

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.

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).

Heavy Chain Antibodies

- [0004] In a conventional IgG antibody, the association of the heavy chain and light chain is due in part to a hydrophobic interaction between the light chain constant region and the CH1 constant domain of the heavy chain. There are additional residues in the heavy chain framework 2 (FR2) and framework 4 (FR4) regions that also contribute to this hydrophobic interaction between the heavy and light chains.
- [0005] It is known, however, that sera of camelids (sub-order Tylopoda which includes camels, dromedaries and llamas) contain a major type of antibodies composed solely of paired H-chains (heavy-chain only antibodies or UniAbs™). The UniAbs™ of *Camelidae* (*Camelus dromedarius*, *Camelus bactrianus*, *Lama glama*, *Lama guanaco*, *Lama alpaca* and *Lama vicugna*) have a unique structure consisting of a single variable domain (VHH), a hinge region and two constant domains (CH2 and CH3), which are highly homologous to the CH2 and CH3 domains of classical antibodies. These UniAbs™ lack the first domain of the constant region (CH1) which is present in the genome, but is spliced out during mRNA processing. The absence of the CH1 domain explains the absence of the light chain in the UniAbs™, since this domain is the anchoring place for the constant domain of the light chain. Such UniAbs™ naturally evolved to confer antigen-binding specificity and high affinity by three CDRs from conventional antibodies or fragments thereof (Muyldermans, 2001; *J Biotechnol* 74:277–302; Revets et al., 2005; *Expert Opin Biol Ther* 5:111–124). Cartilaginous fish, such as sharks, have also evolved a distinctive type of immunoglobulin, designated as IgNAR, which lacks the light polypeptide chains and is composed entirely by heavy chains. IgNAR molecules can be manipulated by molecular engineering to produce the variable domain of a single heavy chain polypeptide (vNARs) (Nuttall et al. *Eur. J. Biochem.* 270, 3543-3554 (2003); Nuttall et al. *Function and Bioinformatics* 55, 187-197 (2004); Dooley et al., *Molecular Immunology* 40, 25-33 (2003)).
- [0006] The ability of heavy chain-only antibodies devoid of light chain to bind antigen was established in the 1960s (Jaton *et al.* (1968) *Biochemistry*, 7, 4185-4195). Heavy chain immunoglobulin physically separated from light chain retained 80% of antigen-binding activity relative to the tetrameric antibody. Sitia et al. (1990) *Cell*, 60, 781-790 demonstrated that removal of the CH1 domain from a rearranged mouse μ gene results in the production of a heavy chain-only antibody, devoid of light chain, in mammalian cell culture. The antibodies produced retained VH binding specificity and effector functions.
- [0007] Heavy chain antibodies with a high specificity and affinity can be generated against a variety of antigens through immunization (van der Linden, R. H., et al. *Biochim. Biophys. Acta.* 1431, 37-46 (1999)) and the VHH portion can be readily cloned and expressed in yeast (Frenken, L. G. J., et al. *J. Biotechnol.* 78, 11-21 (2000)). Their levels of expression, solubility and stability are significantly

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 OF THE INVENTION

[0009] Aspects of the invention relate to heavy chain antibodies, including but not limited to UniAbs™, with binding affinity to PSMA. Further aspects of the invention 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.

[0010] In some embodiments, 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 embodiments, 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 embodiments, the CDR1, CDR2, and CDR3 sequences are present in a human framework. In some embodiments, an antibody further comprises a heavy chain constant region sequence in the absence of a CH1 sequence.

[0011] In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, an antibody comprises a heavy chain variable region sequence selected from the group consisting of SEQ ID NOs: 24 to 58. In some embodiments, 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 embodiments, an antibody binding to PSMA comprises a first heavy chain variable region comprising: (a) a CDR1 sequence of the formula:

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

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 embodiments, a, 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 embodiments, 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 X_1 is S or I or T; X_2 is S or T or R or I; and X_3 is R or S; and (b) a CDR2 sequence of the formula:

I X_4 Y D G S N X_5 (SEQ ID NO: 71)

where X_4 is W or S; and X_5 is R or K; and (c) a CDR3 sequence of the formula:

A R E P R X_6 G Y Y Y X_7 X_8 S G Y X_9 S L D Y (SEQ ID NO: 72)

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.

- [0018] In some embodiments, 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 embodiments, the first heavy chain variable region is located nearer to the C-terminus relative to the second heavy chain variable region.
- [0019] In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the first heavy chain variable region is located nearer to the C-terminus relative to the second heavy chain variable region.

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

[0027] Aspects of the invention 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] Aspects of the invention 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] Aspects of the invention 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] Aspects of the invention 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 invention 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 embodiments, the first antigen-binding region is located nearer to the N-terminus relative to the second antigen-binding region. In certain other embodiments, the first antigen-binding region is located nearer to the C-terminus relative to the second antigen-binding region.

[0032] Aspects of the invention 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 embodiments, the polypeptide linker is a GS linker. In some embodiments, the GS linker consists of the sequence of SEQ ID NO: 73 or SEQ ID NO: 74. In some embodiments, 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 embodiments, 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 embodiments, an antibody is biparatopic and has increased affinity for PSMA as compared to a monoparatopic anti-PSMA antibody. In some embodiments, an antibody is biparatopic and has increased effector function as compared to a monoparatopic anti-PSMA antibody.
- [0034] Aspects of the invention relate to pharmaceutical compositions comprising an antibody described herein.
- [0035] Aspects of the invention 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 invention 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 aspects, the invention relates to an antibody described herein for use in the treatment of a disorder characterized by expression of PSMA. In certain other aspects, the invention 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 aspects, and in some embodiments, the disorder is prostate cancer.
- [0036] Aspects of the invention relate to polynucleotides encoding an antibody described herein, vectors comprising such polynucleotides, and cells comprising such vectors.
- [0037] Aspects of the invention 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] Aspects of the invention 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 aspects 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.

- [0045] FIG. 4, panel B 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 biparatopic according to an embodiment of the invention.
- [0046] FIG. 5, panels A-C, provide schematic illustrations of: an anti-CD3 x monovalent, monospecific anti-PSMA antibody (panel A); an anti-CD3 x bivalent, monospecific anti-PSMA antibody (panel B); and an anti-CD3 x bivalent, biparatopic anti-PSMA antibody (panel C) in accordance with embodiments of the invention.
- [0047] FIG. 6 is a graph depicting T-cell mediated lysis of PSMA positive cells using pre-activated T-cells.
- [0048] FIG. 7 is a graph depicting T-cell mediated lysis of PSMA positive cells using unstimulated T-cells.
- [0049] FIG. 8 is a graph depicting percent specific lysis of PSMA negative DU145 cells as a function of multi-specific antibody concentration in the presence of pre-activated T-cells.
- [0050] FIG. 9 is a graph showing binding of PSMA x CD3 bispecific antibodies to PSMA positive and negative cells.
- [0051] FIG. 10 is a graph showing T-cell mediated lysis of PSMA positive cells.
- [0052] FIG. 11, panel A, is a graph depicting T-cell proliferation as a function of antibody concentration.
- [0053] FIG. 11, panel B, is a graph depicting T-cell proliferation as a function of antibody concentration.
- [0054] FIG. 11, panel C, is a graph depicting the ratio of CD8 to CD4 of proliferated T-cells.
- [0055] FIG. 11, panel D, is a graph depicting the ratio of CD8 to CD4 of proliferated T-cells.
- [0056] FIG. 12, panel A, is a graph depicting T-cell mediated lysis of PSMA positive cells as a function of antibody concentration.
- [0057] FIG. 12, panel B, is a graph depicting cytokine (IFN γ) release as a function of antibody concentration.
- [0058] FIG. 12, panel C, is a graph depicting cytokine (IL-2) release as a function of antibody concentration.
- [0059] FIG. 13 is a graph depicting inhibition of 22Rv1 tumor growth in a tumor xenograft model.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

- [0060] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology,

biochemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook et al., 1989); "Oligonucleotide Synthesis" (M. J. Gait, ed., 1984); "Animal Cell Culture" (R. I. Freshney, ed., 1987); "Methods in Enzymology" (Academic Press, Inc.); "Current Protocols in Molecular Biology" (F. M. Ausubel et al., eds., 1987, and periodic updates); "PCR: The Polymerase Chain Reaction", (Mullis et al., ed., 1994); "A Practical Guide to Molecular Cloning" (Perbal Bernard V., 1988); "Phage Display: A Laboratory Manual" (Barbas et al., 2001); Harlow, Lane and Harlow, Using Antibodies: A Laboratory Manual: Portable Protocol No. I, Cold Spring Harbor Laboratory (1998); and Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory; (1988).

[0061] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0062] Unless indicated otherwise, antibody residues herein are numbered according to the Kabat numbering system (*e.g.*, Kabat *et al.*, Sequences of Immunological Interest. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)).

[0063] In the following description, numerous specific details are set forth to provide a more thorough understanding of the present invention. However, it will be apparent to one of skill in the art that the present invention may be practiced without one or more of these specific details. In other instances, well-known features and procedures well known to those skilled in the art have not been described in order to avoid obscuring the invention.

[0064] All references cited throughout the disclosure, including patent applications and publications, are incorporated by reference herein in their entirety.

I. Definitions

[0065] By "comprising" it is meant that the recited elements are required in the composition/method/kit, but other elements may be included to form the composition/method/kit etc. within the scope of the claim.

[0066] By "consisting essentially of", it is meant a limitation of the scope of composition or method described to the specified materials or steps that do not materially affect the basic and novel characteristic(s) of the subject invention.

- [0067] By “consisting of”, it is meant the exclusion from the composition, method, or kit of any element, step, or ingredient not specified in the claim.
- [0068] Antibody residues herein are numbered according to the Kabat numbering system and the EU numbering system. The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-113 of the heavy chain) (*e.g.*, Kabat *et al.*, Sequences of Immunological Interest. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The “EU numbering system” or “EU index” is generally used when referring to a residue in an immunoglobulin heavy chain constant region (*e.g.*, the EU index reported in Kabat *et al.*, *supra*). The “EU index as in Kabat” refers to the residue numbering of the human IgG1 EU antibody. Unless stated otherwise herein, references to residue numbers in the variable domain of antibodies mean residue numbering by the Kabat numbering system. Unless stated otherwise herein, references to residue numbers in the constant domain of antibodies mean residue numbering by the EU numbering system.
- [0069] Antibodies, also referred to as immunoglobulins, conventionally comprise at least one heavy chain and one light chain, where the amino terminal domain of the heavy and light chains is variable in sequence, hence is commonly referred to as a variable region domain, or a variable heavy (VH) or variable light (VL) domain. The two domains conventionally associate to form a specific binding region, although as will be discussed here, specific binding can also be obtained with heavy chain-only variable sequences, and a variety of non-natural configurations of antibodies are known and used in the art.
- [0070] A “functional” or “biologically active” antibody or antigen-binding molecule (including heavy chain-only antibodies and multi-specific (*e.g.*, bispecific) three-chain antibody-like molecules (TCAs, described herein) is one capable of exerting one or more of its natural activities in structural, regulatory, biochemical or biophysical events. For example, a functional antibody or other binding molecule, *e.g.*, a TCA, may have the ability to specifically bind an antigen and the binding may in turn elicit or alter a cellular or molecular event such as signal transduction or enzymatic activity. A functional antibody or other binding molecule, *e.g.*, a TCA, may also block ligand activation of a receptor or act as an agonist or antagonist. The capability of an antibody or other binding molecule, *e.g.*, a TCA, to exert one or more of its natural activities depends on several factors, including proper folding and assembly of the polypeptide chains.
- [0071] The term “antibody” herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, monomers, dimers, multimers, multispecific antibodies (*e.g.*, bispecific antibodies), heavy chain-only antibodies, three chain antibodies, TCAs, single chain Fv (scFv), nanobodies, etc., and also includes antibody fragments, so long as they exhibit the desired

biological activity (Miller et al (2003) Jour. of Immunology 170:4854-4861). Antibodies may be murine, human, humanized, chimeric, or derived from other species.

[0072] The term antibody may reference a full-length heavy chain, a full length light chain, an intact immunoglobulin molecule; or an immunologically active portion of any of these polypeptides, i.e., a polypeptide that comprises an antigen binding site that immunospecifically binds an antigen of a target of interest or part thereof, such targets including but not limited to, cancer cell or cells that produce autoimmune antibodies associated with an autoimmune disease. The immunoglobulin disclosed herein can be of any type (e.g., IgG, IgE, IgM, IgD, and IgA), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule, including engineered subclasses with altered Fc portions that provide for reduced or enhanced effector cell activity. Light chains of the subject antibodies can be kappa light chains (Vkappa) or lambda light chains (Vlambda). The immunoglobulins can be derived from any species. In one aspect, the immunoglobulin is of largely human origin.

[0073] The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. Monoclonal antibodies in accordance with the present invention can be made by the hybridoma method first described by Kohler et al. (1975) *Nature* 256:495, and can also be made via recombinant protein production methods (see, e.g., U.S. Patent No. 4,816,567), for example.

