG) in a total amount of 1.0 – 10 mol% of the total lipid content of the LNP composition; and (e) a conjugated lipid in a total amount of 0.5 – 2.5 mol% of the total lipid content of the LNP composition.

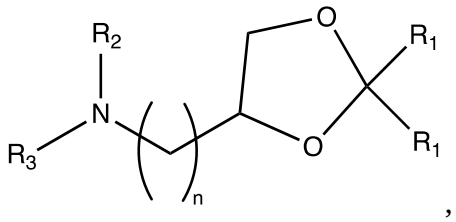
The disclosure relates to a nucleic acid lipid nanoparticle (LNP) composition comprising: (a) a nucleic acid; (b) an ionizable cationic lipid at a N/P ratio of 3 to 8 relative to the nucleic acid in a total amount of 40-65 mol% of the total lipid content of the LNP composition; (c) a sterol in a total amount of 25-45 mol% of the total lipid content of the LNP composition; (d)one or more phospholipids in a total amount of phospholipids of 5-25 mol% of the total lipid content of the LNP composition, and comprising a phosphatidylserine (PS) lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition; and (e) a conjugated lipid in a total amount of 0.5 – 2.5 mol% of the total lipid content of the LNP composition. In some examples, the nucleic acid is mRNA; the ionizable cationic lipid is present in the LNP composition at a N/P ratio of 4 to 7 relative to the nucleic acid; the sterol is cholesterol; and the conjugated lipid is a PEG-containing conjugated lipid.

In some examples, the one or more phospholipids comprise at least two phospholipids having mismatched acyl chain lengths.

In some examples, the phosphatidylserine (PS) lipid is dipalmitoylphosphatidyl-L-serine ((L-serine)DPPS).

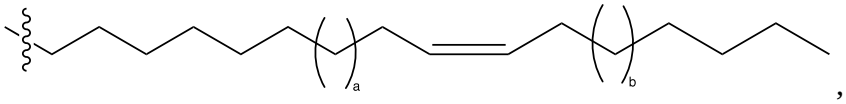
In some examples, the one or more phospholipids comprise a phospholipid selected from the group consisting of: distearoylphosphatidylcholine (DSPC) and hydrogenated soy phosphatidylcholine (HSPC). In some examples, the one or more phospholipids consist of distearoylphosphatidylcholine (DSPC) and dipalmitoylphosphatidyl-L-serine ((L-serine)DPPS). In some examples, the PEG-containing conjugated lipid is PEG(2000)-dimyristoylglycerol (PEG-DMG).

The disclosure relates to an LNP composition wherein the ionizable lipid has the chemical structure:



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wherein R_1 is



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wherein a is 0 or 1; b is 1, 2, 3 or 4, provided the sum a+b is 1, 2, 3 or 4;

R_2 and R_3 are each independently (C_1-C_4) alkyl optionally substituted with hydroxyl; and

n is an integer equal to 2, 3 or 4.

In some examples, R_2 and R_3 are each methyl; and n is 3 or 4.

In some examples, the ionizable cationic lipid is one or more compounds selected from the group consisting of: KC3-OA, KC3-PA, KC3-C17 (8:1), and KC3-C15 (C8:1). In some examples, the ionizable cationic lipid is KC3-PA. In some examples, the ionizable cationic lipid is KC3-OA. In some examples, the ionizable cationic lipid is KC3-C17 (C8:1).

The disclosure relates to an ionizable cationic lipid selected from the group consisting of KC3-PA, KC3-C17 (8:1), and KC3-C15 (C8:1).

The disclosure relates to a nucleic acid lipid nanoparticle (LNP) composition comprising: (a) a nucleic acid; (b) an ionizable cationic lipid at a N/P ratio of 3 to 8 relative to the nucleic acid in a total amount of 40-65 mol% of the total lipid content of the LNP composition; (c) a sterol in a total amount of 25-45 mol% of the total lipid content of the LNP composition; (d) one or more phospholipids in a total amount of phospholipids of 5-25 mol% of the total lipid content of the LNP composition, and comprising a phosphatidylserine (PS) lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition; and (d) a conjugated lipid in a total amount of 0.5 – 2.5 mol% of the total lipid content of the LNP composition.

