

CD8 BINDING AGENTS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit of United States Provisional Application No. 62/895,865 filed September 4, 2019, the contents of which are incorporated herein by reference in their entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name:

146392049240SEQLIST.txt, date recorded: August 19, 2020, size: 14 KB).

FIELD

[0003] The present application relates to CD8 binding agents based on anti-CD8 VHH domains and methods of using such CD8 binding agents for imaging CD8⁺ T-cells in vivo.

BACKGROUND

[0004] Characterization of the number, types, and spatial distribution of immune cells in tumor tissues can provide crucial information regarding cancer diagnosis, prognosis, therapy selection, and response to therapy. Specifically, CD8⁺ cytotoxic lymphocytes have been consistently reported as having diagnostic and prognostic significance in various cancers. Current methods of detecting CD8⁺ cells entail the isolation of cells from the peripheral blood or a tissue of interest. Such sampling methods are prone to error and do not provide dynamic information that reflects the number, localization, and movement of CD8⁺ cells in vivo. One exemplary noninvasive method for detecting immune cells in vivo is positron emission tomography (PET) using radiolabeled tracers. However, the use of such tracers is limited by radioisotope half-life and cell division, which leads to probe dilution in vivo. Accordingly, there remains a need in the art for methods and reagents for monitoring changes in the quantity and temporal distribution of CD8⁺ cells in vivo.

BRIEF SUMMARY

[0004a] In one aspect, provided herein is 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 KD of about 1 nM or less.

[0004b] In another aspect, provided herein is an isolated nucleic acid encoding the CD8 binding agent as described herein.

[0004c] In another aspect, provided herein is an expression vector comprising the nucleic acid as described herein.

[0004d] In another aspect, provided herein is a host cell comprising the nucleic acid as described herein or the expression vector as described herein.

[0004e] In another aspect, the present disclosure refers to a method of making a CD8 binding agent, the method comprising: culturing the host cell as described herein under conditions where the agent is produced; and recovering the CD8 binding agent produced by the host cell.

[0004f] In another aspect, the present disclosure refers to a method of detecting CD8⁺ cells in a subject, the method comprising: administering the labeled CD8 binding agent as described herein to the subject; and 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.

[0004g] In another aspect, the present disclosure refers to 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: administering the labeled CD8 binding agent as described herein to the subject and; 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.

[0004h] In another aspect, the present disclosure refers to a method of monitoring disease progression in a subject having cancer, the method comprising: administering the labeled CD8 binding agent as described herein to the subject, and 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.

[0004i] In another aspect, the present disclosure refers to a 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: administering the labeled CD8 binding agent as described herein to the subject in conjunction with the immunotherapeutic agent, the cell therapy, or the cancer vaccine, and 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.

[0004j] In another aspect, the present disclosure refers to 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: administering the labeled CD8 binding agent as described herein to the subject and 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.

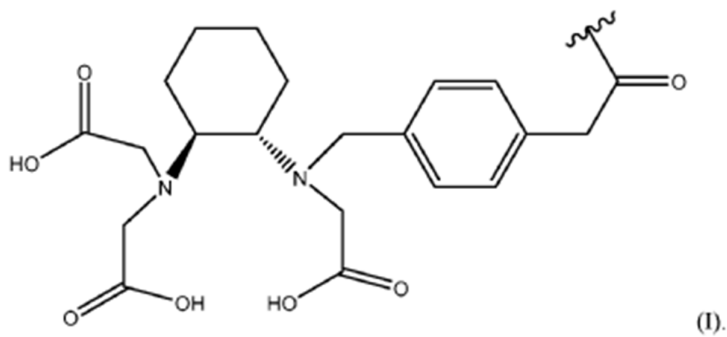
[0004k] In another aspect, the present disclosure refers to a method of monitoring disease progression in a subject having an autoimmune disease or condition, transplant rejection, or graft-versus-host disease, the method comprising: administering the labeled CD8 binding agent as described herein to the subject, and 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.

[0004l] In another aspect, the present disclosure refers to 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: administering the labeled CD8 binding agent as described herein to the subject in conjunction with the immunotherapeutic agent, and 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.

[0004m] In another aspect, the present disclosure refers to a method of identifying gut microbial strains associated with responsiveness to treatment with an immunotherapeutic agent, comprising: 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, 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 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 as described herein 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.