[0074] The term “variable”, as used in connection with antibodies, refers to the fact that certain portions of the antibody variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FRs). The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β -sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β -sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of

Health, Bethesda, MD. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

[0075] The term “hypervariable region” when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a “complementarity determining region” or “CDR” (e.g., residues 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a “hypervariable loop” residues 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). In some embodiments, “CDR” means a complementary determining region of an antibody as defined in Lefranc, MP et al., IMGT, the international ImMunoGeneTics database, *Nucleic Acids Res.*, 27:209-212 (1999). “Framework Region” or “FR” residues are those variable domain residues other than the hypervariable region/CDR residues as herein defined.

[0076] Exemplary CDR designations are shown herein, however one of skill in the art will understand that a number of definitions of the CDRs are commonly in use, including the Kabat definition (see “Zhao et al. A germline knowledge based computational approach for determining antibody complementarity determining regions.” *Mol Immunol.* 2010;47:694–700), which is based on sequence variability and is the most commonly used. The Chothia definition is based on the location of the structural loop regions (Chothia et al. “Conformations of immunoglobulin hypervariable regions.” *Nature.* 1989; 342:877–883). Alternative CDR definitions of interest include, without limitation, those disclosed by Honegger, “Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool.” *J Mol Biol.* 2001;309:657–670; Ofra et al. “Automated identification of complementarity determining regions (CDRs) reveals peculiar characteristics of CDRs and B-cell epitopes.” *J Immunol.* 2008;181:6230–6235; Almagro “Identification of differences in the specificity-determining residues of antibodies that recognize antigens of different size: implications for the rational design of antibody repertoires.” *J Mol Recognit.* 2004;17:132–143; and Padlan et al. “Identification of specificity-determining residues in antibodies.” *Faseb J.* 1995;9:133–139., each of which is herein specifically incorporated by reference.

[0077] The terms “heavy chain-only antibody,” and “heavy chain antibody” are used interchangeably herein and refer, in the broadest sense, to antibodies, or more or more portions of an antibody, e.g., one or more arms of an antibody, lacking the light chain of a conventional antibody. The terms specifically include, without limitation, homodimeric antibodies comprising the VH antigen-binding domain and the CH2 and CH3 constant domains, in the absence of the CH1 domain; functional (antigen-binding) variants of such antibodies, soluble VH variants, Ig-NAR comprising a homodimer

of one variable domain (V-NAR) and five C-like constant domains (C-NAR) and functional fragments thereof; and soluble single domain antibodies (sUniDabsTM). In one embodiment, a heavy chain-only antibody is composed of a variable region antigen-binding domain composed of framework 1, CDR1, framework 2, CDR2, framework 3, CDR3, and framework 4. In another embodiment, a heavy chain-only antibody is composed of an antigen-binding domain, at least part of a hinge region and CH2 and CH3 domains. In another embodiment, a heavy chain-only antibody is composed of an antigen-binding domain, at least part of a hinge region and a CH2 domain. In a further embodiment, a heavy chain-only antibody is composed of an antigen-binding domain, at least part of a hinge region and a CH3 domain. Heavy chain-only antibodies in which the CH2 and/or CH3 domain is truncated are also included herein. In a further embodiment, a 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 or otherwise, covalently or non-covalently, attached with each other. The heavy chain-only 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, a heavy chain antibody is of the IgG1, IgG2, IgG3, or IgG4 subtype, in particular the IgG1 subtype. In one embodiment, the heavy chain-only antibodies herein are used as a binding (targeting) domain of a chimeric antigen receptor (CAR). The definition specifically includes human heavy chain-only antibodies produced by human immunoglobulin transgenic rats (UniRatTM), called UniAbsTM. The variable regions (VH) of UniAbsTM are called UniDabsTM, and are versatile building blocks that can be linked to Fc regions or serum albumin for the development of novel therapeutics with multi-specificity, increased potency and extended half-life. Since the homodimeric UniAbsTM lack a light chain and thus a VL domain, the antigen is recognized by one single domain, i.e., the variable domain of the heavy chain of a heavy-chain antibody (VH or VHH).

[0078] An “intact antibody chain” as used herein is one comprising a full length variable region and a full length constant region (Fc). An intact “conventional” antibody comprises an intact light chain and an intact heavy chain, as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, hinge, CH2 and CH3 for secreted IgG. Other isotypes, such as IgM or IgA may have different CH domains. The constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variants thereof. The intact antibody may have one or more “effector functions” which refer to those biological activities attributable to the Fc constant region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC);

phagocytosis; and down regulation of cell surface receptors. Constant region variants include those that alter the effector profile, binding to Fc receptors, and the like.

[0079] Depending on the amino acid sequence of the Fc (constant domain) of their heavy chains, antibodies and various antigen-binding proteins can be provided as different classes. There are five major classes of heavy chain Fc regions: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into “subclasses” (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The Fc constant domains that correspond to the different classes of antibodies may be referenced as α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. Ig forms include hinge-modifications or hingeless forms (Roux et al (1998) J. Immunol. 161:4083-4090; Lund et al (2000) Eur. J. Biochem. 267:7246-7256; US 2005/0048572; US 2004/0229310). The light chains of antibodies from any vertebrate species can be assigned to one of two types, called κ (kappa) and λ (lambda), based on the amino acid sequences of their constant domains. Antibodies in accordance with embodiments of the invention can comprise kappa light chain sequences or lambda light chain sequences.

[0080] A “functional Fc region” possesses an “effector function” of a native-sequence Fc region. Non-limiting examples of effector functions include C1q binding; CDC; Fc-receptor binding; ADCC; ADCP; down-regulation of cell-surface receptors (e.g., B-cell receptor), etc. Such effector functions generally require the Fc region to interact with a receptor, e.g., the Fc γ RI; Fc γ RIIA; Fc γ RIIB1; Fc γ RIIB2; Fc γ RIIIA; Fc γ RIIIB receptors, and the low affinity FcRn receptor; and can be assessed using various assays known in the art. A “dead” or “silenced” Fc is one that has been mutated to retain activity with respect to, for example, prolonging serum half-life, but which does not activate a high affinity Fc receptor, or which has a reduced affinity to an Fc receptor.

[0081] A “native-sequence Fc region” comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native-sequence human Fc regions include, for example, a native-sequence human IgG1 Fc region (non-A and A allotypes); native-sequence human IgG2 Fc region; native-sequence human IgG3 Fc region; and native-sequence human IgG4 Fc region, as well as naturally occurring variants thereof.

[0082] A “variant Fc region” comprises an amino acid sequence that differs from that of a native-sequence Fc region by virtue of at least one amino acid modification, preferably one or more amino acid substitution(s). Preferably, the variant Fc region has at least one amino acid substitution compared to a native-sequence Fc region or to the Fc region of a parent polypeptide, e.g., from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native-sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% homology with a native-sequence Fc

region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% homology therewith, more preferably at least about 95% homology therewith.

[0083] Variant Fc sequences may include three amino acid substitutions in the CH2 region to reduce FcγRI binding at EU index positions 234, 235, and 237 (see Duncan et al., (1988) Nature 332:563). Two amino acid substitutions in the complement C1q binding site at EU index positions 330 and 331 reduce complement fixation (see Tao et al., J. Exp. Med. 178:661 (1993) and Canfield and Morrison, J. Exp. Med. 173:1483 (1991)). Substitution into human IgG1 or IgG2 residues at positions 233-236 and IgG4 residues at positions 327, 330 and 331 greatly reduces ADCC and CDC (see, for example, Armour KL. *et al.*, 1999 Eur J Immunol. 29(8):2613-24; and Shields RL. *et al.*, 2001. J Biol Chem. 276(9):6591-604). The human IgG4 Fc amino acid sequence (UniProtKB No. P01861) is provided herein as SEQ ID NO: 76. Silenced IgG1 is described, for example, in Boesch, A.W., et al., "Highly parallel characterization of IgG Fc binding interactions." MAbs, 2014. 6(4): p. 915-27, the disclosure of which is incorporated herein by reference in its entirety.

[0084] Other Fc variants are possible, including, without limitation, one in which a region capable of forming a disulfide bond is deleted, or in which certain amino acid residues are eliminated at the N-terminal end of a native Fc, or a methionine residue is added thereto. Thus, in some embodiments, one or more Fc portions of an antibody can comprise one or more mutations in the hinge region to eliminate disulfide bonding. In yet another embodiment, the hinge region of an Fc can be removed entirely. In still another embodiment, an antibody can comprise an Fc variant.

[0085] Further, an Fc variant can be constructed to remove or substantially reduce effector functions by substituting (mutating), deleting or adding amino acid residues to effect complement binding or Fc receptor binding. For example, and not limitation, a deletion may occur in a complement-binding site, such as a C1q-binding site. Techniques for preparing such sequence derivatives of the immunoglobulin Fc fragment are disclosed in International Patent Publication Nos. WO 97/34631 and WO 96/32478. In addition, the Fc domain may be modified by phosphorylation, sulfation, acylation, glycosylation, methylation, farnesylation, acetylation, amidation, and the like.

[0086] In some embodiments, an antibody comprises a variant human IgG4 CH3 domain sequence comprising a T366W mutation, which can optionally be referred to herein as an IgG4 CH3 knob sequence. In some embodiments, an antibody comprises a variant human IgG4 CH3 domain sequence comprising a T366S mutation, an L368A mutation, and a Y407V mutation, which can optionally be referred to herein as an IgG4 CH3 hole sequence. The IgG4 CH3 mutations described herein can be utilized in any suitable manner so as to place a "knob" on a first heavy chain constant region of a first monomer in an antibody dimer, and a "hole" on a second heavy chain constant region of a second monomer in an antibody dimer, thereby facilitating proper pairing (heterodimerization) of the desired pair of heavy chain polypeptide subunits in the antibody.

[0087] In some embodiments, an antibody comprises a heavy chain polypeptide subunit comprising a variant human IgG4 Fc region comprising an S228P mutation, an F234A mutation, an L235A mutation, and a T366W mutation (knob). In some embodiments, an antibody comprises a heavy chain polypeptide subunit comprising a variant human IgG4 Fc region comprising an S228P mutation, an F234A mutation, an L235A mutation, a T366S mutation, an L368A mutation, and a Y407V mutation (hole).

[0088] The term “Fc-region-comprising antibody” refers to an antibody that comprises an Fc region. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during purification of the antibody or by recombinant engineering of the nucleic acid encoding the antibody. Accordingly, an antibody having an Fc region according to this invention can comprise an antibody with or without K447.

[0089] Aspects of the invention include antibodies comprising a heavy chain-only variable region in a monovalent or bivalent configuration. As used herein, the term “monovalent configuration” as used in reference to a heavy chain-only variable region domain means that only one heavy chain-only variable region domain is present, having a single binding site (see FIG. 5, Panel A, right arm of antibody). In contrast, the term “bivalent configuration” as used in reference to a heavy chain-only variable region domain means that two heavy chain-only variable region domains are present (each having a single binding site), and are connected by a linker sequence (see FIG. 5, Panels B and C, right arms of antibodies). Non-limiting examples of linker sequences are discussed further herein, and include, without limitation, GS linker sequences of various lengths. When a heavy chain-only variable region is in a bivalent configuration, each of the two heavy chain-only variable region domains can have binding affinity to the same antigen, or to different antigens (e.g., to different epitopes on the same protein; to two different proteins, etc.). However, unless specifically noted otherwise, a heavy chain-only variable region denoted as being in a “bivalent configuration” is understood to contain two identical heavy chain-only variable region domains, connected by a linker sequence, wherein each of the two identical heavy chain-only variable region domains have binding affinity to the same target antigen.

[0090] Aspects of the invention include antibodies having multi-specific configurations, which include, without limitation, bispecific, trispecific, etc. A large variety of methods and protein configurations are known and used in bispecific monoclonal antibodies (BsMAB), tri-specific antibodies, etc.

[0091] Various methods for the production of multivalent artificial antibodies have been developed by recombinantly fusing variable domains of two or more antibodies. In some embodiments, a first and a second antigen-binding domain on a polypeptide are connected by a polypeptide linker. One non-limiting example of such a polypeptide linker is a GS linker, having an amino acid sequence of

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. Non-limiting examples of such linkers include GGGGS (SEQ ID NO: 73) ($n=1$) and GGGGSGGGGS (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

particular the IgG1 subtype or the IgG4 subtype. Non-limiting examples of a TCA binding compound are described in, for example, WO2017/223111 and WO2018/052503, the disclosures of which are incorporated herein by reference in their entirety.

