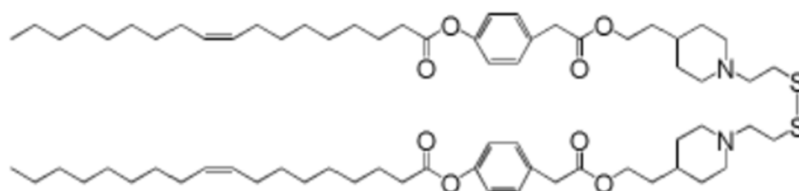


In one aspect, the present disclosure refers to pharmaceutical composition comprising lipid nanoparticle (LNP), wherein the LNP comprises a SS-cleavable lipid and a closed-ended DNA (ceDNA).

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.

5 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:

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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 1-(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),
30 dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyl-oleoylphosphatidylcholine (POPC), palmitoyl-oleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE),
35 monomethyl-phosphatidylethanolamine (such as 16-O-monomethyl PE), dimethyl-phosphatidylethanolamine (such as 16-O-dimethyl PE), 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), hydrogenated soy phosphatidylcholine (HSPC), egg phosphatidylcholine (EPC), dioleoylphosphatidylserine (DOPS), sphingomyelin (SM), dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), distearoylphosphatidylglycerol (DSPG), dierucoylphosphatidylcholine (DEPC), palmitoyl-oleoylphosphatidylglycerol (POPG), dielaidoyl-phosphatidylethanolamine (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, phosphatidic acid, cerebrosides, dicetylphosphate, lysophosphatidylcholine, dilinoleoylphosphatidylcholine, or mixtures thereof. According to some examples, the non-cationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE).

According to some examples, the PEG or PEG-lipid conjugate is present at about 1.5% to about 3%, for example about 1.5% to about 2.75%, about 1.5% to about 2.5%, about 1.5% to about 2.25%, about 1.5% to about 2%, about 1.5% to about 1.75%, about 2% to about 3%, about 2% to about 2.75%, about 2% to about 2.5%, about 2% to about 2.25%. According to some examples, the PEG or PEG-lipid conjugate is present at about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, or about 3%. According to some examples, the cholesterol is present at a molar percentage of about 20% to about 40%, for example about 20% to about 35%, about 20% to about 30%, about 20% to about 25%, about 25% to about 35%, about 25% to about 30%, or about 30% to about 35%, and the SS-cleavable lipid is present at a molar percentage of about 80% to about 60%, for example about 80% to about 65%, about 80% to about 70%, about 80% to about 75%, about 75% to about 60%, about 75% to about 65%, about 75% to about 70%, about 70% to about 60%, or about 70% to about 60%. According to some examples, the cholesterol is present at a molar percentage of about 20% to about 40%, for example about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, or about 40%, and wherein the SS-cleavable lipid is present at a molar percentage of about 80% to about 60%, for example about 80%, about 79%, about 78%, about 77%, about 76%, about 75%, about 74%, about 73%, about 72%, about 71%, about 70%, about 69%, about 68%, about 67%, about 66%, about 65%, about 64%, about 63%, about 62%, about 61%, or about 60%. According to some examples, the cholesterol is present at a molar percentage of about 40%, and wherein the SS-cleavable lipid is present at a molar percentage of about 50%. According to some examples of any of the aspects or examples herein, the composition further comprises a cholesterol, a PEG or PEG-lipid conjugate, and a non-cationic lipid. According to some examples, the PEG or PEG-lipid conjugate is present at about 1.5% to about 3%, for example about 1.5% to about 2.75%, about 1.5% to about 2.5%, about 1.5% to about 2.25%, about 1.5% to about 2%, about 2% to about 3%, about 2% to about 2.75%, about 2% to about 2.5%, about 2% to about 2.25%, about 2.25% to about 3%, about 2.25% to about 2.75%, or about 2.25% to about 2.5%. According to some examples, the PEG or PEG-lipid conjugate is present at about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about

