

higher than those of classical F(ab) or Fv fragments (Ghahroudi, M. A. et al. *FEBS Lett.* 414, 521-526 (1997)).

[0008] Mice in which the λ (lambda) light (L) chain locus and/or the λ and κ (kappa) L chain loci have been functionally silenced and antibodies produced by such mice are described in U.S. Patent Nos. 7,541,513 and 8,367,888. Recombinant production of heavy chain-only antibodies in mice and rats has been reported, for example, in WO2006008548; U.S. Application Publication No. 20100122358; Nguyen et al., 2003, *Immunology*; 109(1), 93-101; Brüggemann et al., *Crit. Rev. Immunol.*; 2006, 26(5):377-90; and Zou et al., 2007, *J Exp Med*; 204(13): 3271–3283. The production of knockout rats via embryo microinjections of zinc-finger nucleases is described in Geurts et al., 2009, *Science*, 325(5939):433. Soluble heavy chain-only antibodies and transgenic rodents comprising a heterologous heavy chain locus producing such antibodies are described in U. S. Patent Nos. 8,883,150 and 9,365,655. CAR-T structures comprising single-domain antibodies as binding (targeting) domain are described, for example, in Iri-Sofla et al., 2011, *Experimental Cell Research* 317:2630-2641 and Jamnani et al., 2014, *Biochim Biophys Acta*, 1840:378-386.

SUMMARY

[0009] Examples of the disclosure relate to heavy chain antibodies, including but not limited to UniAbs™, with binding affinity to PSMA. Further examples of the disclosure relate to methods of making such antibodies, compositions comprising such antibodies, and their use in the treatment of disorders that are characterized by the expression of PSMA.

[0009a] In one aspect, the present disclosure refers to ~~an~~ multi-specific antibody that binds to to a CD3 protein and a PSMA protein, comprising ~~a first heavy chain variable region comprising:~~

(a) a heavy chain variable region of an anti-PSMA heavy chain antibody comprising:

(i) a CDR1 sequence of SEQ ID NO: 1, having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 1 to 10; a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18; and/or

(ii) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 18; or

(iii) a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18; and

(b) a CD3-binding VH domain that is paired with a light chain variable domain;

(b) ~~— a CDR2 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 11 to 17; and/or~~

(c) ~~— a CDR3 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 18 to 23; and~~

~~optionally further comprising a second heavy chain variable region comprising:~~

(a) ~~— a CDR1 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 1 to 10; and/or~~

(b) ~~— a CDR2 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 11 to 17; and/or~~

(c) ~~— a CDR3 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 18 to 23.~~

~~{0009b}~~—In another aspect, the present disclosure refers to an antibody that binds to PSMA, comprising:

~~a first heavy chain variable region comprising:~~

~~(a) — a CDR1 sequence of the formula:~~

~~G G S I S S X1 X2 Y X3 (SEQ ID NO: 67)~~

~~where — X1 is S or N;~~

~~X2 is S or N; and~~

~~X3 is Y or F; and~~

~~(b) — a CDR2 sequence of the formula:~~

~~X4 X5 X6 S G X7 T (SEQ ID NO: 68)~~

~~where — X4 is I or V;~~

~~X5 is D or Y;~~

~~X6 is Y or D; and~~

~~X7 is Y or S; and~~

~~(c) — a CDR3 sequence of the formula:~~

~~A R H K A A T A D F D Y (SEQ ID NO: 69), and~~

~~optionally further comprising a second heavy chain variable region comprising:~~

~~(a) — a CDR1 sequence of the formula:~~

~~G F X1 F X2 X3 Y G (SEQ ID NO: 70)~~

~~where — X1 is S or I or T;~~

~~X2 is S or T or R or I; and~~

~~X3 is R or S; and~~

~~(b) — a CDR2 sequence of the formula:~~

~~I X4 Y D G S N X5 (SEQ ID NO: 71)~~

~~where — X4 is W or S; and~~

~~X5 is R or K; and~~

(e) ~~a CDR3 sequence of the formula:~~

~~A R E P R X6 G Y Y Y X7 X8 S G Y X9 S L D Y (SEQ ID NO: 72)~~

~~where X6 is I or V;~~

~~X7 is E or D;~~

~~X8 is S or T; and~~

~~X9 is Y or D~~

~~[0009c] In another aspect, the present disclosure refers to an antibody that binds to PSMA, comprising a heavy chain variable region comprising a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18 in a human VH framework.~~