- [0094] Heavy-chain antibodies constitute about one fourth of the IgG antibodies produced by the camelids, e.g., camels and llamas (Hamers-Casterman C., et al. *Nature*. 363, 446-448 (1993)). These antibodies are formed by two heavy chains but are devoid of light chains. As a consequence, the variable antigen binding part is referred to as the VHH domain and it represents the smallest naturally occurring, intact, antigen-binding site, being only around 120 amino acids in length (Desmyter, A., et al. *J. Biol. Chem.* 276, 26285-26290 (2001)). Heavy chain antibodies with a high specificity and affinity can be generated against a variety of antigens through immunization (van der Linden, R. H., et al. *Biochim. Biophys. Acta.* 1431, 37-46 (1999)) and the VHH portion can be readily cloned and expressed in yeast (Frenken, L. G. J., et al. *J. Biotechnol.* 78, 11-21 (2000)). Their levels of expression, solubility and stability are significantly higher than those of classical F(ab) or Fv fragments (Ghahroudi, M. A. et al. *FEBS Lett.* 414, 521-526 (1997)). Sharks have also been shown to have a single VH-like domain in their antibodies, termed VNAR. (Nuttall et al. *Eur. J. Biochem.* 270, 3543-3554 (2003); Nuttall et al. *Function and Bioinformatics* 55, 187-197 (2004); Dooley et al., *Molecular Immunology* 40, 25-33 (2003)).
- [0095] The term “PSMA” as used herein refers to a type II transmembrane protein that has N-acetylated-alpha-linked acidic depeptidase, folate hydrolase and dipeptidyl-peptidase activity. The term “PSMA” includes a PSMA protein of any human and non-human animal species, and specifically includes human PSMA as well as PSMA of non-human mammals.
- [0096] The term “human PSMA” as used herein includes any variants, isoforms and species homologs of human PSMA (UniProt Q04609), regardless of its source or mode of preparation. Thus, “human PSMA” includes human PSMA naturally expressed by cells and PSMA expressed on cells transfected with the human PSMA gene.
- [0097] The terms “anti-PSMA heavy chain-only antibody,” “PSMA heavy chain-only antibody,” “anti-PSMA heavy chain antibody” and “PSMA heavy chain antibody” are used herein interchangeably to refer to a heavy chain-only antibody as hereinabove defined, immunospecifically binding to PSMA, including human PSMA, as hereinabove defined. The definition includes, without limitation, human heavy chain antibodies produced by transgenic animals, such as transgenic rats or transgenic mice expressing human immunoglobulin, including UniRatsTM producing human anti-PSMA UniAbTM antibodies, as hereinabove defined.
- [0098] “Percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and

introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2.

[0099] An “isolated” antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0100] Antibodies of the invention include multi-specific antibodies. Multi-specific antibodies have more than one binding specificity. The term “multi-specific” specifically includes “bispecific” and “trisppecific,” as well as higher-order independent specific binding affinities, such as higher-order polypepitopic specificity, as well as tetravalent antibodies and antibody fragments. The terms “multi-specific antibody,” “multi-specific heavy chain-only antibody,” “multi-specific heavy chain antibody,” and “multi-specific UniAb™” are used herein in the broadest sense and cover all antibodies with more than one binding specificity. The multi-specific heavy chain anti-PSMA antibodies of the present invention specifically include antibodies immunospecifically binding to two or more non-overlapping epitopes on a PSMA protein, such as a human PSMA (i.e., bivalent and biparatopic). The multi-specific heavy chain anti-PSMA antibodies of the present invention also specifically include antibodies immunospecifically binding to an epitope on a PSMA protein, such as human PSMA and to an epitope on a different protein, such as, for example, a CD3 protein, such as human CD3 (i.e., bivalent and biparatopic). The multi-specific heavy chain anti-PSMA antibodies of the present invention also specifically include antibodies immunospecifically binding to two or more non-overlapping or partially overlapping epitopes on a PSMA protein, such as a human PSMA

protein, and to an epitope on a different protein, such as, for example, a CD3 protein, such as human CD3 protein (i.e., trivalent and biparatopic).

[0101] Antibodies of the invention include monospecific antibodies, having one binding specificity. Monospecific antibodies specifically include antibodies comprising a single binding specificity, as well as antibodies comprising more than one binding unit having the same binding specificity. The terms “monospecific antibody,” “monospecific heavy chain-only antibody,” “monospecific heavy chain antibody,” and “monospecific UniAbTM” are used herein in the broadest sense and cover all antibodies with one binding specificity. The monospecific heavy chain anti-PSMA antibodies of the present invention specifically include antibodies immunospecifically binding to one epitope on a PSMA protein, such as a human PSMA (monovalent and monospecific). The monospecific heavy chain anti-PSMA antibodies of the present invention also specifically include antibodies having more than one binding unit (e.g., multivalent antibodies) immunospecifically binding to an epitope on a PSMA protein, such as human PSMA. For example, a monospecific antibody in accordance with embodiments of the invention can include a heavy chain variable region comprising two antigen-binding domains, wherein each antigen-binding domain binds to the same epitope on a PSMA protein (i.e., bivalent and monospecific).

[0102] An “epitope” is the site on the surface of an antigen molecule to which a single antibody molecule binds. Generally, an antigen has several or many different epitopes and reacts with many different antibodies. The term specifically includes linear epitopes and conformational epitopes.

[0103] “Epitope mapping” is the process of identifying the binding sites, or epitopes, of antibodies on their target antigens. Antibody epitopes may be linear epitopes or conformational epitopes. Linear epitopes are formed by a continuous sequence of amino acids in a protein. Conformational epitopes are formed of amino acids that are discontinuous in the protein sequence, but which are brought together upon folding of the protein into its three-dimensional structure.

[0104] “Polyepitopic specificity” refers to the ability to specifically bind to two or more different epitopes on the same or different target(s). As noted above, the present invention specifically includes anti-PSMA heavy chain antibodies with polyepitopic specificities, i.e., anti-PSMA heavy chain antibodies binding to one or more non-overlapping epitopes on a PSMA protein, such as a human PSMA; and anti-PSMA heavy chain antibodies binding to one or more epitopes on a PSMA protein and to an epitope on a different protein, such as, for example, a CD3 protein. The term “non-overlapping epitope(s)” or “non-competitive epitope(s)” of an antigen is defined herein to mean epitope(s) that are recognized by one member of a pair of antigen-specific antibodies but not the other member. Pairs of antibodies, or antigen-binding regions targeting the same antigen on a multi-specific antibody, recognizing non-overlapping epitopes, do not compete for binding to that antigen and are able to bind that antigen simultaneously.

- [0105] An antibody binds “essentially the same epitope” as a reference antibody, when the two antibodies recognize identical or sterically overlapping epitopes. The most widely used and rapid methods for determining whether two epitopes bind to identical or sterically overlapping epitopes are competition assays, which can be configured in all number of different formats, using either labeled antigen or labeled antibody. Usually, the antigen is immobilized on a 96-well plate, and the ability of unlabeled antibodies to block the binding of labeled antibodies is measured using radioactive or enzyme labels.
- [0106] The term “valent” as used herein refers to a specified number of binding sites in an antibody molecule.
- [0107] A “monovalent” antibody has one binding site. Thus, a monovalent antibody is also monospecific.
- [0108] A “multi-valent” antibody has two or more binding sites. Thus, the terms “bivalent”, “trivalent”, and “tetravalent” refer to the presence of two binding sites, three binding sites, and four binding sites, respectively. Thus, a bispecific antibody according to the invention is at least bivalent and may be trivalent, tetravalent, or otherwise multi-valent. A bivalent antibody in accordance with embodiments of the invention may have two binding sites to the same epitope (i.e., bivalent, monoparatopic), or to two different epitopes (i.e., bivalent, biparatopic).
- [0109] A large variety of methods and protein configurations are known and used for the preparation of bispecific monoclonal antibodies (BsMAB), tri-specific antibodies, and the like.
- [0110] 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 chain 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 an antigen binding domain 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. A TCA protein makes use of a heavy chain-only antibody as hereinabove defined.
- [0111] The term “chimeric antigen receptor” or “CAR” is used herein in the broadest sense to refer to an engineered receptor, which grafts a desired binding specificity (e.g., the antigen-binding region of a monoclonal antibody or other ligand) to membrane-spanning and intracellular-signaling domains.

Typically, the receptor is used to graft the specificity of a monoclonal antibody onto a T-cell to create a chimeric antigen receptors (CAR). (*J Natl Cancer Inst*, 2015; 108(7):dvj439; and Jackson et al., *Nature Reviews Clinical Oncology*, 2016; 13:370–383). CAR-T cells are T-cells that have been genetically engineered to produce an artificial T-cell receptor for use in immunotherapy. In one embodiment, “CAR-T cell” means a therapeutic T-cell expressing a transgene encoding one or more chimeric antigen receptors comprised minimally of an extracellular domain, a transmembrane domain, and at least one cytosolic domain.

[0112] The term “human antibody” is used herein to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies herein may include amino acid residues not encoded by human germline immunoglobulin sequences, e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*. The term “human antibody” specifically includes heavy chain-only antibodies having human heavy chain variable region sequences, produced by transgenic animals, such as transgenic rats or mice, in particular UniAbsTM produced by UniRatsTM, as defined above.

[0113] By a “chimeric antibody” or a “chimeric immunoglobulin” is meant an immunoglobulin molecule comprising amino acid sequences from at least two different Ig loci, e.g., a transgenic antibody comprising a portion encoded by a human Ig locus and a portion encoded by a rat Ig locus. Chimeric antibodies include transgenic antibodies with non-human Fc-regions or artificial Fc-regions, and human idiotypes. Such immunoglobulins can be isolated from animals of the invention that have been engineered to produce such chimeric antibodies.

[0114] As used herein, the term “effector cell” refers to an immune cell which is involved in the effector phase of an immune response, as opposed to the cognitive and activation phases of an immune response. Some effector cells express specific Fc receptors and carry out specific immune functions. In some embodiments, an effector cell such as a natural killer cell is capable of inducing antibody-dependent cellular cytotoxicity (ADCC). For example, monocytes and macrophages, which express FcR, are involved in specific killing of target cells and presenting antigens to other components of the immune system, or binding to cells that present antigens. In some embodiments, an effector cell may phagocytose a target antigen or target cell.

[0115] “Human effector cells” are leukocytes which express receptors such as T-cell receptors or FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include natural killer (NK) cells, monocytes, cytotoxic T-cells and neutrophils; with NK cells being preferred. The effector cells may be isolated from a native source thereof, e.g., from blood or PBMCs as described herein.

[0116] The term “immune cell” is used herein in the broadest sense, including, without limitation, cells of myeloid or lymphoid origin, for instance lymphocytes (such as B-cells and T-cells including

cytolytic T-cells (CTLs)), killer cells, natural killer (NK) cells, macrophages, monocytes, eosinophils, polymorphonuclear cells, such as neutrophils, granulocytes, mast cells, and basophils.

[0117] Antibody “effector functions” refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B-cell receptor; BCR), etc.

[0118] “Antibody-dependent cell-mediated cytotoxicity” and “ADCC” refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (e.g., Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in an animal model such as that disclosed in Clynes *et al. PNAS (USA)* 95:652-656 (1998).

[0119] “Complement dependent cytotoxicity” or “CDC” refers to the ability of a molecule to lyse a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g. an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g., as described in Gazzano-Santoro *et al., J. Immunol. Methods* 202:163 (1996), may be performed.

[0120] “Binding affinity” refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_d). Affinity can be measured by common methods known in the art. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound.

[0121] As used herein, the “K_d” or “K_d value” refers to a dissociation constant determined by BioLayer Interferometry, using an Octet QK384 instrument (Fortebio Inc., Menlo Park, CA) in kinetics mode. For example, anti-mouse Fc sensors are loaded with mouse-Fc fused antigen and then dipped into antibody-containing wells to measure concentration dependent association rates (k_{on}).

Antibody dissociation rates (k_{off}) are measured in the final step, where the sensors are dipped into wells containing buffer only. The K_d is the ratio of k_{off}/k_{on} . (For further details see, Concepcion, J, et al., *Comb Chem High Throughput Screen*, 12(8), 791-800, 2009).

[0122] The terms “treatment”, “treating” and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. “Treatment” as used herein covers any treatment of a disease in a mammal, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; or (c) relieving the disease, i.e., causing regression of the disease. The therapeutic agent may be administered before, during or after the onset of disease or injury. The treatment of ongoing disease, where the treatment stabilizes or reduces the undesirable clinical symptoms of the patient, is of particular interest. Such treatment is desirably performed prior to complete loss of function in the affected tissues. The subject therapy may be administered during the symptomatic stage of the disease, and in some cases after the symptomatic stage of the disease.

[0123] A “therapeutically effective amount” is intended for an amount of active agent which is necessary to impart therapeutic benefit to a subject. For example, a “therapeutically effective amount” is an amount which induces, ameliorates or otherwise causes an improvement in the pathological symptoms, disease progression or physiological conditions associated with a disease or which improves resistance to a disorder.

[0124] The term “prostate cancer,” as used herein, refers to a malignant tumor of glandular origin in the prostate gland.

[0125] The term “characterized by expression of PSMA” broadly refers to any disease or disorder in which PSMA expression is associated with or involved with one or more pathological processes that are characteristic of the disease or disorder. Such disorders include, but are not limited to, prostate cancer.

[0126] The terms “subject,” “individual,” and “patient” are used interchangeably herein to refer to a mammal being assessed for treatment and/or being treated. In an embodiment, the mammal is a human. The terms “subject,” “individual,” and “patient” encompass, without limitation, individuals having cancer, individuals with autoimmune diseases, with pathogen infections, and the like. Subjects may be human, but also include other mammals, particularly those mammals useful as laboratory models for human disease, e.g., mouse, rat, etc.

[0127] The term “pharmaceutical formulation” refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional

components which are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile. "Pharmaceutically acceptable" excipients (vehicles, additives) are those which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient employed.

[0128] A "sterile" formulation is aseptic or free or essentially free from all living microorganisms and their spores. A "frozen" formulation is one at a temperature below 0 °C.