In some examples, the sterol is cholesterol. In some examples, the one or more phospholipids comprise phospholipids having mismatched acyl chain lengths. In some examples, the PS lipid is dipalmitoylphosphatidyl-L-serine ((L-serine)DPPS). In some examples, the one or more phospholipids comprise a phospholipid selected from the group consisting of: distearoylphosphatidylcholine (DSPC) and hydrogenated soy phosphatidylcholine

(HSPC). In some examples, the one or more phospholipids comprise distearoylphosphatidylcholine (DSPC) and the phosphatidylserine (PS). In some examples, the ionizable cationic lipid is 3-((*S*)-2,2-di((*Z*)-octadec-9-en-1-yl)-1,3-dioxolan-4-yl)-*N*,*N*-dimethylpropan-1-amine **(**KC3-OA). In some examples, the ionizable cationic lipid is 3-((*S*)-2,2-di((*Z*)-octadec-9-en-1-yl)-1,3-dioxolan-4-yl)-*N*,*N*-dimethylpropan-1-amine **(**KC3-OA). In some examples, the one or more phospholipids consist of distearoylphosphatidylcholine (DSPC) and dipalmitoylphosphatidyl-L-serine ((L-serine)DPPS), and the phosphatidylserine (PS) is (L-Serine)DPPS.

In some examples, the conjugated lipid is a PEG-containing conjugated lipid, and wherein the PEG-containing conjugated lipid is selected from the group consisting of: PEG(2000)-dimyristoylglycerol (PEG-DMG), 1,2-dilauroyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG-DLPE), and PEG(2000)-dilauroylglycerol (PEG-DLG).

In some examples, the nucleic acid is mRNA; the one or more phospholipids comprise phosphatidylserine (PS) and one or more phospholipids selected from the group consisting of: distearoylphosphatidylcholine (DSPC), hydrogenated soy phosphatidylcholine (HSPC), and dipalmitoylphosphatidyl-L-serine ((L-serine)DPPS); and the conjugated lipid comprises a polyethylene glycol (PEG). In some examples, the conjugated lipid is PEG(2000)-dimyristoylglycerol (PEG-DMG). In some examples, the ionizable cationic lipid is 3-((*S*)-2,2-di((*Z*)-octadec-9-en-1-yl)-1,3-dioxolan-4-yl)-*N*,*N*-dimethylpropan-1-amine **(**KC3-OA). In some examples, the one or more phospholipids consist of distearoylphosphatidylcholine (DSPC) and dipalmitoylphosphatidyl-L-serine ((L-serine)DPPS).

In some examples, the dipalmitoylphosphatidyl-L-serine ((L-serine)DPPS) is an ammonium salt of (L-Serine)DPPS.

In some examples, (a) the nucleic acid is mRNA; (b) the sterol is a cholesterol sterol in a total amount of 25-45 mol% of the total lipid content of the LNP composition; (c) the ionizable cationic lipid is at a N/P ratio of 3 to 8 relative to the nucleic acid in a total amount of 40-65 mol% of the total lipid content of the LNP composition; (d) the one or more phospholipids is in a total amount of phospholipids of 5-25 mol% of the total lipid content of the LNP composition, the one or more phospholipids consisting of: (i)dipalmitoylphosphatidyl-L-serine ((L-serine)DPPS) lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition; and (ii) a

distearoylphosphatidylcholine (DSPC) phospholipid in a total amount of 5-25 mol% of the total lipid content of the LNP composition; (e) the conjugated lipid is a PEG-containing conjugated lipid in a total amount of 0.5 – 2.5 mol% of the total lipid content of the LNP composition.

In some examples, the PEG-containing conjugated lipid is selected from the group consisting of: PEG(2000)-dimyristoylglycerol (PEG-DMG), 1,2-dilauroyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG-DLPE), and PEG(2000)-dilauroylglycerol (PEG-DLG).

