

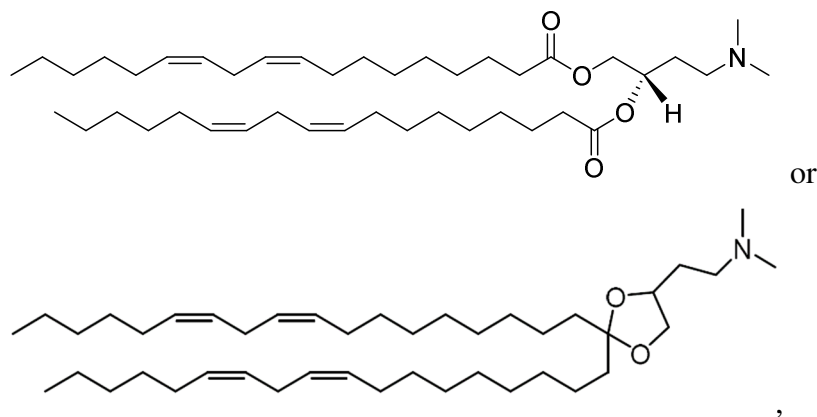
CLAIMS

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 CD3, CD4, CD7, CD8, or a combination thereof.

3. 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 CD7, CD8, CD56, or a combination thereof.

4. The LNP of any one of claims 1 to 3, wherein the immune cell targeting group is covalently coupled to a lipid in the lipid blend via a polyethylene glycol (PEG) containing linker, and 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 wherein the PEG is PEG 2000 or PEG 3400.

5. The LNP of any one of claims 1-4, 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).

6. The LNP of any one of claims 1 to 5, 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.

7. The LNP of any one of claims 1 to 6, wherein the ionizable cationic lipid is present in the lipid blend in a range of 30-70 (e.g., 40-60) mole percent.

8. The LNP of claim 6, wherein the sterol is present in the lipid blend in a range of 20-70 (e.g., 30-50) mole percent, and wherein the sterol is cholesterol.

9. The LNP of any one of claims 6 to 8, 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 is present in the lipid blend in a range of 1-10 mole percent.

10. The LNP of any one of claims 6 to 9, 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.

11. The LNP of any one of claims 6 to 9, wherein the free PEG-lipid comprises a diacylphosphatidylethanolamine comprising Dipalmitoyl (C16) chain or Distearoyl (C18) chain, and is present in the lipid blend in a range of 1-4 mole percent.

12. The LNP of any one of claims 6 to 11, wherein the free PEG-lipid comprises the same or a different lipid as the lipid in the lipid-immune cell targeting group conjugate.

13. The LNP of any one of claims 1 to 12, wherein the LNP has a mean diameter in the range of 50-200 nm, preferably about 100 nm, a polydispersity index in a range from 0.05 to 1, or a zeta potential of from about -10 mV to about + 30 mV at pH 5.

14. The LNP of any one of claims 1 to 13, wherein the nucleic acid is DNA or RNA.

15. The LNP of claim 14, wherein the RNA is an mRNA that encodes a receptor, a growth factor, a hormone, a cytokine, an antibody, an antigen, an enzyme, or a vaccine; 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)

16. The LNP of any one of claims 1 to 15, 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).

17. The LNP of any one of claims 1 to 15, wherein the immune cell targeting group comprises a Fab, F(ab')₂, Fab'-SH, Fv, or scFv fragment.

18. The LNP of claim 16 or claim 17, 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 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.

19. The LNP of any one of claims 17-18, wherein the immune cell targeting group comprises a Fab that has a non-natural interchain disulfide bond (e.g., an engineered, buried interchain disulfide bond), and wherein the Fab comprises F174C substitution in the heavy chain fragment, and S176C substitution in the light chain fragment, numbering according to Kabat.

20. The LNP of claims 17 to 19, 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 one or more amino acids between the heavy chain fragment of the Fab and the C-terminal cysteine.

21. The LNP of claim 16, wherein the immune cell targeting group comprises an immunoglobulin single variable domain comprising a cysteine at the C-terminus, a VHH domain, and a spacer comprising one or more amino acids between the VHH domain and the C-terminal cysteine.

22. The LNP of any one of claims 17 and 21, wherein the immune cell targeting group comprises two or more VHH domains that are linked by an amino acid linker.

23. The LNP of claim 22, 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.

24. The LNP of any one of claims 16 and 21, 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.

25. The LNP of claim 23 or 24, wherein the CH1 domain comprises F174C and C233S substitutions, and the light chain constant domain comprises S176C and C214S substitutions, numbering according to Kabat.

26. The LNP of any one of claims 1 to 15, 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.

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.

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.

29. 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.

30. The LNP of claim 29, wherein the Fab is engineered to knock out the natural interchain disulfide at the C-terminus, and comprises a heavy chain fragment that comprises C233S substitutions, and a light chain fragment that comprises C214S substitutions.