[0004n] In another aspect, the present disclosure refers to a kit comprising the labeled CD8 binding agent as described herein.

[0004a] In another aspect, the present disclosure refers to a method of preparing a labeled CD8 binding agent, comprising conjugating a chelating moiety to the VHH domain of the CD8 binding agent as described herein 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):



[0005] Provided herein is 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 KD of about 1nM or less. In some ~~embodiment~~examples, the CD8 binding agent

specifically binds a human CD8 with a KD of about 500 pM or less, about 250 pM or less, or about 100 pM or less. In some [embodimentexamples](#), the CD8 binding agent specifically binds a human CD8 with a KD of about 132 pM, or about 50 pM. In some [embodimentexamples](#), the CD8 binding agent binds human CD8 with a k_{off} of about 0.002/s or less, or about 0.001/s or less. In some [embodimentexamples](#), the CD8 binding agent binds human CD8 with a k_{off} of about 0.0018/s, or about 0.00085/s. In some [embodimentexamples](#), the CD8 binding agent binds cynomolgus CD8 with a KD of about 1 nM or less. In some [embodimentexamples](#), the CD8 binding agent binds cynomolgus CD8 with a KD of about 500 pM or less, about 250 pM or less, or about 150 pM or less. In some [embodimentexamples](#), the CD8 binding agent binds cynomolgus CD8 with a KD of about 344 pM or about 137 pM. In some [embodimentexamples](#), the CD8 binding agent binds cynomolgus CD8 with a k_{off} of about 0.004/s or less, or about 0.002/s or less. In some [embodimentexamples](#), the CD8 binding agent binds cynomolgus CD8 with a k_{off} of about 0.0037/s, or about 0.0019/s. In some [embodimentexamples](#), the CD8 binding agent has a CD8-binding half-life (e.g., in an in vitro binding assay) of at least about 30 minutes, such as at least about 1 hour, 2 hours or more. In some [embodimentexamples](#), the CD8 binding agent specifically binds a rhesus monkey CD8 with a KD of about 1 nM or less. In some [embodimentexamples](#), the CD8 binding agent does not bind mouse or rat CD8.

[0006] In some [embodimentexamples](#) according to (or as applied to) any of the [embodimentexamples](#) above, the CD8 binding agent does not stimulate or inhibit the activation of CD8⁺ T cells. In some [embodimentexamples](#), the CD8 binding agent does not induce CD8⁺ T cell proliferation. In some [embodimentexamples](#), the CD8 binding agent does not bind CD4⁺ T cells.

[0007] In some [embodimentexamples](#) according to (or as applied to) any of the [embodimentexamples](#) above, the VHH domain is a camelid VHH, such as a llama VHH. In some [embodimentexamples](#), the VHH domain is chimeric. In some [embodimentexamples](#), the VHH is humanized. In some [embodimentexamples](#), the VHH is affinity matured.

[0008] In some [embodimentexamples](#) according to (or as applied to) any of the [embodimentexamples](#) above, the VHH domain specifically binds a human CD8a 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. In some [embodimentexamples](#), the amino acid residues in the human CD8a epitope are within about 4.5 Å from one or more amino

acid residues of the VHH domain in a crystal structure of the CD8 binding agent or the VHH domain bound to the human CD8a.

[0009] In some [embodimentexamples](#) according to (or as applied to) any of the [embodimentexamples](#) above, 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.

[0010] In some [embodimentexamples](#) according to (or as applied to) any of the [embodimentexamples](#) above, 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: 8, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 10. In some [embodimentexamples](#) according to (or as applied to) any of the [embodimentexamples](#) above, 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: 11. In some [embodimentexamples](#) according to (or as applied to) any of the [embodimentexamples](#) above, the VHH domain comprises 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. In some [embodimentexamples](#) according to (or as applied to) any of the [embodimentexamples](#) above, 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.

[0011] In some [embodimentexamples](#) according to (or as applied to) any of the [embodimentexamples](#) above, wherein the VHH domain comprises L49A, wherein the numbering is according to Rabat numbering. In some [embodimentexamples](#), the CD 8 binding agent can be purified using Protein A affinity chromatography.

