

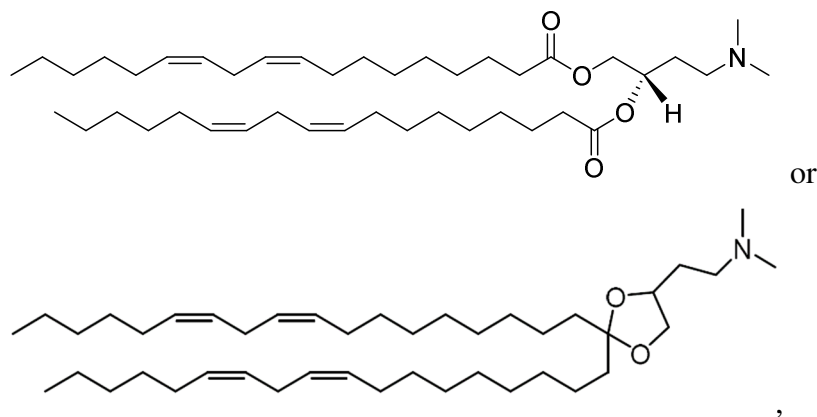
CLAIMS

What is claimed is:

1. A lipid nanoparticle (LNP) comprising a lipid blend for targeted delivery of a nucleic acid into an immune cell, the lipid blend comprising:

(a) a lipid-immune cell targeting group conjugate comprising the compound of Formula IV: [Lipid] – [optional linker] – [immune cell targeting group], and

(b) an ionizable cationic lipid comprising



wherein the LNP further comprises a nucleic acid disposed therein.

2. The LNP of claim 1, wherein the immune cell targeting group comprises an antibody that binds a T cell antigen that is selected from the group consisting of -

~~3. The LNP of claim 2, wherein the T cell antigen is CD3, CD4, CD7, CD8, or a combination thereof (e.g., both CD3 and CD8, both CD4 and CD8, or both CD7 and CD8).~~

~~43.~~ The LNP of claim 2, wherein the immune cell targeting group comprises an antibody that binds a Natural Killer (NK) cell antigen that is selected from the group consisting of -

~~5. The LNP of claim 4, wherein the NK cell antigen is CD7, CD8, CD56, or a combination thereof (e.g., both CD7 and CD8).~~

~~64.~~ The LNP of any one of claims 1 to ~~53~~, wherein the immune cell targeting group is covalently coupled to a lipid in the lipid blend via a polyethylene glycol (PEG) containing linker, and -

~~7. The LNP of claim 6,~~ wherein the lipid covalently coupled to the immune cell targeting group via a PEG containing linker is distearoylglycerol (DSG), distearoyl-phosphatidylethanolamine (DSPE), dimyrstoyl-phosphatidylethanolamine (DMPE), distearoyl-glycero-phosphoglycerol (DSPG), dimyrstoyl-glycerol (DMG), dipalmitoyl-phosphatidylethanolamine (DPPE), dipalmitoyl-glycerol (DPG), or ceramide, and -

~~8. The LNP of claim 6 or 7,~~ wherein the PEG is PEG 2000 or PEG 3400.

95. The LNP of any one of claims 1-~~84~~, wherein the lipid-immune cell targeting group conjugate is present in the lipid blend in a range of 0.001 -0.5 mole percent (e.g., 0.002-0.2 mole percent).

~~106.~~ The LNP of any one of claims 1 to 95, wherein the lipid blend further comprises one or more of a structural lipid (e.g., a sterol), a neutral phospholipid, and a free PEG-lipid.

~~117.~~ The LNP of any one of claims 1 to ~~106~~, wherein the ionizable cationic lipid is present in the lipid blend in a range of 30-70 (e.g., 40-60) mole percent.

~~128.~~ The LNP of claim ~~106~~, wherein the sterol is present in the lipid blend in a range of 20-70 (e.g., 30-50) mole percent, and -

~~13. The LNP of claim 10 or 12,~~ wherein the sterol is cholesterol.

~~149.~~ The LNP of any one of claims ~~10-6~~ to ~~138~~, wherein the neutral phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), and sphingomyelin, and -

~~15. The LNP of any one of claims 10 to 14, wherein the neutral phospholipid~~ is present in the lipid blend in a range of 1-10 mole percent.

~~1610.~~ The LNP of any one of claims ~~10-6~~ to ~~159~~, wherein the free PEG-lipid is selected from the group consisting of PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, and PEG-modified dialkylglycerols. ~~For example, a PEG lipid may be PEG-dioleoylglycerol (PEG-DOG), PEG-dimyrstoyl-glycerol (PEG-DMG), PEG-dipalmitoyl-glycerol (PEG-DPG), PEG-dilinoleoyl-glycero-phosphatidyl ethanolamine (PEG-DLPE), PEG-~~

~~dimyrstoyl-phosphatidylethanolamine (PEG-DMPE), PEG-dipalmitoyl-phosphatidylethanolamine (PEG-DPPE), PEG-distearoylglycerol (PEG-DSG), PEG-diacylglycerol (PEG-DAG, e.g., PEG-DMG, PEG-DPG, and PEG-DSG), PEG-ceramide, PEG-distearoyl-glycero-phosphoglycerol (PEG-DSPG), PEG-dioleoyl-glycero-phosphoethanolamine (PEG-DOPE), 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, or a PEG-distearoyl-phosphatidylethanolamine (PEG-DSPE) lipid.~~

~~1711.~~ The LNP of any one of claims ~~10-6~~ to ~~159~~, wherein the free PEG-lipid comprises a diacylphosphatidylethanolamine comprising Dipalmitoyl (C16) chain or Distearoyl (C18) chain, and -

~~18. The LNP of any one of claims 10 to 17, wherein the free PEG lipid~~ is present in the lipid blend in a range of 1-4 mole percent.

