

CLAIMS

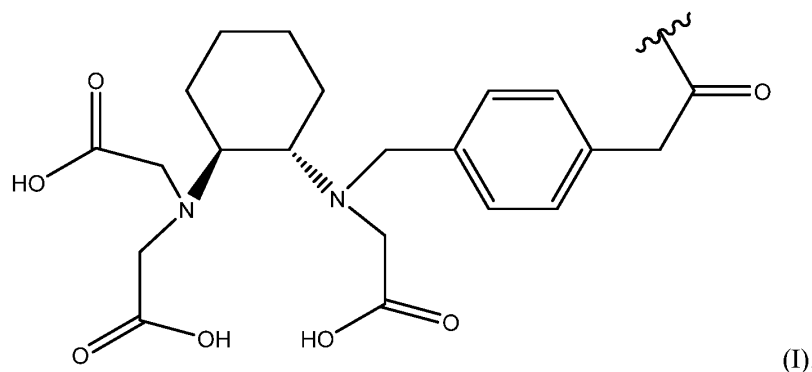
What is claimed is:

1. A CD8 binding agent comprising a variable domain of the heavy chain of a heavy chain antibody (VHH domain), wherein the CD8 binding agent specifically binds a human CD8 with a K_D of about 1 nM or less.
2. The CD8 binding agent of claim 1, wherein the CD8 binding agent binds human CD8 with a k_{off} of about 0.002/s or less.
3. The CD8 binding agent of claim 1 or 2, wherein the CD8 binding agent binds cynomolgus CD8 with a K_D of about 1 nM or less.
4. The CD8 binding agent of any one of claims 1-3, wherein the CD8 binding agent binds cynomolgus CD8 with a k_{off} of about 0.004/s or less.
5. The CD8 binding agent of any one of claims 1-4, wherein the CD8 binding agent does not stimulate or inhibit the activation of CD8⁺ T cells.
6. The CD8 binding agent of any one of claims 1-5, wherein the CD8 binding agent does not induce CD8⁺ T cell proliferation.
7. The CD8 binding agent of any one of claims 1-6, wherein the CD8 binding agent does not bind CD4⁺ T cells.
8. The CD8 binding agent of any one of claims 1-7, wherein the VHH domain is a llama VHH.
9. The CD8 binding agent of any one of claims 1-8, wherein the VHH domain is humanized.
10. The CD8 binding agent of any one of claims 1-9, wherein the VHH domain specifically binds a human CD8 α epitope comprising Arg25, Lys42, Gln44, Val45, Leu46, Leu47, Ser48, Pro50, Thr51, Ser52, Gln75, Arg93, Leu94, Gly95, Asp96, and Thr97, wherein the amino acid numbering is according to SEQ ID NO: 13.

11. The CD8 binding agent of claim 10, wherein the VHH domain comprises a complementarity determining region (CDR) 1 comprising an amino acid sequence of SEQ ID NO: 6 or 7; a CDR2 comprising an amino acid sequence of SEQ ID NO: 8 or 9; and a CDR3 comprising an amino acid sequence of any one of SEQ ID NOs: 10-12.
12. The CD8 binding agent of claim 11, wherein the VHH domain comprises:
- (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 6, a CDR2 comprising the amino acid sequence of SEQ ID NO: 8, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 10;
 - (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 6, a CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 11;
 - (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 7, a CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 11; or
 - (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 6, a CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 12.
13. The CD8 binding agent of claim 11, wherein the VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 6, a CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 12.
14. The CD8 binding agent of any one of claims 1-13, wherein the VHH domain comprises L49A, wherein the numbering is according to Kabat numbering.
15. The CD8 binding agent of any one of claims 1-14, wherein the VHH domain comprises one or more amino acid modifications selected from the group consisting of V89T substitution, T110Q substitution, S112Q substitution and A114 addition, wherein the numbering is according to Kabat numbering.
16. The CD8 binding agent of any one of claims 1-15, wherein the VHH domain comprises the amino acid sequence of any one of SEQ ID NOs: 1-4.

