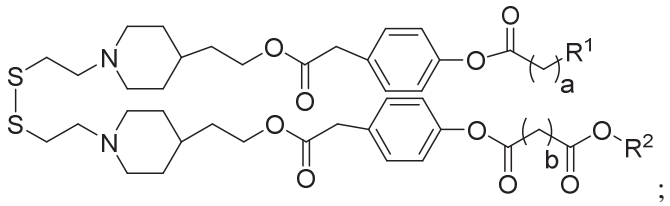
Some widely used ionizable lipids are CLinDMA, DLinDMA (also known as DODAP), and cationic lipid such as DOTAP. Of note, these lipids have been employed for siRNA delivery to liver but suffer from non-optimal delivery efficiency along with liver toxicity at higher doses. In view of the shortcomings of the current cationic lipids, there is a need in the field to provide lipid scaffolds that not only demonstrate enhanced efficacy along with reduced toxicity, but with improved pharmacokinetics and intracellular kinetics such as cellular uptake and nucleic acid release from the lipid carrier.

## SUMMARY

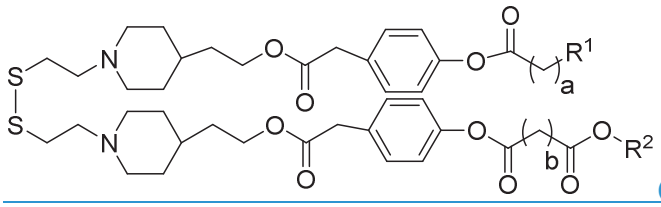
In one ~~aspect~~example, provided herein are ionizable lipids having the Formula (**I**):



;

as well as pharmaceutically acceptable salts thereof, wherein $R^1$, $R^2$, a, and b are as defined herein.

In one aspect, the present disclosure refers to a lipid having the Formula (**I**):



(**I**);

or a pharmaceutically acceptable salt thereof, wherein:

a is an integer ranging from 1 to 20;

b is an integer ranging from 2 to 10;

$R^1$ is absent or is selected from the group consisting of ($C_2$-$C_{20}$)alkenyl, -C(O)O($C_2$-$C_{20}$)alkyl, and cyclopropyl substituted with ($C_2$-$C_{20}$)alkyl; and

$R^2$ is ($C_2$-$C_{20}$)alkyl.

Also provided are pharmaceutical compositions comprising a disclosed ionizable lipid, or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier.

In another aspect, the present disclosure refers to a lipid nanoparticle (LNP) comprising the lipid as disclosed herein, or a pharmaceutically acceptable salt thereof; and a nucleic acid.

Another ~~aspect~~ example of the present disclosure relates to a composition comprising a lipid nanoparticle (LNP) comprising an ionizable lipid described herein, or a pharmaceutically acceptable salt thereof, and a nucleic acid. In one ~~embodiment~~example of any of the aspects or ~~embodiment~~examples herein, the nucleic acid is encapsulated in the ionizable lipid. In a particular ~~embodiment~~example, the nucleic acid is a closed-ended DNA (ceDNA).

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the LNP further comprises a sterol. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the sterol can be a cholesterol, or beta-sitosterol.

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, 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 ionizable 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, 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 ionizable 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the cholesterol is present at a molar percentage of about 40%, and wherein the ionizable lipid is present at a molar percentage of about 50%.

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the composition further comprises a cholesterol, a PEG-lipid conjugate, and a non-cationic lipid. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, 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 20% to about 40%, about 40% to about 50%, or about 45% to about 50%. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the LNP further comprises a polyethylene glycol (PEG)-lipid. According to some ~~embodiment~~examples

of any of the aspects or ~~embodiment~~examples herein, the PEG-lipid is l-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG). According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the LNP further comprises a non-cationic lipid. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, 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-phosphoethanolamine (DPHyPE); lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidicacid,cerebrosides, dicetylphosphate, lysophosphatidylcholine, dilinoleoylphosphatidylcholine, or mixtures thereof. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the non-cationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE).

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the PEG-lipid conjugate is present at about 1.5% to about 4%, for example about 1.5% to about 3%, about 2% to about 3%, about 2.5% to about 3%, 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples

herein, the 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the ionizable lipid is present at a molar percentage of about 42.5% to about 62.5%. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the ionizable 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the non-cationic lipid is present at a molar percentage of about 2.5% to about 12.5%. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the cholesterol is present at a molar percentage of about 40%, the ionizable 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-lipid is present at about 3%.