~~1912.~~ The LNP of any one of claims ~~10-6~~ to ~~1811~~, wherein the free PEG-lipid comprises the same or a different lipid as the lipid in the lipid-immune cell targeting group conjugate.

~~2013.~~ The LNP of any one of claims 1 to ~~1912~~, wherein the LNP has a mean diameter in the range of 50-200 nm, preferably -

~~21. The LNP of claim 20, where the LNP has a mean diameter of~~ about 100 nm, -

~~22. The LNP of any one of claims 1 to 21, wherein the LNP has~~ a polydispersity index in a range from 0.05 to 1, or -

~~23. The LNP of any one of claims 1 to 22, wherein the LNP has~~ a zeta potential of from about -10 mV to about + 30 mV at pH 5.

~~2414.~~ The LNP of any one of claims 1 to ~~2313~~, wherein the nucleic acid is DNA or RNA.

~~2515.~~ The LNP of claim ~~2414~~, wherein the RNA is an mRNA that -

~~26. The LNP of claim 25, wherein the mRNA~~ encodes a receptor, a growth factor, a hormone, a cytokine, an antibody, an antigen, an enzyme, or a vaccine; -

~~27. The LNP of claim 25, wherein the mRNA~~ or encodes a polypeptide capable of regulating immune response in the immune cell, preferably a synthetic T cell receptor (synTCR) or a Chimeric Antigen Receptor (CAR).

~~28. The LNP of claim 25, wherein the mRNA encodes a polypeptide capable of reprogramming the immune cell.~~

~~29. The LNP of claim 27, wherein the mRNA encodes a synthetic T cell receptor (synTCR) or a Chimeric Antigen Receptor (CAR).~~

~~3016.~~ The LNP of any one of claims 1 to ~~2915~~, wherein the immune cell targeting group comprises an antibody, and the antibody is a Fab or an immunoglobulin single variable domain (e.g., a Nanobody).

~~3417.~~ The LNP of any one of claims 1 to ~~2915~~, wherein the immune cell targeting group comprises a Fab, F(ab')₂, Fab'-SH, Fv, or scFv fragment.

~~3218.~~ The LNP of claim ~~3016~~ or claim ~~3417~~, wherein the immune cell targeting group comprises a Fab that is engineered to knock out the natural interchain disulfide bond at the C-terminus, and -

~~33.~~ ~~The LNP of claim 32,~~ wherein the Fab comprises a heavy chain fragment that comprises C233S substitution, and a light chain fragment that comprises C214S substitution, numbering according to Kabat.

~~3419.~~ The LNP of any one of claims ~~3417-3318~~, wherein the immune cell targeting group comprises a Fab that has a non-natural interchain disulfide bond (e.g., a engineered, buried interchain disulfide bond)-), and

~~35.~~ ~~The LNP of claim 34,~~ wherein the Fab comprises F174C substitution in the heavy chain fragment, and S176C substitution in the light chain fragment, numbering according to Kabat.

~~3620.~~ The LNP of claims ~~3417~~ to ~~3519~~, wherein the immune cell targeting group comprises a Fab that comprises a cysteine at the C-terminus of the heavy or light chain fragment and -

~~37.~~ ~~The LNP of claim 36, wherein the Fab further comprises~~ one or more amino acids between the heavy chain fragment of the Fab and the C-terminal cysteine.

~~3821.~~ The LNP of claim ~~3016~~, wherein the immune cell targeting group comprises an immunoglobulin single variable domain-

~~39. The LNP of claim 30 or claim 38, wherein the immunoglobulin single variable domain comprises comprising a cysteine at the C-terminus, -~~

~~40. The LNP of claim 39, wherein the immunoglobulin single variable domain comprises a VHH domain, and further comprises a spacer comprising one or more amino acids between the VHH domain and the C-terminal cysteine.~~

~~4122.~~ The LNP of any one of claims ~~31-17~~ and ~~38-4021~~, wherein the immune cell targeting group comprises two or more VHH domains that -

~~42. The LNP of claim 41, wherein the two or more V_{HH} domains are linked by an amino acid linker.~~

~~4323.~~ The LNP of claim ~~4122~~, wherein the immune cell targeting group comprises a first V_{HH} domain linked to an antibody CH1 domain and a second V_{HH} domain linked to an antibody light chain constant domain, and wherein the antibody CH1 domain and the antibody light chain constant domain are linked by one or more disulfide bonds.

~~4424.~~ The LNP of any one of claims ~~30-16~~ and ~~38-4021~~, wherein the immune cell targeting group comprises a VHH domain linked to an antibody CH1 domain, and wherein the antibody CH1 domain is linked to an antibody light chain constant domain by one or more disulfide bonds.

~~4525.~~ The LNP of claim ~~43-23~~ or ~~4424~~, wherein the CH1 domain comprises F174C and C233S substitutions, and the light chain constant domain comprises S176C and C214S substitutions, numbering according to Kabat.

~~4626.~~ The LNP of any one of claims 1 to ~~2715~~, wherein the immune cell targeting group comprises a Fab that comprises:

(a) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 1 and a light chain fragment comprising the amino acid sequence of SEQ ID NO:2 or 3; or

(b) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 6 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 7.