~~[0009d] In another aspect, the present disclosure refers to an antibody that binds to PSMA, comprising a heavy chain variable region comprising a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18 in a human VH framework, in a monovalent or bivalent configuration.~~

~~[0009e] In yet another aspect, the present disclosure refers to a three-chain antibody-like molecule comprising:~~

~~a first polypeptide comprising SEQ ID NO: 86;~~

~~a second polypeptide comprising SEQ ID NO: 87; and~~

~~a third polypeptide comprising SEQ ID NO: 88, 89, 90, 91, 92, or 93.~~

~~[0009f] In yet another aspect, the present disclosure refers to a three-chain antibody-like molecule comprising:~~

~~a first polypeptide comprising SEQ ID NO: 86;~~

~~a second polypeptide comprising SEQ ID NO: 87; and~~

~~a third polypeptide comprising SEQ ID NO: 88.~~

[0009g] In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising:

an multi-specific antibody as described herein; and

a pharmaceutically acceptable carrier.

[0009h] In yet another aspect, the present disclosure refers to a polynucleotide encoding a multi-specific antibody as described herein.

[0009i] In yet another aspect, the present disclosure refers to a vector comprising a polynucleotide as described herein.

[0009j] In yet another aspect, the present disclosure refers to a cell comprising a vector as described herein.

[0009k] In yet another aspect, the present disclosure refers to the multi-specific antibody as described herein for use in therapy.

[0010] In some examples, an antibody binding to PSMA comprises a first heavy chain variable region comprising: (a) a CDR1 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 1 to 10; and/or (b) a CDR2 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 11 to 17; and/or (c) a CDR3 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 18 to 23. In some examples, the antibody further comprises a second heavy chain variable region comprising: (a) a CDR1 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 1 to 10; and/or (b) a CDR2 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 11 to 17; and/or (c) a CDR3 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 18 to 23. In some examples, the CDR1, CDR2, and CDR3 sequences are present in a human framework. In some examples, an antibody further comprises a heavy chain constant region sequence in the absence of a CH1 sequence.

[0011] In some examples, the first heavy chain variable region of the antibody comprises: (a) a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1 to 10; and/or (b) a CDR2

[0025] In some examples, an antibody binding to PSMA comprises: a first heavy chain variable region comprising: a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18; and a second heavy chain variable region comprising: a CDR1 sequence of SEQ ID NO: 7, a CDR2 sequence of SEQ ID NO: 15, and a CDR3 sequence of SEQ ID NO: 20, in a human VH framework. In some examples, the antibody comprises a first heavy chain variable region which is located nearer to the N-terminus relative to the second heavy chain variable region. In some examples, the first heavy chain variable region is located nearer to the C-terminus relative to the second heavy chain variable region.

[0026] In some examples, an antibody is monospecific. In some examples, an antibody is multi-specific. In some examples, an antibody is bispecific. In some examples, an antibody has binding affinity to a CD3 protein and a PSMA protein. In some examples, an antibody has binding affinity to two different epitopes on the same PSMA protein. In some examples, an antibody has binding affinity to an effector cell. In some examples, an antibody has binding affinity to a T-cell antigen. In some examples, an antibody has binding affinity to CD3. In some examples, an antibody is in a CAR-T format.