17. The CD8 binding agent of any one of claims 1-16, wherein the agent does not comprise an Fc region.
18. An isolated nucleic acid encoding the CD8 binding agent of any one claims 1-17.
19. An expression vector comprising the nucleic acid of claim 18.
20. A host cell comprising the nucleic acid of claim 18 or the expression vector of claim 19.
21. The host cell of claim 20, wherein the host cell is a eukaryotic cell such as a mammalian cell (*e.g.*, an Expi293 cell), or a prokaryotic cell.
22. A method of making a CD8 binding agent, the method comprising:
 - a) culturing the host cell of claim 20 or 21 under conditions where the agent is produced; and
 - b) recovering the CD8 binding agent produced by the host cell.
23. The CD8 binding agent of any one of claims 1-17, wherein the VHH domain is conjugated to a label.
24. The CD8 binding agent of claim 23, wherein the label is a fluorescent dye, a radionuclide, or an enzyme.
25. The CD8 binding agent of claim 24, wherein the label is a radionuclide.
26. The CD8 binding agent of claim 25, wherein the radionuclide is ^{18}F , ^{89}Zr , $^{99\text{m}}\text{Tc}$, ^{67}Ga , ^{68}Ga , ^{64}Cu , ^{52}Mn , ^{111}In , or ^{124}I .
27. The CD8 binding agent of any one of claims 23-26, wherein the VHH domain is conjugated to a label via a chelating moiety.
28. The CD8 binding agent of claim 27, wherein the chelating moiety is covalently linked to the VHH domain via a lysine residue.
29. The CD8 binding agent of claim 27 or 28, wherein the label forms a complex with a metal, wherein the complex is chelated by the chelating moiety.

30. The CD8 binding agent of claim 29, wherein the label is ^{18}F and the metal is aluminum.
31. The CD8 binding agent of claim 30, wherein the chelating moiety is a compound of Formula (I):



32. A method of detecting CD8^+ cells in a subject, the method comprising:
- a) administering the labeled CD8 binding agent of any one of claims 23-31 to the subject;
- and
- b) detecting binding of the labeled CD8 binding agent to CD8^+ cells in the subject, wherein the detection of the binding indicates the presence of CD8^+ cells.
33. The method of claim 32, wherein detecting binding of the labeled CD8 binding agent to CD8^+ cells in the subject comprises imaging CD8^+ cells in the subject.
34. The method of claim 33, wherein imaging CD8^+ cells in the subject comprises performing a positron emission tomography (PET) scan or positron emission tomography/computed tomography (PET/CT) scan on the subject.
35. The method of any one of claims 32-34, wherein the CD8^+ cells are CD8^+ T cells or CD8^+ tumor cells.
36. The method of any one of claims 32-35, wherein the detecting is carried out within about 1 day or less after the administering.

37. The method of any one of claims 32-36, wherein the method is repeated for one or more times.
38. The method of claim 37, wherein the method is repeated after about 1 day after the prior administration of the CD8 binding agent.
39. The method of claim 37 or 38, wherein the method is repeated for 1 to 4 times per year.
40. The method of any one of claims 37-39, wherein the method is repeated for more than 1 year.
41. The method of any one of claims 32-40, wherein the method has a sensitivity of about 1nM to about 30 nM.
42. The method of any one of claims 32-41, wherein the subject is a human or a non-human primate.
43. The method of claim 42, wherein the non-human primate is a cynomolgus monkey or a rhesus monkey.
44. The method of any one of claims 32-43, wherein the subject has cancer.
45. The method of any one of claims 32-43, wherein the subject has an autoimmune disease or condition, transplant rejection, or graft-versus-host disease.
46. A method of predicting responsiveness of a subject having a cancer to an immunotherapeutic agent, a cell therapy, or a cancer vaccine, the method comprising:
- a) administering the labeled CD8 binding agent of any one of claims 23-31 to the subject and;
 - b) detecting binding of the labeled CD8 binding agent to CD8⁺ T cells in a tumor tissue in the subject, wherein the detection of the binding indicates that the subject is likely to respond to the immunotherapeutic agent, the cell therapy, or the cancer vaccine.