31. The LNP of claim 30, wherein the Fab comprises a non-natural interchain disulfide, or F174C substitution in the heavy chain fragment, and S176C substitution in the light chain fragment.

32. The LNP of claim 29, wherein the antibody is an immunoglobulin single variable (ISV) domain, and the ISV domain is an Nanobody® ISV.

33. The LNP of claim 32, wherein the free PEG lipid comprises a PEG having a molecular weight of at least 2000 daltons, preferably 3000 to 5000 daltons.

34. The LNP of claim 29, wherein the antibody is a Fab that binds CD3, and the free PEG lipid in the LNP comprises a PEG having a molecular weight of about 2000 daltons.

35. The LNP of claim 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.

36. The LNP of claim 29, wherein the immune cell targeting group comprises two or more VHH domains that are linked by an amino acid linker.

37. The LNP of claim 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.

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.

39. The LNP of claim 38, 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 one conjugate, and the conjugate comprises a bispecific antibody that binds both CD3 and CD11a; or one conjugate, and the conjugate comprises a bispecific antibody that binds both CD3 and CD18, wherein the bispecific antibody is an immunoglobulin single variable domain or Fab-ScFv.

40. 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.

41. The LNP of claim 40, wherein the LNP comprises two conjugates, wherein the first conjugate comprises an antibody that binds CD7, and a second conjugate that binds CD8; or one conjugate, wherein the conjugate comprises a bispecific antibody that binds CD7 and CD8, and wherein the bispecific antibody is an immunoglobulin single variable domain or Fab-ScFv.

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.

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

44. The LNP of claim 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 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 the bispecific antibody is an immunoglobulin single variable domain or a Fab-ScFv.

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.

46. 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.

47. 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.

48. The LNP of any one of claims 27 to 47, wherein the immune cell targeting group is covalently coupled to a lipid in the lipid blend via a polyethylene glycol (PEG) containing linker, and 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),

dimyristoyl-glycerol (DMG), dipalmitoyl-phosphatidylethanolamine (DPPE), dipalmitoyl-glycerol (DPG), or ceramide.

49. The LNP of any one of claims 27 to 41, wherein the lipid-immune cell targeting group conjugate is present in the lipid blend in a range of 0.002-0.2 mole percent.

50. The LNP of any one of claims 27 to 49, 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.

51. The LNP of any one of claims 27 to 50, wherein the ionizable cationic lipid is present in the lipid blend in a range of 40-60 mole percent.

52. The LNP of claim 51, wherein the sterol is present in the lipid blend in a range of 30-50 mole percent, and wherein the sterol is cholesterol.

53. The LNP of any one of claims 47 to 52, 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 is present in the lipid blend in a range of 1-10 mole percent.

54. The LNP of any one of claims 27 to 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.

55. The LNP of any one of claims 27 to 54, wherein the free PEG-lipid comprises a diacylphosphatidylethanolamines comprising Dipalmitoyl (C16) chain or Distearoyl (C18) chain, and is present in the lipid blend in a range of 1-2 mole percent.

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

57. The LNP of any one of claims 27 to 59, wherein the LNP has a mean diameter in the range of 50-200 nm, preferably about 100 nm; a polydispersity index in a range from 0.05 to 1; or a zeta potential of from about -10 mV to about + 30 mV at pH 5.

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

59. The LNP of claim 58, wherein the RNA is an mRNA that encodes a receptor, a growth factor, a hormone, a cytokine, an antibody, an antigen, an enzyme, or a vaccine; or a polypeptide capable of regulating immune response in the immune cell, preferably a synthetic T cell receptor (synTCR) or a Chimeric Antigen Receptor (CAR).

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.

61. The LNP of claim 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.
65. Use of a lipid nanoparticle (LNP) of any one of claims

to 1-64 in the manufacture of a medicament for targeting the delivery of a nucleic acid to an immune cell of a subject, wherein the immune cell is to be contacted with the LNP.

66. The use of claim 65, wherein NK cells are to be targeted, and wherein the immune cell targeting group binds to CD56.

67. The use of claim 65, wherein 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.

68. The use of claim 65, wherein 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.

69. Use of a lipid nanoparticle (LNP) of any one of claims to 1-64 in the manufacture of a medicament for expressing a polypeptide of interest in a targeted immune cell of a subject, wherein the immune cell is to be contacted with the LNP.

70. Use of a lipid nanoparticle (LNP) of any one of claims to 1-64 in the manufacture of a medicament for modulating cellular function of a target immune cell of a subject, wherein the LNP is to be administered to the subject.

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

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).

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

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

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

76. A polypeptide comprising the ISVD of claim 72.

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

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

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

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

81. A composition comprising the ISVD of any one of claims 72 to 74, or the polypeptide of any one of claims 75 to 80.

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

83. Use of the pharmaceutical composition of claim 82 in the manufacture of a medicament for treating a disease or disorder related to CD8 in a subject.

84. The use of claim 83, wherein the disease or disorder is cancer.