2.7%, about 2.8%, about 2.9%, or about 3%. According to some examples, the cholesterol is present at a molar percentage of about 30% to about 50%, for example about 30% to about 45%, about 30% to about 40%, about 30% to about 35%, about 35% to about 50%, about 35% to about 45%, about 35% to about 40%, about 40% to about 50%, or about 45% to about 50%. According to some examples, the cholesterol is present at a molar percentage of about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, or about 50%. According to some examples, the SS-cleavable lipid is present at a molar percentage of about 42.5% to about 62.5%. According to some examples, the SS-cleavable lipid is present at a molar percentage of about 42.5%, about 43%, about 43.5%, about 44%, about 44.5%, about 45%, about 45.5%, about 46%, about 46.5%, about 47%, about 47.5%, about 48%, about 48.5%, about 49%, about 49.5%, about 50%, about 50.5%, about 51%, 51.5%, about 52%, about 52.5%, about 53%, about 53.5%, about 54%, about 54.5%, about 55%, about 55.5%, about 56%, about 56.5%, about 57%, 57.5%, about 58%, about 58.5%, about 59%, about 59.5%, about 60%, about 60.5%, about 61%, about 61.5%, about 62%, or about 62.5%. According to some examples of any of the aspects or examples herein, the non-cationic lipid is present at a molar percentage of about 2.5% to about 12.5%. According to some examples of any of the aspects or examples herein, 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 wherein the PEG is present at about 3%. According to some examples of any of the aspects or examples herein, the composition further comprises dexamethasone palmitate. According to some examples of any of the aspects or examples herein, the LNP is in size ranging from about 50 nm to about 110 nm in diameter, for example about 50 nm to about 100 nm, about 50 nm to about 95 nm, about 50 nm to about 90 nm, about 50 nm to about 85 nm, about 50 nm to about 80 nm, about 50 nm to about 75 nm, about 50 nm to about 70 nm, about 50 nm to about 65 nm, about 50 nm to about 60 nm, about 50 nm to about 55 nm, about 60 nm to about 110 nm, about 60 nm to about 100 nm, about 60 nm to about 95 nm, about 60 nm to about 90 nm, about 60 nm to about 85 nm, about 60 nm to about 80 nm, about 60 nm to about 75 nm, about 60 nm to about 70 nm, about 60 nm to about 65 nm, about 70 nm to about 110 nm, about 70 nm to about 100 nm, about 70 nm to about 95 nm, about 70 nm to about 90 nm, about 70 nm to about 85 nm, about 70 nm to about 80 nm, about 70 nm to about 75 nm, about 80 nm to about 110 nm, about 80 nm to about 100 nm, about 80 nm to about 95 nm, about 80 nm to about 90 nm, about 80 nm to about 85 nm, about 90 nm to about 110 nm, or about 90 nm to about 100 nm. According to some examples of any of the aspects or examples herein, the LNP is less than about 100 nm in size, for example less than about 105 nm, less than about 100 nm, less than about 95 nm, less than about 90 nm, less than about 85 nm, less than about 80 nm, less than about 75 nm, less than about 70 nm, less than about 65 nm, less than about 60 nm, less than about 55 nm, less than about 50 nm, less than about 45 nm, less than about 40