[0027] Aspects-Examples of the disclosure include a bispecific antibody comprising: (i) a heavy chain variable region having binding affinity to CD3, comprising a CDR1 sequence of SEQ ID NO: 59, a CDR2 sequence of SEQ ID NO: 60, and CDR3 sequence of SEQ ID NO: 61, in a human VH framework; (ii) a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 62, a CDR2 sequence of SEQ ID NO: 63, and CDR3 sequences of SEQ ID NO: 64, in a human VL framework; and (iii) an antigen-binding domain of an anti-PSMA heavy chain antibody, comprising a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18, in a human VH framework.

[0028] Examples of the disclosure include a bispecific antibody comprising: (i) a heavy chain variable region having binding affinity to CD3, comprising a CDR1 sequence of SEQ ID NO: 59, a CDR2 sequence of SEQ ID NO: 60, and CDR3 sequence of SEQ ID NO: 61, in a human VH framework; (ii) a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 62, a CDR2 sequence of SEQ ID NO: 63, and CDR3 sequences of SEQ ID NO: 64, in a human VL framework; and (iii) an antigen-binding domain of an anti-PSMA heavy chain antibody, comprising a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18, in a human VH framework, in a monovalent or bivalent configuration.

[0029] Examples of the disclosure include a bispecific antibody comprising: (i) a heavy chain variable region having binding affinity to CD3, comprising a CDR1 sequence of SEQ ID NO: 59, a CDR2 sequence of SEQ ID NO: 60, and CDR3 sequence of SEQ ID NO: 61, in a human VH framework; (ii) a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 62, a CDR2 sequence of

SEQ ID NO: 63, and CDR3 sequences of SEQ ID NO: 64, in a human VL framework; and (iii) an antigen-binding domain of an anti-PSMA heavy chain antibody, comprising a CDR1 sequence of SEQ ID NO: 7, a CDR2 sequence of SEQ ID NO: 15, and a CDR3 sequence of SEQ ID NO: 20, in a human VH framework.

[0030] Examples of the disclosure include a bispecific antibody comprising: (i) a heavy chain variable region having binding affinity to CD3, comprising a CDR1 sequence of SEQ ID NO: 59, a CDR2 sequence of SEQ ID NO: 60, and CDR3 sequence of SEQ ID NO: 61, in a human VH framework; (ii) a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 62, a CDR2 sequence of SEQ ID NO: 63, and CDR3 sequences of SEQ ID NO: 64, in a human VL framework; and (iii) an antigen-binding domain of an anti-PSMA heavy chain antibody, comprising a CDR1 sequence of SEQ ID NO: 7, a CDR2 sequence of SEQ ID NO: 15, and a CDR3 sequence of SEQ ID NO: 20, in a human VH framework, in a monovalent or bivalent configuration.

[0031] ~~Aspects-Examples~~ of the disclosure include a multi-specific antibody comprising: (i) a heavy chain variable region having binding affinity to CD3, comprising a CDR1 sequence of SEQ ID NO: 59, a CDR2 sequence of SEQ ID NO: 60, and CDR3 sequence of SEQ ID NO: 61, in a human VH framework; (ii) a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 62, a CDR2 sequence of SEQ ID NO: 63, and CDR3 sequences of SEQ ID NO: 64, in a human VL framework; and (iii) an antigen-binding domain of an anti-PSMA heavy chain antibody, wherein the antigen-binding domain comprises a first and a second antigen-binding region, in a bivalent configuration, wherein: the first antigen-binding region comprises a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18, in a human VH framework; and the second antigen-binding region comprises a CDR1 sequence of SEQ ID NO: 7, a CDR2 sequence of SEQ ID NO: 15, and a CDR3 sequence of SEQ ID NO: 20, in a human VH framework. In certain examples, the first antigen-binding region is located nearer to the N-terminus relative to the second antigen-binding region. In certain other examples, the first antigen-binding region is located nearer to the C-terminus relative to the second antigen-binding region.