~~47. A method of targeting the delivery of a nucleic acid to an immune cell of a subject, comprising contacting the immune cell with a lipid nanoparticle (LNP), wherein the LNP comprises:~~

- ~~(a) — An ionizable cationic lipid;~~
- ~~(b) — A conjugate comprising the compound of the following formula:~~
~~[Lipid] — [optional linker] — [immune cell targeting group];~~
- ~~(c) — A sterol or other structural lipid;~~
- ~~(d) — A neutral phospholipid~~
- ~~(e) — A free Polyethylene glycol (PEG) lipid, and~~
- ~~(f) — the nucleic acid;~~

~~wherein the LNP provides at least one of the following benefits:~~

- ~~(i) — increased specificity of targeted delivery to the immune cell compared to a reference LNP;~~
- ~~(ii) — increased half life of the nucleic acid or a polypeptide encoded by the nucleic acid in the immune cell compared to a reference LNP;~~
- ~~(iii) — increased transfection rate compared to a reference LNP; and~~
- ~~(iv) — a low level of dye accessible mRNA (<15%) and high RNA encapsulation efficiencies, wherein at least 80% mRNA was recovered in final formulation relative to the total RNA used in LNP batch preparation.~~

~~48. A method of expressing a polypeptide of interest in a targeted immune cell of a subject, comprising contacting the immune cell with a lipid nanoparticle (LNP), wherein the LNP comprises:~~

- ~~(a) An ionizable cationic lipid;~~
- ~~(b) A conjugate comprising the following structure:~~
~~[Lipid] — [optional linker] — [immune cell targeting group];~~
- ~~(c) A sterol or other structural lipid;~~

- ~~(d) A neutral phospholipid~~
- ~~(e) A free Polyethylene glycol (PEG) lipid, and~~
- ~~(f) a nucleic acid encoding the polypeptide.~~

49. The method of claim 48, wherein the LNP provides at least one of the following benefits:

- ~~(i) increased expression level in the immune cell compared to a reference LNP;~~
- ~~(ii) increased specificity of expression in the immune cell compared to a reference LNP;~~
- ~~(iii) increased half life of the nucleic acid or a polypeptide encoded by the nucleic acid in the immune cell compared to a reference LNP;~~
- ~~(iv) increased transfection rate compared to a reference LNP; and~~
- ~~(v) a low level of dye accessible mRNA (<15%) and high RNA encapsulation efficiencies, wherein at least 80% mRNA was recovered in final formulation relative to the total RNA used in LNP batch preparation.~~

50. A method of modulating cellular function of a target immune cell of a subject, comprising administering to the subject a lipid nanoparticle (LNP), wherein the LNP comprises:

- ~~(a) — An ionizable cationic lipid,~~
- ~~(b) — A conjugate comprising the following structure:~~
~~[Lipid] — [optional linker] — [immune cell targeting group];~~
- ~~(c) — A sterol or other structural lipid;~~
- ~~(d) — A neutral phospholipid;~~
- ~~(e) — A free Polyethylene glycol (PEG) lipid, and~~
- ~~(f) — a nucleic acid encoding a polypeptide for modulating the cellular function of the immune cell.~~

51. The method of claim 50, wherein the LNP provides at least one of the following benefits:

- ~~(i) increased expression level in the immune cell compared to a reference LNP;~~

(ii) increased specificity of expression in the immune cell compared to a reference LNP;

(iii) increased half life of the nucleic acid or a polypeptide encoded by the nucleic acid in the immune cell compared to a reference LNP;

(iv) increased transfection rate compared to a reference LNP; and

(v) the LNP can be administered at a lower dose compared to a reference LNP to reach the same biologic effect in the immune cell; and

(vi) a low level of dye accessible mRNA (<15%) and high RNA encapsulation efficiencies, wherein at least 80% mRNA was recovered in final formulation relative to the total RNA used in LNP batch preparation.

52. The method of claim 50 or 51, wherein the modulation of cell function comprises reprogramming the immune cells to initiate an immune response.

53. The method of claim 50 or 51, wherein the modulation of cell function comprises modulating antigen specificity of the immune cell.

54. A method of treating, ameliorating, or preventing a symptom of a disorder or disease in a subject in need thereof, comprising administering to the subject a lipid nanoparticle (LNP) for delivering a nucleic acid into an immune cell of the subject, wherein the LNP comprises:

- (a) — An ionizable cationic lipid;
- (b) — A conjugate comprising the following structure:
[Lipid]—[optional linker]—[immune cell targeting group];
- (c) — A sterol or other structural lipid;
- (d) — A neutral phospholipid;
- (e) — A free Polyethylene glycol (PEG) lipid, and
- (f) — the nucleic acid;

Wherein the nucleic acid modulates the immune response of the immune cell, therefore to treat or ameliorate the symptom.

55. The method of claim 50, wherein the LNP provides at least one of the following benefits:

- ~~61. The method of claim 60, wherein the NK cell antigen is CD7, CD8, or CD56.~~
- ~~62. The method of any one of claims 58-61, wherein the antibody is a human or humanized antibody.~~
- ~~63. The method of any one of claims 44 to 62, wherein the immune cell targeting group is covalently coupled to a lipid in the lipid blend via a polyethylene glycol (PEG)-containing linker.~~
- ~~64. The method of claim 63, wherein the lipid covalently coupled to the immune cell targeting group via a PEG-containing linker is distearoylglycerol (DSG), distearoylphosphatidylethanolamine (DSPE), dimyrstoyl phosphatidylethanolamine (DMPE), distearoylglycero phosphoglycerol (DSPG), dimyrstoyl glycerol (DMG), dipalmitoyl phosphatidylethanolamine (DPPE), dipalmitoyl glycerol (DPG), or ceramide.~~
- ~~65. The method of claim 63 or 64, wherein the PEG is PEG 2000.~~
- ~~66. The method of any one of claims 46 to 65, wherein the lipid-immune cell targeting group conjugate is present in the lipid blend in a range of 0.002-0.2 mole percent.~~
- ~~67. The method of any one of claims 46 to 66, wherein the ionizable cationic lipid is present in the lipid blend in a range of 40-60 mole percent.~~
- ~~68. The method of clam 44 to 67, wherein the sterol is cholesterol.~~
- ~~69. The method of any one of claims 44 to 68, wherein the sterol is present in the lipid blend in a range of 30-50 mole percent.~~
- ~~70. The method of clam 44 to 69, wherein the neutral phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, distearoyl sn-glycero-3-phosphoethanolamine (DSPE), 1,2-distearoyl sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dioleoyl sn-glycero-3-phosphocholine (DOPC), sphingomyelin (SM).~~
- ~~71. The method of clam 44 to 70, wherein the neutral phospholipid is present in the lipid blend in a range of 1-10 mole percent.~~
- ~~72. The method of any one of claims 44 to 71, wherein the free PEG lipid is selected from the group consisting of PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified~~