[0129] A "stable" formulation is one in which the protein therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. Preferably, the formulation essentially retains its physical and chemical stability, as well as its biological activity upon storage. The storage period is generally selected based on the intended shelf-life of the formulation. Various analytical techniques for measuring protein stability are available in the art and are reviewed in Peptide and Protein Drug Delivery, 247-301. Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones. A. Adv. Drug Delivery Rev. 10: 29-90 (1993), for example. Stability can be measured at a selected temperature for a selected time period. Stability can be evaluated qualitatively and/or quantitatively in a variety of different ways, including evaluation of aggregate formation (for example using size exclusion chromatography, by measuring turbidity, and/or by visual inspection); by assessing charge heterogeneity using cation exchange chromatography, image capillary isoelectric focusing (icIEF) or capillary zone electrophoresis; amino-terminal or carboxy-terminal sequence analysis; mass spectrometric analysis; SDS-PAGE analysis to compare reduced and intact antibody; peptide map (for example tryptic or LYS-C) analysis; evaluating biological activity or antigen binding function of the antibody; etc. Instability may involve any one or more of: aggregation, deamidation (e.g., Asn deamidation), oxidation (e.g., Met oxidation), isomerization (e.g., Asp isomerization), clipping/hydrolysis/fragmentation (e.g., hinge region fragmentation), succinimide formation, unpaired cysteine(s), N-terminal extension, C-terminal processing, glycosylation differences, etc.

II. Detailed Description

Anti-PSMA Antibodies

[0130] The present invention provides a family of closely related antibodies that bind to human PSMA. The antibodies of this family comprise a set of CDR sequences as defined herein and shown in Table 1, and are exemplified by the provided heavy chain variable region (VH) sequences of SEQ ID NOs: 24 to 54 set forth in Table 2. The family of antibodies provides a number of benefits that contribute to utility as clinically therapeutic agent(s). The antibodies include members with a range of binding affinities, allowing the selection of a specific sequence with a desired binding affinity.

[0131] Table 1: Anti-PSMA heavy chain antibody unique CDR amino acid sequences.

SEQ_aa_CDR1	SEQ_aa_CDR2	SEQ_aa_CDR3
GGSISSSSYY (SEQ ID NO: 1)	IDYSGYT (SEQ ID NO: 11)	ARHKAATADFDY (SEQ ID NO: 18)
GGSISSSNFY (SEQ ID NO: 2)	VDYSGYT (SEQ ID NO: 12)	AREPRIGYYYESSGYDSLIDY (SEQ ID NO: 19)
GGSISSNSYY (SEQ ID NO: 3)	IYDSGST (SEQ ID NO: 13)	AREPRIGYYYESSGYYSIDY (SEQ ID NO: 20)
GFSFRSYG (SEQ ID NO: 4)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYDSSGYDSLIDY (SEQ ID NO: 21)
GFSFSSYG (SEQ ID NO: 5)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYDSSGYYSIDY (SEQ ID NO: 22)
GFIFRSYG (SEQ ID NO: 6)	ISYDGSNK (SEQ ID NO: 16)	AREPRVGYYYETSGYYSIDY (SEQ ID NO: 23)
GFSFSRYG (SEQ ID NO: 7)	ISYDGSNR (SEQ ID NO: 17)	
GFSFTSYG (SEQ ID NO: 8)		
GFTFISYG (SEQ ID NO: 9)		
GFTFSSYG (SEQ ID NO: 10)		

[0132] Table 2: Anti-PSMA heavy chain antibody variable domain amino acid sequences.

Clone ID #	SEQ_aa_FR1_FR4	SEQ ID NO.
325920	QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWRQPPGKGLEWIGSIDYS GYTYYNPSLQSRVTISVDTSKNQFSLKLSSVTAADTAVYNCARHKAATADFDYR GQGLTVTVSS	24
346181	QLQLQESGPGLVKPSETLSLTCTVSGGSISSSNFYWGWRQSPGKGLEWIGSIDYS GYTYYNPSLQSRVTISVDTSKNQFSLKLSSVTAADTAVYNCARHKAATADFDYR GQGLTVTVSS	25
346165	QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWRQPPGKGLEWIGSVDYS GYTYYNPSLQSRVTISVDTSKNQFSLKLSSVTAADTAVYNCARHKAATADFDYR GQGLTVTVSS	26
346172	QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWRQPPGKGLEWIGSIDYS GYTYYNPSLQSRVTISVDTSKNQFSLKLSSVTAADTAVYNCARHKAATADFDYR GQGLTVTVSS	27
326109	QLQLQESGPGLVKPSETLSLTCTVSGGSISSNSYYWGWRQSPGKGLEWLGSIDYS GSTHYNPSLQSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARHKAATADFDYR GQGLTVTVSS	28
325867	QVQLVESGGGVVQPGRLRLSCAASGFSFRSYGMHWVRQAPGKGLEWVAVIY DGSNKYYADSVKGRFTISRDIYSKNTLYLQMNSLRAEDTAVYYCAREPRIGY SSGYDSLIDYRGQGLTVTVSS	29
325742	QVQLVESGGGVVQPGRLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVAVIY DGSNKYYADSVKGRFTISRDIYSKNTLYLQMNSLRAEDTAVYYCAREPRIGY SSGYDSLIDYRGQGLTVTVSS	30

Clone ID #	SEQ_aa_FR1_FR4	SEQ ID NO.
325748	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEGVAVIWY DGSNRYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGYYYYE SSGYYSLDYRGQGTLVTVSS	31
325940	QVQLVESGGGVVQPGRSLRLSCAASGFIFRSYGMHWVRQAPGKGPEWVAVIWY DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGYYYYE SSGYYSLDYRGQGTLVTVSS	32
325836	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVAVIWY DGSNKYYADSVKGRFTISR DY SKNTLYLQMNSLRAEDTAVYYCAREPRIGYYYYD SSGYDSL DYRGQGTLVTVSS	33
326027	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVAVIWY DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGYYYYD SSGYDSL DYRGQGTLVTVSS	34
326087	QVQLVESGGGVVQPGRSLRLSCAASGFIFRSYGMHWVRQAPGKGPEWVAVIWY DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGYYYYD SSGYDSL DYRGQGTLVTVSS	35
326084	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEGVAVIWY DGSNRYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGYYYYD SSGYYSLDYRGQGTLVTVSS	36
326028	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVAVISY DGSNKYYADSVKGRFTISR DY SKNTLYLQMNSLRAEDTAVYYCAREPRIGYYYYD SSGYDSL DYRGQGTLVTVSS	37
345497	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEGVAVIWY DGSNRYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGYYYYE SSGYYSLDYRGQGTLVTVSS	38
326029	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVAVISY DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRVGYYY ETSGYYSLDYRGQGTLVTVSS	39
345461	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEGVAVIWY DGSNRYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGYYYYE SSGYYSLDYRGQGTLVTVSS	40
345493	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVAVIWY DGSNRYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGYYYYE SSGYYSLDYRGQGTLVTVSS	41
345436	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEGVAVIWY DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGYYYYE SSGYYSLDYRGQGTLVTVSS	42
345443	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEGVAVIWY DGSNRYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGYYYYE SSGYYSLDYRGQGTLVTVSS	43
345490	QVQLVESGGGLVKPGGSLRLSCAASGFSFSSYGMHWVRQAPGKGLEGVAVIWY DGSNRYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGYYYYE SSGYYSLDYRGQGTLVTVSS	44
345482	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEGVAVIWY DGSNRYYADSVKGRFTISR DSSKNTLYLQMNSLRAEDTAVYYCAREPRIGYYYYE SSGYYSLDYRGQGTLVTVSS	45
345485	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVAVIWY DGSNKYYADSVKGRFTISRDN SKNTMYLQMNSLRAEDTAVYYCAREPRIGYYYYE SSGYYSLDYRGQGTLVTVSS	46

Clone ID #	SEQ_aa_FR1_FR4	SEQ ID NO.
345463	QVQLVESGGGVVQPGRSLRLSCAASGFI FRSYGMHWVRQAPGKGPEWVAVIWY DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGY YYYE SSGYYSLDYRGQGTLVTVSS	47
325932	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEGVAVIWY DGSNRYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGY YYYE SSGYYSLDYRGQGTLVTVSS	48
345505	QVQLVESGGGVVQPGRSLRLSCAASGFTFISYGMHWVRQAPGKGLEGVAVIWY DGSNRYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGY YYYE SSGYYSLDYRGQGTLVTVSS	49
345508	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGPEWVAVIWY DGSNRYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGY YYYE SSGYYSLDYRGQGTLVTVSS	50
345480	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVAVIWY DGSNRYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGY YYYE SSGYYSLDYRGQGTLVTVSS	51
326116	QVQLVESGGGVVQPGRSLRLSCAASGFSFSRYGMHWVRQAPGKGLEGVAVISY DGSNKYYADSVKGRFTISR DY SKNTLYLQMNSLRAEDTAVYYCAREPRIGY YYYD SSGYDSL DYRGQGTLVTVSS	52
345509	QVQLVESGGGLVQPGGSLRLSCAASGFSFSSYGMHWVRQAPGKGLEGVAVIWY DGSNKYYADSVKGRFTISRDN SKNTMYLQMNSLRAEDTAVYYCAREPRIGY YYYE SSGYYSLDYRGQGTLVTVSS	53
345444	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEGVAVIWY DGSNRYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGY YYYE SSGYYSLDYRGQGTLVTVSS	54
345421	QVQLVESVGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEGVAVIWY DGSNRYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGY YYYE SSGYYSLDYRGQGTLVTVSS	55
345447	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEGVAVISY DGSNRYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGY YYYE SSGYYSLDYRGQGTLVTVSS	56
345510	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVAVIWY DGSNKYYADSVKGRFTISRDN SKNTMYLQMNSLRAEDTAVYYCAREPRIGY YYYE SSGYYSLDYRGQGTLVTVSS	57
345438	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGPEWVAVIWY DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGY YYYE SSGYYSLDYRGQGTLVTVSS	58

[0133] A suitable antibody may be selected from those provided herein for development and therapeutic or other use, including, without limitation, use as a bispecific antibody, e.g., as shown in FIG. 5, panels A-C, or a tri-specific antibody, or part of a CAR-T structure. FIG. 5, panels A-C provide illustrations of anti-CD3 x anti-PSMA multi-specific antibodies, where the anti-PSMA domain is monovalent and monospecific, bivalent and monospecific, or bivalent and bispecific (biparatopic). The anti-CD3 domain contains a CH1 domain and pairs with a light chain, while the anti-PSMA domains are derived from heavy chain-only antibodies and do not contain a CH1 domain

or interact with a light chain. In some embodiments, the two heavy chains are paired using, e.g., knobs-into-holes technology. Turning to the antibodies depicted in FIG. 5, panel A depicts an anti-CD3 x anti-PSMA bispecific antibody wherein the anti-PSMA binding arm is monovalent and monospecific, and the antigen-binding domain of the anti-PSMA arm is in a monovalent configuration, meaning only one antigen-binding domain is present. Panel B depicts an anti-CD3 x anti-PSMA bispecific antibody wherein the anti-PSMA binding arm is bivalent and monospecific, and the antigen-binding domain of the anti-PSMA arm is in a bivalent configuration, meaning there are two identical antigen binding domains placed in tandem. Panel C depicts an anti-CD3 x anti-PSMA bispecific antibody wherein the anti-PSMA binding arm is bivalent and biparatopic, and the antigen-binding domains of the anti-PSMA arm are in a bivalent configuration.

[0134] Determination of affinity for a candidate protein can be performed using methods known in the art, such as Biacore measurements. Members of the antibody family may have an affinity for PSMA with a K_d of from about 10^{-6} to around about 10^{-11} , including without limitation: from about 10^{-6} to around about 10^{-10} ; from about 10^{-6} to around about 10^{-9} ; from about 10^{-6} to around about 10^{-8} ; from about 10^{-8} to around about 10^{-11} ; from about 10^{-8} to around about 10^{-10} ; from about 10^{-8} to around about 10^{-9} ; from about 10^{-9} to around about 10^{-11} ; from about 10^{-9} to around about 10^{-10} ; or any value within these ranges. The affinity selection may be confirmed with a biological assessment for modulating, e.g., blocking, a PSMA biological activity, including *in vitro* assays, pre-clinical models, and clinical trials, as well as assessment of potential toxicity.

[0135] Members of the antibody family herein are not cross-reactive with the PSMA protein of *Cynomolgus* macaque, but can be engineered to provide cross-reactivity with the PSMA protein of *Cynomolgus* macaque, or with the PSMA of any other animal species, if desired.

[0136] The family of PSMA-specific antibodies herein comprises a VH domain, comprising CDR1, CDR2 and CDR3 sequences in a human VH framework. The CDR sequences may be situated, as an example, in the region of around amino acid residues 26-33; 51-58; and 97-116 for CDR1, CDR2 and CDR3, respectively, of the provided exemplary variable region sequences set forth in SEQ ID NOs: 24 to 58. It will be understood by one of ordinary skill in the art that the CDR sequences may be in different positions if a different framework sequence is selected, although generally the order of the sequences will remain the same.