[0012] In some [embodimentexamples](#) according to (or as applied to) any of the [embodimentexamples](#) above, the VHH domain comprises one or more amino acid modifications selected from the group consisting of V89T substitution, T110Q substitution, S112Q substitution and addition of A at position 114 (referred herein after as “A114 addition”), wherein the numbering is according to Rabat numbering. In some [embodimentexamples](#), the VHH domain comprises V89T substitution, T110Q substitution, S112Q substitution and A114 addition, wherein the numbering is according to Rabat numbering. In some

embodimentexamples, the CD8 binding agent does not bind pre-existing anti-VHH antibodies in a subject receiving the CD8 binding agent.

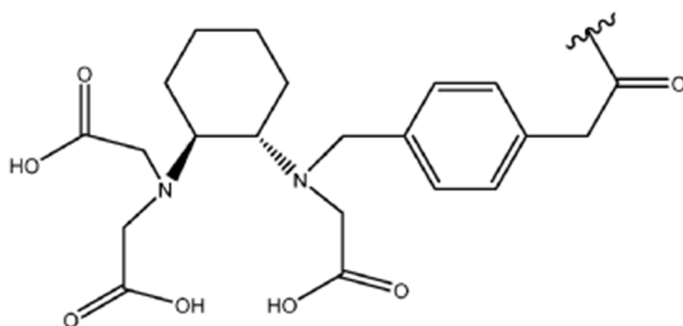
[0013] In some embodimentexamples according to (or as applied to) any of the embodimentexamples above, the VHH domain comprises the amino acid sequence of SEQ ID NO: 1. In some embodimentexamples according to (or as applied to) any of the embodimentexamples above, the VHH domain comprises the amino acid sequence of SEQ ID NO: 2. In some embodimentexamples according to (or as applied to) any of the embodimentexamples above, the VHH domain comprises the amino acid sequence of SEQ ID NO: 3. In some embodimentexamples according to (or as applied to) any of the embodimentexamples above, the VHH domain comprises the amino acid sequence of SEQ ID NO: 4.

[0014] Also provided herein is an isolated nucleic acid encoding the CD8 binding agent according to (or as applied to) any of the embodimentexamples above. In some embodimentexamples, provided is an expression vector comprising the nucleic acid according to (or as applied to) any of the embodimentexamples above. In some embodimentexamples, provided is a host cell comprising the nucleic acid or the expression vector according to (or as applied to) any of the embodimentexamples above. In some embodimentexamples, the host cell is a eukaryotic cell, such as a mammalian cell, for example, CHO cell or Expi293 cell. In some embodimentexamples, the host cell is a prokaryotic cell, such as an E. coli cell.

[0015] Further provided herein is a method of making the CD8 binding agent according to (or as applied to) any of the embodimentexamples above, the method comprising: a) culturing the host cell according to (or as applied to) any of the embodimentexamples above under conditions where the agent is produced; and b) recovering the CD8 binding agent produced by the host cell.

[0016] In some embodimentexamples according to (or as applied to) any of the embodimentexamples above, the CD8 binding agent comprises a label. A CD8 binding agent comprising a label is referred herein as a “labeled CD8 binding agent”.

[0017] In some embodimentexamples, there is provided a method of preparing a labeled CD8 binding agent, comprising conjugating a chelating moiety to the VHH domain of a CD8 binding agent according to (or as applied to) any of the embodimentexamples above 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):



(I). In

some [embodimentexamples](#), the conjugate is contacted with the aluminum fluoride complex in the presence of one or more anti-oxidant compounds, such as methionine and/or N-acetyl-tryptophan.

[0018] Provided herein is a labeled CD8 binding agent comprising an anti-CD8 VHH domain according to (or as applied to) any of the [embodimentexamples](#) above conjugated to a label. In some [embodimentexamples](#), the label is a fluorescent dye, a radionuclide, or an enzyme. In some [embodimentexamples](#) according to (or as applied to) any of the [embodimentexamples](#) above, the label is a radionuclide. In some [embodimentexamples](#), the radionuclide is ^{18}F , ^{89}Zr , $^{99\text{m}}\text{Tc}$, ^{67}Ga , ^{68}Ga , ^{64}Cu , ^{52}Mn , $^{\text{m}}\text{In}$, or ^{124}I . In some [embodimentexamples](#), the VHH domain is conjugated to a label via a chelating moiety. In some [embodimentexamples](#), the chelating moiety is covalently linked to the VHH domain via a lysine residue. In some [embodimentexamples](#), the label forms a complex with a metal, wherein the complex is chelated by the chelating moiety. In some [embodimentexamples](#), the label is ^{18}F and the metal is aluminum. In some [embodimentexamples](#), the chelating moiety is a compound of Formula (I).