diacylglycerols, and PEG-modified dialkylglycerols. For example, a PEG-lipid may be PEG-dioleoylglycerol (PEG-DOG), PEG-dimyristoyl-glycerol (PEG-DMG), PEG-dipalmitoyl-glycerol (PEG-DPG), PEG-dilinoleoyl-glycero-phosphatidyl-ethanolamine (PEG-DLPE), PEG-dimyristoyl-phosphatidylethanolamine (PEG-DMPE), PEG-dipalmitoyl-phosphatidylethanolamine (PEG-DPPE), PEG-distearoylglycerol (PEG-DSG), PEG-diacylglycerol (PEG-DAG, e.g., PEG-DMG, PEG-DPG, and PEG-DSG), PEG-ceramide, PEG-distearoyl-glycero-phosphoglycerol (PEG-DSPG), PEG-dioleoyl-glycero-phosphoethanolamine (PEG-DOPE), 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, or a PEG-distearoyl-phosphatidylethanolamine (PEG-DSPE) lipid.

73. The method of claims 44 to 71, wherein the free PEG-lipid comprises a diacylphosphatidylethanolamines comprising Dipalmitoyl (C16) chain or Distearoyl (C18) chain.

74. The method of any one of claims 44 to 73, wherein the free PEG-lipid is present in the lipid blend in a range of 2-4 mole percent.

75. The method of any one of claims 65 to 74, wherein the free PEG-lipid comprises the same or a different lipid as the lipid in the lipid-immune cell targeting group conjugate.

76. The method of claims 44 to 73, wherein the LNP has a mean diameter in the range of 50-200 nm.

77. The method of claim 74, where the LNP has a mean diameter of about 100 nm.

78. The method of claims 44 to 77, wherein the LNP has a polydispersity index in a range from 0.05 to 1.

79. The method of claims 44 to 78, wherein the LNP has a zeta potential of from about -10 mV to about +30 mV at pH 5.

80. The method of claims 44 to 79, wherein the nucleic acid is DNA or RNA.

81. The method of claim 80, wherein the RNA is an mRNA, tRNA, siRNA, or microRNA.

82. The method of claim 81, wherein the mRNA encodes a receptor, a growth factor, a hormone, a cytokine, an antibody, an antigen, an enzyme, or a vaccine.

~~83. The method of claim 81, wherein the mRNA encodes a polypeptide capable of regulating immune response in the immune cell.~~

~~84. The method of claim 81, wherein the mRNA encodes a polypeptide capable of reprogramming the immune cell.~~

~~85. The method of claim 81, wherein the mRNA encodes a synthetic T cell receptor (synTCR) or a Chimeric Antigen Receptor (CAR).~~

~~86. The method of any one of claims 44 to 85, wherein the immune cell targeting group comprises an antibody, and the antibody is a Fab or an immunoglobulin single variable domain.~~

~~87. The method of any one of claims 44 to 85, wherein the immune cell targeting group comprises an antibody fragment selected from the group consisting of a Fab, F(ab')₂, Fab' SH, Fv, and scFv fragment.~~

~~88. The method of claim 86 or 87, wherein the immune cell targeting group comprises a Fab that comprises one or more interchain disulfide bonds.~~

~~89. The method of claim 88, wherein the Fab comprises a heavy chain fragment that comprises F174C and C233S substitutions, and a light chain fragment that comprises S176C and C214S substitutions, numbering according to Kabat.~~

~~90. The method of any one of claims 86 to 89, wherein the immune cell targeting group comprises a Fab that comprises a cysteine at the C-terminus of the heavy or light chain fragment.~~

~~91. The method of claim 86, wherein the Fab further comprises one or more amino acids between the heavy chain fragment of the Fab and the C-terminal cysteine.~~

~~92. The method of any one of claims 87-91, wherein the Fab comprises a heavy chain variable domain linked to an antibody CH1 domain and a light chain variable domain linked to an antibody light chain constant domain, wherein the CH1 domain and the light chain constant domain are linked by one or more interchain disulfide bonds, and wherein the immune cell targeting group further comprises a single chain variable fragment (scFv) linked to the C-terminus of the light chain constant domain by an amino acid linker.~~

~~93. The method of claim 86, wherein the immune cell targeting group comprises an immunoglobulin single variable domain.~~

~~94. The method of claim 86 or 93, wherein the immunoglobulin single variable domain comprises a cysteine at the C-terminus.~~

~~95. The method of claim 94, wherein the immunoglobulin single variable domain comprises a VHH domain and further comprises a spacer comprising one or more amino acids between the VHH domain and the C-terminal cysteine.~~