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the LNP composition further comprises dexamethasone palmitate.

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, 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 ~~embodiment~~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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the LNP composition has a total lipid to nucleic acid ratio of about 10:1. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the LNP composition has a total lipid to nucleic acid ratio of about 20:1. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the composition has a total lipid to nucleic acid ratio of about 30:1. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the composition has a total lipid to nucleic acid ratio of about 40:1. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the composition has a total lipid to nucleic acid ratio of about 50:1.

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the LNP further comprises a tissue targeting moiety. The tissue targeting moiety can be a peptide, oligosaccharide or the like, which can be used for the delivery of the LNP to one or more specific tissues such as cancer, the liver, the CNS, or the muscle. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the tissue targeting moiety is linked to the PEG-lipid conjugate. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the tissue targeting moiety is a ligand for liver specific receptors. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the ligand of liver specific receptors used for liver targeting is an oligosaccharide such as N-Acetylgalactosamine (GalNAc).

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the GalNAc-linked GalNAc-linked PEG-lipid conjugate is present in the lipid nanoparticle at a molar percentage of 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1%. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the GalNAc-linked PEG-lipid conjugate is present in the LNP at a molar percentage of 0.2%. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the GalNAc-linked PEG-lipid conjugate is present in the LNP at a molar percentage of 0.3%. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the GalNAc-linked PEG-lipid conjugate is present in the LNP at a molar percentage of 0.4%. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the GalNAc-linked PEG-lipid conjugate is present in the LNP at a molar percentage of 0.5%. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the GalNAc-linked PEG-lipid conjugate is present in the LNP at a molar percentage of 0.6%. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the GalNAc- linked PEG-lipid conjugate is present in the LNP at a molar percentage of 0.7%. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, GalNAc-linked PEG-lipid conjugate is present in the LNP at a molar percentage of 0.8%. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the GalNAc-linked PEG-lipid conjugate is present in the LNP at a molar percentage of 0.9%. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the GalNAc-linked PEG-lipid conjugate is present in the LNP at a molar percentage of 1.0%. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the GalNAc-linked PEG-lipid conjugate is present in the LNP at a molar percentage of about 1.5%. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the GalNAc-linked PEG-lipid conjugate is present in the LNP at a molar percentage of 2.0%.

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the LNP composition is prepared in a buffer such as malic acid. In some ~~embodiment~~examples of any of the aspects and ~~embodiment~~examples herein, the composition is prepared in 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the composition is prepared in 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the composition comprises about 20 mM malic acid.

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the LNP composition is prepared in a solution having 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the LNP composition is prepared in a solution having about 30 mM NaCl, about 35 mM NaCl, about 40 mM NaCl, or about 45 mM NaCl. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the LNP composition is prepared in a solution having about 40 mM NaCl.

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the LNP composition is prepared in a solution having about 20 mM to about 100 mM $MgCl_2$, for example about 20 mM to about 90 mM $MgCl_2$, about 20 mM to about 80 mM $MgCl_2$, about 20 mM to about 70 mM $MgCl_2$, about 20 mM to about 60 mM $MgCl_2$, about 20 mM to about 50 mM $MgCl_2$, about 20 mM to about 40 mM $MgCl_2$, about 20 mM to about 30 mM $MgCl_2$, about 320 mM to about 90 mM $MgCl_2$, about 30 mM to about 80 mM $MgCl_2$, about 30 mM to about 70 mM $MgCl_2$, about 30 mM to about 60 mM $MgCl_2$, about 30 mM to about 50 mM $MgCl_2$, about 30 mM to about 40 mM $MgCl_2$, about 40 mM to about 90 mM $MgCl_2$, about 40 mM to about 80 mM $MgCl_2$, about 40 mM to about 70 mM $MgCl_2$, about 40 mM to about 60 mM $MgCl_2$, about 40 mM to about 50 mM $MgCl_2$, about 50 mM to about 90 mM $MgCl_2$, about 50 mM to about 80 mM $MgCl_2$, about 50 mM to about 70 mM $MgCl_2$, about 50 mM to about 60 mM $MgCl_2$, about 60 mM to about 90 mM $MgCl_2$, about 60 mM to about 80 mM $MgCl_2$, about 60 mM to about 70 mM $MgCl_2$, about 70 mM to about 90 mM $MgCl_2$, about 70 mM to about 80 mM $MgCl_2$, or about 80 mM to about 90 mM $MgCl_2$.

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the ceDNA is closed-ended linear duplex DNA. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the ceDNA comprises an expression cassette comprising a promoter sequence and a transgene.