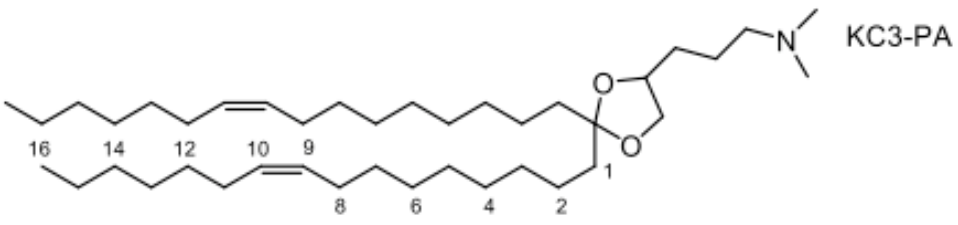
The disclosure relates to a nucleic acid lipid nanoparticle (LNP) human vaccine composition comprising: (a) a nucleic acid; (b) an ionizable cationic lipid at a N/P ratio of 3 to 8 relative to the nucleic acid in a total amount of 40-65 mol% of the total lipid content of the LNP composition; (c) a sterol in a total amount of 25-45 mol% of the total lipid content of the LNP composition; (d) one or more phospholipids in a total amount of phospholipids of 5-25 mol% of the total lipid content of the LNP composition, and comprising a phosphatidylglycerol (PG) in a total amount of 1.0 – 10 mol% of the total lipid content of the LNP composition; and (e) a conjugated lipid in a total amount of 0.5 – 2.5 mol% of the total lipid content of the LNP composition.

In some examples, the sterol is cholesterol; and the phosphatidylglycerol (PG) is an anionic phospholipid selected from the group consisting of: distearoylphosphatidylglycerol (DSPG) and dipalmitoyphosphatidylglycerol (DPPG).

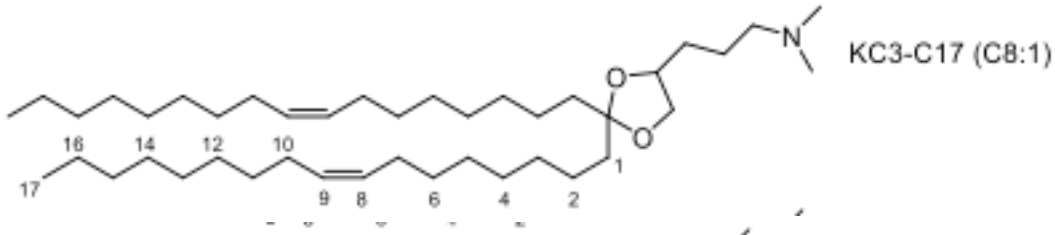
In some examples, the nucleic acid is mRNA and the ionizable cationic lipid is present at a N/P ratio of 4 to 7 relative to the nucleic acid.

The disclosure relates to a nucleic acid lipid nanoparticle (LNP) composition comprising an ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP composition, wherein the ionizable cationic lipid is selected from the group consisting of: KC3-PA, KC3-C17 (8:1), and KC3-C15 (C8:1), KC3-OA, and KC3-01

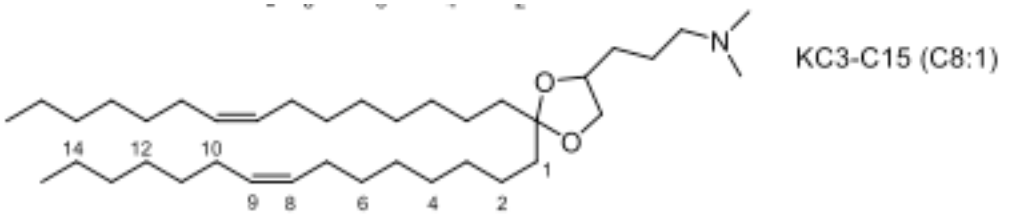
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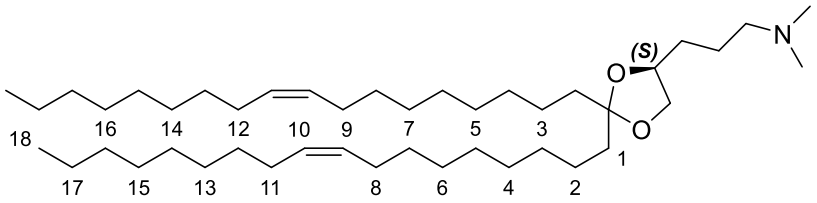
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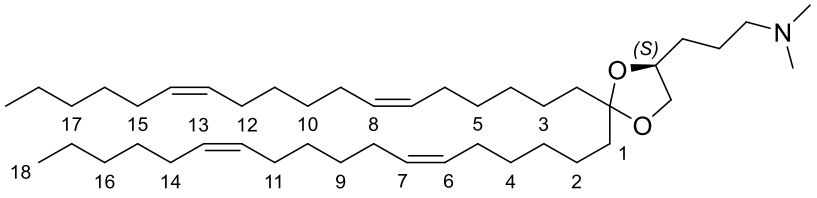


