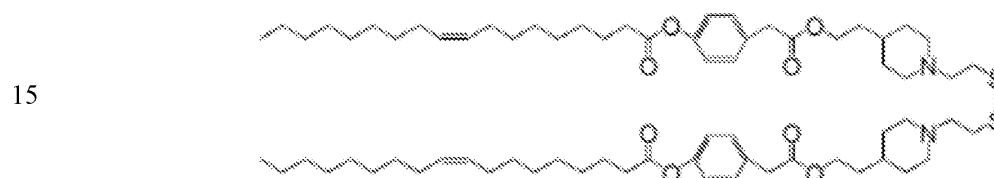


CLAIMS

What is Claimed is:

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



4. The pharmaceutical composition of any one of the previous claims, wherein the LNP
20 further comprises a sterol.
5. The pharmaceutical composition of claim 4, wherein the sterol is a cholesterol.
6. The pharmaceutical composition of any one of the previous claims, wherein the LNP
25 further comprises a polyethylene glycol (PEG).
7. The pharmaceutical composition of claim 6, wherein the PEG is 1-(monomethoxy-
polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG).
- 30 8. The pharmaceutical composition of any one of the previous claims, wherein the LNP
further comprises a non-cationic lipid.
9. The pharmaceutical composition of claim 8, wherein the non-cationic lipid is selected
from the group consisting of distearoyl-sn-glycero-phosphoethanolamine,
35 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), dimyristoylphosphoethanolamine (DMPE),
 distearoyl-phosphatidyl-ethanolamine (DSPE), monomethyl-phosphatidylethanolamine (such as 16-
 5 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), dierycylphosphatidylcholine (DEPC),
 10 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,
 15 lysophosphatidylcholine, dilinoleoylphosphatidylcholine, or mixtures thereof.

10. The pharmaceutical composition of claim 9, wherein the non-cationic lipid is
 selected from the group consisting of dioleoylphosphatidylcholine (DOPC),
 distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE).

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11. The pharmaceutical composition of claim 10, wherein the PEG or PEG-lipid
 conjugate is present at about 1.5% to about 3%.

12. The pharmaceutical composition of any one of claims 10 and 11, wherein the
 25 cholesterol is present at a molar percentage of about 20% to about 40%, and wherein the SS-cleavable
 lipid is present at a molar percentage of about 80% to about 60%.

13. The pharmaceutical composition of claim 12, wherein the cholesterol is present at a
 molar percentage of about 40%, and wherein the SS-cleavable lipid is present at a molar percentage of
 30 about 50%.

14. The pharmaceutical composition of any one of claims 1-3, wherein the composition
 further comprises a cholesterol, a PEG or PEG-lipid conjugate, and a non-cationic lipid.

15. The pharmaceutical composition of claim 14, wherein the PEG or PEG-lipid
 conjugate is present at about 1.5% to about 3%.

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16. The pharmaceutical composition of claim 14 or claim 15, wherein the cholesterol is present at a molar percentage of about 30% to about 50%.

17. The pharmaceutical composition of any one of claims 14-16, wherein the SS-cleavable lipid is present at a molar percentage of about 42.5% to about 62.5%.

18. The pharmaceutical composition of any one of claims 14-17, wherein the non-cationic lipid is present at a molar percentage of about 2.5% to about 12.5%.

19. The pharmaceutical composition of any one of claims 14-18, wherein the cholesterol is present at a molar percentage of about 40%, the SS-cleavable lipid is present at a molar percentage of about 52.5%, the non-cationic lipid is present at a molar percentage of about 7.5%, and wherein the PEG is present at about 3%.

20. The pharmaceutical composition of any of the previous claims, wherein the composition further comprises dexamethasone palmitate.

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

22. The pharmaceutical composition of any one of claims 1-20, wherein the LNP is less than about 100 nm in size.

23. The pharmaceutical composition of claim 22, wherein the LNP is less than about 70 nm in size.

24. The pharmaceutical composition of claim 23, wherein the LNP is less than about 60 nm in size.

25. The pharmaceutical composition of any one of the previous claims, wherein the composition has a total lipid to ceDNA ratio of about 15:1.