[0032] Examples of the disclosure include multispecific or bispecific antibodies wherein the first and second antigen-binding regions of the antigen-binding domain of the anti-PSMA heavy chain antibody are connected by a polypeptide linker. In some examples, the polypeptide linker is a GS linker. In some examples, the GS linker consists of the sequence of SEQ ID NO: 73 or SEQ ID NO: 74. In some examples, the antigen-binding domain of the anti-PSMA heavy chain antibody is monoparatopic, and induces less cytokine production as compared to a biparatopic antigen-binding domain. In some examples, the antigen-binding domain of the anti-PSMA heavy chain antibody is monoparatopic, and expands CD8⁺ T-cells to a greater extent than a biparatopic antigen-binding domain.

- [0033] In some examples, an antibody is biparatopic and has increased affinity for PSMA as compared to a monoparatopic anti-PSMA antibody. In some examples, an antibody is biparatopic and has increased effector function as compared to a monoparatopic anti-PSMA antibody.
- [0034] Examples of the disclosure relate to pharmaceutical compositions comprising an antibody described herein.
- [0035] Examples of the disclosure relate to methods for the treatment of a disorder characterized by expression of PSMA, comprising administering to a subject with said disorder an antibody or a pharmaceutical composition described herein. In certain other aspects, the disclosure relates to uses of an multi-specific antibody described herein, in the preparation of a medicament for the treatment of a disorder characterized by expression of PSMA. In yet other examples, the disclosure relates to an antibody described herein for use in the treatment of a disorder characterized by expression of PSMA. In certain other examples, the disclosure relates to methods of treatment, comprising administering to an individual in need an effective dose of an antibody or a pharmaceutical composition described herein. With respect to these examples, and in some examples, the disorder is prostate cancer.
- [0036] Examples of the disclosure relate to polynucleotides encoding an antibody described herein, vectors comprising such polynucleotides, and cells comprising such vectors.
- [0037] Aspects of the disclosure relate to methods of producing an multi-specific antibody described herein, comprising growing a cell described herein under conditions permissive for expression of the antibody, and isolating the antibody from the cell.
- [0038] Examples of the disclosure relate to methods of making an antibody described herein, comprising immunizing a UniRat animal with a PSMA protein and identifying PSMA-binding antibody sequences.
- [0039] These and further examples will be further explained in the rest of the disclosure, including the Examples.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0040] FIG. 1, panels A-B, provide a series of graphs showing serum titer as a function of dilution.
- [0041] FIG. 2, panel A is a graph showing cell binding to human PSMA.
- [0042] FIG. 2, panel B is a graph showing cell binding to cynomolgus monkey PSMA.
- [0043] FIG. 3 is a graph showing binding competition between two antibody families in accordance with an embodiment of the invention.
- [0044] FIG. 4, panel A is a Scatchard plot showing binding affinity to cell surface expressed PSMA of a bispecific antibody having binding affinity to CD3 and PSMA, wherein the PSMA arm is monoparatopic and monovalent according to an embodiment of the invention.

CLAIMS:

1. ~~— An antibody that binds to PSMA, comprising a first heavy chain variable region comprising:~~
 - ~~(a) — a CDR1 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 1 to 10; and/or~~
 - ~~(b) — a CDR2 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 11 to 17; and/or~~
 - ~~(c) — a CDR3 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 18 to 23; and~~
~~optionally further comprising a second heavy chain variable region comprising:~~
 - ~~(a) — a CDR1 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 1 to 10; and/or~~
 - ~~(b) — a CDR2 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 11 to 17; and/or~~
 - ~~(c) — a CDR3 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 18 to 23.~~
2. ~~— The antibody of claim 1, wherein:~~
 - ~~the CDR1, CDR2, and CDR3 sequences are present in a human VH framework; and/or~~
 - ~~the antibody further comprises a heavy chain constant region sequence in the absence of a CH1 sequence.~~
3. ~~— The antibody of claim 1 or 2, wherein the first heavy chain variable region comprises:~~
 - ~~(a) — a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1 to 10; and/or~~
 - ~~(b) — a CDR2 sequence selected from the group consisting of SEQ ID NOs: 11 to 17; and/or~~
 - ~~(c) — a CDR3 sequence selected from the group consisting of SEQ ID NOs: 18 to 23.~~
4. ~~— The antibody of any one of claims 1 to 3, wherein the antibody comprises the second heavy chain variable region, wherein the second heavy chain variable region comprises:~~
 - ~~(a) — a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1 to 10; and/or~~
 - ~~(b) — a CDR2 sequence selected from the group consisting of SEQ ID NOs: 11 to 17; and/or~~
 - ~~(c) — a CDR3 sequence selected from the group consisting of SEQ ID NOs: 18 to 23.~~