;



AKG-KC3-OA

; and



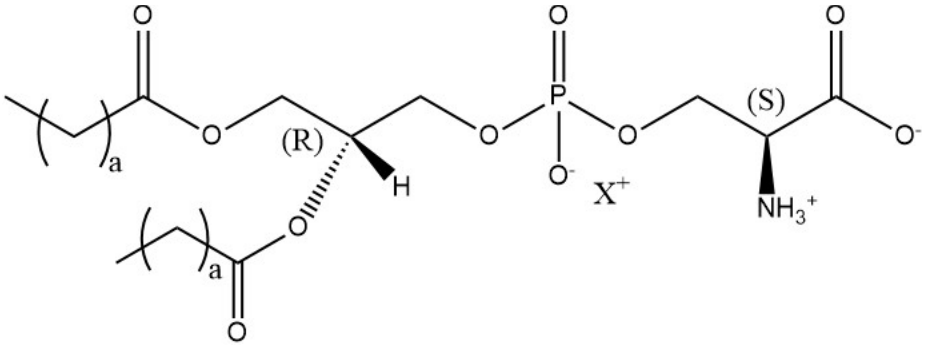
AKG-KC3-01

The disclosure relates to a nucleic acid lipid nanoparticle (LNP) composition comprising: (a) a nucleic acid; (b) an ionizable cationic lipid at a N/P ratio of 3 to 8 relative to the nucleic acid in a total amount of 40-65 mol% of the total lipid content of the LNP composition; (c) a sterol in a total amount of 25-45 mol% of the total lipid content of the LNP composition; (d) one or more phospholipids in a total amount of phospholipids of 5-25 mol% of the total lipid content of the LNP composition; and (d) a conjugated lipid in a total amount of 0.5 – 2.5 mol% of the total lipid content of the LNP composition, wherein the conjugated lipid which is a PEG-lipid having a poly(ethylene glycol) chain terminally attached to a linking moiety and two hydrocarbon chains terminally attached to the same linking moiety, wherein the two hydrocarbon chains are

saturated C_{12}-chains independently selected from n-dodecyl (lauryl) group and n-dodecanoyl (lauroyl) group.

In some examples, the linking moiety is a glyceryl group, N-oxycarbonyl glycerophoshoryl ethanolaminocarbonyl group, oxycarbonylamide group, or oxyacetamide group. In some examples, the poly(ethyene glycol) chain is methoxy-poly(ethyene glycol) with an average molecular weight of 2000. In some examples, the PEG-lipid is mPEG-1,2-dilauroylglycerol (PEG-DLG), mPEG-1,2-dilaurylglycerol (PEG-DLG), PEG-1,2-dilaurylglycerol, PEG-DLPE, PEG-oxycarbonyl-N,N-didodecylamide, or mPEG-N,N-didodecylacetamide.

In another aspect, the present disclosure relates to a salt of an anionic phospholipid of Formula (V-A-1):



wherein X^+ is an ammonium cation or a sodium (Na^+) cation; and a is 14, 15 or 16;

wherein the ammonium cation is selected from the group consisting of: ammonium, alkyammonium, dialkylammonium, trialkylammonium, and tetraalkylammonium.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a depiction of the oxidative degradation mechanisms of lipid esters of linoleic acid containing conjugated multiple unsaturations that are particularly sensitive to oxidation.

FIG. 2 shows the reaction of the reduced c-terminal cysteine of a Fab' antibody fragment with a maleimide terminated-poly(ethylene glycol) 2000 derivatized distearoylphosphatidylethanolamine. R1 and R2 are stearic acid. The final antibody lipopolymer conjugate is an intermediate that is subsequently inserted into the outer lipid layer of lipidic nanoparticle to make it actively targeted.