26. The pharmaceutical composition of any one of the previous claims, wherein the composition has a total lipid to ceDNA ratio of about 30:1.

27. The pharmaceutical composition of any one of the previous claims, wherein the composition has a total lipid to ceDNA ratio of about 40:1.

28. The pharmaceutical composition of any one of the previous claims, wherein the composition has a total lipid to ceDNA ratio of about 50:1.

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

30. The pharmaceutical composition of Claim 29, wherein the GalNAc is present in the LNP at a molar percentage of 0.5% of the total lipid.

10 31. The pharmaceutical composition of any one of the previous claims, wherein the composition further comprises about 10 mM to about 30 mM malic acid.

32. The pharmaceutical composition of claim 31, wherein the composition comprises about 20 mM malic acid.

15 33. The pharmaceutical composition of any one of the previous claims, wherein the composition further comprises about 30 mM to about 50 mM NaCl.

20 34. The pharmaceutical composition of claim 33, wherein the composition comprises about 40 mM NaCl.

35. The pharmaceutical composition of any one of claims 1-33, wherein the composition further comprises about 20 mM to about 100 mM MgCl₂.

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

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

30 38. The pharmaceutical composition of claim 37, wherein the ceDNA comprises expression cassette comprising a polyadenylation sequence.

39. The pharmaceutical composition of any one of claims 36-38, wherein the ceDNA
35 comprises at least one inverted terminal repeat (ITR) flanking either 5' or 3' end of said expression cassette.

40. The pharmaceutical composition of claim 39, wherein said expression cassette is flanked by two ITRs, wherein the two ITRs comprise one 5' ITR and one 3' ITR.

41. The pharmaceutical composition of claim 39, wherein the expression cassette is
5 connected to an ITR at 3' end (3' ITR).

42. The pharmaceutical composition of claim 39, wherein the expression cassette is connected to an ITR at 5' end (5' ITR).

10 43. The pharmaceutical composition of claim 39, wherein at least one of 5' ITR and 3' ITR is a wild-type AAV ITR.

44. The pharmaceutical composition of claim 39, wherein at least one of 5' ITR and 3' ITR is a modified ITR.
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45. The pharmaceutical composition of claim 39, wherein the ceDNA further comprises a spacer sequence between a 5' ITR and the expression cassette.

46. The pharmaceutical composition of claim 39, wherein the ceDNA further comprises a
20 spacer sequence between a 3' ITR and the expression cassette.

47. The pharmaceutical composition of claim 45 or claim 46, wherein the spacer sequence is at least 5 base pairs long in length.

25 48. The pharmaceutical composition of claim 47, wherein the spacer sequence is 5 to 100 base pairs long in length.

49. The pharmaceutical composition of claim 47, wherein the spacer sequence is 5 to 500
30 base pairs long in length.

50. The pharmaceutical composition of any one of the previous claims, wherein the ceDNA has a nick or a gap.

51. The pharmaceutical composition of claim 39, wherein the ITR is an ITR derived from
35 an AAV serotype, derived from an ITR of goose virus, derived from a B19 virus ITR, a wild-type ITR from a parvovirus.

52. The pharmaceutical composition of claim 51, wherein said AAV serotype is selected from the group comprising of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 and AAV12.

5 53. The pharmaceutical composition of claim 39, wherein the ITR is a mutant ITR, and the ceDNA optionally comprises an additional ITR which differs from the first ITR.

54. The pharmaceutical composition of claim 39, 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.

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

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56. The pharmaceutical composition of any one of the previous claims, further comprising a pharmaceutically acceptable excipient.

20 57. 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 previous claims.

58. The method of claim 50, wherein the subject is a human.

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59. The method of claim 57 or claim 58, 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 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-

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- 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
- 10 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.
60. The method of claim 59, wherein the genetic disorder is Leber congenital amaurosis
- 15 (LCA).
61. The method of claim 60, wherein the LCA is LCA10.
62. The method of claim 59, wherein the genetic disorder is Niemann-Pick disease.
- 20 63. The method of claim 59, wherein the genetic disorder is Stargardt macular dystrophy.
64. The method of claim 59, wherein the genetic disorder is glucose-6-phosphatase (G6Pase) deficiency (glycogen storage disease type I) or Pompe disease (glycogen storage disease
- 25 type II).
65. The method of claim 59, wherein the genetic disorder is hemophilia A (Factor VIII deficiency).
- 30 66. The method of claim 59, wherein the genetic disorder is hemophilia B (Factor IX deficiency).
67. The method of claim 59, wherein the genetic disorder is hunter syndrome (Mucopolysaccharidosis II).
- 35 68. The method of claim 59, wherein the genetic disorder is cystic fibrosis.