~~96. The method of any one of claims 86, and 93 to 95, wherein the immune cell targeting group comprises two or more VHH domains.~~

~~97. The method of claim 96, wherein the two or more VHH domains are linked by an amino acid linker.~~

~~98. The method of claim 96, wherein the immune cell targeting group comprises a first VHH domain linked to an antibody CH1 domain and a second VHH domain linked to an antibody light chain constant domain, and wherein the antibody CH1 domain and the antibody light chain constant domain are linked by one or more disulfide bonds.~~

~~99. The method of any one of claims 86, and 93 to 95, wherein the immune cell targeting group comprises a VHH domain linked to an antibody CH1 domain, and wherein the antibody CH1 domain is linked to an antibody light chain constant domain by one or more disulfide bonds.~~

~~100. The method of claim 96 or 97, wherein the CH1 domain comprises F174C and C233S substitutions, and the light chain constant domain comprises S176C and C214S substitutions, numbering according to Kabat.~~

~~101. The method of any one of claims 44 to 85, wherein the immune cell targeting group comprises a Fab that comprises:~~

~~—— (a) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 1 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 2 or 3;~~

~~—— (b) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 6 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 7.~~

~~102. The method of any one of claims 44 to 101, wherein no more than 5% non-immune cells are transfected by the LNP.~~

~~103. The method of any one of claims 44 to 102, wherein half life of the nucleic acid delivered by the LNP or a polypeptide encoded by the nucleic acid delivered by the LNP is at least 10% longer than half life of nucleic acid delivered by a reference LNP or a polypeptide encoded by the nucleic acid delivered by the reference LNP.~~

~~104. The method of any one of claims 44 to 103, wherein at least 10% immune cells are transfected by the LNP.~~

~~105. The method of any one of claims 44 to 104, wherein expression level of the nucleic acid delivered by the LNP is at least 10% higher than expression level of nucleic acid delivered by a reference LNP.~~

~~106~~27. A lipid nanoparticle (LNP) for delivering a nucleic acid into an immune cell of the subject, wherein the LNP comprises:

- (a) An ionizable cationic lipid,
- (b) A conjugate comprising the following structure:
[Lipid] – [optional linker] – [immune cell targeting group];
- (c) A sterol or other structural lipid;
- (d) A neutral phospholipid;
- (e) A free Polyethylene glycol (PEG) lipid, and
- (f) the nucleic acid,

wherein the immune cell is an NK cell, and the immune cell targeting group comprises an antibody that binds CD56.

~~107~~28. A lipid nanoparticle (LNP) for delivering a nucleic acid into an immune cell of the subject, wherein the LNP comprises:

- (a) An ionizable cationic lipid,
- (b) A conjugate comprising the following structure:
[Lipid] – [optional linker] – [immune cell targeting group];
- (c) A sterol or other structural lipid;

- (d) A neutral phospholipid;
- (e) A free Polyethylene glycol (PEG) lipid, and
- (f) the nucleic acid,

wherein the immune cell targeting group comprises an antibody that binds CD7 or CD8, and the free PEG lipid is DMG-PEG.

10829. A lipid nanoparticle (LNP) for delivering a nucleic acid into an immune cell of the subject, wherein the LNP comprises:

- (a) An ionizable cationic lipid,
- (b) A conjugate comprising the following structure:
[Lipid] – [optional linker] – [immune cell targeting group];
- (c) A sterol or other structural lipid;
- (d) A neutral phospholipid;
- (e) A free Polyethylene glycol (PEG) lipid, and
- (f) the nucleic acid,

wherein the immune cell targeting group comprises an antibody, and the antibody is a Fab or an immunoglobulin single variable domain.

10930. The LNP of claim **10829**, wherein the Fab is engineered to knock out the natural interchain disulfide at the C-terminus, and -

~~**110.** The LNP of claim **109**, wherein the Fab comprises a heavy chain fragment that comprises C233S substitutions, and a light chain fragment that comprises C214S substitutions.~~

~~**11131.** The LNP of claim **11030**, wherein the Fab comprises a non-natural interchain disulfide, or -~~

~~**112.** The LNP of claim **110**, wherein the Fab comprises F174C substitution in the heavy chain fragment, and S176C substitution in the light chain fragment.~~

11332. The LNP of claim **10829**, wherein the antibody is an immunoglobulin single variable (ISV) domain, and the ISV domain is an Nanobody® ISV.

~~114~~33. The LNP of claim ~~113~~32, wherein the free PEG lipid comprises a PEG having a molecular weight of at least 2000 daltons, preferably

~~115. The LNP of claim 114, wherein the PEG has a molecular weight of about 3000 to 5000 daltons.~~

~~116~~34. The LNP of claim ~~108~~29, wherein the antibody is a Fab,

~~117. The LNP of claim 116, wherein the Fab that~~ binds CD3, and the free PEG lipid in the LNP comprises a PEG having a molecular weight of about 2000 daltons.

~~118~~35. The LNP of claim ~~116~~34, wherein the Fab is an anti-CD4 antibody, and the free PEG lipid in the LNP comprises a PEG having a molecular weight of about 3000 to 3500 daltons.

~~119~~36. The LNP of claim ~~108~~29, wherein the immune cell targeting group comprises two or more VHH domains that -

~~120. The LNP of claim 119, wherein the two or more VHH domains~~ are linked by an amino acid linker.

~~121~~37. The LNP of claim ~~120~~36, wherein the immune cell targeting group comprises a first VHH domain linked to an antibody CH1 domain and a second VHH domain linked to an antibody light chain constant domain.