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the ceDNA comprises expression cassette comprising a polyadenylation sequence.

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the expression cassette is flanked by two ITRs, wherein the two ITRs comprise one 5' ITR and one 3' ITR. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the expression cassette is connected to an ITR at 3' end (3' ITR). According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the expression cassette is connected to an ITR at 5' end (5' ITR). According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, at least one of 5' ITR and 3' ITR is a wild-type AAV ITR. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, at least one of 5' ITR and 3' ITR is a modified ITR. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the ceDNA further comprises a spacer sequence between a 5' ITR and the expression cassette.

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the ceDNA further comprises a spacer sequence between a 3' ITR and the expression cassette. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the spacer sequence is at least 5 base pairs long in length. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the spacer sequence is 5 to 100 base pairs long in length. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the spacer sequence is 5 to 500 base pairs long in length. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the ceDNA has a nick or a gap.

According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the ITR is a mutant ITR, and the ceDNA optionally comprises an additional ITR which differs from the first ITR. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the ceDNA is a CELiD, DNA-based minicircle, a MIDGE, a ministering 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the pharmaceutical composition further comprises a pharmaceutically acceptable excipient.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising the lipid as disclosed herein or the lipid nanoparticle as disclosed herein and a pharmaceutically acceptable excipient. In yet another aspect, the present disclosure refers to the use of the lipid nanoparticle as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a genetic disorder in a subject in need thereof.

According to some ~~aspects~~examples, 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 ~~embodiment~~examples herein. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the subject is a human. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, 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 disease, phenylketonuria (PKU), congenital hepatic porphyria,

inherited disorders of hepatic metabolism, Lesch Nyhan syndrome, sickle cell anemia, thalassemia, 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, 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), ornithine transcarbamylase (OTC) deficiency, Usher syndrome, alpha-1 antitrypsin deficiency, progressive familial intrahepatic cholestasis (PFIC) type I (ATP8B1 deficiency), type II (ABCB11), type III (ABCB4), or type IV (TJP2) and Cathepsin A deficiency. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is Leber congenital amaurosis (LCA). According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the LCA is LCA10. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is Niemann-Pick disease. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is Stargardt macular dystrophy. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, 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 ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is hemophilia A (Factor VIII deficiency). According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is hemophilia B (Factor IX deficiency). According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is hunter syndrome (Mucopolysaccharidosis II). According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is cystic fibrosis. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is dystrophic epidermolysis bullosa (DEB). According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is phenylketonuria (PKU). According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is progressive familial intrahepatic cholestasis (PFIC). According to some ~~embodiment~~examples of any of the aspects or

~~embodiment~~examples herein, the genetic disorder is Wilson disease. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is Gaucher disease Type I, II or III. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is age related macular degeneration. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is ornithine transcarbamylase deficiency. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is retinitis pigmentosa (RP1). According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is Usher syndrome. According to some ~~embodiment~~examples of any of the aspects or ~~embodiment~~examples herein, the genetic disorder is Lysosomal Acid Lipase (LAL) deficiency.

## 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.

**FIG. 1** shows the improvements in ceDNA-luc expression achieved by employing disclosed lipid nanoparticles (*e.g.,* LNP 5 comprising Lipid 1 and LNP 6 comprising Lipid 3) compared to SS-OP (*e.g.*, LNPs 1, 2, and 7-12) as observed in Study A.

**FIG. 2** shows the improvements in ceDNA-luc expression achieved by employing disclosed lipid nanoparticles (*e.g.,* LNP 16 comprising Lipid 2, LNP 17 comprising Lipid 1, and LNP 18 comprising Lipid 3) compared to SS-OP (*i.e.*, LNP 13), as observed in Study B.

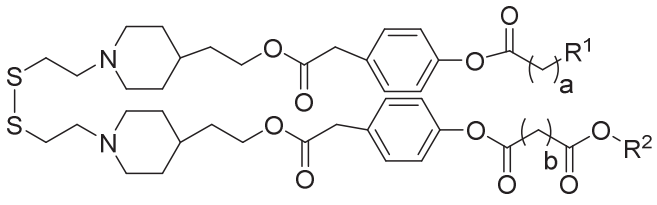
**FIG. 3** shows the improvements in responsiveness to increased dosage levels, whereby an increased dosage administered to the mice leads to a greater increase in ceDNA-luc expression, achieved by employing disclosed lipid nanoparticles (*e.g.,* LNP 20 comprising Lipid 1) compared to SS-OP (*i.e.*, LNP 19), as observed in Study C.

