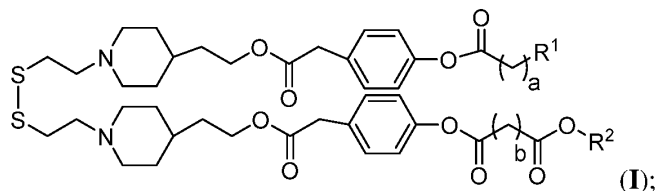


## CLAIMS

What is Claimed is:

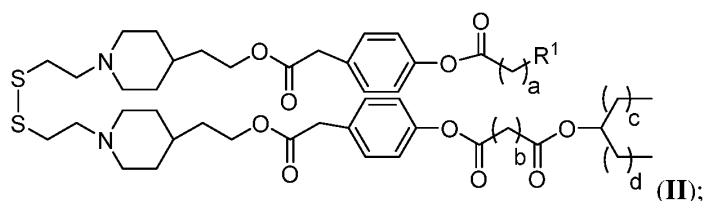
- 5           1.       A lipid having the Formula (I):



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;  
 10       R<sup>1</sup> is absent or is selected from (C<sub>2</sub>-C<sub>20</sub>)alkenyl, -C(O)O(C<sub>2</sub>-C<sub>20</sub>)alkyl, and  
           cyclopropyl substituted with (C<sub>2</sub>-C<sub>20</sub>)alkyl; and  
           R<sup>2</sup> is (C<sub>2</sub>-C<sub>20</sub>)alkyl.

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



- 15           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  
 20       and d are each independently integers ranging from 2 to 8.

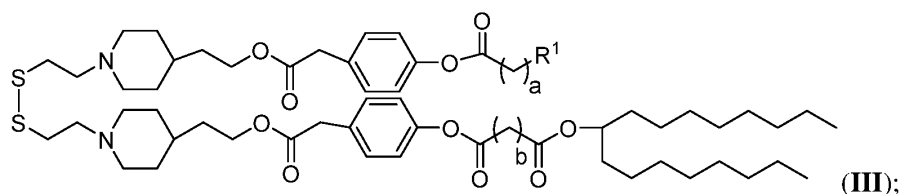
4.       The lipid of claim 2 or 3, or a pharmaceutically acceptable salt thereof,  
           wherein c and d are each independently integers ranging from 4 to 8.

- 25       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.

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

7. The lipid of any one of claims 2 to 6, or a pharmaceutically acceptable salt thereof, wherein at least one of c and d is 7.

8. The lipid of any one of claims 1 to 7, wherein the lipid is of the Formula (III):



or a pharmaceutically acceptable salt thereof.

10

9. The lipid of any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof, wherein b is an integer ranging from 3 to 9.

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.

11. The lipid of any one of claims 1 to 10, or a pharmaceutically acceptable salt thereof, wherein b is 5 or 7.

12. The lipid of any one of claims 1 to 11, or a pharmaceutically acceptable salt thereof, wherein a is an integer ranging from 2 to 18.

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.

25

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.

30

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.

5 17. The lipid of claim 16, or a pharmaceutically acceptable salt thereof, wherein a is 3.

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.

10

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  
15 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.

20 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.

25

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

25. The lipid of any one of claims 1 to 24, or a pharmaceutically acceptable salt  
30 thereof, wherein R<sup>1</sup> is absent or is selected from (C<sub>5</sub>-C<sub>15</sub>)alkenyl, -C(O)O(C<sub>4</sub>-C<sub>18</sub>)alkyl, and cyclopropyl substituted with (C<sub>4</sub>-C<sub>16</sub>)alkyl.

26. The lipid of any one of claims 1 to 25, or a pharmaceutically acceptable salt thereof, wherein R<sup>1</sup> is absent or is selected from (C<sub>5</sub>-C<sub>12</sub>)alkenyl, -C(O)O(C<sub>4</sub>-C<sub>12</sub>)alkyl, and cyclopropyl substituted with (C<sub>4</sub>-C<sub>12</sub>)alkyl.

5           27. The lipid of any one of claims 1 to 26, or a pharmaceutically acceptable salt thereof, wherein R<sup>1</sup> is absent or is selected from (C<sub>5</sub>-C<sub>10</sub>)alkenyl, -C(O)O(C<sub>4</sub>-C<sub>10</sub>)alkyl, and cyclopropyl substituted with (C<sub>4</sub>-C<sub>10</sub>)alkyl.