nm, less than about 35 nm, less than about 30 nm, less than about 25 nm, less than about 20 nm, less than about 15 nm, or less than about 10 nm in size. According to some examples, the LNP is less than about 70 nm in size., for example less than about 65 nm, less than about 60 nm, less than about 55 nm, less than about 50 nm, less than about 45 nm, less than about 40 nm, less than about 35 nm, less than about 30 nm, less than about 25 nm, less than about 20 nm, less than about 15 nm, or less than about 10 nm in size. According to some examples, the LNP is less than about 60 nm in size, for example less than about 55 nm, less than about 50 nm, less than about 45 nm, less than about 40 nm, less than about 35 nm, less than about 30 nm, less than about 25 nm, less than about 20 nm, less than about 15 nm, or less than about 10 nm in size. According to some examples of any of the aspects or examples herein, the composition has a total lipid to ceDNA ratio of about 15:1. According to some examples of any of the aspects or examples herein, the composition has a total lipid to ceDNA ratio of about 30:1. According to some examples of any of the aspects or examples herein, the composition has a total lipid to ceDNA ratio of about 40:1. According to some examples of any of the aspects or examples herein, the composition has a total lipid to ceDNA ratio of about 50:1. According to some examples of any of the aspects or examples herein, the composition further comprises N-Acetylgalactosamine (GalNAc). According to some examples, the GalNAc is present in the LNP at a molar percentage of 0.2% of the total lipid. According to some examples, the GalNAc is present in the LNP at a molar percentage of 0.3% of the total lipid. According to some examples, the GalNAc is present in the LNP at a molar percentage of 0.4% of the total lipid. According to some examples, the GalNAc is present in the LNP at a molar percentage of 0.5% of the total lipid. According to some examples, the GalNAc is present in the LNP at a molar percentage of 0.6% of the total lipid. According to some examples, the GalNAc is present in the LNP at a molar percentage of 0.7% of the total lipid. According to some examples, the GalNAc is present in the LNP at a molar percentage of 0.8% of the total lipid. According to some examples, the GalNAc is present in the LNP at a molar percentage of 0.9% of the total lipid. According to some examples, the GalNAc is present in the LNP at a molar percentage of 1.0% of the total lipid. According to some examples, the GalNAc is present in the LNP at a molar percentage of about 1.5% of the total lipid. According to some examples, the GalNAc is present in the LNP at a molar percentage of 2.0% of the total lipid. According to some examples of any of the aspects or examples herein, the composition further comprises about 10 mM to about 30 mM malic acid, for example about 10 mM to about 25 mM, about 10 mM to about 20 mM, about 10 mM to about 15 mM, about 15 mM to about 25 mM, about 15 mM to about 20 mM, about 20 mM to about 25 mM. According to some examples of any of the aspects or examples herein, the composition further comprises about 10 mM malic acid, about 11 mM malic acid, about 12 mM malic acid, about 13 mM malic acid, about 14 mM malic acid, about 15 mM malic acid, about 16 mM malic acid, about 17 mM malic acid, about 18 mM malic acid, about 19 mM malic acid, about 20 mM malic acid, about 21 mM malic acid, about 22 mM malic acid, about 23 mM malic

acid, about 24 mM malic acid, about 25 mM malic acid, about 26 mM malic acid, about 27 mM malic acid, about 28 mM malic acid, about 29 mM malic acid, or about 30 mM malic acid. According to some examples, the composition comprises about 20 mM malic acid. According to some examples of any of the aspects or examples herein, the composition further comprises about 30 mM to about 50 mM NaCl, for example about 30 mM to about 45 mM NaCl, about 30 mM to about 40 mM NaCl, about 30 mM to about 35 mM NaCl, about 35 mM to about 45 mM NaCl, about 35 mM to about 40 mM NaCl, or about 40 mM to about 45 mM NaCl. According to some examples of any of the aspects or examples herein, the composition further comprises about 30 mM NaCl, about 35 mM NaCl, about 40 mM NaCl, or about 45 mM NaCl. According to some examples, the composition comprises about 40 mM NaCl. According to some examples, the composition further comprises about 20 mM to about 100 mM MgCl₂, for example about 20 mM to about 90 mM MgCl₂, about 20 mM to about 80 mM MgCl₂, about 20 mM to about 70 mM MgCl₂, about 20 mM to about 60 mM MgCl₂, about 20 mM to about 50 mM MgCl₂, about 20 mM to about 40 mM MgCl₂, about 20 mM to about 30 mM MgCl₂, about 30 mM to about 90 mM MgCl₂, about 30 mM to about 80 mM MgCl₂, about 30 mM to about 70 mM MgCl₂, about 30 mM to about 60 mM MgCl₂, about 30 mM to about 50 mM MgCl₂, about 30 mM to about 40 mM MgCl₂, about 40 mM to about 90 mM MgCl₂, about 40 mM to about 80 mM MgCl₂, about 40 mM to about 70 mM MgCl₂, about 40 mM to about 60 mM MgCl₂, about 40 mM to about 50 mM MgCl₂, about 50 mM to about 90 mM MgCl₂, about 50 mM to about 80 mM MgCl₂, about 50 mM to about 70 mM MgCl₂, about 50 mM to about 60 mM MgCl₂, about 60 mM to about 90 mM MgCl₂, about 60 mM to about 80 mM MgCl₂, about 60 mM to about 70 mM MgCl₂, about 70 mM to about 90 mM MgCl₂, about 70 mM to about 80 mM MgCl₂, or about 80 mM to about 90 mM MgCl₂. According to some examples of any of the aspects or examples herein, the ceDNA is closed-ended linear duplex DNA. According to some examples of any of the aspects or examples herein, the ceDNA comprises an expression cassette comprising a promoter sequence and a transgene. According to some examples, the ceDNA comprises expression cassette comprising a polyadenylation sequence. According to some examples of any of the aspects or examples herein, the ceDNA comprises at least one inverted terminal repeat (ITR) flanking either 5' or 3' end of said expression cassette. According to some examples, the expression cassette is flanked by two ITRs, wherein the two ITRs comprise one 5' ITR and one 3' ITR. According to some examples, the expression cassette is connected to an ITR at 3' end (3' ITR). According to some examples, the expression cassette is connected to an ITR at 5' end (5' ITR). According to some examples, at least one of 5' ITR and 3' ITR is a wild-type AAV ITR. According to some examples, at least one of 5' ITR and 3' ITR is a modified ITR. According to some examples, the ceDNA further comprises a spacer sequence between a 5' ITR and the expression cassette. According to some examples, the ceDNA further comprises a spacer sequence between a 3' ITR and the expression cassette. According to some examples, the spacer sequence is at least 5 base pairs long in length. According to some examples, the spacer sequence

