

HEAVY CHAIN ANTIBODIES BINDING TO PSMA

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit of the filing date of U.S. Provisional Patent Application No. 62/830,130, filed on April 5, 2019, the disclosure of which application is herein incorporated by reference in its entirety.

SEQUENCE LISTING

[0001a] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 9, 2020, is named TNO-0016-WO_SL.txt and is 121,310 bytes in size.

FIELD OF THE INVENTION

[0002] The present invention concerns human heavy chain antibodies (e.g., UniAbs™) binding to PSMA. The invention further concerns methods of making such antibodies, compositions, including pharmaceutical compositions, comprising such antibodies, and their use to treat disorders that are characterized by the expression of PSMA.

BACKGROUND OF THE INVENTION

PSMA

[0003] PSMA, also known as Prostate Specific Membrane Antigen and Glutamate Carboxypeptidase II (UniProt Q04609), is a type II transmembrane protein that has N-acetylated-alpha-linked-acidic dipeptidase, folate hydrolase and dipeptidyl-peptidase activity. It is encoded by the FOLH1 gene in humans and consists of a 19 amino acid cytoplasmic domain, a 24 amino acid transmembrane portion, and a 707 amino acid extracellular portion. The protein is enzymatically active as a non-covalent homodimer. PSMA is expressed on prostate epithelium tissue and is upregulated in prostate cancer and the neovasculature of solid tumors. It is also expressed at low levels in healthy tissues such as the brain, kidney, and salivary glands, but its overexpression in malignant prostate tissue makes it an attractive target for the therapeutic treatment of prostate cancer. It may also be relevant for therapy or imaging of solid tumors, given its high expression in malignant neovasculature. Monoclonal antibodies, antibody-drug conjugates and chimeric antigen receptor T-cells targeting PSMA have been described for treatment of metastatic prostate cancer (Hernandez-Hoyos et al 2016, PMID: 27406985, DiPippo et al 2014, PMID: 25327986, Serganova et al 2016, PMID: 28345023). In

addition, radionuclide conjugates specific to PSMA are being investigated for imaging and treatment of prostate cancer (e.g., Hofman et al., 2018 PMID: 29752180).

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 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.

[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

(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

[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 antibody as described herein; and

a pharmaceutically acceptable carrier.

[0009h] In yet another aspect, the present disclosure refers to a polynucleotide encoding an 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 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

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.

[0012] In some examples, the antibody further comprises a second heavy chain variable region which 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.

[0013] In some examples, the antibody comprises: (a) a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1 to 10; and (b) a CDR2 sequence selected from the group consisting of SEQ ID NOs: 11 to 17; and (c) a CDR3 sequence selected from the group consisting of SEQ ID NOs: 18 to 23. In some examples, the antibody comprises a second heavy chain variable region which comprises: (a) a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1 to 10; and (b) a CDR2 sequence selected from the group consisting of SEQ ID NOs: 11 to 17; and (c) a CDR3 sequence selected from the group consisting of SEQ ID NOs: 18 to 23.

[0014] In some examples, the antibody comprises: (a) 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; or (b) 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 some examples, the antibody comprises 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. In some examples, an antibody comprises a heavy chain variable region sequence selected from the group consisting of SEQ ID NOs: 24 to 58. In some examples, the antibody comprises a heavy chain variable region sequence is selected from the group consisting of: SEQ ID NO: 25 and SEQ ID NO: 38.

[0015] In some examples, an antibody binding to PSMA comprises a first heavy chain variable region comprising: (a) a CDR1 sequence of the formula:

$$G G S I S S X_1 X_2 Y X_3 \text{ (SEQ ID NO: 67)}$$

where X_1 is S or N; X_2 is S or N; and X_3 is Y or F; and (b) a CDR2 sequence of the formula:

$$X_4 X_5 X_6 S G X_7 T \text{ (SEQ ID NO: 68)}$$

where X_4 is I or V; X_5 is D or Y; X_6 is Y or D; and X_7 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), in a monovalent or bivalent format.

[0016] In some examples, an antibody binding to PSMA comprises a first 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, in a monovalent or bivalent format.

[0017] In some examples, an antibody binding PSMA comprises: 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 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.

[0018] In some examples, the antibody comprises a first and a second heavy chain variable region, wherein the first heavy chain variable region 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.

[0019] In some examples, an antibody binding to PSMA comprises a heavy chain variable region comprising CDR1, CDR2 and CDR3 sequences in a human VH framework, wherein the CDR sequences comprise a sequence having two or fewer substitutions in a CDR sequence selected from the group consisting of SEQ ID NOs: 1-23.

[0020] In some examples, an antibody binding to PSMA comprises a heavy chain variable region comprising CDR1, CDR2 and CDR3 sequences in a human VH framework, wherein the CDR sequences are selected from the group consisting of SEQ ID NOs: 1-23.

