

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 VHH 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: 9, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 12; or

(2) 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.

[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, preferably wherein the host cell is a eukaryotic cell.

[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 pharmaceutical composition comprising the CD8 binding agent as described herein, optionally comprising pharmaceutically acceptable excipients, further optionally comprising one or more anti-oxidant compounds.

[0004g] In another aspect, the present disclosure refers to a kit comprising the CD8 binding agent as described herein or the pharmaceutical composition as described herein, optionally further comprising reagents for preparation of the labeled CD8 binding agent.

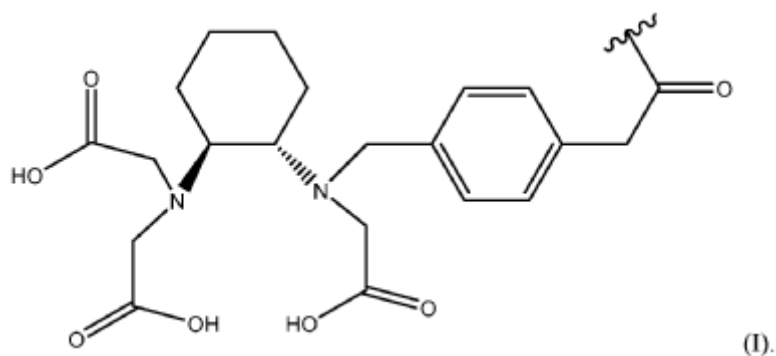
[0004h] In another aspect, the present disclosure refers to the labeled CD8 binding agent as described herein, or the pharmaceutical composition as described herein, for use in treatment, diagnosis, prognosis, therapy selection or of monitoring response to therapy; optionally wherein monitoring response to therapy comprises monitoring the progression/remission of a disease.

[0004i] In another aspect, the present disclosure refers to a method of detecting CD8⁺ cells in a subject, the method comprising:

detecting binding of the labeled CD8 binding agent as described herein or the pharmaceutical composition as described herein to CD8⁺ cells in the subject, wherein the labeled CD8 binding agent

was administered to the subject before detecting the binding of the labeled CD8 binding agent or the pharmaceutical composition to CD8⁺ cells in the subject, wherein the detection of the binding indicates the presence of CD8⁺ cells, preferably wherein detecting binding of the labeled CD8 binding agent to CD8⁺ cells comprises imaging CD8⁺ cells; more preferably wherein imaging CD8⁺ cells comprises performing a positron emission tomography (PET) scan or positron emission tomography/computed tomography (PET/CT) scan on the subject.

[0004j] 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 18F 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 examples, the CD8 binding agent

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 VHH 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: 9, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 12; or

(2) 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.

2. The CD8 binding agent of claim 1, 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.

3. The CD8 binding agent of claim 1 or claim 2, wherein:

(a) the VHH domain comprises L49A, wherein the numbering is according to Kabat numbering; and/or

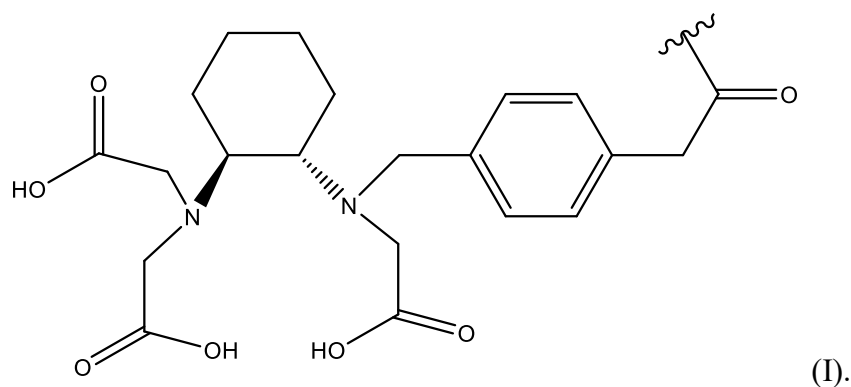
(b) the VHH domain comprises one or more amino acid modifications selected from the group consisting of a V89T substitution, a T110Q substitution, a S112Q substitution and an A114 addition, wherein the numbering is according to Kabat numbering; and/or

(c) the agent does not comprise an Fc region.

4. The CD8 binding agent of any one of claims 1-3, wherein the VHH domain comprises an amino acid sequence that has at least 80% amino acid sequence identity with the amino acid sequence of any one of SEQ ID NOs: 1-4, preferably wherein the VHH domain comprises an amino acid sequence that has at least 90% amino acid sequence identity with the amino acid sequence SEQ ID NOs: 1-4.