~~122~~38. A lipid nanoparticle (LNP) for delivering a nucleic acid into an immune cell of the subject, wherein the LNP comprises:

- (a) An ionizable cationic lipid,
- (b) A conjugate comprising the following structure:
[Lipid] – [optional linker] – [immune cell targeting group];
- (c) A sterol or other structural lipid;
- (d) A neutral phospholipid;
- (e) A free Polyethylene glycol (PEG) lipid, and
- (f) the nucleic acid,

wherein the LNP binds CD3, and also binds CD11a or CD18.

~~12339~~. The LNP of claim ~~12238~~, wherein the LNP comprises two conjugates, wherein the first conjugate comprises an antibody that binds CD3, and the second conjugate comprises an antibody that binds CD11a or CD18; or

~~124~~. The LNP of claim ~~122~~, wherein the LNP comprises one conjugate, and the conjugate comprises a bispecific antibody that binds both CD3 and CD11a; or -

~~125~~. The LNP of claim ~~122~~, wherein the LNP comprises one conjugate, and the conjugate comprises a bispecific antibody that binds both CD3 and CD18; -

~~126~~. The LNP of claim ~~124 or 125~~, wherein the bispecific antibody is an immunoglobulin single variable domain or Fab-ScFv.

~~12740~~. A lipid nanoparticle (LNP) for delivering a nucleic acid into an immune cell of the subject, wherein the LNP comprises:

- (a) An ionizable cationic lipid,
- (b) A conjugate comprising the following structure:
[Lipid] – [optional linker] – [immune cell targeting group];
- (c) A sterol or other structural lipid;
- (d) A neutral phospholipid;
- (e) A free Polyethylene glycol (PEG) lipid, and
- (f) the nucleic acid,

wherein the LNP binds CD7 and CD8 of the immune cell.

~~12841~~. The LNP of claim ~~12740~~, wherein the LNP comprises two conjugates, wherein the first conjugate comprises an antibody that binds CD7, and a second conjugate that binds CD8; or

~~129~~. The LNP of claim ~~127~~, wherein the LNP comprises one conjugate, wherein the conjugate comprises a bispecific antibody that binds CD7 and CD8; and -

~~130~~. The LNP of claim ~~129~~, wherein the bispecific antibody is an immunoglobulin single variable domain or Fab-ScFv.

~~131~~42. A lipid nanoparticle (LNP) for delivering a nucleic acid into two different types of immune cells of the subject, wherein the LNP comprises:

- (a) An ionizable cationic lipid,
- (b) A conjugate comprising the following structure:
[Lipid] – [optional linker] – [immune cell targeting group];
- (c) A sterol or other structural lipid;
- (d) A neutral phospholipid;
- (e) A free Polyethylene glycol (PEG) lipid, and
- (f) the nucleic acid,

wherein the LNP binds to a first antigen on the surface of the first type of immune cell, and also binds to a second antigen on the surface of the second type of immune cell.

~~132~~43. The LNP of claim ~~131~~42, wherein the two different types of immune cells are CD4+ T cells and CD8+ T cell.

~~133~~44. The LNP of claim ~~131~~42, wherein the LNP comprises two conjugates, and the first conjugate comprises a first antibody that binds to the first antigen of the first type of immune cell, and the second conjugate comprises a second antibody that binds to the second antigen of the second type of immune cell; or

~~134. The LNP of claim 131, wherein the LNP comprises one conjugate, and the conjugate comprises a bispecific antibody, and the bispecific antibody binds to both the first antigen on the first type of immune cell, and the second antigen on the second type of immune cells; wherein~~

~~135. The LNP of claim 134, wherein the bispecific antibody is an immunoglobulin single variable domain or a Fab-ScFv.~~

~~136~~45. A lipid nanoparticle (LNP) for delivering a nucleic acid into both CD4+ and CD8+ T cells of a subject, wherein the LNP comprises:

- (a) An ionizable cationic lipid,
- (b) A conjugate comprising the following structure:

[Lipid] – [optional linker] – [immune cell targeting group];

- (c) A sterol or other structural lipid;
- (d) A neutral phospholipid;
- (e) A free Polyethylene glycol (PEG) lipid, and
- (f) the nucleic acid,

wherein the immune cell targeting group comprises a single antibody that binds to CD3 or CD7.

13746. A lipid nanoparticle (LNP) for delivering a nucleic acid into both T cells and NK cells of a subject, wherein the LNP comprises:

- (a) An ionizable cationic lipid,
- (b) A conjugate comprising the following structure:

[Lipid] – [optional linker] – [immune cell targeting group];

- (c) A sterol or other structural lipid;
- (d) A neutral phospholipid;
- (e) A free Polyethylene glycol (PEG) lipid, and
- (f) the nucleic acid,

wherein the immune cell targeting group binds to CD7, CD8, or both CD7 and CD8.

13847. A lipid nanoparticle (LNP) for delivering a nucleic acid into both T cells and NK cells of a subject, wherein the LNP comprises:

- (a) An ionizable cationic lipid,
- (b) A conjugate comprising the following structure:

[Lipid] – [optional linker] – [immune cell targeting group];

- (c) A sterol or other structural lipid;
- (d) A neutral phospholipid;
- (e) A free Polyethylene glycol (PEG) lipid, and

(f) the nucleic acid,

wherein the immune cell targeting group binds to

- (i) both CD3 and CD56;
- (ii) both CD8 and CD56; or
- (iii) both CD7 and CD56.