[0137] The CDR1, CDR2, and CDR3 sequences of the anti-PSMA antibodies of the present invention may be encompassed by the following structural formulae, where an X indicates a variable amino acid, which may be the specific amino acids as indicated below.

CDR1

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

CDR2

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

CDR3

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

[0138] The CDR1, CDR2, and CDR3 sequences of the anti-PSMA antibodies of the present invention may be encompassed by the following structural formulas, where an X indicates a variable amino acid, which may be the specific amino acids as indicated below.

CDR1

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

CDR2

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

CDR3

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 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)	AREPRIGYYYESSGYDSL DY (SEQ ID NO: 19)
325742	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYESSGYDSL DY (SEQ ID NO: 19)
325748	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
325940	GFIFRSYG (SEQ ID NO: 6)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
325836	GFSFRSYG (SEQ ID NO: 4)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYDSSGYDSL DY (SEQ ID NO: 21)
326027	GFSFRSYG (SEQ ID NO: 4)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYDSSGYDSL DY (SEQ ID NO: 21)
326087	GFIFRSYG (SEQ ID NO: 6)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYDSSGYDSL DY (SEQ ID NO: 21)
326084	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYDSSGYSLDY (SEQ ID NO: 22)
326028	GFSFRSYG (SEQ ID NO: 4)	ISYDGSNK (SEQ ID NO: 16)	AREPRIGYYYDSSGYDSL DY (SEQ ID NO: 21)
345497	GFSFSRYG (SEQ ID NO: 7)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
326029	GFSFSRYG (SEQ ID NO: 7)	ISYDGSNK (SEQ ID NO: 16)	AREPRVGYYYETSGYYSLDY (SEQ ID NO: 23)
345461	GFSFTSYG (SEQ ID NO: 8)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)

Clone ID #	SEQ_aa_CDR1	SEQ_aa_CDR2	SEQ_aa_CDR3
345493	GFSFSRYG (SEQ ID NO: 7)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
345436	GFSFSRYG (SEQ ID NO: 7)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
345443	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
345490	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
345482	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
345485	GFSFRSYG (SEQ ID NO: 4)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
345463	GFIFRSYG (SEQ ID NO: 6)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
325932	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
345505	GFTFISYG (SEQ ID NO: 9)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
345508	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
345480	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
326116	GFSFSRYG (SEQ ID NO: 7)	ISYDGSNK (SEQ ID NO: 16)	AREPRIGYYYDSSGYDSLIDY (SEQ ID NO: 21)
345509	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
345444	GFTFSSYG (SEQ ID NO: 10)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
345421	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNR (SEQ ID NO: 15)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
345447	GFSFSSYG (SEQ ID NO: 5)	ISYDGSNR (SEQ ID NO: 17)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
345510	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)
345438	GFSFSSYG (SEQ ID NO: 5)	IWYDGSNK (SEQ ID NO: 14)	AREPRIGYYYESSGYSLDY (SEQ ID NO: 20)

[0140] In some embodiments, an anti-PSMA antibody comprises a CDR1 sequence of any one of SEQ ID NOs: 1-10. In a particular embodiment, the CDR1 sequence is SEQ ID NO: 2 or 7.

[0141] In some embodiments, an anti-PSMA antibody comprises a CDR2 sequence of any one of SEQ ID NOs: 11-17. In a particular embodiment, the CDR2 sequence is SEQ ID NO: 11 or 15.

[0142] In some embodiments, an anti-PSMA antibody comprises a CDR3 sequence of any one of SEQ ID NOs: 18-23. In a particular embodiment, the CDR3 sequence is SEQ ID NO: 18 or 20.

- [0143] In a further embodiment, an anti-PSMA heavy chain-only antibody comprises the CDR1 sequence of SEQ ID NO: 2; the CDR2 sequence of SEQ ID NO: 11; and the CDR3 sequence of SEQ ID NO: 18.
- [0144] In a further embodiment, an anti-PSMA antibody comprises the CDR1 sequence of SEQ ID NO: 7; the CDR2 sequence of SEQ ID NO: 15; and the CDR3 sequence of SEQ ID NO: 20.
- [0145] In a further embodiment, an anti-PSMA antibody comprises any of the heavy chain variable region amino acid sequences of SEQ ID NOs: 24 to 58 (Table 2).
- [0146] In a still further embodiment, an anti-PSMA antibody comprises the heavy chain variable region sequence of SEQ ID NO: 25.
- [0147] In a still further embodiment, an anti-PSMA antibody comprises the heavy chain variable region sequence of SEQ ID NO: 38.
- [0148] In some embodiments, a CDR sequence in an anti-PSMA antibody of the invention comprises one or two amino acid substitutions relative to a CDR1, CDR2 and/or CDR3 sequence or set of CDR1, CDR2 and CDR3 sequences in any one of SEQ ID NOs: 1 to 23 (Table 1).
- [0149] In some embodiments, an anti-PSMA antibody preferably comprises a heavy chain variable domain (VH) in which the CDR3 sequence has greater than or equal to 80%, such as at least 85%, at least 90%, at least 95%, or at least 99% sequence identity at the amino acid level to a CDR3 sequence of any one of the antibodies whose CDR3 sequences are provided in Table 1, and binds to PSMA.
- [0150] In some embodiments, an anti-PSMA antibody preferably comprises a heavy chain variable domain (VH) in which the full set of CDRs 1, 2, and 3 (combined) has greater than or equal to eighty-five percent (85%) sequence identity at the amino acid level to the CDRs 1, 2, and 3 (combined) of the antibodies whose CDR sequences are provided in Table 1, and binds to PSMA.
- [0151] In some embodiments, an anti-PSMA antibody preferably comprises a heavy chain variable domain (VH) in which the full set of CDRs 1, 2, and 3 (combined) has greater than or equal to eighty-five percent (85%) sequence identity at the amino acid level to the CDRs 1, 2, and 3 (combined) of the antibodies whose CDR sequences are provided in Table 3, and binds to PSMA.
- [0152] In some embodiments, an anti-PSMA antibody comprises a heavy chain variable region sequence with at least about 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 98% identity, or at least 99% identity to any of the heavy chain variable region sequences of SEQ ID NOs: 24 to 58 (shown in Table 2), and binds to PSMA.
- [0153] In some embodiments, bispecific or multi-specific antibodies are provided, which may have any of the configurations discussed herein, including, without limitation, a bispecific three-chain antibody like molecule (TCA). In some embodiments, a multi-specific antibody can comprise at least one heavy chain variable region having binding specificity for PSMA, and at least one heavy chain variable region having binding specificity for a protein other than PSMA. In some embodiments, a

multi-specific antibody can comprise a heavy chain variable region comprising at least two antigen-binding domains, wherein each of the antigen-binding domains has binding specificity for PSMA. In some embodiments, a multi-specific antibody can comprise a heavy chain/light chain pair that has binding specificity for a first antigen (e.g., CD3), and a heavy chain from a heavy chain-only antibody. In certain embodiments, the heavy chain from the heavy chain-only antibody comprises an Fc portion comprising CH2 and/or CH3 and/or CH4 domains, in the absence of a CH1 domain. In one particular embodiment, a bispecific antibody comprises a heavy chain/light chain pair that has binding specificity for an antigen on an effector cell (e.g., a CD3 protein on a T-cell), and a heavy chain from a heavy chain-only antibody comprising an antigen-binding domain that has binding specificity for PSMA.

[0154] In some embodiments, a multi-specific antibody comprises a CD3-binding VH domain that is paired with a light chain variable domain. In certain embodiments, the light chain is a fixed light chain. In some embodiments, 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, in a human VH framework. In some embodiments, the fixed light chain 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, in a human VL framework. Together, the CD3-binding VH domain and the light chain variable domain have binding affinity for CD3. In some embodiments, a CD3-binding VH domain comprises a heavy chain variable region sequence of SEQ ID NO: 65. In some embodiments, a CD3-binding VH domain comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% percent identity to the heavy chain variable region sequence of SEQ ID NO: 65. In some embodiments, a fixed light chain comprises a light chain variable region sequence of SEQ ID NO: 66. In some embodiments, a fixed light chain comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% percent identity to the heavy chain variable region sequence of SEQ ID NO: 66.

[0155] Multi-specific antibodies comprising the above-described CD3-binding VH domain and light chain variable domain have advantageous properties, for example, as described in published PCT application publication number WO2018/052503, the disclosure of which is incorporated by reference herein in its entirety. Any of the multi-specific antibodies and antigen-binding domains described herein, having binding affinity to PSMA, can be combined with the CD3-binding domains and fixed light chain domains described herein (see, e.g., Table 4 and Table 5), as well as additional sequences, such as those provided in Table 6 and Table 7, to generate multi-specific antibodies having binding affinity to one or more PSMA epitopes, as well as CD3.

Table 4. Anti-CD3 Heavy and Light Chain CDR1, CDR2, CDR3 amino acid sequences.

	SEQ_aa_CDR1	SEQ_aa_CDR2	SEQ_aa_CDR3
Heavy Chain	GFTFDDYA (SEQ ID NO: 59)	ISWNSGSI (SEQ ID NO: 60)	AKDSRGYGDYRLGGAY (SEQ ID NO: 61)
Light Chain	QSVSSN (SEQ ID NO: 62)	GAS (SEQ ID NO: 63)	QQYNNWPWT (SEQ ID NO: 64)

Table 5. Anti-CD3 heavy and light chain variable region amino acid sequences.

VH	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEW VSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYC AKDSRGYGDYRLGGAYWGQGTLLTVSS (SEQ ID NO: 65)
VL	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYG ASTRATGIPARFSGSGSGTEFTLTISLQSEDAVYYCQQYNNWPWTFGQ GTKVEIK (SEQ ID NO: 66)

Table 6: Human IgG1 and IgG4 Fc region sequences.

Human IgG1 (UniProt No. P01857)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSGGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK (SEQ ID NO: 75)
Human IgG4 (UniProt No. P01861)	ASTKGPSVFP LAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKT YTCNVNHNKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVD GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKS LSLSLGK (SEQ ID NO: 76)

Human IgG1 with silencing mutations (Fc region)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 77)
Human IgG4 with silencing mutations (Fc region)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNDHKPSNTKVDK RVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVD VSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLY SRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 78)

Table 7: additional sequences.

Anti-CD3 light chain constant region sequence (kappa light chain)	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC (SEQ ID NO: 79)
Anti-CD3 heavy chain sequence (VH + wt IgG1 Fc)	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLE WVSGISWNSGSGIGYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAL YYCAKDSRGYGDYRLGGAYWGQGLTVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 80)
Anti-CD3 heavy chain sequence (with silenced IgG1 Fc)	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLE WVSGISWNSGSGIGYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAL YYCAKDSRGYGDYRLGGAYWGQGLTVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 81)

Anti-CD3 heavy chain constant region sequence (with wt IgG4 Fc)	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLE WVSGISWNSGSIGYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAL YYCAKDSRGYGDYRLGGAYWGQGLTVTVSSASTKGPSVFPLAPCSRS TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVTVTPSSSLGTTKTYTCNVDPHKPSNTKVDKRVESKYGPPCPSCPAPE FLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLK (SEQ ID NO: 82)
Anti-CD3 heavy chain constant region sequence (with silenced IgG4 Fc)	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLE WVSGISWNSGSIGYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAL YYCAKDSRGYGDYRLGGAYWGQGLTVTVSSASTKGPSVFPLAPCSRS TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVTVTPSSSLGTTKTYTCNVDPHKPSNTKVDKRVESKYGPPCPSCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVD GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLK (SEQ ID NO: 83)
Silenced IgG4 (hinge – CH2 – CH3; hole (S228P, F234A, L235A; T366S, L368A, Y407V))	ESKYGPPCP <u>P</u> CPAPE <u>AA</u> GGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL <u>S</u> <u>A</u> VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLK (SEQ ID NO: 84)
Silenced IgG4 (hinge – CH2 – CH3; knob (S228P, F234A, L235A; T366W))	ESKYGPPCP <u>P</u> CPAPE <u>AA</u> GGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL <u>W</u> CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLK (SEQ ID NO: 85)
Anti-CD3 full length light chain (VL + kappa CL)	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNNWPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 86)