FIG. 3A Impact of DSPS inclusion from 0-2.5 mol % on transfection efficiency of dendritic cells (MutuDC1940) using mCherry mRNA LNPs formulated with DLin-KC2-DMA as the ionizable cationic lipid. ICL was kept at 50 mol%, cholesterol at 38.5 mol%, PEG-DMG at 1.5 mol% and the DSPS content varied. Inclusion of DSPS was made by reducing the DSPC content by the same mol% of DSPS that was added. Cells were incubated with each formulation at a concentration of 1 ug mRNA/mL for 24 h. UT sample corresponds to cells where no LNPs were added. Lipofect refers to Lipofectamine treated sample.

FIG. 3B Impact of DSPS inclusion from 0-7.5 mol % on transfection efficiency of dendritic cells (MutuDC1940) using mCherry mRNA LNPs formulated with DLin-KC2-DMA as the ionizable cationic lipid. ICL was kept at 50 mol%, cholesterol at 38.5 mol%, PEG-DMG at 1.5 mol% and the DSPS content varied. Inclusion of DSPS was made by reducing the DSPC content by the same mol% of DSPS that was added. Cells were incubated with each formulation at a concentration of 1 ug mRNA/mL for 24 h. UT sample corresponds to cells where no LNPs were added. Lipofect refers to Lipofectamine treated sample.

CLAIMS

What is claimed is:

1. A nucleic acid lipid nanoparticle (LNP) composition comprising:
   a. a nucleic acid;
   b. an ionizable cationic lipid at a N/P ratio of 3 to 8 relative to the nucleic acid in a total amount of 40-65 mol% of the total lipid content of the LNP composition;
   c. a sterol in a total amount of 25-45 mol% of the total lipid content of the LNP composition;
   d. one or more phospholipids in a total amount of phospholipids of 5-25 mol% of the total lipid content of the LNP composition, and comprising an anionic targeting ligand for targeting of the LNP to dendritic cells, selected from the group consisting of: (i) a phosphatidylserine (PS) lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition, or (ii) a phosphatidylglycerol (PG) in a total amount of 1.0 – 10 mol% of the total lipid content of the LNP composition; and
   e. a conjugated lipid in a total amount of 0.5 – 2.5 mol% of the total lipid content of the LNP composition.
2. The composition of claim 1, wherein the ionizable cationic lipid is selected from the group KC3-PA, KC3-C17 (8:1), and KC3-C15 (C8:1).
3. The composition of claim 1, wherein the anionic targeting ligand is a phosphatidylglycerol (PG) in a total amount of 1.0 – 10 mol% of the total lipid content of the LNP composition.
4. The composition of claim 1, wherein the nucleic acid is mRNA.
5. The composition of claim 1, wherein the sterol is cholesterol.
6. The composition of claim 1, wherein the ionizable cationic lipid is selected from the group KC3-PA, KC3-C17 (8:1), and KC3-C15 (C8:1).
7. The composition of claim 6, wherein the anionic targeting ligand is a phosphatidylserine (PS) lipid selected from the group consisting of dipalmitoylphosphatidyl-L-serine (DPPS), and distearoylphosphatidyl-L-serine (DSPS).
8. The composition of claim 1, wherein the anionic targeting ligand is a phosphatidylglycerol (PG) selected from the group consisting of: distearoylphosphatidylglycerol (DSPG) and dipalmitoyphosphatidylglycerol (DPPG).

9. The composition of claim 1, wherein the one or more phospholipids comprise a second phospholipid is selected from the group consisting of: distearoylphosphatidylcholine (DSPC), hydrogenated soy phosphatidylcholine (HSPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), and sphingomyelin.

10. The composition of claim 1, wherein the conjugated lipid comprises a PEG-lipid having a poly(ethylene glycol) chain terminally attached to a linking moiety and two hydrocarbon chains terminally attached to the same linking moiety, wherein the hydrocarbon chains are saturated C_{12}-chains independently selected from n-dodecyl (lauryl) group and n-dodecanoyl (lauroyl) group.