is 5 to 100 base pairs long in length. According to some examples, the spacer sequence is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 base pairs long in length. According to some examples, the spacer sequence is 5 to 500 base pairs long in length. According to some examples, the spacer sequence is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 405, 410, 415, 420, 425, 430, 435, 440, 445, 450, 455, 460, 465, 470, 475, 480, 485, 490, or 495 base pairs long in length.

According to some examples of any of the aspects or examples herein, the ceDNA has a nick or a gap.

According to some examples, the ITR is an ITR derived from an AAV serotype, derived from an ITR of goose virus, derived from a B19 virus ITR, a wild-type ITR from a parvovirus. According to some examples, the AAV serotype is selected from the group comprising of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 and AAV12. According to some examples, the ITR is a mutant ITR, and the ceDNA optionally comprises an additional ITR which differs from the first ITR. According to some examples, the ceDNA comprises two mutant ITRs in both 5' and 3' ends of the expression cassette, optionally wherein the two mutant ITRs are symmetric mutants. According to some examples of any of the aspects or examples herein, the ceDNA is a CELiD, DNA-based minicircle, a MIDGE, a minitering 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. According to some examples of any of the aspects or examples herein, the pharmaceutical composition further comprises a pharmaceutically acceptable excipient.

According to some aspects, the disclosure provides a method of treating a genetic disorder in a subject, the method comprising administering to the subject an effective amount of the pharmaceutical composition according to any of the aspects or examples herein. According to some examples, the subject is a human. According to some examples, the genetic disorder is selected from the group consisting of sickle-cell anemia, melanoma, hemophilia A (clotting factor VIII (FVIII) deficiency) and hemophilia B (clotting factor IX (FIX) deficiency), cystic fibrosis (CFTR), familial hypercholesterolemia (LDL receptor defect), hepatoblastoma, Wilson's disease, phenylketonuria (PKU), congenital hepatic porphyria, inherited disorders of hepatic metabolism, Lesch Nyhan syndrome, sickle cell anemia, thalassaemias, xeroderma pigmentosum, Fanconi's anemia, retinitis pigmentosa, ataxia telangiectasia, Bloom's 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, Mucopolysaccharidosis 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),

5 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

10 (GACI), Leber Congenital Amaurosis, Stargardt macular dystrophy (ABCA4), ornithine transcarbamylase (OTC) deficiency, Usher syndrome, alpha-1 antitrypsin deficiency, and Cathepsin A deficiency.