[0019] Provided herein is a labeled CD8 binding agent comprising an anti-CD8 VHH domain comprising 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, wherein the VHH domain is conjugated to a radionuclide (e.g., ^{18}F) via a chelating moiety. In some [embodimentexamples](#), the chelating moiety is a compound of Formula (I), and the radionuclide is ^{18}F complexed with aluminum. In some [embodimentexamples](#), the VHH domain comprises the amino acid sequence of SEQ ID NO: 3.

[0020] Provided herein is a labeled CD8 binding agent comprising an anti-CD8 VHH domain comprising 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, wherein the VHH domain is conjugated to a radionuclide (e.g., ^{18}F) via a chelating moiety. In some [embodimentexamples](#), the chelating moiety is a compound of Formula (I), and the radionuclide is ^{18}F complexed with aluminum. In some [embodimentexamples](#), the VHH domain comprises the amino acid sequence of SEQ ID NO: 4.

[0021] Also provided herein is a pharmaceutical composition comprising a CD8 binding agent (including a labeled CD8 binding agent) according to (or as applied to) any of the [embodimentexamples](#) above, and a pharmaceutically acceptable carrier.

[0022] Further provided herein are use of a CD8 binding agent (including a labeled CD8 binding agent) according to (or as applied to) any of the [embodimentexamples](#) above for treatment or diagnosis of a disease or condition in a subject, and use of a CD8 binding agent (including a labeled CD8 binding agent) according to (or as applied to) any of the [embodimentexamples](#) above in the preparation of a medicament for treatment or diagnosis of a disease or condition in a subject.

[0023] Further provided herein is a pharmaceutical formulation comprising a CD8 binding agent (including a labeled CD8 binding agent) according to (or as applied to) any of the [embodimentexamples](#) above and one or more anti-oxidant compounds. In some [embodimentexamples](#), the one or more anti-oxidant compounds are methionine and/or N-acetyl tryptophan. In some [embodimentexamples](#), the pharmaceutical formulation comprises methionine and N-acetyl tryptophan. In some [embodimentexamples](#), the pharmaceutical formulation further comprises histidine and sucrose.

[0024] Provided herein is a method of detecting CD8⁺ cells in a subject, the method comprising: a) administering a labeled CD8 binding agent according to (or as applied to) any of the [embodimentexamples](#) above, 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. In some [embodimentexamples](#), detecting binding of the labeled CD8 binding agent to CD8⁺ cells in the subject comprises imaging CD8⁺ cells in the subject. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the CD8⁺ cells are CD8⁺ T cells. In some [embodimentexamples](#), the CD8⁺ cells are CD8⁺ tumor cells. In some [embodimentexamples](#), the detecting is carried out within about 1 day or less (e.g., within about 6 hours, 4 hours, 2 hours, 90 minutes, 1 hour, 30 minutes or less) after the administering. In some [embodimentexample](#), the method is repeated for one or more times, such as about 1 to 4 times per year. In

some [embodimentexamples](#), the method is repeated after about 1 day after the prior administration of the CD8 binding agent. In some [embodimentexamples](#), the method is repeated for more than 1 year. In some [embodimentexample](#), the method has a sensitivity of about 1 nM to about 30 nM. In some [embodimentexamples](#), the subject is a human or a non-human primate. In some [embodimentexamples](#), the subject is a cynomolgus monkey or a rhesus monkey. In some [embodimentexamples](#), the subject is human. In some [embodimentexamples](#), the subject has cancer. In some [embodimentexample](#), the subject has an autoimmune disease or condition, transplant rejection, or graft- versus-host disease.