**FIG. 4A** shows the improvements in ceDNA-luc expression achieved by employing disclosed lipid nanoparticles (*e.g.,* LNP 24 comprising Lipid 6, LNP 25 comprising Lipid 7, and LNP 26 comprising Lipid 8) compared to SS-OP (*i.e.*, LNP 23), as observed in Study D. **FIG. 4B** shows the improvements in tolerability (as measured by change in body weight) in mice by employing disclosed lipid nanoparticles (*e.g.,* LNP 24 comprising Lipid 6, LNP 25 comprising Lipid 7, and LNP 26 comprising Lipid 8) compared to Ionizable Lipid A (*i.e.*, LNP 22) being used as control.

## CLAIMS

*What is Claimed is:*

1. A lipid having the Formula (**I**):



(**I**);

or a pharmaceutically acceptable salt thereof, wherein:

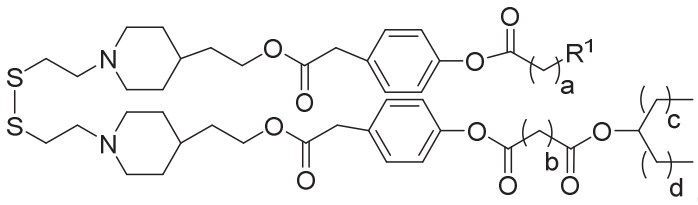
a is an integer ranging from 1 to 20;

b is an integer ranging from 2 to 10;

$R^1$ is absent or is selected from the group consisting of ($C_2$-$C_{20}$)alkenyl, -C(O)O($C_2$-$C_{20}$)alkyl, and cyclopropyl substituted with ($C_2$-$C_{20}$)alkyl; and

$R^2$ is ($C_2$-$C_{20}$)alkyl.

2. The lipid of claim 1, wherein the lipid is of the Formula (**II**):



(**II**);

or a pharmaceutically acceptable salt thereof, wherein c and d are each independently integers ranging from 1 to 8.

3. The lipid of claim 2, or a pharmaceutically acceptable salt thereof, wherein c and d are each independently integers ranging from 2 to 8, or from 4 to 8, or from 6 to 8.

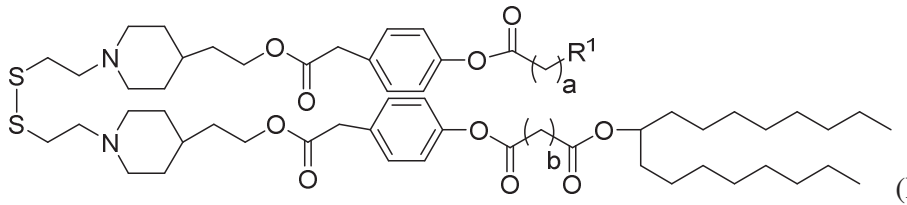
~~4. The lipid of claim 2 or 3, or a pharmaceutically acceptable salt thereof, wherein c and d are each independently integers ranging from 4 to 8.~~

~~5. The lipid of any one of claims 2 to 4, or a pharmaceutically acceptable salt thereof, wherein c and d are each independently integers ranging from 6 to 8.~~

46. The lipid of claim 2, or a pharmaceutically acceptable salt thereof, wherein c and d are each independently 1, 3, 5, or 7.

57. The lipid of any one of claims 2 to 64, or a pharmaceutically acceptable salt thereof, wherein at least one of c and d is 7.

68. The lipid of any one of claims 1 to 57, wherein the lipid is of the Formula (**III**):




(**III**);

or a pharmaceutically acceptable salt thereof.

79. The lipid of any one of claims 1 to 68, or a pharmaceutically acceptable salt thereof, wherein b is an integer ranging from 3 to 9, or from 5 to 7.

~~10. The lipid of any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, wherein b is an integer ranging from 5 to 7.~~

811. The lipid of any one of claims 1 to 710, or a pharmaceutically acceptable salt thereof, wherein b is 5 or 7.

912. The lipid of any one of claims 1 to 811, or a pharmaceutically acceptable salt thereof, wherein a is an integer ranging from 2 to 18, or from 3 to 17, or from 6 to 18, or from 4 to 12, or from 2 to 5, or from 6 to 8, or from 16 to 18, or from 9 to 11.