5. ~~The antibody of any one of claims 1 to 4, comprising a heavy chain variable region sequence having at least 95% sequence identity to any one of the sequences of SEQ ID NOs: 24 to 58.~~

6. ~~The antibody of any one of claims 1 to 5, comprising a heavy chain variable region sequence selected from the group consisting of SEQ ID NOs: 24 to 58.~~

7. ~~An antibody that binds to PSMA, comprising:
a first heavy chain variable region comprising:
(a) a CDR1 sequence of the formula:~~

~~G-G-S-I-S-S-X₁-X₂-Y-X₃ (SEQ ID NO: 67)~~

~~where X₁ is S or N;
X₂ is S or N; and
X₃ is Y or F; and
(b) a CDR2 sequence of the formula:~~

~~X₄-X₅-X₆-S-G-X₇-T (SEQ ID NO: 68)~~

~~where X₄ is I or V;
X₅ is D or Y;
X₆ is Y or D; and
X₇ is Y or S; and
(c) a CDR3 sequence of the formula:~~

~~A-R-H-K-A-A-T-A-D-F-D-Y (SEQ ID NO: 69), and
optionally further comprising a second heavy chain variable region comprising:
(a) a CDR1 sequence of the formula:~~

~~G-F-X₁-F-X₂-X₃-Y-G (SEQ ID NO: 70)~~

~~where X₁ is S or I or T;
X₂ is S or T or R or I; and~~

_____X₃ is R or S; and

_____ (b) _____ a CDR2 sequence of the formula:

I X₄ Y D G S N X₅ (SEQ ID NO: 71)

where _____ X₄ is W or S; and

_____ X₅ is R or K; and

(c) _____ a CDR3 sequence of the formula:

A R E P R X₆ G Y Y Y X₇ X₈ S G Y X₉ S L D Y (SEQ ID NO: 72)

where _____ X₆ is I or V;

_____ X₇ is E or D;

_____ X₈ is S or T; and

_____ X₉ is Y or D.

8. _____ The antibody of claim 7, wherein:

the antibody comprises the second heavy chain variable region; and

the first heavy chain variable region is located nearer to the N-terminus relative to the second heavy chain variable region.

9. _____ The antibody of claim 7, wherein:

the antibody comprises the second heavy chain variable region; and

the first heavy chain variable region is located nearer to the C-terminus relative to the second heavy chain variable region.

10. _____ An antibody that binds to PSMA, comprising a heavy chain variable region comprising a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18 in a human VH framework.

11. _____ An antibody that binds to PSMA, comprising a heavy chain variable region comprising a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18 in a human VH framework, in a monovalent or bivalent configuration.

~~12. The antibody of claim 10 or 11, wherein the heavy chain variable region sequence has at least 95% sequence identity to SEQ ID NO: 25.~~

~~13. The antibody of any one of claims 10 to 12, wherein the heavy chain variable region sequence is SEQ ID NO: 25.~~

~~14. The antibody of any one of claims 1 to 6 or 10 to 13, which is monospecific.~~

~~151. The A multi-specific antibody that binds to a CD3 protein and a PSMA protein, comprising:~~

~~(a) a heavy chain variable region of an anti-PSMA heavy chain antibody comprising:~~

~~(i) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18; or~~

~~(ii) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 18; or~~

~~(iii) a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18; and~~

~~(b) a CD3-binding VH domain that is paired with a light chain variable domain of any one of claims 1 to 13, which is multi-specific.~~