~~13948~~. The LNP of any one of claims ~~106-27~~ to ~~13847~~, wherein the immune cell targeting group is covalently coupled to a lipid in the lipid blend via a polyethylene glycol (PEG) containing linker, and -

~~140. The LNP of claim 139~~, wherein the lipid covalently coupled to the immune cell targeting group via a PEG containing linker is distearoylglycerol (DSG), distearoyl-phosphatidylethanolamine (DSPE), dimyrstoyl-phosphatidylethanolamine (DMPE), distearoyl-glycero-phosphoglycerol (DSPG), dimyrstoyl-glycerol (DMG), dipalmitoyl-phosphatidylethanolamine (DPPE), dipalmitoyl-glycerol (DPG), or ceramide.

~~14149~~. The LNP of any one of claims ~~106-27~~ to ~~13041~~, wherein the lipid-immune cell targeting group conjugate is present in the lipid blend in a range of 0.002-0.2 mole percent.

~~14250~~. The LNP of any one of claims ~~106-27~~ to ~~14149~~, wherein the lipid blend further comprises one or more of a structural lipid (e.g., a sterol), a neutral phospholipid, and a free PEG-lipid.

~~14351~~. The LNP of any one of claims ~~106-27~~ to ~~14250~~, wherein the ionizable cationic lipid is present in the lipid blend in a range of 40-60 mole percent.

~~14452~~. The LNP of claim ~~14251~~, wherein the sterol is present in the lipid blend in a range of 30-50 mole percent, and -

~~145. The LNP of claim 142 or 144~~, wherein the sterol is cholesterol.

~~14653~~. The LNP of any one of claims ~~138-47~~ to ~~14552~~, wherein the neutral phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), and -

~~147. The LNP of any one of claims 106 to 146, wherein the neutral phospholipid is present in the lipid blend in a range of 1-10 mole percent.~~

~~148~~54. The LNP of any one of claims ~~106-27~~ to ~~146~~53, wherein the free PEG-lipid is selected from the group consisting of PEG-distearoyl-phosphatidylethanolamine (PEG-DSPE) or PEG-dimyristoyl-phosphatidylethanolamine (PEG-DMPE), N-(Methylpolyoxyethylene oxycarbonyl)-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE-PEG) 1,2-Dimyristoyl-rac-glycero-3-methylpolyoxyethylene (PEG-DMG), 1,2-Dipalmitoyl-rac-glycero-3-methylpolyoxyethylene (PEG-DPG), 1,2-Dioleoyl-rac-glycerol, methoxypolyethylene Glycol (DOG-PEG) 1,2-Distearoyl-rac-glycero-3-methylpolyoxyethylene (PEG-DSG), N-palmitoyl-sphingosine-1-{succinyl[methoxy(polyethylene glycol)] (PEG-ceramide), and DSPE-PEG-cysteine, or a derivative thereof.

~~149~~55. The LNP of any one of claims ~~106-27~~ to ~~148~~54, wherein the free PEG-lipid comprises a diacylphosphatidylethanolamines comprising Dipalmitoyl (C16) chain or Distearoyl (C18) chain, and ~~;~~

~~150. The LNP of any one of claims 106 to 149, wherein the free PEG-lipid is present in the lipid blend in a range of 1-2 mole percent.~~

~~151~~56. The LNP of any one of claims ~~106-27~~ to ~~150~~55, wherein the free PEG-lipid comprises the same or a different lipid as the lipid in the lipid-immune cell targeting group conjugate.

~~152~~57. The LNP of any one of claims ~~106-27~~ to ~~151~~56, wherein the LNP has a mean diameter in the range of 50-200 nm, preferably ~~;~~

~~153. The LNP of claim 152, where the LNP has a mean diameter of about 100 nm;~~

~~154. The LNP of any one of claims 106 to 153, wherein the LNP has a polydispersity index in a range from 0.05 to 1;~~ or

~~155. The LNP of any one of claims 106 to 154, wherein the LNP has a zeta potential of from about -10 mV to about + 30 mV at pH 5.~~

~~156~~58. The LNP of any one of claims ~~106-27~~ to ~~155~~57, wherein the nucleic acid is DNA or RNA.

~~157~~59. The LNP of claim ~~156~~58, wherein the RNA is an mRNA that -

~~158. The LNP of claim 157, wherein the mRNA~~ encodes a receptor, a growth factor, a hormone, a cytokine, an antibody, an antigen, an enzyme, or a vaccine; or -

~~159. The LNP of claim 157, wherein the mRNA encodes~~ a polypeptide capable of regulating immune response in the immune cell, preferably -

~~160. The LNP of claim 159, wherein the mRNA encodes a polypeptide capable of reprogramming the immune cell.~~

~~161. The LNP of claim 160, wherein the mRNA encodes~~ a synthetic T cell receptor (synTCR) or a Chimeric Antigen Receptor (CAR).

~~162~~60. A lipid nanoparticle (LNP) for delivering a nucleic acid into an immune cell of a subject, wherein the LNP comprises:

- (a) An ionizable cationic lipid,
- (b) A conjugate comprising the following structure:
[Lipid] – [optional linker] – [immune cell targeting group];
- (c) A sterol or other structural lipid;
- (d) A neutral phospholipid;
- (e) A free Polyethylene glycol (PEG) lipid, and
- (f) the nucleic acid,

wherein the immune cell targeting group comprises a Fab lacking the native interchain disulfide bond.

~~163~~61. The LNP of claim ~~162~~60, wherein the Fab is engineered to replace one or both cysteines on the native constant light chain and the native constant heavy chain that form the native interchain disulfide with a non-cysteine amino acid, therefor to remove the native interchain disulfide bond in the Fab.

62. A lipid nanoparticle (LNP) for delivering a nucleic acid into an immune cell of a subject, wherein the LNP comprises:

- (a) An ionizable cationic lipid,

(b) A conjugate comprising the following structure:

[Lipid] – [optional linker] – [immune cell targeting group];

(c) A sterol or other structural lipid;

(d) A neutral phospholipid;

(e) A free Polyethylene glycol (PEG) lipid, and

(f) the nucleic acid.