10           28. The lipid of any one of claims 1 to 27, or a pharmaceutically acceptable salt thereof, wherein R<sup>1</sup> is C<sub>10</sub> alkenyl.

15           29. The lipid of any one of claims 1 to 27, or a pharmaceutically acceptable salt thereof, wherein the alkyl in -C(O)O(C<sub>2</sub>-C<sub>20</sub>)alkyl, -C(O)O(C<sub>4</sub>-C<sub>18</sub>)alkyl, -C(O)O(C<sub>4</sub>-C<sub>12</sub>)alkyl, or -C(O)O(C<sub>4</sub>-C<sub>10</sub>)alkyl for R<sup>1</sup> is an unbranched alkyl.

            30. The lipid of claim 29, or a pharmaceutically acceptable salt thereof, wherein R<sup>1</sup> is -C(O)O(C<sub>9</sub> alkyl).

20           31. The lipid of any one of claims 25 to 27, or a pharmaceutically acceptable salt thereof, wherein the alkyl in -C(O)O(C<sub>4</sub>-C<sub>18</sub>)alkyl, -C(O)O(C<sub>4</sub>-C<sub>12</sub>)alkyl, or -C(O)O(C<sub>4</sub>-C<sub>10</sub>)alkyl is a branched alkyl.

            32. The lipid of claim 31, or a pharmaceutically acceptable salt thereof, wherein R<sup>1</sup> is -C(O)O(C<sub>17</sub> alkyl).

25           33. The lipid of any one of claims 1 to 24, or a pharmaceutically acceptable salt thereof, wherein R<sup>1</sup> is selected from any group listed in Table 1.

30           34. The lipid of claim 1, or a pharmaceutically acceptable salt thereof, wherein R<sup>2</sup> is selected from any group listed in Table 2.

            35. The lipid of claim 1, wherein the lipid is selected from any lipid listed in Table 3, or a pharmaceutically acceptable salt thereof.

36. A lipid nanoparticle (LNP) comprising the lipid of any one of claims 1 to 35, or a pharmaceutically acceptable salt thereof; and a nucleic acid.

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

38. The lipid nanoparticle of claim 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,  
10 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).  
15

40. The lipid nanoparticle of any one of claims 36 to 39, further comprising a sterol.  
20

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  
25 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.  
30

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  
 5 (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-  
 10 phosphatidylethanolamine (SOPE), hydrogenated soy phosphatidylcholine (HSPC), egg  
 phosphatidylcholine (EPC), dioleoylphosphatidylserine (DOPS), sphingomyelin (SM),  
 dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG),  
 distearoylphosphatidylglycerol (DSPG), dierycylphosphatidylcholine (DEPC),  
 palmitoyloleoylphosphatidylglycerol (POPG), dielaidoyl-phosphatidylethanolamine (DEPE),  
 15 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, and mixtures  
 20 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).

25

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  
 30 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 is DMG-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%.

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  
10 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.  
15

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

66. The lipid nanoparticle of any one of claims 36 to 64, wherein the nanoparticle  
20 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.

25 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.  
30

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  
5 ceDNA ratio of about 40:1.

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

10 74. The lipid nanoparticle of claim 73, wherein the tissue specific targeting moiety is N-acetylgalactosamine (GalNAc); wherein GalNAc is linked to the PEG-lipid conjugate; and the 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  
15 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%.

20 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.

25 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.

30 80. The lipid nanoparticle of any one of claims 36 to 79, further comprising about 20 mM to about 100 mM MgCl<sub>2</sub>.

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.

5

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.

10

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.

15

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).

20

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.

25

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

30

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  
5 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.

10 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-  
15 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.

20

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  
25 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-  
30 ended DNA comprising two hairpin structures of ITRs in the 5' and 3' ends of an expression cassette, or a doggybone™ DNA.

101. A pharmaceutical composition comprising the lipid nanoparticle of any one of claims 36 to 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.

5 103. A method of treating a genetic disorder in a subject, the method comprising administering to the subject an effective amount of the lipid nanoparticle of any one of claims 36 to 100, or an effective amount of the pharmaceutical composition according to claim 101.

104. The method of claim 103, wherein the subject is a human.

10

105. The method claim 103 or claim 104, 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, 15 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- 20 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 25 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, 30 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.

5

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.

10

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.

15

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).

20

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).

25

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.

30

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  
5 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  
10 Type I, II or III.