[0025] Provided herein is a method of predicting responsiveness of a subject having cancer to an immunotherapeutic agent, a cell therapy, or a cancer vaccine, the method comprising: a) administering the labeled CD8 binding agent according to (or as applied to) any of the [embodimentexamples](#) above 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. In some [embodimentexamples](#), detecting binding of the labeled CD 8 binding agent to CD8⁺ cells in the subject comprises imaging CD8⁺ cells in the subject. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the method further comprises 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. In some [embodimentexamples](#), the detecting is carried out within about 1 day or less (e.g ., within about 6 hours, 4 hours, 2 hours, 90 minutes, 1 hour, 30 minutes or less) after the administering. In some [embodimentexamples](#), the method is repeated for one or more times, such as about 1 to 4 times per year. In some [embodimentexamples](#), the method is repeated after at least 1 day after the prior administration of the CD8 binding agent. In some [embodimentexamples](#), the method is repeated for more than 1 year.

[0026] Also provided herein is a method of monitoring disease progression in a subject having cancer, the method comprising: a) administering the labeled CD8 binding agent according to (or as applied to) any of the [embodimentexamples](#) above 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. In some [embodimentexamples](#), detecting binding of the labeled CD8 binding agent to CD8⁺

cells in the subject comprises imaging CD8⁺ cells in the subject. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the method further comprises 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. In some [embodimentexamples](#), the detecting is carried out within about 1 day or less (e.g ., within about 6 hours, 4 hours, 2 hours, 90 minutes, 1 hour, 30 minutes or less) after the administering. In some [embodimentexamples](#), the method is repeated for one or more times, such as about 1 to 4 times per year. In some [embodimentexamples](#), the method is repeated after at least 1 day after the prior administration of the CD 8 binding agent. In some [embodimentexamples](#), the subject is monitored for more than 1 year.

[0027] Provided herein is 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 according to (or as applied to) any of the [embodimentexamples](#) above 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. In some [embodimentexamples](#), detecting binding of the labeled CD8 binding agent to CD8⁺ cells in the subject comprises imaging CD8⁺ cells in the subject. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), 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 CD 8 binding agent, and wherein the second time point is after the

first time point. In some [embodimentexamples](#), the detecting is carried out within about 1 day or less (e.g., within about 6 hours, 4 hours, 2 hours, 90 minutes, 1 hour, 30 minutes or less) after the administering. In some [embodimentexamples](#), the method is repeated for one or more times, such as about 1 to 4 times per year. In some [embodimentexamples](#), the method is repeated after at least 1 day after the prior administration of the CD8 binding agent. In some [embodimentexamples](#), the subject is monitored for more than 1 year.

[0028] In some [embodimentexamples](#) according to (or as applied to) any of the methods of predicting or methods of monitoring above, the immunotherapeutic agent is administered to the subject. In some [embodimentexamples](#), the immunotherapeutic agent is an anti-PDL1 antibody, an anti-PD 1 antibody, an anti-TIGIT antibody, a TIGIT antagonist, an anti-CSF-IR antibody, an anti-CSF-IR antagonist, an anti-CEA antibody, an anti-CEA antagonist, an anti-CTLA4 antibody, a CTLA4 antagonist, an anti-OX40 antibody, or an OX40 agonist. In some [embodimentexamples](#), the immunotherapeutic agent is an anti-PD-L1 antibody. In some [embodimentexamples](#), the anti-PD-L1 antibody is administered in combination with one or more therapeutic agents. In some [embodimentexamples](#), the one or more therapeutic agents is TARCEVA® (erlotinib), ZELBORAF® (vemurafenib), GAZYVA® (obinituzumab), 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-IR antibody, an anti-CEA antibody, an IDO inhibitor, or an anti-TIGIT antibody. In some [embodimentexamples](#), the immunotherapeutic agent is a cytokine. In some [embodimentexamples](#), the cytokine is IL2, an engineered IL2, IL15, or an engineered IL15. In some [embodimentexamples](#), the immunotherapeutic agent is a bispecific antigen-binding molecule that specifically binds CD3. In some [embodimentexamples](#), the bispecific antigen-binding molecule is an antibody or an antigen-binding fragment thereof. In some [embodimentexamples](#), the immunotherapeutic agent is a bispecific antigen-binding molecule that specifically binds CD 16. In some [embodimentexamples](#), the bispecific antigen-binding molecule is an antibody or an antigen-binding fragment thereof. In some [embodimentexamples](#), the bispecific antigen-binding molecule specifically binds CD16A. In some [embodimentexamples](#), the immunotherapeutic agent is a dendritic cell modulator, such as dendritic cell activator or dendritic cell growth factor.