According to some examples, the genetic disorder is Leber congenital amaurosis (LCA). According to some examples, the LCA is LCA10. According to some examples, the genetic disorder is Niemann-Pick disease. According to some examples, the genetic disorder is Stargardt macular dystrophy. According to

15 some examples, the genetic disorder is glucose-6-phosphatase (G6Pase) deficiency (glycogen storage disease type I) or Pompe disease (glycogen storage disease type II). According to some examples, the genetic disorder is hemophilia A (Factor VIII deficiency). According to some examples, the genetic disorder is hemophilia B (Factor IX deficiency). According to some examples, the genetic disorder is Hunter syndrome (Mucopolysaccharidosis II). According to some examples, the genetic disorder is cystic

20 fibrosis. According to some examples, the genetic disorder is dystrophic epidermolysis bullosa (DEB). According to some examples, the genetic disorder is phenylketonuria (PKU). According to some examples, the genetic disorder is hyaluronidase deficiency. According to some examples of any of the aspects or examples herein, the method further comprises administering an immunosuppressant.

According to some examples, the immunosuppressant is dexamethasone. According to some examples of

25 any of the aspects or examples herein, 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. According to some examples, the immune response is measured by detecting the levels of a pro-inflammatory cytokine or

30 chemokine. According to some examples, the pro-inflammatory cytokine or chemokine is selected from the group consisting of IL-6, IFN α , IFN γ , IL-18, TNF α , IP-10, MCP-1, MIP1 α , MIP1 β , and RANTES. According to some examples, at least one of the pro-inflammatory cytokines is under a detectable level in serum of the subject at 6 hours after the administration of the pharmaceutical composition. According to some examples of any of the aspects or examples herein, the LNP comprising the SS-cleavable lipid and

35 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. According to some examples, the SS-cleavable lipid is ss-OP of Formula I. According to some examples, the LNP further comprises cholesterol and a PEG-lipid conjugate. According to some examples, the LNP further comprises a noncationic lipid. According to some examples, the noncationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE). According to some examples, the LNP further comprises N-Acetylgalactosamine (GalNAc). According to some examples, the GalNAc is present in the LNP at a molar percentage of 0.5% of the total lipid.

According to another example, the disclosure provides a method of mitigating a complement response in a subject in need of treatment with a therapeutic nucleic acid, the method comprising administering to the subject an effective amount of a lipid nanoparticle LNP comprising therapeutic nucleic acid, ss-cleavable lipid, sterol, and polyethylene glycol (PEG) and N-Acetylgalactosamine (GalNAc). According to some examples, the subject is suffering from a genetic disorder. According to some examples, the genetic disorder is selected from the group consisting of sickle-cell anemia, melanoma, hemophilia A (clotting factor VIII (FVIII) deficiency) and hemophilia B (clotting factor IX (FIX) deficiency), cystic fibrosis (CFTR), familial hypercholesterolemia (LDL receptor defect), hepatoblastoma, Wilson's disease, phenylketonuria (PKU), congenital hepatic porphyria, inherited disorders of hepatic metabolism, Lesch Nyhan syndrome, sickle cell anemia, thalassaemias, xeroderma pigmentosum, Fanconi's anemia, retinitis pigmentosa, ataxia telangiectasia, Bloom's 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, Mucopolipidosis 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), ornithine transcarbamylase

(OTC) deficiency, Usher syndrome, alpha-1 antitrypsin deficiency, and Cathepsin A deficiency.

According to some examples, the therapeutic nucleic acid is selected from the group consisting of minigenes, plasmids, minicircles, small interfering RNA (siRNA), microRNA (miRNA), antisense oligonucleotides (ASO), ribozymes, ceDNA, ministring, doggybone™, protelomere closed ended DNA, or dumbbell linear DNA, dicer-substrate dsRNA, small hairpin RNA (shRNA), asymmetrical interfering RNA (aiRNA), microRNA (miRNA), mRNA, tRNA, rRNA, DNA viral vectors, viral RNA vector, non-viral vector and any combination thereof. According to some examples, the ceDNA is selected from the group consisting of a CELiD, 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, wherein the ceDNA is capsid free and linear duplex DNA. According to some examples, the PEG is 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG).