63. The LNP of claim 62, wherein the LNP provides at least one of the following benefits:

(i) increased specificity of targeted delivery to the immune cell compared to a reference LNP;

(ii) increased half-life of the nucleic acid or a polypeptide encoded by the nucleic acid in the immune cell compared to a reference LNP;

(iii) increased transfection rate compared to a reference LNP; and

(iv) a low level of dye accessible mRNA (<15%) and high RNA encapsulation efficiencies, wherein at least 80% mRNA was recovered in final formulation relative to the total RNA used in LNP batch preparation.

64. The LNP of claim 62, wherein the LNP provides at least one of the following benefits:

(i) increased expression level in the immune cell compared to a reference LNP;

(ii) increased specificity of expression in the immune cell compared to a reference LNP;

(iii) increased half-life of the nucleic acid or a polypeptide encoded by the nucleic acid in the immune cell compared to a reference LNP;

(iv) increased transfection rate compared to a reference LNP; and

(v) the LNP can be administered at a lower dose compared to a reference LNP to reach the same biologic effect in the immune cell; and

(vi) a low level of dye accessible mRNA (<15%) and high RNA encapsulation efficiencies, wherein at least 80% mRNA was recovered in final formulation relative to the total RNA used in LNP batch preparation.

16465. Use of a lipid nanoparticle (LNP) of any one of claims to 1-64 in the manufacture of a medicament for A method of targeting the delivery of a nucleic acid to an immune cell of a subject, wherein ~~comprising contacting~~ the immune cell is to be contacted with a lipid nanoparticle (the LNP) of any one of claims to 106 to 163.

16566. The ~~method use~~ of claim 16465, wherein ~~the method is for targeting~~ NK cells are to be targeted, and wherein the immune cell targeting group binds to CD56.

16667. The ~~method use~~ of claim 16465, wherein ~~the method is for targeting~~ both T cells and NK cells are to be targeted simultaneously, and wherein the immune cell targeting group binds to CD7, CD8, or both CD7 and CD8.

16768. The ~~method use~~ of claim 16465, wherein ~~the method is for targeting~~ both CD4+ and CD8+ T cells are to be targeted simultaneously, and wherein the immune cell targeting group comprises a polypeptide that binds to CD3 or CD7.

16869. Use of a lipid nanoparticle (LNP) of any one of claims to 1-64 in the manufacture of a medicament for A method of expressing a polypeptide of interest in a targeted immune cell of a subject, wherein ~~comprising contacting~~ the immune cell is to be contacted with ~~the a lipid nanoparticle (LNP) of any one of claims to 106 to 163.~~

16970. Use of a lipid nanoparticle (LNP) of any one of claims to 1-64 in the manufacture of a medicament for A method of modulating cellular function of a target immune cell of a subject, wherein ~~comprising administering to the subject a the lipid nanoparticle (LNP) of any one of claims to 106 to 159~~ is to be administered to the subject.

17071. Use of a lipid nanoparticle (LNP) of any one of claims to 1-64 in the manufacture of a medicament for A method of treating, ameliorating, or preventing a symptom of a disorder or disease in a subject in need thereof, wherein ~~the comprising administering to the subject a lipid nanoparticle (LNP) of any one of claims to 106 to 163~~ disorder is an immune disorder, an inflammatory disorder, or cancer.

~~171~~72. An immunoglobulin single variable domain (ISVD) that binds to human CD8, wherein the ISVD comprises three complementarity determining domains CDR1, CDR2, and CDR3, wherein

(a) the CDR1 comprises GSTFSDYG (SEQ ID NO: 100),

(b) the CDR2 comprises IDWNGEHT (SEQ ID NO: 101), and

(c) the CDR3 comprises AADALPYTVRKYNY (SEQ ID NO: 102).

~~172~~73. The ISVD of claim ~~171~~72, wherein the ISVD is humanized.

~~173~~74. The ISVD of claim ~~172~~73, wherein the ISVD comprises SEQ ID NO: 77.

~~174~~75. A polypeptide comprising GSTFSDYG (SEQ ID NO: 100), IDWNGEHT (SEQ ID NO: 101), and AADALPYTVRKYNY (SEQ ID NO: 102).

~~175~~76. A polypeptide comprising the ISVD of claim ~~171~~72.

~~176~~77. The polypeptide of claim ~~175~~76, further comprising a second binding moiety, wherein the second binding moiety binds to CD8 or another different target.

~~177~~78. The polypeptide of claim ~~176~~77, wherein the second binding moiety is also an ISVD.

~~178~~79. The polypeptide of claim ~~175~~76, further comprising a detectable marker.

~~179~~80. The polypeptide of claim ~~175~~76, further comprising a therapeutic agent.

~~180~~81. A composition comprising the ISVD of any one of claims ~~171~~72 to ~~173~~74, or the polypeptide of any one of claims ~~174~~75 to ~~179~~80.

~~181~~82. A pharmaceutical composition comprising the ISVD of any one of claims ~~171~~72 to ~~173~~74, or the polypeptide of any one of claims ~~174~~75 to ~~179~~80, and a pharmaceutically acceptable carrier.

~~182~~83. Use of the pharmaceutical composition of claim 82 in the manufacture of a medicament for ~~A method of~~ treating a disease or disorder related to CD8 in a subject, ~~comprising administering the pharmaceutical composition of claim 181 to the subject.~~

~~183~~84. The ~~use~~method of claim ~~182~~83, wherein the disease or disorder is cancer.