~~13. The lipid of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, wherein a is an integer ranging from 3 to 17.~~

~~14. The lipid of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, wherein a is an integer ranging from 6 to 18.~~

~~15. The lipid of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, wherein a is an integer ranging from 4 to 12.~~

~~16. The lipid of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, wherein a is an integer ranging from 2 to 5.~~

10~~17~~. The lipid of claim 9~~16~~, or a pharmaceutically acceptable salt thereof, wherein a is 3, 7, 8, 10 or 17.

~~18. The lipid of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, wherein a is an integer ranging from 6 to 8.~~

~~19. The lipid of claim 18, or a pharmaceutically acceptable salt thereof, wherein a is 7.~~

~~20. The lipid of claim 18, or a pharmaceutically acceptable salt thereof, wherein a is 8.~~

~~21. The lipid of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, wherein a is an integer ranging from 16 to 18.~~

~~22. The lipid of claim 21, or a pharmaceutically acceptable salt thereof, wherein a is 17.~~

~~23. The lipid of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, wherein a is an integer ranging from 9 to 11.~~

~~24. The lipid of claim 23, or a pharmaceutically acceptable salt thereof, wherein a is 10.~~

11~~25~~. The lipid of any one of claims 1 to 10~~24~~, or a pharmaceutically acceptable salt thereof, wherein $R^1$ is absent or is selected from the group consisting of $(C_5\text{-}C_{15})$alkenyl, -C(O)O$(C_4\text{-}C_{18})$alkyl, and cyclopropyl substituted with $(C_4\text{-}C_{16})$alkyl.

12~~26~~. The lipid of any one of claims 1 to 11~~25~~, or a pharmaceutically acceptable salt thereof, wherein $R^1$ is absent or is selected from the group consisting of ($C_5$-$C_{12}$)alkenyl, -C(O)O($C_4$-$C_{12}$)alkyl, and cyclopropyl substituted with ($C_4$-$C_{12}$)alkyl.

13~~27~~. The lipid of any one of claims 1 to 12~~26~~, or a pharmaceutically acceptable salt thereof, wherein $R^1$ is absent or is selected from the group consisting of ($C_5$-$C_{10}$)alkenyl, -C(O)O($C_4$-$C_{10}$)alkyl, and cyclopropyl substituted with ($C_4$-$C_{10}$)alkyl.

14~~28~~. The lipid of any one of claims 1 to 13~~27~~, or a pharmaceutically acceptable salt thereof, wherein $R^1$ is $C_{10}$ alkenyl.

15~~29~~. The lipid of any one of claims 1 to 13~~27~~, or a pharmaceutically acceptable salt thereof, wherein the alkyl in -C(O)O($C_2$-$C_{20}$)alkyl, -C(O)O($C_4$-$C_{18}$)alkyl, -C(O)O($C_4$-$C_{12}$)alkyl, or -C(O)O($C_4$-$C_{10}$)alkyl ~~for $R^1$~~ is an unbranched alkyl.

16~~30~~. The lipid of claim 15~~29~~, or a pharmaceutically acceptable salt thereof, wherein $R^1$ is -C(O)O($C_9$ alkyl).

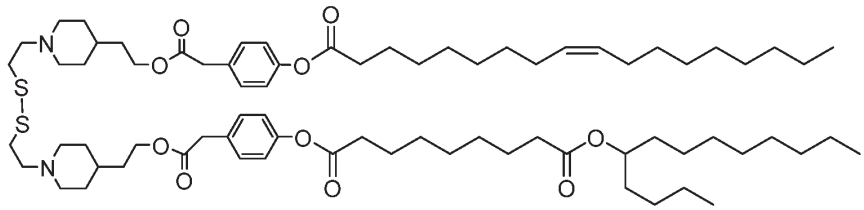
17~~31~~. The lipid of any one of claims 11~~25~~ to 13~~27~~, or a pharmaceutically acceptable salt thereof, wherein the alkyl in -C(O)O($C_4$-$C_{18}$)alkyl, -C(O)O($C_4$-$C_{12}$)alkyl, or -C(O)O($C_4$-$C_{10}$)alkyl is a branched alkyl.

18~~32~~. The lipid of claim 17~~-31~~, or a pharmaceutically acceptable salt thereof, wherein $R^1$ is -C(O)O($C_{17}$ alkyl).

19~~33~~. The lipid of any one of claims 1 to 10~~24~~, or a pharmaceutically acceptable salt thereof, wherein $R^1$ is selected from the group consisting of any group listed in Table 1; and/or wherein $R^2$ is selected from the group consisting of any group listed in Table 2.