~~162. The multi-specific antibody of claim 151, which is bispecific.~~

~~3. The multi-specific antibody of claim 1 or claim 2, wherein the CD3-binding VH domain comprises a CDR1 sequence of SEQ ID NO: 59, a CDR2 sequence of SEQ ID NO: 60, and a CDR3 sequence of SEQ ID NO: 61.~~

~~4. The multi-specific antibody of any one of claims 1 to 3, wherein the light chain variable domain comprises a CDR1 sequence of SEQ ID NO: 62, a CDR2 sequence of SEQ ID NO: 63, and a CDR3 sequence of SEQ ID NO: 64.~~

~~5. The multi-specific antibody of any one of claims 1 to 4, wherein:~~

~~the CD3-binding VH domain comprises a CDR1 sequence of SEQ ID NO: 59, a CDR2 sequence of SEQ ID NO: 60, and a CDR3 sequence of SEQ ID NO: 61; and~~

~~the light chain variable domain comprises a CDR1 sequence of SEQ ID NO: 62, a CDR2 sequence of SEQ ID NO: 63, and a CDR3 sequence of SEQ ID NO: 64.~~

~~17. The antibody of claim 15 or 16, which has binding affinity to a CD3 protein and a PSMA protein.~~

~~186. The multi-specific antibody of any one of claims 171 to 5, wherein:~~

~~comprising the~~ CD3-binding VH domain ~~comprising comprises~~ a CDR1 sequence of SEQ ID NO: 59, a CDR2 sequence of SEQ ID NO: 60, and a CDR3 sequence of SEQ ID NO: 61, in a human VH framework; and

~~the light chain variable domain optionally further comprising a fixed light chain~~ comprising a CDR1 sequence of SEQ ID NO: 62, a CDR2 sequence of SEQ ID NO: 63, and a CDR3 sequence of SEQ ID NO: 64, in a human VL framework.

~~197. The multi-specific antibody of claim 17 or 18 any one of claims 1 to 6, comprising wherein:~~

~~the a~~-CD3-binding VH domain ~~comprising comprises~~ a sequence having at least 95% sequence identity to the heavy chain variable region sequence of SEQ ID NO: 65; and/or

~~the light chain variable domain ; and optionally further comprising a fixed light chain~~ comprising a sequence having at least 95% sequence identity to the light chain variable region of SEQ ID NO: 66.

~~8. The multi-specific antibody of any one of claims 1 to 7, wherein:~~

~~the CD3-binding VH domain comprises the heavy chain variable region sequence of SEQ ID NO: 65, and/or~~

~~the light chain variable domain comprises the light chain variable region of SEQ ID NO: 66.~~

~~9. The multi-specific antibody of any one of claims 1 to 8, wherein the heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18.~~

~~10. The multi-specific antibody of any one of claims 1 to 9, wherein the heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18 in a monovalent configuration.~~

~~20. The antibody of claim 15 or 16, which has binding affinity to two different epitopes on the same PSMA protein.~~

11. The multi-specific antibody of any one of claims 1 to 10, wherein the heavy chain variable region comprises a sequence having at least 95% sequence identity to the heavy chain variable region sequence of SEQ ID NO: 25.

12. The multi-specific antibody of any one of claims 1 to 11, wherein the heavy chain variable region comprises the heavy chain variable region sequence of SEQ ID NO: 25.

13. The multi-specific antibody of any one of claims 1 to 12, further comprising a heavy chain constant region sequence in the absence of a CH1 sequence.

~~24~~14. The multi-specific antibody of any one of claims 1 to ~~20~~13, further comprising a silenced Fc region.

~~22. The antibody of any one of claims 1 to 20, which is in a CAR-T format.~~

~~23~~15. A multi-specific antibody of claim 2, wherein~~bispecific antibody comprising:~~

(i) ~~_____ a heavy chain variable region having binding affinity to CD3, the CD3-binding VH domain comprising~~ comprises a CDR1 sequence of SEQ ID NO: 59, a CDR2 sequence of SEQ ID NO: 60, and a CDR3 sequence of SEQ ID NO: 61, in a human VH framework;

(ii) ~~_____ the a-light chain variable region domain~~ comprising a CDR1 sequence of SEQ ID NO: 62, a CDR2 sequence of SEQ ID NO: 63, and a CDR3 sequence of SEQ ID NO: 64, in a human VL framework; and

(iii) ~~_____ the heavy chain variable region an antigen-binding domain of an anti-PSMA heavy chain antibody, comprising~~ es a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18, in a human VH framework.

