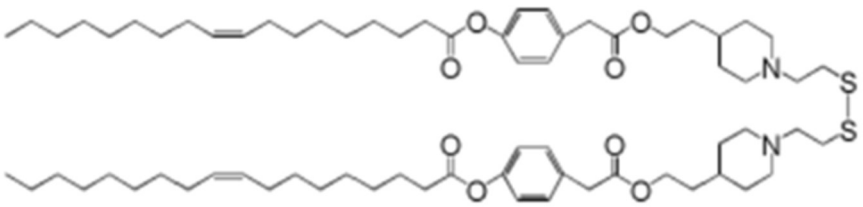
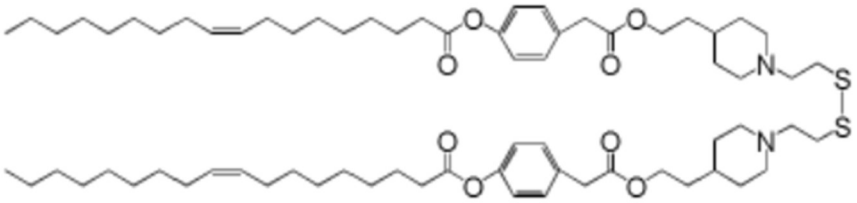
In one aspect, the present disclosure refers to pharmaceutical composition comprising lipid nanoparticle (LNP), wherein the LNP comprises a SS-cleavable lipid and a closed-ended DNA (ceDNA), wherein the SS-cleavable lipid comprises an ss-OP lipid of Formula I:



.

In another aspect, the present disclosure refers to the use of the pharmaceutical composition as disclosed herien in the manufacture of a medicament for treating a genetic disorder in a subject.

In one example, disclosed herein is a pharmaceutical composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises a SS-cleavable lipid and a therapeutic nucleic acid (TNA). In another example, disclosed herein is a pharmaceutical composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises a SS-cleavable lipid and an mRNA. In one example, disclosed herein is a pharmaceutical composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises a SS-cleavable lipid and a closed-ended DNA (ceDNA). According to some examples, the SS-cleavable lipid comprises a disulfide bond and a tertiary amine. According to some examples of any of the aspects or examples herein, the SS-cleavable lipid comprises an ss-OP lipid of Formula I:

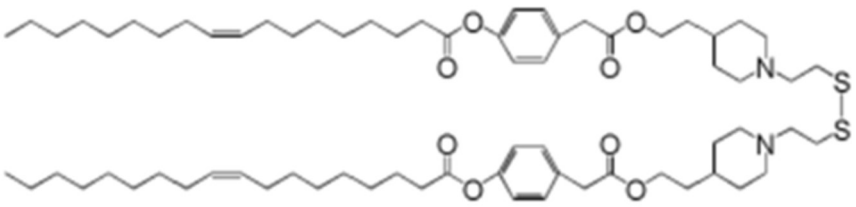


According to some examples of any of the aspects or examples herein, the LNP further comprises a sterol. According to some examples, the sterol is a cholesterol. According to some examples of any of the aspects or examples herein, the LNP further comprises a polyethylene glycol (PEG). According to some examples, the PEG is l-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG). According to some examples of any of the aspects or examples herein, the LNP further comprises a non-cationic lipid. According to some examples, the non-cationic lipid is selected from the group consisting of distearoyl-sn-glycero-phosphoethanolamine, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), monomethyl-phosphatidylethanolamine (such as 16-O-monomethyl PE), dimethyl-phosphatidylethanolamine (such as 16-O-dimethyl PE), 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE), hydrogenated soy phosphatidylcholine (HSPC), egg phosphatidylcholine (EPC), dioleoylphosphatidylserine (DOPS), sphingomyelin (SM), dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), distearoylphosphatidylglycerol (DSPG), dierucoylphosphatidylcholine (DEPC), palmitoyloleyolphosphatidylglycerol (POPG), dielaidoyl-phosphatidylethanolamine (DEPE), 1,2-dilauroyl-sn-glycero-3 -pho sphoethanolamine (DLPE); 1,2-diphytanoyl-sn-glycero-3-

CLAIMS

What is Claimed is:

1. A pharmaceutical composition comprising lipid nanoparticle (LNP), wherein the LNP comprises a SS-cleavable lipid and a closed-ended DNA (ceDNA), wherein the SS-cleavable lipid comprises an ss-OP lipid of Formula I:



.