~~34. The lipid of claim 1, or a pharmaceutically acceptable salt thereof, wherein $R^2$ is selected from any group listed in Table 2.~~

2035. The lipid of claim 1, wherein the lipid is selected from the group consisting of any lipid listed in Table 3, or a pharmaceutically acceptable salt thereof; or wherein the lipid is Lipid 7:



**Lipid 7**

1-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-(oleoyloxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) 9-(tridecan-5-yl) nonanedioate, or a pharmaceutically acceptable salt thereof.

2136. A lipid nanoparticle (LNP) comprising the lipid of any one of claims 1 to 2035, or a pharmaceutically acceptable salt thereof; and a nucleic acid.

~~37. The lipid nanoparticle of claim 36, wherein the nucleic acid is encapsulated in the lipid.~~

2238. The lipid nanoparticle of claim 21 ~~36 or claim 37~~, wherein the 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.

~~39. The lipid nanoparticle of claim 38, wherein the nucleic acid is a closed-ended DNA (ceDNA).~~

2340. The lipid nanoparticle of claim 21 or 22~~any one of claims 36 to 39~~, further comprising at least one lipid selected from the group consisting of a sterol, a PEG-lipid conjugate, and a non-cationic lipid.

41. The lipid nanoparticle of claim 40, wherein the sterol is a cholesterol or beta-sitosterol.

42. The lipid nanoparticle of any one of claims 36 to 41, further comprising a PEG-lipid conjugate.

43. The lipid nanoparticle of claim 42, wherein the PEG-lipid conjugate is 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG).

44. The lipid nanoparticle of any one of claims 36 to 43, further comprising a non-cationic lipid.

45. The lipid nanoparticle of claim 44, wherein 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-phosphoethanolamine (DPHyPE); lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidicacid,cerebrosides,

dicetylphosphate, lysophosphatidylcholine, dilinoleoylphosphatidylcholine, and mixtures thereof.

46. The lipid nanoparticle of claim 45, wherein the non-cationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl phosphatidylethanolamine (DOPE).

47. The lipid nanoparticle of claim 46, wherein the PEG-lipid conjugate is present at a molar percentage of about 1.5% to about 4%.

48. The lipid nanoparticle of claim 47, wherein the PEG-lipid conjugate is present at a molar percentage of about 2% to about 3%.

49. The lipid nanoparticle of claim 48, wherein the PEG-lipid conjugate is present at a molar percentage of about 2.5 to about 3%.

50. The lipid nanoparticle of claim 49, wherein the PEG-lipid conjugate is present at a molar percentage of about 3%.

51. The lipid nanoparticle of any one of claims 42 to 50, wherein the PEG-lipid conjugate isDMG-PEG.

52. The lipid nanoparticle of any one of claims 36 to 51, wherein the cholesterol or beta-sitosterol is present at a molar percentage of about 20% to about 40%, and wherein the lipid is present at a molar percentage of about 80% to about 60%.

53. The lipid nanoparticle of claim 52, wherein the cholesterol or beta-sitosterol is present at a molar percentage of about 40%, and wherein the lipid is present at a molar percentage of about 50%.

54. The lipid nanoparticle of any one of claims 36 to 39, further comprising a cholesterol, PEG-lipid conjugate, and a non-cationic lipid.

~~55. The lipid nanoparticle of claim 54, wherein the PEG-lipid conjugate is present at about 1.5% to about 4%.~~

~~56. The lipid nanoparticle of claim 55, wherein the PEG-lipid conjugate is present at about 2% to about 3%.~~

~~57. The lipid nanoparticle of claim 56, wherein the PEG-lipid conjugate is present at about 2.5 to about 3%.~~

~~58. The lipid nanoparticle of claim 57, wherein the PEG-lipid conjugate is present at about 3%.~~

~~59. The lipid nanoparticle of any one of claims 42 to 47, wherein the cholesterol is present at a molar percentage of about 30% to about 50%.~~

~~60. The lipid nanoparticle of any one of claims 54 to 60, wherein the PEG-lipid conjugate is DMG-PEG2000.~~

~~61. The lipid nanoparticle of any one of claims 53 to 60, wherein the lipid is present at a molar percentage of about 42.5% to about 62.5%.~~

~~62. The lipid nanoparticle of any one of claims 53 to 60, wherein the non-cationic lipid is present at a molar percentage of about 2.5% to about 12.5%.~~

~~63. The lipid nanoparticle of any one of claims 53 to 60, wherein the cholesterol is present at a molar percentage of about 40%, the 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-lipid conjugate is present at about 3%.~~

~~64. The lipid nanoparticle of any one of claims 36 to 63, further comprising dexamethasone palmitate.~~

24~~65~~. The lipid nanoparticle of any one of claims 21~~36~~ to 23~~64~~, wherein the nanoparticle has a diameter ranging from about 50 nm to about 110 nm, or wherein the

nanoparticle is less than about 100 nm in size, less than about 70 nm in size or less than about 60 nm in size.