69. The method of claim 59, wherein the genetic disorder is dystrophic epidermolysis bullosa (DEB).

70. The method of claim 59, wherein the genetic disorder is phenylketonuria (PKU).

71. The method of claim 59, wherein the genetic disorder is hyaluronidase deficiency.

72. The method of any one of claims 57-71, further comprising administering an immunosuppressant.

73. The method of claim 72, wherein the immunosuppressant is dexamethasone.

74. The method of any one of claims 57-73, 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.

75. The method of claim 74, wherein the immune response is measured by detecting the levels of a pro-inflammatory cytokine or chemokine.

76. The method of claim 75, wherein 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.

77. The method of claim 76, wherein 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.

78. The method of any one of claims 57-77, 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.

79. The method of claim 78, wherein the SS-cleavable lipid is ss-OP of Formula I.

80. The method of claim 79, wherein the LNP further comprises cholesterol and a PEG-lipid conjugate.

81. The method of claim 80, wherein the LNP further comprises a noncationic lipid.

82. The method of claim 81, wherein the noncationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE).

83. The method of any of claim 80 or claim 81, wherein the LNP further comprises N-Acetylgalactosamine (GalNAc).

84. The method of claim 83, wherein the GalNAc is present in the LNP at a molar percentage of 0.5% of the total lipid.

85. A method of increasing therapeutic nucleic acid targeting to the liver of a subject in need of treatment, 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).

86. The method of claim 85, wherein the PEG is 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG).

87. The method of claim 85, wherein the LNP further comprises a non-cationic lipid.

88. The method of claim 87, wherein the non-cationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE).

89. The method of claim 85, wherein the GalNAc is present in the LNP at a molar percentage of 0.5% of the total lipid.

90. The method of claim 85, wherein the subject is suffering from a genetic disorder.

91. The method of claim 90, wherein the genetic disorder is hemophilia A (Factor VIII deficiency).

92. The method of claim 90, wherein the genetic disorder is hemophilia B (Factor IX deficiency).

93. The method of claim 90, wherein the genetic disorder is phenylketonuria (PKU).

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94. The method of claim 85, wherein 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.

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95. The method of claim 85, wherein the therapeutic nucleic acid is ceDNA.

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96. The method of claim 95, wherein the ceDNA comprises an expression cassette comprising a promoter sequence and a transgene.

97. The method of claim 96, wherein the ceDNA comprises at least one inverted terminal repeat (ITR) flanking either 5' or 3' end of said expression cassette.

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98. The method of claim 95, wherein 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.

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99. A method of mitigating a complement response in a subject in need of treatment with a therapeutic nucleic acid (TNA), the method comprising administering to the subject an effective amount of a lipid nanoparticle (LNP) comprising the TNA, a ss-cleavable lipid, a sterol, polyethylene glycol (PEG), and N-Acetylgalactosamine (GalNAc).

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100. The method of claim 99, wherein the subject is suffering from a genetic disorder.

101. The method of claim 100, 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

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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, Mucopolidosis 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.

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102. The method of claim 99, wherein 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.

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103. The method of claim 102, wherein 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.

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104. The method of claim 99, wherein the PEG is 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG).

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105. The method of claim 104, wherein the PEG is present in the LNP at a molecular percentage of about 2 to 4%.

106. The method of claim 105, wherein the PEG is present in the LNP at a molecular percentage of about 3%.

107. The method of claim 99, wherein the LNP further comprises a non-cationic lipid.

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108. The method of claim 107, wherein the non-cationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE).

109. The method of claim 99, wherein the GalNAc is present in the LNP at a molar percentage of about 0.3 to 1% of the total lipid.

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110. The method of claim 107, wherein the GalNAc is present in the LNP at a molar percentage of about 0.5% of the total lipid.

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