11. The composition of claim 1, wherein

a. the nucleic acid is mRNA;

b. the sterol is cholesterol;

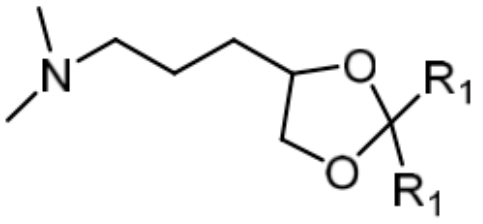
c. the phosphatidylserine (PS) lipid is dipalmitoylphosphatidyl-L-serine (DPPS), or distearoylphosphatidyl-L-serine (DSPS); and the phosphatidylglycerol (PG) is distearoylphosphatidylglycerol (DSPG) or dipalmitoyphosphatidylglycerol (DPPG); and

d. the conjugated lipid comprises a PEG-lipid having a poly(ethylene glycol) chain terminally attached to a linking moiety and two hydrocarbon chains terminally attached to the same linking moiety, wherein the hydrocarbon chains are saturated C_{12}-chains independently selected from n-dodecyl (lauryl) group and n-dodecanoyl (lauroyl) group.

12. The composition of claim 11, wherein

a. the nucleic acid is a mRNA;

b. the ionizable cationic lipid has the chemical structure:



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wherein R_1 is alkyl group of C_{15}-C_{19} containing one or two olefins;

c. the one or more phospholipids comprise a second phospholipid is selected from the group consisting of: distearoylphosphatidylcholine (DSPC), hydrogenated soy phosphatidylcholine (HSPC), and sphingomyelin; and

d. the linking moiety of the conjugated lipid is glyceryl group, N-oxycarbonyl glycerophoshoryl ethanolaminocarbonyl group, oxycarbonylamide group, or oxyacetamide group; and the poly(ethyene glycol) chain of the conjugated lipid is methoxy-poly(ethyene glycol) with an average molecular weight of 2000.

13. The composition of claim 12, wherein the conjugated lipid is mPEG-1,2-dilauroylglycerol (PEG- DLG), mPEG-1,2-dilaurylglycerol (PEG-DLG), PEG-1,2-dilaurylglycerol, PEG-DLPE, PEG- oxycarbonyl-N,N-didodecylamide, or mPEG-N,N-didodecylacetamide.

14. The composition of any one of claims 1-13, wherein the LNP is a human vaccine composition having a z-average particle size of 60-150 nm and is freeze-thaw stable.

15. A method of making the composition of claim 14, wherein the one or more phospholipids comprise phosphatidyl-(L-serine), the method comprising a step of dissolving the phosphatidyl-(L-serine) in ethanol wherein the phosphatidyl-(L-serine) is in the form of an ammonium salt of the phosphatidyl-(L-serine).

16. The method of claim 15, wherein

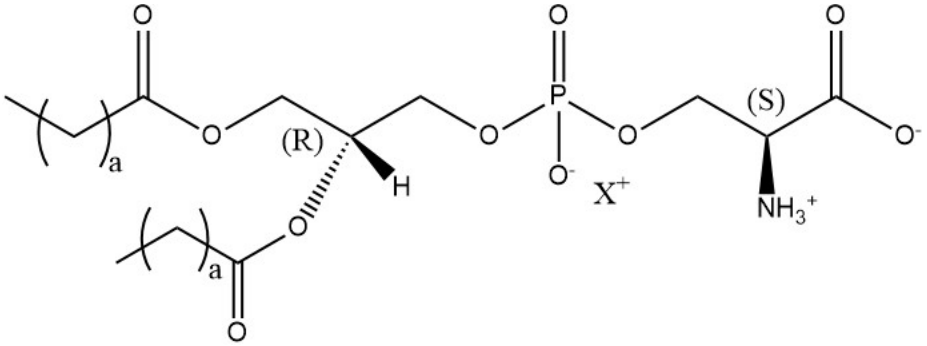
a. the phosphatidylserine is (L-serine)-DPPS and the phosphatidylserine is dissolved in ethanol to the concentration of more than 0.2 mM;

b. the ammonium salt is a salt comprising an ammonium selected from the group consisting of: ammonium, alkyammonium, dialkylammonium, trialkylammonium, and tetraalkylammonium.

17. The composition of claim 4, claim 11 or claim 12, wherein the nucleic acid is chemically modified RNA.

18. The composition of claim 4, claim 11 or claim 12, wherein the nucleic acid is a mRNA chemically modified with N-methylpseudouridine.

19. A salt of an anionic phospholipid of Formula (V-A-1):



wherein X^+ is an ammonium cation or a sodium (Na^+) cation; and a is 14, 15 or 16;

wherein the ammonium cation is selected from the group consisting of: ammonium, alkyammonium, dialkylammonium, trialkylammonium, and tetraalkylammonium.