[0021] In some examples, an antibody binding to PSMA comprises: 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.

[0022] In some examples, an antibody binding to PSMA comprises: 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.

[0023] In some examples, an antibody binding to PSMA comprises: a 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.

[0024] In some examples, an antibody binding to PSMA comprises: a 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 monovalent or bivalent configuration.

[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 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 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 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 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.

four glycine residues, followed by one serine residue, and wherein the sequence is repeated n times, where n is an integer ranging from 1 to about 10, such as 2, 3, 4, 5, 6, 7, 8, or 9 (SEQ ID NO: 94). Non-limiting examples of such linkers include GGGGS (SEQ ID NO: 73) (n=1) and GGGSGGGGS (SEQ ID NO: 74 (n=2). Other suitable linkers can also be used, and are described, for example, in Chen et al., *Adv Drug Deliv Rev.* 2013 October 15; 65(10): 1357-69, the disclosure of which is incorporated herein by reference in its entirety.

[0092] The term “three-chain antibody like molecule” or “TCA” is used herein to refer to antibody-like molecules comprising, consisting essentially of, or consisting of three polypeptide subunits, two of which comprise, consist essentially of, or consist of one heavy and one light chain of a monoclonal antibody, or functional antigen-binding fragments of such antibody chains, comprising an antigen-binding region and at least one CH domain. This heavy chain/light chain pair has binding specificity for a first antigen. The third polypeptide subunit comprises, consists essentially of, or consists of a heavy-chain only antibody comprising an Fc portion comprising CH2 and/or CH3 and/or CH4 domains, in the absence of a CH1 domain, and one or more antigen binding domains (e.g., two antigen binding domains) that binds an epitope of a second antigen or a different epitope of the first antigen, where such binding domain is derived from or has sequence identity with the variable region of an antibody heavy or light chain. Parts of such variable region may be encoded by V_H and/or V_L gene segments, D and J_H gene segments, or J_L gene segments. The variable region may be encoded by rearranged V_HDJ_H, V_LDJ_H, V_HJ_L, or V_LJ_L gene segments.

[0093] A TCA binding compound makes use of a “heavy chain only antibody” or “heavy chain antibody” or “heavy chain polypeptide” which, as used herein, mean a single chain antibody comprising heavy chain constant regions CH2 and/or CH3 and/or CH4 but no CH1 domain. In one embodiment, the heavy chain antibody is composed of an antigen-binding domain, at least part of a hinge region and CH2 and CH3 domains. In another embodiment, the heavy chain antibody is composed of an antigen-binding domain, at least part of a hinge region and a CH2 domain. In a further embodiment, the heavy chain antibody is composed of an antigen-binding domain, at least part of a hinge region and a CH3 domain. Heavy chain antibodies in which the CH2 and/or CH3 domain is truncated are also included herein. In a further embodiment, the heavy chain is composed of an antigen binding domain, and at least one CH (CH1, CH2, CH3, or CH4) domain but no hinge region. The heavy chain only antibody can be in the form of a dimer, in which two heavy chains are disulfide bonded other otherwise covalently or non-covalently attached with each other, and can optionally include an asymmetric interface between one or more of the CH domains to facilitate proper pairing between polypeptide chains. The heavy-chain antibody may belong to the IgG subclass, but antibodies belonging to other subclasses, such as IgM, IgA, IgD and IgE subclass, are also included herein. In a particular embodiment, the heavy chain antibody is of the IgG1, IgG2, IgG3, or IgG4 subtype, in

where X_6 is I or V;
 X_7 is E or D;
 X_8 is S or T; and
 X_9 is Y or D.

[0139] Representative CDR1, CDR2 and CDR3 sequences are shown in Tables 1 and 3.

Table 3: Anti-PSMA heavy chain antibody CDR1, CDR2 and CDR3 amino acid sequences.