Anti-CD3 full length heavy chain (VH + silenced IgG4 Fc + knob (S228P, F234A, L235A; T366W))	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLE WVSGISWNSGSIGYADSVKGRFTISRDNKNSLYLQMNSLRAEDTAL YYCAKDSRGYGDYRLGGAYWGQGLTVTVSSASTKGPSVFPLAPCSRS TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCP <u>P</u> CPAPE <u>AA</u> GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV DGEVHNNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL <u>W</u> CLVKGF YPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQE GNVFSCSVMHLEAHNHYTQKSLSLSLGK (SEQ ID NO: 87)
PSMA monovalent heavy chain (clone ID 346181) + silenced IgG4 Fc, hole (S228P, F234A, L235A, T366S, L368A, Y407V	QLQLQESGPGLVKPSETLSLTCTVSGGSISSSNYFWGWIRQSPGKGLE WIGSIDYSGYTYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYN CARHKAATADFDYRGQGLTVTVSSESKYGPPCP <u>P</u> CPAPE <u>AA</u> GGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK TISKAKGQPREPQVYTLPPSQEEMTKNQVSL <u>SCA</u> VKGFYPSDIAVEW ESNGQPENNYKTPPVLDSDGSFFL <u>V</u> SRLTVDKSRWQEGNVFSCSVM HEALHNHYTQKSLSLSLGK (SEQ ID NO: 88)
PSMA bivalent heavy chain (clone ID 346181) + silenced IgG4 Fc, hole (S228P, F234A, L235A, T366S, L368A, Y407V	QLQLQESGPGLVKPSETLSLTCTVSGGSISSSNYFWGWIRQSPGKGLE WIGSIDYSGYTYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYN CARHKAATADFDYRGQGLTVTVSSESKYGPPCP <u>P</u> CPAPE <u>AA</u> GGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK TISKAKGQPREP QVYTLPPSQEEMTKNQVSL <u>SCA</u> VKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFL <u>V</u> SRLTVDKSRWQEGNVFSCSVMHLEAHNHYTQ KSLSLSLGK (SEQ ID NO: 89)
PSMA monovalent heavy chain (clone ID 345497) + silenced IgG4 Fc, hole (S228P, F234A, L235A, T366S, L368A, Y407V	QVQLVESGGGVVQPGRSLRLSCAASGFSFSRYGMHWVRQAPGKGLE GVAVIWYDGSNRYADSVKGRFTISRDNKNTLYLQMNSLRAEDTA VYYCAREPRIGYYYESSGYISLDYRGQGLTVTVSSESKYGPPCP <u>P</u> CP APE <u>AA</u> GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL <u>SCA</u> VK GFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL <u>V</u> SRLTVDKSRW QEGNVFSCSVMHLEAHNHYTQKSLSLSLGK (SEQ ID NO: 90)

PSMA bivalent heavy chain (clone ID 345497) + silenced IgG4 Fc, hole (S228P, F234A, L235A, T366S, L368A, Y407V)	QVQLVESGGGVVQPGRSLRLSCAASGFSFSRYGMHWVRQAPGKGLE GVAVIWYDGSNRYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTA VYYCAREPRIGYYYESSGYSLDYRGQGT LVTVSSGGGSGGGGSQV QLVESGGGVVQPGRSLRLSCAASGFSFSRYGMHWVRQAPGKLEGV AVIWYDGSNRYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCAREPRIGYYYESSGYSLDYRGQGT LVTVSSSESKYGPPCP <u>P</u> CPAPE <u>AA</u> GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL <u>SCA</u> VKGFY PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL <u>V</u> SRLTVDKSRWQEG NVFSCSVMH EALHNHYTQKSLSLGLGK (SEQ ID NO: 91)
PSMA bivalent heavy chain (clone ID 346181 x clone ID 345497) + silenced IgG4 Fc, hole (S228P, F234A, L235A, T366S, L368A, Y407V)	QLQLQESGPGLVKPSSETLSLTCTVSGGSISSSNYFWGWIRQSPGKGLE WIGSIDYSGYTYNPSLKSRTVISVDT SKNQFSLKLSSVTAADTAVYN CARHKAATADFDYRGQGT LVTVSSGGGSGGGGSQVQLVESGGGVV QPGRSLRLSCAASGFSFSRYGMHWVRQAPGKLEGVAVIWYDGSNR YYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREPRIGYY YESSGYSLDYRGQGT LVTVSSSESKYGPPCP <u>P</u> CPAPE <u>AA</u> GGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS KAKGQPREPQVYTLPPSQEEMTKNQVSL <u>SCA</u> VKGFYPSDIAVEWES NGQPENNYKTTTPVLDSDGSFFL <u>V</u> SRLTVDKSRWQEGNVFSCSVMH EALHNHYTQKSLSLGLGK (SEQ ID NO: 92)
PSMA bivalent heavy chain (clone ID 345497 x clone ID 346181) + silenced IgG4 Fc, hole (S228P, F234A, L235A, T366S, L368A, Y407V)	QVQLVESGGGVVQPGRSLRLSCAASGFSFSRYGMHWVRQAPGKGLE GVAVIWYDGSNRYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTA VYYCAREPRIGYYYESSGYSLDYRGQGT LVTVSSGGGSGGGGSQV QLQESGPGLVKPSSETLSLTCTVSGGSISSSNYFWGWIRQSPGKGLEWIG SIDYSGYTYNPSLKSRTVISVDT SKNQFSLKLSSVTAADTAVYN CAR HKAATADFDYRGQGT LVTVSSSESKYGPPCP <u>P</u> CPAPE <u>AA</u> GGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS KAKGQPREPQVYTLPPSQEEMTKNQVSL <u>SCA</u> VKGFYPSDIAVEWES NGQPENNYKTTTPVLDSDGSFFL <u>V</u> SRLTVDKSRWQEGNVFSCSVMH EALHNHYTQKSLSLGLGK (SEQ ID NO: 93)

[0156] In some embodiments, bispecific or multi-specific antibodies are provided, which may have any of the configurations discussed herein, including, without limitation, a bispecific three-chain antibody like molecule (TCA). In some embodiments, a bispecific antibody can comprise at least one heavy chain variable region having binding specificity for PSMA, and at least one heavy chain variable region having binding specificity for a protein other than PSMA. In some embodiments, a bispecific antibody can comprise a heavy chain/light chain pair that has binding specificity for a first antigen, and a heavy chain from 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 an antigen binding domain that binds an epitope of a second antigen or a different epitope of the first antigen. In one particular embodiment, a bispecific antibody comprises a heavy chain/light chain pair that has binding specificity for an antigen on an effector cell (e.g., a CD3 protein on a T-cell), and a heavy chain from a heavy chain-only antibody comprising an antigen-binding domain that has binding specificity for PSMA.

[0157] In some embodiments, where an antibody of the invention is a bispecific antibody, one arm of the antibody (one binding moiety, or one binding unit) is specific for human PSMA, while the other arm may be specific for target cells, tumor-associated antigens, targeting antigens, e.g., integrins, etc., pathogen antigens, checkpoint proteins, and the like. Target cells specifically include cancer cells, including, without limitation, cells from solid tumors, e.g., prostate tumors, as discussed below. In some embodiments, one arm of the antibody (one binding moiety, or one binding unit) is specific for human PSMA, while the other arm is specific for CD3.

[0158] In some embodiments, an antibody comprises an anti-CD3 light chain polypeptide comprising the sequence of SEQ ID NO: 66 linked to the sequence of SEQ ID NO: 79, an anti-CD3 heavy chain polypeptide comprising the sequence of any one of SEQ ID NOs: 80, 81, 82, 83, 84 or 85, and an anti-PSMA heavy chain polypeptide comprising the sequence of any one of SEQ ID NOs: 24-58, in a monovalent or bivalent configuration, linked to the sequence of any one of SEQ ID NOs: 75, 76, 77, 78, 84 or 85. These sequences can be combined in various ways to produce a bispecific antibody of a desired IgG subclass, e.g., IgG1, IgG4, silenced IgG1, silenced IgG4. In one preferred embodiment, an antibody is a TCA 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. In one preferred embodiment, an antibody is a TCA consisting of 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, 89, 90, 91, 92 or 93.

[0159] Various formats of multi-specific antibodies are within the ambit of the invention, including, without limitation, single chain polypeptides, two chain polypeptides, three chain polypeptides, four chain polypeptides, and multiples thereof. The multi-specific antibodies herein specifically include T-cell multi-specific (e.g., bispecific) antibodies binding to PSMA and CD3 (anti-PSMA x anti-CD3 antibodies). Such antibodies induce potent T-cell mediated killing of cells expressing PSMA.

Preparation of anti-PSMA antibodies

[0160] The antibodies of the present invention can be prepared by methods known in the art. In a preferred embodiment, the antibodies herein are produced by transgenic animals, including transgenic mice and rats, preferably rats, in which the endogenous immunoglobulin genes are knocked out or

disabled. In a preferred embodiment, the heavy chain antibodies herein are produced in UniRat™. UniRat™ have their endogenous immunoglobulin genes silenced and use a human immunoglobulin heavy-chain translocus to express a diverse, naturally optimized repertoire of fully human HCAs. While endogenous immunoglobulin loci in rats can be knocked out or silenced using a variety of technologies, in UniRat™ the zinc-finger (endo)nuclease (ZNF) technology was used to inactivate the endogenous rat heavy chain J-locus, light chain C κ locus and light chain C λ locus. ZNF constructs for microinjection into oocytes can produce IgH and IgL knock out (KO) lines. For details see, e.g., Geurts et al., 2009, Science 325:433. Characterization of Ig heavy chain knockout rats has been reported by Menoret et al., 2010, Eur. J. Immunol. 40:2932-2941. Advantages of the ZNF technology are that non-homologous end joining to silence a gene or locus via deletions up to several kb can also provide a target site for homologous integration (Cui et al., 2011, Nat Biotechnol 29:64-67). Human heavy chain antibodies produced in UniRat™ are called UniAbs™ and can bind epitopes that cannot be attacked with conventional antibodies. Their high specificity, affinity, and small size make them ideal for mono- and poly-specific applications.

[0161] In addition to UniAbs™, specifically included herein are heavy chain-only antibodies lacking the camelid VHH framework and mutations, and their functional VH regions. Such heavy chain-only antibodies can, for example, be produced in transgenic rats or mice which comprise fully human heavy chain-only gene loci as described, e.g., in WO2006/008548, but other transgenic mammals, such as rabbit, guinea pig, rat can also be used, rats and mice being preferred. Heavy chain-only antibodies, including their VHH or VH functional fragments, can also be produced by recombinant DNA technology, by expression of the encoding nucleic acid in a suitable eukaryotic or prokaryotic host, including, for example, mammalian cells (e.g., CHO cells), E. coli or yeast.

[0162] Domains of heavy chain-only antibodies combine advantages of antibodies and small molecule drugs: can be mono- or multi-valent; have low toxicity; and are cost-effective to manufacture. Due to their small size, these domains are easy to administer, including oral or topical administration, are characterized by high stability, including gastrointestinal stability; and their half-life can be tailored to the desired use or indication. In addition, VH and VHH domains of HCAs can be manufactured in a cost-effective manner.

[0163] In a particular embodiment, the heavy chain antibodies of the present invention, including UniAbs™, have the native amino acid residue at the first position of the FR4 region (amino acid position 101 according to the Kabat numbering system), substituted by another amino acid residue, which is capable of disrupting a surface-exposed hydrophobic patch comprising or associated with the native amino acid residue at that position. Such hydrophobic patches are normally buried in the interface with the antibody light chain constant region but become surface exposed in HCAs and are, at least partially, for the unwanted aggregation and light chain association of HCAs. The substituted

amino acid residue preferably is charged, and more preferably is positively charged, such as lysine (Lys, K), arginine (Arg, R) or histidine (His, H), preferably arginine (R). In a preferred embodiment the heavy chain-only antibodies derived from the transgenic animals contain a Trp to Arg mutation at position 101. The resultant HCAs preferably have high antigen-binding affinity and solubility under physiological conditions in the absence of aggregation.

[0164] As part of the present invention, human IgG anti-PSMA heavy chain antibodies with unique sequences from UniRatTM animals (UniAbTM) were identified that bind to human PSMA in ELISA protein and cell-binding assays. The identified heavy chain variable region (VH) sequences are positive for human PSMA protein binding and/or for binding to PSMA+ cells, and are all negative for binding to cells that do not express PSMA. See, e.g., Table 8.

[0165] Heavy chain antibodies binding to non-overlapping epitopes on a PSMA protein, e.g., UniAbsTM can be identified by competition binding assays, such as enzyme-linked immunoassays (ELISA assays) or flow cytometric competitive binding assays. For example, one can use competition between known antibodies binding to the target antigen and the antibody of interest. By using this approach, one can divide a set of antibodies into those that compete with the reference antibody and those that do not. The non-competing antibodies are identified as binding to a distinct epitope that does not overlap with the epitope bound by the reference antibody. Often, one antibody is immobilized, the antigen is bound, and a second, labeled (e.g., biotinylated) antibody is tested in an ELISA assay for ability to bind the captured antigen. This can be performed also by using surface plasmon resonance (SPR) platforms, including ProteOn XPR36 (BioRad, Inc), Biacore 2000 and Biacore T200 (GE Healthcare Life Sciences), and MX96 SPR imager (Ibis technologies B.V.), as well as on biolayer interferometry platforms, such as Octet Red384 and Octet HTX (ForteBio, Pall Inc). For further details see the examples herein.

[0166] Typically, an antibody “competes” with a reference antibody if it causes about 15-100% reduction in the binding of the reference antibody to the target antigen, as determined by standard techniques, such as by the competition binding assays described above. In various embodiments, the relative inhibition is at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50% at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95% or higher.

Pharmaceutical Compositions, Uses and Methods of Treatment

[0167] It is another aspect of the present invention to provide pharmaceutical compositions comprising one or more antibodies of the present invention in admixture with a suitable pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers as used herein are

exemplified, but not limited to, adjuvants, solid carriers, water, buffers, or other carriers used in the art to hold therapeutic components, or combinations thereof.