~~66. The lipid nanoparticle of any one of claims 36 to 64, wherein the nanoparticle is less than about 100 nm in size.~~

~~67. The lipid nanoparticle of claim 66, wherein the particle is less than about 70 nm in size.~~

~~68. The lipid nanoparticle of claim 67, wherein the particle is less than about 60 nm in size.~~

~~69. The lipid nanoparticle of claim 39, wherein the particle has a total lipid to ceDNA ratio of about 10:1.~~

~~70. The lipid nanoparticle of claim 39, wherein the particle has a total lipid to ceDNA ratio of about 20:1.~~

~~71. The lipid nanoparticle of claim 39, wherein the particle has a total lipid to ceDNA ratio of about 30:1.~~

~~72. The lipid nanoparticle of claim 39, wherein the particle has a total lipid to ceDNA ratio of about 40:1.~~

25~~73~~. The lipid nanoparticle of any one of claims 21~~36~~ to 24~~72~~, further comprising a tissue specific targeting moiety.

26~~74~~. The lipid nanoparticle of claim 25~~73~~, wherein the tissue specific targeting moiety is N-acetylgalactosamine (GalNAc); wherein ~~GalNac~~ GalNAc is linked to ~~the~~a second PEG-lipid conjugate to form a GalNAc-linked PEG-lipid conjugate; and the ~~GalNac~~GalNAc-linked PEG-lipid conjugate is present in the particle at a molar percentage of about 1.5%, about 1.4%, about 1.3%, about 1.2%, about 1.1%, about 1.0%, about 0.9%, about 0.8%, about 0.7%, about 0.6%, about 0.5%, about 0.4%, about 0.3%, about 0.2%, or about 0.1%.

75. The lipid nanoparticle of claim 74, wherein the GalNac-linked PEG-lipid conjugate is present in the particle at a molar percentage of about 0.5%.

76. The lipid nanoparticle of any one of claims 36 to 75, further comprising about 10 mM to about 30 mM malic acid.

77. The lipid nanoparticle of claim 76, comprising about 20 mM malic acid.

78. The lipid nanoparticle of any one of claims 36 to 77, further comprising about 30 mM to about 50 mM NaCl.

79. The lipid nanoparticle of claim 78, further comprising about 40 mM NaCl.

80. The lipid nanoparticle of any one of claims 36 to 79, further comprising about 20 mM to about 100 mM $MgCl_2$.

81. The lipid nanoparticle of claim 39, wherein the ceDNA is a closed-ended linear duplex DNA.

82. The lipid nanoparticle of claim 39, wherein the ceDNA comprises an expression cassette, and wherein the expression cassette comprises a promoter sequence and a transgene.

83. The lipid nanoparticle of claim 82, wherein the expression cassette comprises a polyadenylation sequence.

84. The lipid nanoparticle of any one of claims 81 to 83, wherein the ceDNA comprises at least one inverted terminal repeat (ITR) flanking either 5' or 3' end of said expression cassette.

85. The lipid nanoparticle of claim 84, wherein the expression cassette is flanked by two ITRs, wherein the two ITRs comprise one 5' ITR and one 3' ITR.

~~86. The lipid nanoparticle of claim 84, wherein the expression cassette is connected to an ITR at 3' end (3' ITR).~~

~~87. The lipid nanoparticle of claim 84, wherein the expression cassette is connected to an ITR at 5' end (5' ITR).~~

~~88. The lipid nanoparticle of claim 84, wherein at least one of 5' ITR and 3' ITR is a wild-type AAV ITR.~~

~~89. The lipid nanoparticle of claim 84, wherein at least one of 5' ITR and 3' ITR is a modified ITR.~~

~~90. The lipid nanoparticle of claim 84, wherein the ceDNA further comprises a spacer sequence between a 5' ITR and the expression cassette.~~

~~91. The lipid nanoparticle of claim 84, wherein the ceDNA further comprises a spacer sequence between a 3' ITR and the expression cassette.~~

~~92. The lipid nanoparticle of claim 90 or claim 91, wherein the spacer sequence is at least 5 base pairs long in length.~~