Clone ID #	SEQ aa CDR1	SEQ aa CDR2	SEQ aa CDR3
325920	GGSISSSSYY (SEQ ID NO: 1)	IDYSGYT (SEQ ID NO: 11)	ARHKAATADFDY (SEQ ID NO: 18)
346181	GGSISSSNFY (SEQ ID NO: 2)	IDYSGYT (SEQ ID NO: 11)	ARHKAATADFDY (SEQ ID NO: 18)
346165	GGSISSSSYY (SEQ ID NO: 1)	VDYSGYT (SEQ ID NO: 12)	ARHKAATADFDY (SEQ ID NO: 18)
346172	GGSISSSSYY (SEQ ID NO: 1)	IDYSGYT (SEQ ID NO: 11)	ARHKAATADFDY (SEQ ID NO: 18)
326109	GGSISSNSYY (SEQ ID NO: 3)	IYDSGST (SEQ ID NO: 13)	ARHKAATADFDY (SEQ ID NO: 18)
325867	GFSFRSYG (SEQ ID NO: 4)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYESSGYDSLIDY (SEQ ID NO: 19)
325742	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYESSGYDSLIDY (SEQ ID NO: 19)
325748	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYISLDY (SEQ ID NO: 20)
325940	GFIFRSYG (SEQ ID NO: 6)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYESSGYISLDY (SEQ ID NO: 20)
325836	GFSFRSYG (SEQ ID NO: 4)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYDSSGYDSLIDY (SEQ ID NO: 21)
326027	GFSFRSYG (SEQ ID NO: 4)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYDSSGYDSLIDY (SEQ ID NO: 21)
326087	GFIFRSYG (SEQ ID NO: 6)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYDSSGYDSLIDY (SEQ ID NO: 21)
326084	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYDSSGYISLDY (SEQ ID NO: 22)
326028	GFSFRSYG (SEQ ID NO: 4)	ISYDGSNK (SEQ ID NO: 16)	AREPRIGYYYDSSGYDSLIDY (SEQ ID NO: 21)
345497	GFSFSRYG (SEQ ID NO: 7)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYISLDY (SEQ ID NO: 20)
326029	GFSFSRYG (SEQ ID NO: 7)	ISYDGSNK (SEQ ID NO: 16)	AREPRVGYYYETSGYISLDY (SEQ ID NO: 23)
345461	GFSFTSYG (SEQ ID NO: 8)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYISLDY (SEQ ID NO: 20)

Column 1: CLONE_ID	Column 2: LNCaP
345497	112
326029	109
345461	102
345493	101
345436	87
345443	84
345490	80
345482	80
345485	71
345463	68
325932	64
345505	59
345508	55
345480	47
326116	38
345509	37
345444	23
345421	22
345447	14
345510	13
345438	13

[0187] The differences in binding to cynomolgus PSMA, as shown in FIG. 2, panels A and B, supports the difference in human PSMA epitope recognized by HCAbs 346181 and 345497.

Example 3: Recombinant Protein Binding by Biolayer Interferometry (BLI)

[0188] Using Biolayer interferometry, binding competition was assessed between the two antibody families of which clone ID 345497 and clone ID 346181 are members. Antigen-antibody epitope binning analysis was performed on the Octet QK-384 (ForteBio). Briefly, anti-Penta HIS Capture (HIS1K) sensors ("Penta HIS" disclosed as SEQ ID NO: 95) were used to immobilize the antigen - recombinant human PSMA (R&D Systems Cat No.: 4234-ZN) for 120 seconds. After baseline readings, sensors were dipped into solutions containing Antibody 1 (325867) for 300 seconds and another baseline was set for 60 secs. Then sensors were dipped into wells containing either Antibody 1 as a positive control for blocking or Antibody 2 (325920). Association and dissociation rates were measured for 300 and 600 seconds, respectively. Data analysis was performed with Octet Data Analysis HT v11.0 (ForteBio). As shown in FIG. 3, 325920 bound the PSMA protein pre-bound with the 325867 antibody, informing that these two antibodies recognize non-overlapping epitopes on PSMA. The shift in binding signal is reported in nanometers.

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; andoptionally 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.
15. The antibody of any one of claims 1 to 13, which is multi-specific.
16. The antibody of claim 15, which is bispecific.
17. The antibody of claim 15 or 16, which has binding affinity to a CD3 protein and a PSMA protein.
18. The antibody of claim 17, comprising a CD3-binding VH domain comprising 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 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.
19. The antibody of claim 17 or 18, comprising a CD3-binding VH domain comprising a sequence having at least 95% sequence identity to the heavy chain variable region sequence of SEQ ID NO: 65; 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.
20. The antibody of claim 15 or 16, which has binding affinity to two different epitopes on the same PSMA protein.
21. The antibody of any one of claims 1 to 20, 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. 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 a CDR3 sequence 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.
24. The antibody of claim 23, further comprising a silenced Fc region.
25. 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.
26. The three-chain antibody-like molecule of claim 25, wherein the third polypeptide comprises SEQ ID NO: 88.
27. 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.

30. A pharmaceutical composition comprising:
an antibody of any one of claims 1 to 29; and
a pharmaceutically acceptable carrier.
31. Use of an antibody of any one of claims 1 to 29, in the preparation of a medicament for the treatment of a disorder characterized by expression of PSMA.
32. The use of claim 31, wherein the disorder is prostate cancer.
33. The use of claim 31, wherein the disorder is a solid tumor.
34. A polynucleotide encoding an antibody of any one of claims 1 to 29.
35. A vector comprising a polynucleotide of claim 34.
36. A cell comprising a vector of claim 35.
37. A method of producing an antibody of any one of claims 1 to 29, comprising growing a cell according to claim 36 under conditions permissive for expression of the antibody, and isolating the antibody from the cell.
38. The antibody of any one of claims 1 to 29 for use in therapy.