[0168] In one embodiment, a pharmaceutical composition comprises a heavy chain antibody (e.g., UniAb™) that binds to PSMA. In another embodiment, a pharmaceutical composition comprises a multi-specific (including bispecific) heavy chain antibody (e.g., UniAb™) with binding specificity for two or more non-overlapping epitopes on a PSMA protein. In a preferred embodiment, a pharmaceutical composition comprises a multi-specific (including bispecific and TCA) heavy chain antibody (e.g., UniAb™) with binding specificity to PSMA and with binding specificity to a binding target on an effector cell (e.g., a binding target on a T-cell, such as, e.g., a CD3 protein on a T-cell).

[0169] Pharmaceutical compositions of the antibodies used in accordance with the present invention are prepared for storage by mixing proteins having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (see, e.g. Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), such as in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[0170] Pharmaceutical compositions for parenteral administration are preferably sterile and substantially isotonic and manufactured under Good Manufacturing Practice (GMP) conditions. Pharmaceutical compositions can be provided in unit dosage form (i.e., the dosage for a single administration). The formulation depends on the route of administration chosen. The antibodies herein can be administered by intravenous injection or infusion or subcutaneously. For injection administration, the antibodies herein can be formulated in aqueous solutions, preferably in physiologically-compatible buffers to reduce discomfort at the site of injection. The solution can contain carriers, excipients, or stabilizers as discussed above. Alternatively, antibodies can be in lyophilized form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0171] Antibody formulations are disclosed, for example, in U.S. Patent No. 9,034,324. Similar formulations can be used for the heavy chain antibodies, including UniAbs™, of the present invention. Subcutaneous antibody formulations are described, for example, in US20160355591 and US20160166689.

Methods of Use

[0172] The anti-PSMA antibodies and pharmaceutical compositions described herein can be used for the treatment of diseases and conditions characterized by the expression of PSMA, including, without limitation, the conditions and diseases described further herein.

[0173] PSMA is a type II transmembrane protein that 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).

[0174] In one aspect, the anti-PSMA antibodies (e.g., UniAbs™) and pharmaceutical compositions herein can be used to treat disorders characterized by the expression of PSMA, including, without limitation, prostate cancer and solid tumors.

[0175] Effective doses of the compositions of the present invention for the treatment of disease vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human, but nonhuman mammals may also be treated, e.g., companion animals such as dogs, cats, horses, etc., laboratory mammals such as rabbits, mice, rats, etc., and the like. Treatment dosages can be titrated to optimize safety and efficacy.

[0176] Dosage levels can be readily determined by the ordinarily skilled clinician, and can be modified as required, e.g., as required to modify a subject's response to therapy. The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the host treated and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

[0177] In some embodiments, the therapeutic dosage the agent may range from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example, dosages can be 1 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment regime entails administration once every two weeks or once a month or once every 3 to 6 months. Therapeutic entities of the present invention are usually administered on multiple occasions. Intervals between single dosages can be weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of the therapeutic entity in the patient. Alternatively, therapeutic entities of the present invention can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the polypeptide in the patient.

[0178] Typically, compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The pharmaceutical compositions herein are suitable for intravenous or subcutaneous administration, directly or after reconstitution of solid (e.g., lyophilized) compositions. The preparation also can be emulsified or encapsulated in liposomes or micro particles such as polylactide, polyglycolide, or copolymer for enhanced adjuvant effect, as discussed above. Langer, Science 249: 1527, 1990 and Hanes, Advanced Drug Delivery Reviews 28: 97-119, 1997. The agents of this invention can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained or pulsatile release of the active ingredient. The pharmaceutical compositions are generally formulated as sterile, substantially isotonic and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

[0179] Toxicity of the antibodies and antibody structures described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the LD50 (the dose lethal to 50% of the population) or the LD100 (the dose lethal to 100% of the population). The dose ratio between toxic and therapeutic effect is the therapeutic index. The data obtained from these cell culture assays and animal studies can be used in formulating a dosage range that is not toxic for use in humans. The dosage of the antibodies described herein lies preferably within a range of circulating concentrations that include the effective dose with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition.

[0180] The compositions for administration will commonly comprise an antibody or other ablative agent dissolved in a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., buffered saline and the like. These solutions are sterile and

generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, e.g., sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of active agent in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs (e.g., Remington's Pharmaceutical Science (15th ed., 1980) and Goodman & Gillman, The Pharmacological Basis of Therapeutics (Hardman et al., eds., 1996)).

[0181] Also within the scope of the invention are kits comprising the active agents and formulations thereof, of the invention and instructions for use. The kit can further contain a least one additional reagent, e.g. a chemotherapeutic drug, etc. Kits typically include a label indicating the intended use of the contents of the kit. The term "label" as used herein includes any writing, or recorded material supplied on or with a kit, or which otherwise accompanies a kit.

[0182] The invention now being fully described, it will be apparent to one of ordinary skill in the art that various changes and modifications can be made without departing from the spirit or scope of the invention.

EXAMPLES

Materials and Methods

Example 1: UniRat™ Immunization with Recombinant Human PSMA

[0183] Twelve UniRat™ animals were immunized with recombinant human PSMA protein fused to a his tag (R&D Systems Cat No.: 4234-ZN). The animals were immunized twice per week for eight weeks. After a 35-day immunization time course, serum was collected from the rats to determine serum titers.

Serum titer results

[0184] Serum titer summary information is shown in FIG 1, panels A-B. In the graphs depicted in FIG. 1, panels A-B, each line represents an individual animal. The legends of the graphs show the ID number of each individual animal. Binding activity for a 12-point dilution series of serum was tested by ELISA against a huPSMA+His tag protein and a His tag off-target protein. Among this group of animals, a range of serum reactivity levels to human PSMA protein was observed. No serum response to the His tag off-target protein was observed.

Example 2: Flow cytometry analysis of binding to PSMA positive and negative cells by anti-PSMA UniAbs™

[0185] Binding to PSMA-positive cells was assessed by flow cytometry (Guava easyCyte 8HT, EMD Millipore) using the LNCaP cell line (ATCC: CRL-1740), 22Rv1 cell line (ATCC CRL-2505), a PC3 cell line (ATCC CRL-1435) stably transfected to express human PSMA, or the DU-145 cell line (ATCC HTB-81). Briefly, 50,000 target cells were stained with a dilution series of purified UniAbs™ for 30 minutes at 4°C. Following incubation, the cells were washed twice with flow cytometry buffer (1X PBS, 1% BSA, 0.1% NaN₃) and stained with goat F(ab')₂ anti-human IgG conjugated to R-phycoerythrin (PE) (Southern Biotech, cat. #2042-09) to detect cell-bound antibodies. After a 20-minute incubation at 4°C, the cells were washed twice with flow cytometry buffer and the mean fluorescence intensity (MFI) was measured by flow cytometry. The MFI of cells stained with secondary antibody alone were used for determination of background signal and binding of each antibody was converted to fold over background. Binding to cynomolgus PSMA positive cells was determined using the same protocol with the following modifications: the target cells were from Freestyle 293-F cells (ThermoFisher R79007) transiently transfected to express the extracellular domain of cynomolgus PSMA. In some experiments EC50 values were calculated using GraphPad Prism 7.

[0186] Table 8 summarizes target binding activity of several of the anti-PSMA heavy-chain antibodies (HCAb) described herein. Column 1 indicates the clone ID of the HCAb. Column 2 indicates binding to LNCaP cells measured as fold over the background MFI signal.

Table 8: Binding to PSMA-expressing cell line

Column 1: CLONE ID	Column 2: LNCaP
325920	282
346181	264
346165	243
346172	216
326109	25
325867	210
325742	200
325748	193
325940	169
325836	163
326027	138
326087	129
326084	125
326028	117

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 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.

Example 4: Composition of biparatopic and bivalent anti-PSMA antibodies

[0189] As shown in Table 9, clone ID 350123 is composed of clone ID 346181 sequence linked to clone ID 345497 sequence with the bridging sequence GGGGSGGGGS (SEQ ID NO: 74). Clone ID 350122 is composed of two repeats of clone ID 346181 joined by the same linker sequence. Clone ID 350123 is biparatopic as it is composed of two anti-PSMA domains recognizing different epitopes on PSMA. Clone ID 350122 is bivalent but not biparatopic, as it is composed of the same anti-PSMA domain in tandem. Schematic illustrations of various anti-PSMA x anti-CD3 antibodies are depicted in FIG. 5, panels A-C.

Table 9: Description of amino acid sequence of biparatopic and bivalent anti-PSMA antibodies

Clone ID	Sequence 1	Linker sequence	Sequence 2
350123	346181	GGGGSGGGGS (SEQ ID NO: 74)	345497
350122	346181	GGGGSGGGGS (SEQ ID NO: 74)	346181

Example 5: Determination of affinity to cell surface expressed human PSMA

[0190] PSMA cell surface affinity was determined by Scatchard analysis using the human prostate carcinoma cell line 22Rv1. First, PSMAxCD3 multi-specific antibodies were labeled with Alexa Fluor 488 using the Alexa Fluor 488 5-SDP Ester kit (ThermoFisher A30052). Binding to 22Rv1s was then assessed by flow cytometry (Guava easyCyte 8HT, EMD Millipore). Briefly, 100,000 target cells were stained with a dilution series of Alexa Fluor 488 labeled multi-specific antibodies for 1 hour at 4°C. Following incubation, the cells were washed twice with flow cytometry buffer and the mean fluorescence intensity was measured by flow cytometry.

[0191] To establish a standard curve for the calculation of molecules of equivalent soluble fluorophores (MESF), Bangs Lab Quantum Alex Fluor 488 MESF bead populations 1 through 4 were combined into a single tube and run on the Guava easyCyte 8HT. Blank beads were analyzed in a separate tube. The MFI of each bead population was measured for the FITC-channel. A linear regression of Log10(MFI) against Log10(MESF) was plotted using GraphPad Prism 7.

[0192] The MFI of each experimental sample was interpolated on the calibration curve and MESF was determined for each sample. Subsequently, the average number of antibodies bound per cell (ABC) was calculated by dividing the average MESF by the degree of labeling (DOL) of the antibody. The number of ABC was multiplied by the cell concentration to determine total

concentration of bound antibody. The free antibody concentration was calculated by subtracting the bound antibody concentration from the staining concentration (starting dose). Free antibody concentration was plotted against bound antibody concentration in GraphPad Prism 7. The resulting plot was fitted to a non-linear regression, one-site specific binding function to determine affinity, as shown in FIG. 4, panels A and B.

Example 6: Multi-specific antibody mediated killing of PSMA positive prostate tumor cells through T-cell redirection

Assays using resting T-cells

[0193] Target cells were seeded at 15,000 cells per well in a 96-well plate and grown overnight at 37°C. Following incubation, increasing amounts of multi-specific antibody were added together with resting human T-cells at a 10:1 effector to target cell ratio and incubated for an additional 48 or 72 hours at 37°C (48 hours for assays with LNCaP, MDA-PCa-2b and PC3-PSMA cells and 72 hours for assays with 22Rv1 cells). Cell death was measured using either the cell proliferation reagent WST-1 (Sigma Cat No.: 11644807001) or flow cytometry. In some experiments, a small sample of each supernatant was collected after incubation but prior to analysis of target cell viability and saved for analysis of cytokine production. When cell viability was analyzed with WST-1 reagent, the reagent stock was added to each well at a 1:10 dilution and incubated for 90 minutes at 37°C. The absorbance was then measured at 450 nm (reference 690 nm), and the percent specific lysis was calculated.

[0194] If target cell viability was analyzed by flow cytometry, then the target cells were labeled before initiating the assay with the membrane dye DiR (ThermoFisher D12731). After incubation with T-cells and antibody, the supernatants were either saved for cytokine analysis or disposed of. Wells were then washed once to collect dead tumor cells and T-cells, which were transferred to a flow cytometry plate. The remaining attached tumor cells were trypsinized and then added to the corresponding wells in the flow cytometry plate. Annexin-V reagent was used to stain dead cells and flow cytometry was conducted (BD FACSCelesta) to quantitate the percent of dead tumor cells in each sample, gated by DiR staining. Wells containing untreated target cells were used to normalize for spontaneous cell death. In some experiments, a negative control antibody was used, consisting of the same CD3-targeting arm as in the PSMAxCD3 multi-specific molecules, but replacing the tumor-targeting arm with a VH specific to the HIV protein gp120.

[0195] FIG. 7 shows T-cell mediated lysis of PSMA positive cells using unstimulated T-cells. Unstimulated human T-cells were incubated with PSMA-expressing cells (LNCaP) and different concentrations of multi-specific antibodies. The biparatopic anti-PSMAxCD3 antibody (350123xCD3) outperformed the monoparatopic PSMAxCD3 antibody (346181xCD3).