According to some examples, the PEG is present in the LNP at a molecular percentage of about 2% to 4%, *e.g.*, about 2% to about 3.5%, about 2% to about 3%, about 2% to about 2.5%, about 2.5% to about 4%, about 2.5% to about 3.5%, about 2.5% to about 3%, about 3% to about 4%, about 3.5% to about 4%, or about 2%, about 2.25%, about 2.5%, about 2.75%, about 3%, about 3.25%, about 3.5%, about 3.75%, or about 4%. According to some examples, the PEG is present in the LNP at a molecular percentage of about 3%. According to some examples, the LNP further comprises a non-cationic lipid. According to some examples, the non-cationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE).

According to some examples, the GalNAc is present in the LNP at a molar percentage of about 0.3 to 1% of the total lipid, *e.g.*, about 0.3% to about 0.9%, about 0.3% to about 0.8%, about 0.3% to about 0.7%, about 0.3% to about 0.6%, about 0.3% to about 0.5%, about 0.3% to about 0.4%, about 0.4% to about 0.9%, about 0.4% to about 0.8%, about 0.4% to about 0.7%, about 0.4% to about 0.6%, about 0.4% to about 0.5%, about 0.5% to about 0.9%, about 0.5% to about 0.8%, about 0.5% to about 0.7%, about 0.5% to about 0.6%, about 0.6% to about 0.9%, about 0.6% to about 0.8%, about 0.6% to about 0.7%, about 0.7% to about 0.9%, about 0.7% to about 0.8%, about 0.8% to about 0.9% of the total lipid, or about 0.3%, about 0.4, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, or about 1% of the total lipid. According to some examples, the GalNAc is present in the LNP at a molar percentage of about 0.5% of the total lipid.

BRIEF DESCRIPTION OF THE DRAWINGS

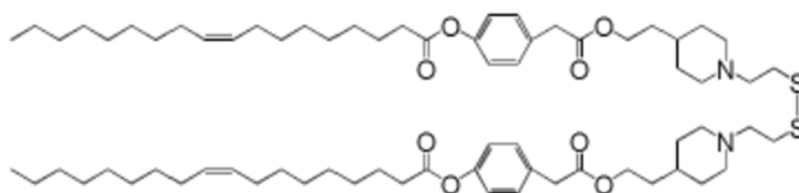
Embodiments of the present disclosure, briefly summarized above and discussed in greater detail below, can be understood by reference to the illustrative embodiments of the disclosure depicted in the appended drawings. However, the appended drawings illustrate only typical embodiments of the disclosure and are therefore not to be considered limiting of scope, for the disclosure may admit to other equally effective embodiments.

CLAIMS

What is Claimed is:

1. A pharmaceutical composition comprising lipid nanoparticle (LNP), wherein the LNP
5 comprises a SS-cleavable lipid and a closed-ended DNA (ceDNA).
2. The pharmaceutical composition of claim 1, wherein the SS-cleavable lipid comprises a disulfide bond and a tertiary amine.
- 10 3. The pharmaceutical composition of any one of the previous claims, wherein the SS-cleavable lipid comprises an ss-OP lipid of Formula I:

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4. The pharmaceutical composition of any one of the previous claims, wherein the LNP
20 further comprises a sterol, a polyethylene glycol (PEG) or PEG-lipid conjugate, and/or a non-cationic lipid.
5. The pharmaceutical composition of claim 4, wherein the sterol is a cholesterol or β -sitosterol;
- 25 the PEG or PEG-lipid conjugate is 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG) or 1,2-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), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyl-oleoylphosphatidylcholine (POPC), palmitoyl-oleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE),
35 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-phosphatidylethanolamine (SOPE), hydrogenated soy phosphatidylcholine (HSPC), egg phosphatidylcholine (EPC), dioleoylphosphatidylserine (DOPS), sphingomyelin (SM),
5 dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), distearoylphosphatidylglycerol (DSPG), dierycophosphatidylcholine (DEPC), palmitoyloleoylphosphatidylglycerol (POPG), dielaidoyl-phosphatidylethanolamine (DEPE), 1,2-dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE); 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPHyPE); lecithin, phosphatidylethanolamine, lysolecithin,
10 lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidic acid, cerebrosides, dicetylphosphate, lysophosphatidylcholine, dilinoleoylphosphatidylcholine, or mixtures thereof.