[0029] In some [embodimentexamples](#) according to (or as applied to) any of the methods of predicting or methods of monitoring above, the cancer vaccine is administered to the subject. In some [embodimentexamples](#), the cancer vaccine is a Personalized Cancer Vaccine (PCV).

[0030] In some [embodimentexamples](#) according to (or as applied to) any of the methods of predicting or methods of monitoring above, the cell therapy is administered to the subject. In some [embodimentexamples](#), the cell therapy is a CAR-T. In some [embodimentexamples](#), the cell therapy is neoantigen-specific T cells.

[0031] Provided herein is 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 according to (or as applied to) any of the [embodimentexamples](#) above 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. In some [embodimentexamples](#), detecting binding of the labeled CD 8 binding agent to CD8⁺ cells in the subject comprises imaging CD8⁺ cells in the subject. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the method further comprises the step of: (c) administering a therapeutically effective amount of an immunotherapeutic agent to the subject in whom the binding has been detected. In some [embodimentexamples](#), the detecting is carried out within about 1 day or less (e.g ., within about 6 hours, 4 hours, 2 hours, 90 minutes, 1 hour, 30 minutes or less) after the administering. In some [embodimentexamples](#), the method is repeated for one or more times, such as about 1 to 4 times per year. In some [embodimentexamples](#), the method is repeated after at least 1 day after the prior administration of the CD8 binding agent. In some [embodimentexamples](#), the method is repeated for more than 1 year.

[0032] Also provided herein is 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 according to (or as applied to) any of the [embodimentexamples](#) above 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. In some [embodimentexamples](#), detecting binding of the labeled CD8 binding agent to CD8⁺ cells in the subject comprises imaging CD8⁺ cells in the subject. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the method further comprises 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. In some [embodimentexamples](#), the detecting is carried out within about 1 day or less (e.g ., within about 6 hours, 4 hours, 2 hours, 90 minutes, 1 hour, 30 minutes or less) after the administering. In some [embodimentexamples](#), the method is repeated for one or more times, such as about 1 to 4 times per year. In some [embodimentexamples](#), the method is repeated after at least 1 day after the prior administration of the CD8 binding agent. In some [embodimentexamples](#), the subject is monitored for more than 1 year.

[0033] Provided herein is 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 according to (or as applied to) any of the [embodimentexamples](#) above 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. In some [embodimentexamples](#), detecting binding of the labeled CD 8 binding agent to CD8⁺ cells in the subject comprises imaging CD8⁺ cells in the subject. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the detecting is carried out within about 1 day or less (e.g., within about 6 hours, 4 hours, 2 hours, 90 minutes, 1 hour, 30 minutes or less) after the administering. In some [embodimentexamples](#), the method is repeated for one or more times, such as about 1 to 4 times per year. In some [embodimentexamples](#), the method is repeated after at least 1 day after the prior administration of the CD8 binding agent. In some [embodimentexamples](#), the subject is monitored for more than 1 year.

[0034] Provided herein is a method of identifying gut microbial strains associated with responsiveness to treatment with an immunotherapeutic agent, the method 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 CD 8 binding agent according to (or as applied to) any of the [embodimentexamples](#) above 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. In some [embodimentexamples](#), the method further comprises preparing a microbiome-based drug comprising gut microbial strains associated with responsiveness to the immunotherapeutic agent. In some [embodimentexamples](#), the immunotherapeutic agent is an anti-PD-1 antibody. In some [embodimentexamples](#), the immunotherapeutic agent is an anti-PD-L1 antibody, such as atezolizumab.

[0035] Further provided herein are kits and articles of manufacture comprising the CD8 binding agent according to (or as applied to) any of the [embodimentexamples](#) above, such as labeled CD8 binding agent. In some [embodimentexamples](#), the kit or article of manufacture comprises an instruction for use of the CD8 binding agent according to any of the methods described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] FIG. 1 provides an alignment of the amino acid sequences of exemplary anti-CD8 VHH domains, including llama VHH wt2C8 (SEQ ID NO: 1), humanized VHHs hu2C8v130 (SEQ ID