5. The CD8 binding agent of claim 4, wherein the VHH domain comprises the amino acid sequence of any one of SEQ ID NOs: 1-4.
6. An isolated nucleic acid encoding the CD8 binding agent of any one claims 1 to 5.
7. An expression vector comprising the nucleic acid of claim 6.
8. A host cell comprising the nucleic acid of claim 6 or the expression vector of claim 7, preferably wherein the host cell is a eukaryotic cell.
9. The host cell of claim 8, wherein the host cell is a mammalian cell (e.g. an Expi293 cell) or a prokaryotic cell.
10. A method of making a CD8 binding agent, the method comprising:
 - a) culturing the host cell of claim 8 or 9 under conditions where the agent is produced; and
 - b) recovering the CD8 binding agent produced by the host cell.
11. The CD8 binding agent of any one of claims 1-5, wherein the VHH domain is conjugated to a chelating moiety.
12. The CD8 binding agent of claim 11, wherein the chelating moiety chelates a complex comprising an ^{18}F label and aluminum.
13. The CD8 binding agent of claim 11 or 12, wherein the chelating moiety is covalently linked to the VHH domain via a lysine residue.
14. The CD8 binding agent of any one of claims 1 to 5, wherein the VHH domain is conjugated to a label; optionally wherein the label is a fluorescent dye, a radionuclide, or an enzyme, preferably wherein the label is a radionuclide, optionally wherein the radionuclide is ^{18}F , ^{32}P , ^{33}P , ^{45}Ti , ^{47}Sc , ^{52}Fe , ^{59}Fe , ^{62}Cu , ^{64}Cu , ^{67}Cu , ^{67}Ga , ^{68}Ga , ^{52}Mn , ^{75}Sc , ^{77}As , ^{86}Y , ^{89}Sr , ^{89}Zr , ^{90}Y , ^{90}Nb , ^{94}Tc , ^{99}Tc , $^{99\text{m}}\text{Tc}$, ^{99}Mo , ^{105}Pd , ^{105}Rh , ^{111}Ag , ^{111}In , ^{123}I , ^{124}I , ^{125}I , ^{131}I , ^{142}Pr , ^{143}Pr , ^{149}Pm , ^{153}Sm , $^{154-158}\text{Gd}$, ^{161}Tb , ^{166}Dy , ^{169}Er , ^{175}Lu , ^{177}Lu , ^{186}Re , ^{188}Re , ^{189}Re , ^{194}Ir , ^{198}Au , ^{199}Au , ^{211}At , ^{211}Pb , ^{212}Bi , ^{212}Pb , ^{213}Bi , ^{223}Ra , or ^{225}Ac ; more preferably 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 ; most preferably wherein the radionuclide is ^{18}F .

19. The CD8 binding agent of any one of claims 11 to 13 or 15 to 18, wherein the chelating moiety is a compound of Formula (I):



22. A pharmaceutical composition comprising the CD8 binding agent of any one of claims 1-4 or 14-22, optionally comprising pharmaceutically acceptable excipients, further optionally comprising one or more anti-oxidant compounds.

23. A kit comprising the CD8 binding agent of any one of claims 1-4 or the pharmaceutical composition of claim 22, optionally further comprising reagents for preparation of the labeled CD8 binding agent.

24. The kit according to claim 23, wherein the chelating agent is of Formula (I) and an [^{18}F]-aluminum fluoride complex.

25. The labeled CD8 binding agent of any of claims 14-21, or the pharmaceutical composition of claim 22, for use in treatment, diagnosis, prognosis, therapy selection or of monitoring response to therapy; optionally wherein monitoring response to therapy comprises monitoring the progression/remission of a disease.

26. The labelled CD8 binding or the pharmaceutical composition of claim 25, wherein the disease is cancer, autoimmune disease or condition, transplant rejection, or graft-versus-host disease.

27. A method of detecting CD8+ cells in a subject, the method comprising:

detecting binding of the labeled CD8 binding agent of any one of claims 14-21 or the pharmaceutical composition of claim 22 to CD8+ cells in the subject, wherein the labeled CD8 binding agent was administered to the subject before detecting the binding of the labeled CD8 binding agent or the pharmaceutical composition to CD8+ cells in the subject, wherein the detection of the binding indicates the presence of CD8+ cells, preferably wherein detecting binding of the labeled CD8 binding agent to CD8+ cells comprises imaging CD8+ cells; more preferably wherein imaging CD8+ cells comprises performing a positron emission tomography (PET) scan or positron emission tomography/computed tomography (PET/CT) scan on the subject.

28. The method of claim 27 wherein the subject is a human, or a non-human mammal, optionally wherein the subject is a rat, mouse, guinea pig, hamster, rabbit, dog, cat, cow, horse, goat, sheep, donkey, pig, monkey, ape, or other non-human primate; preferably wherein the subject is a human.

29. 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 1-4 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):