~~93. The lipid nanoparticle of claim 92, wherein the spacer sequence is 5 to 100 base pairs long in length.~~

~~94. The lipid nanoparticle of claim 92, wherein the spacer sequence is 5 to 500 base pairs long in length.~~

~~95. The lipid nanoparticle of any one of claims 38 to 94, wherein the ceDNA has a nick or a gap.~~

~~96. The lipid nanoparticle of claim 84, wherein 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.~~

~~97. The lipid nanoparticle of claim 96, wherein said AAV serotype is selected from the group comprising of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 and AAV12.~~

~~98. The lipid nanoparticle of claim 84, wherein the ITR is a mutant ITR, and the ceDNA optionally comprises an additional ITR which differs from the first ITR.~~

~~99. The lipid nanoparticle of claim 84, wherein 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.~~

~~100. The lipid nanoparticle of claim 39, wherein the ceDNA is a CELiD, DNA-based minicircle, a MIDGE, a ministering 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.~~

27~~101~~. A pharmaceutical composition comprising the lipid of any one of claims 1 to 20 or the lipid nanoparticle of any one of claims 21~~36~~ to 26~~100~~ and a pharmaceutically acceptable excipient.

~~102. A pharmaceutical composition comprising the lipid of any one of claims 1 to 25 or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable excipient.~~

28~~103~~. ~~A method of treating a genetic disorder in a subject, the method comprising administering to the subject an effective amount of the~~ Use of the lipid nanoparticle of any one of claims 21~~36~~ to 26~~100~~, or ~~an effective amount of~~ the pharmaceutical composition ~~according to~~ of claim ~~101~~27 in the manufacture of a medicament for treating a genetic disorder in a subject in need thereof.

29~~104~~. The ~~method~~ use of claim 28~~103~~, wherein the subject is a human.

30~~105~~. The ~~method claim 103 or claim 104~~use of claim 28 or 29, 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 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, 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), ornithine transcarbamylase (OTC) deficiency, age-related macular degeneration, Usher syndrome, alpha-1 antitrypsin deficiency, progressive familial intrahepatic cholestasis (PFIC) type I (ATP8B1 deficiency), type II (ABCB11), type III (ABCB4), or type IV (TJP2), and Cathepsin A deficiency.

31. The use of claim 30, wherein the genetic disorder is selected from the group consisting of hemophilia A (clotting factor VIII (FVIII) deficiency), hemophilia B (clotting factor IX (FIX) deficiency), Wilson disease, phenylketonuria (PKU), retinitis pigmentosa, Hunter syndrome (MPS Type II), glycogen storage disease types I and II (Pompe disease), Gaucher disease Types I, II and III, Leber Congenital Amaurosis (e.g., LCA10), Stargardt macular dystrophy (ABCA4), ornithine transcarbamylase (OTC) deficiency, age-related macular degeneration, Usher syndrome, alpha-1 antitrypsin deficiency, and progressive familial intrahepatic cholestasis (PFIC) type I (ATP8B1 deficiency).

~~106. The method of claim 105, wherein the genetic disorder is Leber congenital amaurosis (LCA).~~

~~107. The method of claim 106, wherein the LCA is LCA10.~~

~~108. The method of claim 105, wherein the genetic disorder is Niemann-Pick disease.~~

~~109. The method of claim 105, wherein the genetic disorder is Stargardt macular dystrophy.~~

~~110. The method of claim 105, wherein the genetic disorder is glucose-6-phosphatase (G6Pase) deficiency (glycogen storage disease type I) or Pompe disease (glycogen storage disease type II).~~

~~111. The method of claim 105, wherein the genetic disorder is hemophilia A (Factor VIII deficiency).~~

~~112. The method of claim 105, wherein the genetic disorder is hemophilia B (Factor IX deficiency).~~

~~113. The method of claim 105, wherein the genetic disorder is hunter syndrome (Mucopolysaccharidosis II).~~

~~114. The method of claim 105, wherein the genetic disorder is cystic fibrosis.~~

~~115. The method of claim 105, wherein the genetic disorder is dystrophic epidermolysis bullosa (DEB).~~

~~116. The method of claim 105, wherein the genetic disorder is phenylketonuria (PKU).~~

~~117. The method of claim 105, wherein the genetic disorder is progressive familial intrahepatic cholestasis (PFIC).~~

~~118. The method of claim 105, wherein the genetic disorder is Wilson disease.~~

~~119. The method of claim 105, wherein the genetic disorder is Gaucher disease Type I, II or III.~~