Assays using pre-activated T-cells

- [0196] Human pan T-cells were pre-activated with plate-bound OKT3 and IL-2 for three days, followed by an additional day of incubation in fresh IL-2. Target cells were trypsinized, loaded with Calcein-AM (ThermoFisher C3100MP), mixed with activated T-cells to an E:T ratio of 20:1, and added to the wells of a 96-well plate. Dilution series of different multi-specific antibodies were added, followed by incubation for 4 hours at 37°C. Supernatants were then transferred to black 96-well plates and absorbance was measured at 480 nm/520 nm ex/em to quantify release of calcein. Target cells incubated without T-cells were used to normalize for spontaneous calcein release of intact tumor cells. Addition of 2% Triton-X to control wells containing target cells allowed for calculation of the calcein signal corresponding to maximum cell lysis. Using this value, each experimental well was reported as percent of maximum cell lysis. Data analysis was conducted using GraphPad prism 7.
- [0197] FIG. 6 shows T-cell mediated lysis of PSMA positive cells using pre-activated T-cells. Pre-activated human T-cells were incubated with human PSMA-expressing cells (LNCaP) and different concentrations of multi-specific antibodies. Tumor cell death was measured by calcein release and normalized to spontaneous release of tumor cells in the absence of T-cells. The biparatopic anti-PSMAxCD3 antibody (350123xCD3) outperformed both monoparatopic PSMAxCD3 antibodies.
- [0198] FIG. 8 shows that multi-specific antibodies do not lyse PSMA-negative cells. Pre-activated human T-cells were incubated with PSMA-negative prostate cancer cells (DU145) and different concentrations of multi-specific antibodies. No lysis of these cells occurred by any of the antibodies tested.
- [0199] FIG. 9 shows binding of PSMAxCD3 multi-specific antibodies to PSMA positive and negative cells. Multi-specific anti-PSMA x anti-CD3 antibodies show binding to PSMA positive prostate tumor cells (22Rv1), but no binding to PSMA negative prostate tumor cells (DU145). The biparatopic molecule (350123) showed the strongest on-target cell binding.
- [0200] FIG. 10 depicts T-cell mediated lysis of PSMA positive cells. The data in FIG. 10 demonstrates that binding to PSMA via two different epitopes results in increased cell killing as compared to a bivalent but monospecific version of the antibody.

Example 7: A monoparatopic PSMAxCD3 bispecific antibody induces less cytokine production than a biparatopic PSMAxCD3 multi-specific antibody

- [0201] Cytokine production was analyzed in tumor cytotoxicity assays with resting T-cells. The design of these assays is detailed elsewhere. Supernatants were collected upon completion of the assays (after 72 hours of incubation for assays using 22Rv1 cells, 48 hours for all other cell lines). ELISA kits were used for detection of IL-2 (Biolegend 431804) and IFN γ (Biolegend 430104)

according to the manufacturer's protocol. Experimental supernatants were diluted before analysis in the ELISAs such that the levels of cytokines would fall within the linear portion of the standard curve supplied with each kit. In some cases, no cytokines could be detected in the experiment wells, and values were reported as less than or equal to the lower limit of quantification for the assay.

[0202] FIG. 12, panels A-C, show T-cell mediated lysis of PSMA positive cells and comparison with cytokine production. Multispecific PSMAxCD3 antibodies induce T-cell mediated lysis of the PSMA positive prostate cancer cell line LNCaP. The biparatopic molecule (350123) stimulated more potent tumor cell killing as compared to the monoparatopic molecule (346181), but also caused production of higher levels of the cytokines interferon gamma (IFN γ) and interleukin 2 (IL-2), as exemplified by FIG. 12, panel B and panel C.

[0203] Table 10 shows T-cell mediated lysis and cytokine production against four PSMA positive prostate tumor cell lines. The PSMAxCD3 multi-specific antibodies were tested in *in vitro* tumor cell cytotoxicity assays using unstimulated T-cells and a dose series of antibody against a panel of four PSMA positive tumor cell lines. After 72 hours (22Rv1) or 48 hours (MDA-PCa-2b, LNCAP, PC3-PSMA) the percent of tumor cell death was calculated and reported by EC50 as well as the highest percent killing achieved. Supernatants from these experiment wells were collected and analyzed by ELISA for the cytokines interferon-gamma (IFN γ) or interleukin-2 (IL-2). The monoparatopic molecule (346181) induced approximately equivalent levels of tumor cytotoxicity against all four cell lines tested as compared to the biparatopic molecule, but had higher EC50s for cytokine production and in most cases stimulated lower levels of maximum cytokine production.

[0204] Table 10: T-cell mediated lysis and cytokine production against four PSMA positive prostate tumor cell lines.

Cell line	Antibody	Cell binding EC50 (nM)	Max Cytotoxicity (% lysis)	Kill EC50 (nM)	Max IFN γ (pg/mL)	IFN γ EC50 (nM)	Max IL-2 (pg/ml)	IL-2 EC50 (nM)
22Rv1	346181xCD3	58	45	52.8	21,150	173.8	<LLOQ	NA
	350123xCD3	3	53	0.42	73,031	380.2	<LLOQ	NA
MDA-PCa-2b	346181xCD3	28	26	23.6	14,309	116.5	524	38.5
	350123xCD3	2	29	0.41	12,026	0.90	1111	1.11
LNCAP	346181xCD3	17	79	14.6	32,237	63.0	183	575
	350123xCD3	2	75	0.84	60,397	3.29	1057	3.73
PC3-PSMA	346181xCD3	30	42	3.7	7,340	10.1	1569	4.1
	350123xCD3	6	51	0.40	10,136	1.01	3480	1.1

Example 8: PSMAxCD3 multi-specific antibodies induce T-cell proliferation

[0205] PSMA positive tumor cells were seeded at 25,000 cells per well in a 96-well plate and grown overnight at 37°C. Human pan T-cells isolated from resting PBMCs (Miltenyi 130-096-535) were labeled with the lineage tracing dye CFSE according to manufacturer's instructions (ThermoFisher C34554). 100,000 labeled pan T-cells were then added to the wells containing the tumor cells, followed by a dilution series of antibodies, and incubated at 37°C, 8% CO₂. After 5 days of incubation, the cells were mixed gently and transferred to a flow cytometry plate. The cells were pelleted, and the supernatant removed, followed by staining with anti-CD8 conjugated to APC (Biolegend 301049) and anti-CD4 conjugated to PE (Biolegend 317410) for 20 minutes on ice. The cells were then washed and resuspending in flow cytometry buffer for analysis (BD FACSCelesta). Cells were gated on forward and side scatter, and CD4 or CD8 expression. The percent of T-cells that had proliferated, as indicated by CD4 or CD8 positive staining and low or negative CFSE signal, was calculated for the entire T-cell population, as well as the CD4 and CD8 subsets. Flow cytometry data was analyzed using FlowJo and plotted in GraphPad Prism 7.

[0206] FIG. 11, panels A-D, show that PSMAxCD3 multi-specific antibodies stimulated T-cell proliferation in the presence of PSMA positive tumor cells, and that monoparatopic PSMA bispecific antibodies preferentially activate CD3 T-cells. Multi-specific antibodies were incubated together with PSMA expressing tumor cells and T-cells labeled with the lineage tracing dye CFSE. After 5 days of incubation, T-cell proliferation and the composition of proliferated T-cells (CD8+ versus CD4+) were analyzed by flow cytometry. Panels A and B show total T-cell proliferation, while panels C and D indicate the ratio of CD8+ to CD4+ T-cells in the proliferated wells. A dashed horizontal line indicates that CD8:CD4 ratio of the unstimulated T-cells and is approximately 1:2 (actual value = 0.64). The monoparatopic PSMAxCD3 bispecific antibody (346181) preferentially activates CD8 T-cells (CD8:CD4 ratio after expansion of approximately 2:1) whereas the biparatopic PSMAxCD3 multi-specific antibody (350123) less preferentially activates CD8+ T-cells (CD8:CD4 ratio of about 1:1).

Example 9: A multi-specific antibody causes suppression of prostate tumor growth in a xenograft model

[0207] 5-6 week old male immune-deficient CIEA-NOG mice (Taconic) were implanted with 10 million 22Rv1 cells subcutaneously into their lower right flanks, followed by addition of 10 million human PBMCs via tail vein injection one day following tumor implantation. The animals received treatment with 100 µg of multi-specific antibody or vehicle by tail vein injection starting one day after tumor implantation on days 1, 5, 9 and 13. Tumor volume was quantified using calipers and was recorded for 25 days.

[0208] FIG. 13 shows the results of the 22Rv1 tumor xenograft model. The biparatopic PSMAxCD3 molecule (350123) showed inhibition of 22Rv1 tumor growth in a tumor xenograft model. Three mice were tested for each treatment group, and the change in tumor volume for each animal was plotted in millimeters cubed. Animals received PBMCs on day 1 post tumor implantation and were treated with antibody on days 1, 5, 9, and 13. Two out of the three animals treated with multispecific antibody showed delay in tumor progression.

[0209] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

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.
2. The antibody of claim 1, 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.
3. The antibody of any one of claims 1 or 2, wherein said CDR1, CDR2, and CDR3 sequences are present in a human framework.
4. The antibody of any one of claims 1 to 3, further comprising a heavy chain constant region sequence in the absence of a CH1 sequence.
5. The antibody of any one of claims 1 to 4, 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.
6. The antibody of any one of claims 2 to 5, 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.
7. The antibody of any one of claims 5 to 6, comprising:
(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.
8. The antibody of any one of claims 5 to 7, 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
(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.
9. The antibody of any one of claims 1 to 8, comprising:
(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.
10. The antibody of any one of claims 1 to 9, 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.
11. The antibody of any one of claims 1 to 10, comprising a heavy chain variable region sequence selected from the group consisting of SEQ ID NOs: 24 to 58.
12. The antibody of claim 11, wherein the heavy chain variable region sequence is selected from the group consisting of: SEQ ID NO: 25 and SEQ ID NO: 38.
13. 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),

in a monovalent or bivalent format.

14. An antibody that binds to PSMA, comprising 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.

15. 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

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.

16. The antibody of claim 15, wherein the first heavy chain variable region is located nearer to the N-terminus relative to the second heavy chain variable region.
17. The antibody of claim 15, wherein the first heavy chain variable region is located nearer to the C-terminus relative to the second heavy chain variable region.
18. An antibody that binds to PSMA, comprising 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.
19. The antibody of claim 18, comprising 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.
20. 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.
21. 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.

22. An antibody that binds to PSMA, comprising:

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.

23. An antibody that binds to PSMA, comprising:

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 a monovalent or bivalent configuration.

24. An antibody that binds to PSMA, comprising:

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.

25. The antibody of claim 24, wherein the first heavy chain variable region is located nearer to the N-terminus relative to the second heavy chain variable region.

26. The antibody of claim 24, wherein the first heavy chain variable region is located nearer to the C-terminus relative to the second heavy chain variable region.

27. The antibody of any one of claims 1-14 and 18-23, which is monospecific.

28. The antibody of any one of claims 1 to 26, which is multi-specific.

29. The antibody of claim 28, which is bispecific.

30. The antibody of claim 28 or 29, which has binding affinity to a CD3 protein and a PSMA protein.
31. The antibody of claim 28 or 29, which has binding affinity to two different epitopes on the same PSMA protein.
32. The antibody of claim 28 or 29, having binding affinity to an effector cell.
33. The antibody of claim 32, having binding affinity to a T-cell antigen.
34. The antibody of claim 33, having binding affinity to CD3.
35. The antibody of any one of claims 1 to 34, which is in a CAR-T format.
36. 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.
37. 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.

38. 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.

39. 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.

40. 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.

41. The multi-specific antibody of claim 40, wherein the first antigen-binding region is located nearer to the N-terminus relative to the second antigen-binding region.
42. The multi-specific antibody of claim 40, wherein the first antigen-binding region is located nearer to the C-terminus relative to the second antigen-binding region.
43. The multi-specific or bispecific antibody of any one of claims 36 to 42, 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.
44. The multi-specific or bispecific antibody of claim 43, wherein the polypeptide linker is a GS linker.
45. The multi-specific or bispecific antibody of claim 44, wherein the GS linker consists of the sequence of SEQ ID NO: 73 or SEQ ID NO: 74.
46. The bispecific antibody of any one of claims 36-39, wherein 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.
47. The bispecific antibody of any one of claims 36-39, wherein 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.
48. The antibody of any one of claims 1 to 45, wherein the antibody is biparatopic and has increased affinity for PSMA as compared to a monoparatopic anti-PSMA antibody.
49. The antibody of any one of claims 1 to 45, wherein the antibody is biparatopic and has increased effector function as compared to a monoparatopic anti-PSMA antibody.
50. A pharmaceutical composition comprising the antibody of any one of claims 1 to 49.

51. A method for the treatment of a disorder characterized by expression of PSMA, comprising administering to a subject with said disorder an antibody of any one of claims 1 to 49, or the pharmaceutical composition of claim 50.
52. Use of an antibody of any one of claims 1 to 49, in the preparation of a medicament for the treatment of a disorder characterized by expression of PSMA.
53. An antibody of any one of claims 1 to 49, for use in the treatment of a disorder characterized by expression of PSMA.
54. The method, use, or antibody of any one of claims 51 to 53, wherein the disorder is prostate cancer.
55. A polynucleotide encoding an antibody of any one of claims 1 to 49.
56. A vector comprising the polynucleotide of claim 55.
57. A cell comprising the vector of claim 56.
58. A method of producing an antibody of any one of claims 1 to 49, comprising growing a cell according to claim 57 under conditions permissive for expression of the antibody, and isolating the antibody from the cell.
59. A method of making an antibody of any one of claims 1 to 49, comprising immunizing a UniRat animal with a PSMA protein and identifying PSMA-binding antibody sequences.
60. A method of treatment, comprising administering to an individual in need an effective dose of the antibody of any one of claims 1 to 49, or the pharmaceutical composition of claim 50.