2. The pharmaceutical composition of any one of the previous claims, wherein the LNP further comprises a sterol, a polyethylene glycol (PEG) or PEG-lipid conjugate, and/or a non-cationic lipid.

3. The pharmaceutical composition of claim 2, wherein the sterol is a cholesterol or β-sitosterol;

the PEG or PEG-lipid conjugate is l-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG) or l,2-distearoyl-rac-glycero-3-methylpolyoxyethylene (DSG-PEG2000); and/or

the non-cationic lipid is selected from the group consisting of distearoyl-sn-glycero-phosphoethanolamine, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoylphosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoylphosphatidylethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoylphosphatidylethanolamine (DSPE), monomethylphosphatidylethanolamine (such as 16-O-monomethyl PE), dimethylphosphatidylethanolamine (such as 16-O-dimethyl PE), 18-1-trans PE, 1-stearoyl-2-oleoylphosphatidyethanolamine (SOPE), hydrogenated soy phosphatidylcholine (HSPC), egg phosphatidylcholine (EPC), dioleoylphosphatidylserine (DOPS), sphingomyelin (SM),

dimyristoylphosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), distearoylphosphatidylglycerol (DSPG), dierucoylphosphatidylcholine (DEPC), palmitoyloleyolphosphatidylglycerol (POPG), dielaidoylphosphatidylethanolamine (DEPE), 1,2-dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE); 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPHyPE); lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidicacid,cerebrosides, dicetylphosphate, lysophosphatidylcholine, dilinoleoylphosphatidylcholine, or mixtures thereof.

4. The pharmaceutical composition of claim 2, wherein:
the PEG or PEG-lipid conjugate is present at a molar percentage of 1.5% to 3%;
the sterol is cholesterol, wherein the cholesterol is present at a molar percentage of 20% to 40% or 30% to 50%; and/or
the SS-cleavable lipid is present at a molar percentage of 80% to 60%, or about 50%.

5. The pharmaceutical composition of claim 2, wherein the composition comprises a cholesterol, a PEG or PEG-lipid conjugate, and a non-cationic lipid, and wherein:
the SS-cleavable lipid is present at a molar percentage of 42.5% to 62.5%;
the PEG or PEG-lipid conjugate is present at a molar percentage of 1.5% to 3%;
the cholesterol is present at a molar percentage of 30% to 50%; and/or
the non-cationic lipid is present at a molar percentage of 2.5% to 12.5%.

6. The pharmaceutical composition of claim 5, wherein the cholesterol is present at a molar percentage of about 40%, the SS-cleavable lipid is present at a molar percentage of about 52.5%, the non-cationic lipid is present at a molar percentage of about 7.5%, and the PEG or PEG-lipid conjugate is present at a molar percentage of about 3%.

7. The pharmaceutical composition of any one of the previous claims, wherein the LNP is 50 nm to 110 nm in diameter.

8. The pharmaceutical composition of claim 1, wherein the LNP is less than 70 nm in diameter.

9. The pharmaceutical composition of any one of the previous claims, wherein the composition has a total lipid to ceDNA ratio of about 15:1, about 30:1, about 40:1, or about 50:1.

10. The pharmaceutical composition of any one of the previous claims, wherein the composition further comprises N-Acetylgalactosamine (GalNAc).

11. The pharmaceutical composition of any one of the previous claims, wherein the ceDNA is closed-ended linear duplex DNA.

12. The pharmaceutical composition of any one of the previous claims, wherein the ceDNA comprises an expression cassette comprising a promoter sequence and a transgene.

13. The pharmaceutical composition of claim 12, wherein:
the ceDNA comprises at least one inverted terminal repeat (ITR) flanking either 5' or 3' end of said expression cassette.

14. The pharmaceutical composition of claim 13, wherein said expression cassette is flanked by two ITRs, wherein the two ITRs comprise one 5' ITR and one 3' ITR.

15. The pharmaceutical composition of claim 13, wherein:
at least one of 5' ITR and 3' ITR is a wild-type AAV ITR;
at least one of 5' ITR and 3' ITR is a modified ITR; and/or
the 5' ITR and 3' ITR are symmetric ITRs or asymmetric ITRs.