~~24~~16. The multi-specific antibody of claim ~~23~~15, further comprising a silenced Fc region.

17. The multi-specific antibody of any one of claims 1 to 16, which is a three-chain antibody-like molecule.

~~25~~18. ~~A three-chain antibody-like molecule~~The multi-specific antibody of claim 17, comprising:
a first polypeptide comprising SEQ ID NO: 86;
a second polypeptide comprising SEQ ID NO: 87; and

a third polypeptide comprising SEQ ID NO: 88 ~~or; 89, 90, 91, 92, or 93.~~

~~2619.~~ The ~~multi-specific antibody three-chain-antibody-like molecule~~ of claim ~~2518~~, wherein the third polypeptide comprises SEQ ID NO: 88.

~~2720.~~ ~~The multi-specific antibody of any one of claims 17 to 19, A three-chain-antibody-like molecule~~ comprising:

- a first polypeptide consisting of SEQ ID NO: 86;
- a second polypeptide consisting of SEQ ID NO: 87; and
- a third polypeptide consisting of SEQ ID NO: 88.

~~28.~~ ~~The three-chain-antibody-like molecule of any one of claims 25 to 27, wherein the three-chain antibody-like molecule binds to PSMA and CD3.~~

~~29.~~ ~~A multi-specific antibody comprising:~~

- ~~—— (i) a heavy chain variable region having binding affinity to CD3, comprising a CDR1 sequence of SEQ ID NO: 59, a CDR2 sequence of SEQ ID NO: 60, and CDR3 sequence of SEQ ID NO: 61, in a human VH framework;~~
- ~~—— (ii) a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 62, a CDR2 sequence of SEQ ID NO: 63, and CDR3 sequences of SEQ ID NO: 64, in a human VL framework; and~~
- ~~—— (iii) an antigen-binding domain of an anti-PSMA heavy chain antibody, wherein the antigen-binding domain comprises a first and a second antigen-binding region, in a bivalent configuration, wherein:~~

~~the first antigen-binding region comprises a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18, in a human VH framework; and~~

~~the second antigen-binding region comprises a CDR1 sequence of SEQ ID NO: 7, a CDR2 sequence of SEQ ID NO: 15, and a CDR3 sequence of SEQ ID NO: 20, in a human VH framework.~~

~~3021.~~ A pharmaceutical composition comprising:
an ~~multi-specific~~ antibody of any one of claims 1 to ~~2920~~; and
a pharmaceutically acceptable carrier.

~~3122~~. Use of a ~~multi-specific~~ antibody of any one of claims 1 to ~~2920~~, in the preparation of a medicament for the treatment of a disorder characterized by expression of PSMA.

~~3223~~. The use of claim ~~3122~~, wherein the disorder is prostate cancer.

~~3324~~. The use of claim ~~3122~~, wherein the disorder is a solid tumor.

~~3425~~. A polynucleotide encoding a ~~multi-specific~~ antibody of any one of claims 1 to ~~2920~~.

~~3526~~. A vector comprising a polynucleotide of claim ~~3425~~.

~~3627~~. A cell comprising a vector of claim ~~3526~~.

~~3728~~. A method of producing a ~~multi-specific~~ antibody of any one of claims 1 to ~~2920~~, comprising growing a cell according to claim ~~36-27~~ under conditions permissive for expression of the ~~multi-specific~~ antibody, and isolating the ~~multi-specific~~ antibody from the cell.

~~3829~~. The ~~multi-specific~~ antibody of any one of claims 1 to ~~29-20~~ for use in therapy.