15 6. The pharmaceutical composition of claim 4, wherein:
the PEG or PEG-lipid conjugate is present at 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%.

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7. The pharmaceutical composition of claim 4, 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 1.5% to 3%;
25 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%.

8. The pharmaceutical composition of claim 7, wherein the cholesterol is present at a molar percentage of about 40%, the SS-cleavable lipid is present at a molar percentage of about
30 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 about 3%.

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

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10. The pharmaceutical composition of claim 1, wherein the LNP is less than about 70 nm in size.
- 5 11. 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.
12. The pharmaceutical composition of any one of the previous claims, wherein the composition further comprises N-Acetylgalactosamine (GalNAc).
- 10 13. The pharmaceutical composition of any one of the previous claims, wherein the ceDNA is closed-ended linear duplex DNA.
14. The pharmaceutical composition of any one of the previous claims, wherein the
15 ceDNA comprises an expression cassette comprising a promoter sequence and a transgene.
15. The pharmaceutical composition of claim 14, wherein:
the ceDNA comprises at least one inverted terminal repeat (ITR) flanking either 5' or 3' end
of said expression cassette.
- 20 16. The pharmaceutical composition of claim 15, wherein said expression cassette is flanked by two ITRs, wherein the two ITRs comprise one 5' ITR and one 3' ITR.
17. The pharmaceutical composition of claim 15, wherein:
25 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.
18. The pharmaceutical composition of claim 15, wherein at least one ITR is an ITR
30 derived from an AAV serotype, derived from an ITR of goose virus, derived from a B19 virus ITR, or
a wild-type ITR from a parvovirus.
19. The pharmaceutical composition of any one of the previous claims, wherein the
ceDNA is a CELiD, DNA-based minicircle, a MIDGE, a minitering DNA, a dumbbell shaped linear
35 duplex closed-ended DNA comprising two hairpin structures of ITRs in the 5' and 3' ends of an
expression cassette, or a doggybone™ DNA.

20. 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.
- 5 21. The use of claim 20, wherein the genetic disorder is selected from the group consisting of sickle-cell anemia, melanoma, hemophilia A (clotting factor VIII (FVIII) deficiency) and hemophilia B (clotting factor IX (FIX) deficiency), cystic fibrosis (CFTR), familial hypercholesterolemia (LDL receptor defect), hepatoblastoma, Wilson's disease, phenylketonuria (PKU), congenital hepatic porphyria, inherited disorders of hepatic metabolism, Lesch Nyhan
10 syndrome, sickle cell anemia, thalassaemias, xeroderma pigmentosum, Fanconi's anemia, retinitis pigmentosa, ataxia telangiectasia, Bloom's 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
15 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, Mucopolipidosis 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,
20 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),
25 dystrophic epidermolysis bullosa (DEB), ectonucleotide pyrophosphatase 1 deficiency, generalized arterial calcification of infancy (GACI), Leber Congenital Amaurosis, Stargardt macular dystrophy (ABCA4), ornithine transcarbamylase (OTC) deficiency, Usher syndrome, alpha-1 antitrypsin deficiency, and Cathepsin A deficiency.
- 30 22. The use of claim 21, wherein the genetic disorder is Leber congenital amaurosis (LCA).
23. The use of claim 21, wherein the genetic disorder is Stargardt macular dystrophy.
- 35 24. The use of claim 21, wherein the genetic disorder is hemophilia A (Factor VIII deficiency).

25. The use of claim 21, wherein the genetic disorder is dystrophic epidermolysis bullosa (DEB).

5 26. The use of claim 20 or 21, further comprising that an immunosuppressant is to be administered.

27. The use of any one of claims 20, 21 and 26, 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.

10 28. The use of any one of claims 20, 21, 26, and 27, 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.

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