47. The method of claim 46, further comprising the step of:
- (c) administering a therapeutically effective amount of the immunotherapeutic agent, the cell therapy, or the cancer vaccine to the subject in whom the binding has been detected.
48. A method of monitoring disease progression in a subject having cancer, the method comprising:
- a) administering the labeled CD8 binding agent of any one of claims 23-31 to the subject, and
- b) detecting binding of the labeled CD8 binding agent to CD8⁺ T cells in a tumor tissue in the subject at a first time point and a second time point.
49. The method of claim 48, further comprising the step of:
- (c) administering a therapeutically effective amount of an immunotherapeutic agent, a cell therapy, or a cancer vaccine to the subject wherein a level of CD8⁺ T cells in the tumor tissue at the second time point is higher than the level of CD8⁺ T cells in the tumor tissue at the first time point.
50. A method of monitoring treatment progress in a subject having cancer who has or is receiving an immunotherapeutic agent, a cell therapy, or a cancer vaccine, the method comprising:
- i) administering the labeled CD8 binding agent of any one of claims 23-31 to the subject in conjunction with the immunotherapeutic agent, the cell therapy, or the cancer vaccine, and
- ii) detecting binding of the labeled CD8 binding agent to CD8⁺ T cells in a tumor tissue at a first time point and a second time point.
51. The method of claim 50, wherein the labeled CD8 binding agent is administered before the immunotherapeutic agent, the cell therapy, or the cancer vaccine, wherein the first time point is after the administration of the labeled CD8 binding agent and prior to the administration of the immunotherapeutic agent, the cell therapy, or the cancer vaccine, and wherein the second time point is after the administration of the immunotherapeutic agent, the cell therapy, or the cancer vaccine.

52. The method of claim 50, wherein the immunotherapeutic agent, the cell therapy, or the cancer vaccine is administered before the labeled CD8 binding agent, wherein the first time point is after the administration of the immunotherapeutic agent, the cell therapy, or the cancer vaccine and after the administration of the labeled CD8 binding agent, and wherein the second time point is after the first time point.
53. The method of any one of claims 46-47, and 49-52, wherein the immunotherapeutic agent is administered to the subject.
54. The method of claim 53, wherein the immunotherapeutic agent is an anti-PDL1 antibody, an anti-PD1 antibody, an anti-TIGIT antibody, a TIGIT antagonist, an anti-CSF-1R antibody, an anti-CSF-1R antagonist, an anti-CEA antibody, an anti-CEA antagonist, an anti-CTLA4 antibody, a CTLA4 antagonist, an anti-OX40 antibody, or an OX40 agonist.
55. The method of claim 54, wherein the immunotherapeutic agent is an anti-PD-L1 antibody.
56. The method of claim 55, wherein the anti-PD-L1 antibody is atezolizumab.
57. The method of claim 55 or 56, wherein the anti-PD-L1 antibody is administered in combination with one or more therapeutic agents.
58. The method of claim 57, wherein the one or more therapeutic agents is TARCEVA[®] (erlotinib), ZELBORAF[®] (vemurafenib), GAZYVA[®] (obinutuzumab), AVASTIN[®] (bevacizumab), COTELLIC[®] (cobimetinib), ZELBORAF[®] (vemurafenib) and COTELLIC[®] (cobimetinib), ALECENSA[®] (alectinib), KADCYLA[®] (ado-trastuzumab emtansine), HERCEPTIN[®] (trastuzumab), PERJETA[®] (pertuzumab), polatuzumab, IFN-alpha, an anti-CD40 agent, an anti-OX40 antibody, an OX40 agonist, an anti-CSF-1R antibody, an anti-CEA antibody, an IDO inhibitor, or an anti-TIGIT antibody.
59. The method of claim 53, wherein the immunotherapeutic agent is a cytokine, such as IL2, an engineered IL2, IL15, or an engineered IL15

60. The method of claim 53, wherein the immunotherapeutic agent is a dendritic cell modulator, such as a dendritic cell activator or dendritic cell growth factor.
61. The method of claim 53, wherein the immunotherapeutic agent is a bispecific antigen-binding molecule that specifically binds CD3 or CD16 such as CD16A.
62. The method of any one of claims 46-47, and 49-52, wherein the cancer vaccine is administered to the subject.
63. The method of claim 62, wherein the cancer vaccine is a Personalized Cancer Vaccine (PCV).
64. The method of any one of claims 46-47, and 49-52, wherein the cell therapy is administered to the subject.
65. The method of claim 64, wherein the cell therapy is a CAR-T or neoantigen-specific T cells.
66. A method of predicting responsiveness of a subject having an autoimmune disease or condition, transplant rejection, or graft-versus-host disease to an immunotherapeutic agent, the method comprising:
- a) administering the labeled CD8 binding agent of any one of claims 23-31 to the subject and;
 - b) detecting binding of the labeled CD8 binding agent to CD8⁺ T cells in a diseased tissue in the subject, wherein the detection of the binding indicates that the subject is likely to respond to the immunotherapeutic agent.
67. The method of claim 66, further comprising the step of:
- (c) administering a therapeutically effective amount of the immunotherapeutic agent to the subject in whom the binding has been detected.
68. A method of monitoring disease progression in a subject having an autoimmune disease or condition, transplant rejection, or graft-versus-host disease, the method comprising:

a) administering the labeled CD8 binding agent of any one of claims 23-31 to the subject,
and

b) detecting binding of the labeled CD8 binding agent to CD8⁺ T cells in a diseased tissue in the subject at a first time point and a second time point, wherein an increase in CD8⁺ T cells from the first time point and the second time point is an indication that the autoimmune disease or condition, transplant rejection, or graft-versus-host disease has progressed.

69. The method of claim 68, further comprising the step of:

(c) administering a therapeutically effective amount of an immunotherapeutic agent to the subject wherein a level of CD8⁺ T cells in the diseased tissue at the second time point is lower than the level of CD8⁺ T cells in the diseased tissue at the first time point.

70. A method of monitoring treatment progress in a subject having an autoimmune disease or condition, transplant rejection, or graft-versus-host disease who has or is receiving an immunotherapeutic agent, the method comprising:

i) administering the labeled CD8 binding agent of any one of claims 23-31 to the subject in conjunction with the immunotherapeutic agent, and

ii) detecting binding of the labeled CD8 binding agent to CD8⁺ T cells in a diseased tissue at a first time point and a second time point.

71. The method of claim 70, wherein the labeled CD8 binding agent is administered before the immunotherapeutic agent, wherein the first time point is after the administration of the labeled CD8 binding agent and prior to the administration of the immunotherapeutic agent, and wherein the second time point is after the administration of the immunotherapeutic agent.

72. The method of claim 70, wherein the immunotherapeutic agent is administered before the labeled CD8 binding agent, wherein the first time point is after the administration of the immunotherapeutic agent and after the administration of the labeled CD8 binding agent, and wherein the second time point is after the first time point.

73. The method of any one of claims 46-72, wherein detecting binding of the labeled CD8 binding agent to the CD8⁺ T cells in the subject comprises imaging CD8⁺ T cells in the subject.

74. The method of claim 73, wherein imaging the CD8⁺ T cells in the subject comprises performing a positron emission tomography (PET) scan or positron emission tomography/computed tomography (PET/CT) scan on the subject.

75. The method of any one of claims 48-65 and 68-74, wherein the subject is monitored for at least 1 year.

76. A method of identifying gut microbial strains associated with responsiveness to treatment with an immunotherapeutic agent, comprising:

a) obtaining gut microbiome samples from a population of subjects having cancer, which population comprises subjects who are responsive to treatment with the immunotherapeutic agent and subjects who are not responsive to treatment with the immunotherapeutic agent;

b) analyzing the gut microbiome samples of the subjects who are responsive to the treatment and the gut microbiome samples of the subjects who are not responsive to the treatment; and

c) identifying gut microbial strains associated with the subjects who are responsive to the treatment; wherein responsiveness is determined by detecting binding of the labeled CD8 binding agent of any one of claims 23-31 to CD8⁺ T cells in a tumor tissue in the subjects, and wherein the detection of the binding indicates that the subjects are responsive to the immunotherapeutic agent.

77. The method of claim 76, further comprising preparing a microbiome-based drug comprising gut microbial strains associated with responsiveness to the immunotherapeutic agent.

78. The method of claim 76 or 77, wherein the immunotherapeutic agent is an anti-PD-1 antibody or an anti-PD-L1 antibody such as atezolizumab.

79. A kit comprising the labeled CD8 binding agent of any one of claims 23-31.

80. A method of preparing a labeled CD8 binding agent, comprising conjugating a chelating moiety to the VHH domain of the CD8 binding agent of any one of claims 1-17 to provide a conjugate, and contacting the conjugate with an aluminum fluoride complex comprising ^{18}F to provide the labeled CD8 binding agent, wherein the chelating moiety is a compound of Formula (I):