16. The pharmaceutical composition of claim 13, wherein at least one ITR is an ITR derived from an AAV serotype, derived from an ITR of goose virus, derived from a B19 virus ITR, or derived from a wild-type ITR from a parvovirus.

17. The pharmaceutical composition of any one of the previous claims, wherein the ceDNA is a CELiD, a DNA-based minicircle, a MIDGE, a ministring DNA, a dumbbell shaped linear duplex closed-ended DNA comprising two hairpin structures of ITRs in the 5' and 3' ends of an expression cassette, or a doggybone™ DNA.

18. Use of the pharmaceutical composition according to any one of the previous claims in the manufacture of a medicament for treating a genetic disorder in a subject.

19. The use of claim 18, wherein the genetic disorder is selected from the group consisting of melanoma, hemophilia A (clotting factor VIII (FVIII) deficiency) and hemophilia B (clotting factor IX (FIX) deficiency), cystic fibrosis (CFTR defect), familial hypercholesterolemia (LDL receptor defect), hepatoblastoma, Wilson's disease, phenylketonuria (PKU), congenital hepatic porphyria, inherited disorders of hepatic metabolism, LeschNyhan syndrome, sickle cell anemia, thalassaemias, xeroderma pigmentosum, Fanconi anemia, retinitis pigmentosa, ataxia telangiectasia, Bloom syndrome, retinoblastoma, mucopolysaccharide storage diseases (*e.g.*, Hurler syndrome (MPS Type I), Scheie syndrome (MPS Type I S), Hurler-Scheie syndrome (MPS Type I H-S), Hunter syndrome (MPS Type II), Sanfilippo Types A, B, C, and D (MPS Types III A, B, C, and D), Morquio Types A and B (MPS IVA and MPS IVB), Maroteaux-Lamy syndrome (MPS Type VI), Sly syndrome (MPS Type VII), hyaluronidase deficiency (MPS Type IX)), Niemann-Pick Disease Types A/B, C1 and C2, Fabry disease, Schindler disease, GM2-gangliosidosis Type II (Sandhoff Disease), Tay-Sachs disease, Metachromatic Leukodystrophy, Krabbe disease, Mucolipidosis Type I, II/III and IV, Sialidosis Types I and II, Glycogen Storage disease Types I and II (Pompe disease), Gaucher disease Types I, II and III, Fabry disease, cystinosis, Batten disease, Aspartylglucosaminuria, Salla disease, Danon disease (LAMP-2 deficiency), Lysosomal Acid Lipase (LAL) deficiency, neuronal ceroid lipofuscinoses (CLN1-8, INCL, and LINCL), sphingolipidoses, galactosialidosis, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, Huntington's disease, spinocerebellar ataxia, spinal muscular atrophy, Friedreich's ataxia, Duchenne muscular dystrophy (DMD), Becker muscular dystrophies (BMD), dystrophic epidermolysis bullosa (DEB), ectonucleotide pyrophosphatase 1 deficiency, generalized arterial calcification of infancy (GACI), Leber Congenital Amaurosis, Stargardt macular dystrophy (ABCA4 deficiency), ornithine transcarbamylase (OTC) deficiency, Usher syndrome, alpha-1 antitrypsin deficiency, and Cathepsin A deficiency.

20. The use of claim 19, wherein the genetic disorder is Leber congenital amaurosis (LCA).

21. The use of claim 19, wherein the genetic disorder is Stargardt macular dystrophy.

22. The use of claim 19, wherein the genetic disorder is hemophilia A (Factor VIII deficiency).

23. The use of claim 19, wherein the genetic disorder is dystrophic epidermolysis bullosa (DEB).

24. The use of claim 18 or 19, further comprising that an immunosuppressant is to be administered.

25. The use of any one of claims 18, 19 and 24, wherein the subject exhibits a diminished immune response level against the pharmaceutical composition, as compared to an immune response level observed with an LNP comprising MC3 as a main cationic lipid, wherein the immune response level against the pharmaceutical composition is at least 50% lower than the level observed with the LNP comprising MC3.

26. The use of any one of claims 18, 19, 24, and 25, wherein the LNP comprising the SS-cleavable lipid and the closed-ended DNA (ceDNA) is not phagocytosed; or exhibits diminished phagocytic levels by at least 50% as compared to phagocytic levels of LNPs comprising MC3 as a main cationic lipid administered at a similar condition.