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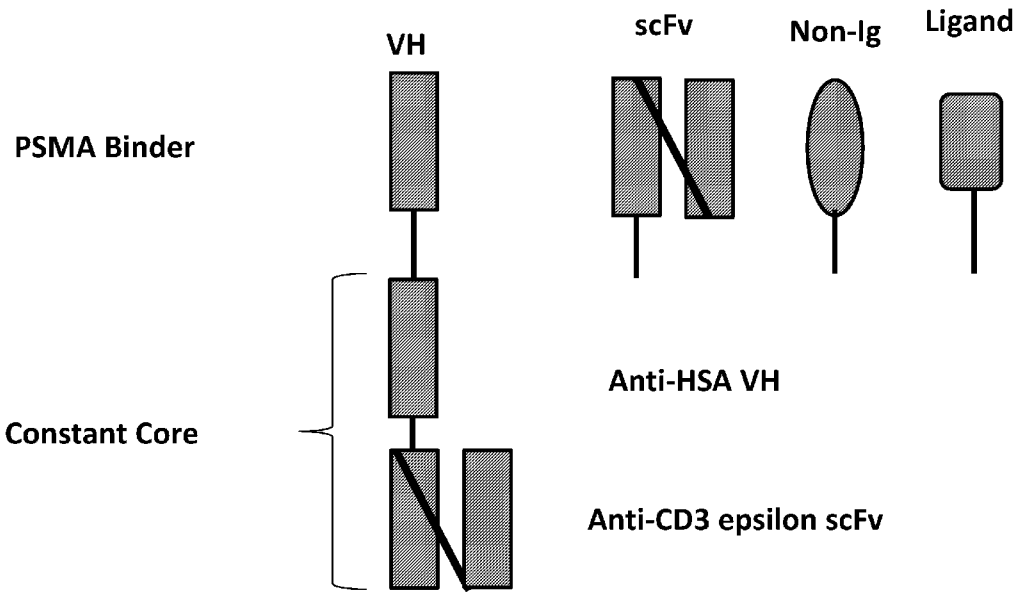
(54) Title: PROSTATE SPECIFIC MEMBRANE ANTIGEN BINDING PROTEIN

Figure 1




(57) Abstract: Disclosed herein are PSMA binding proteins with improved binding affinities, and robust aggregation profiles. Also described are multispecific binding proteins comprising a PSMA binding protein according to the instant disclosure. Pharmaceutical compositions comprising the binding proteins disclosed herein and methods of using such formulations are further provided.

[Continued on next page]

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# PROSTATE SPECIFIC MEMBRANE ANTIGEN BINDING PROTEIN

## CROSS-REFERENCE

**[0001]** This application claims the benefit of U.S. Provisional Application No. 62/426,086 filed November 23, 2016, which is incorporated by reference herein in its entirety.

## SEQUENCE LISTING

**[0002]** The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 22, 2017, is named 47517-707_601_SL.txt and is 148,650 bytes in size.

## INCORPORATION BY REFERENCE

**[0003]** All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference, and as if set forth in their entireties.

## BACKGROUND OF THE INVENTION

**[0004]** The present disclosure provides a prostate specific membrane antigen (PSMA) binding protein which can be used for diagnosing and treating prostate conditions and other indications correlated to expression of PSMA.

## SUMMARY OF THE INVENTION

**[0005]** Provided herein in one embodiment is a prostate specific membrane antigen (PSMA) binding protein, comprising complementarity determining regions CDR1, CDR2, and CDR3, wherein

(a) the amino acid sequence of CDR1 is as set forth in RFMISX1YX2MH (SEQ ID NO: 1);

(b) the amino acid sequence of CDR2 is as set forth in $X_3$INPA$X_4X_5$TDYAE$X_6$VKG (SEQ ID NO: 2); and (c) the amino acid sequence of CDR3 is as set forth in D$X_7$YGY (SEQ ID NO: 3). In some embodiments, the prostate specific membrane antigen binding protein comprises the following formula:f1-r1-f2-r2-f3-r3-f4, wherein, r1 is SEQ ID NO: 1; r2 is SEQ ID NO: 2; and r3 is SEQ ID NO: 3; and wherein $f_1$, $f_2$, $f_3$ and $f_4$ are framework residues selected so that said protein is at least eighty percent identical to the amino acid sequence set forth in SEQ ID NO: 4. In some embodiments, $X_1$ is proline. In some embodiments, $X_2$ is histidine. In some embodiments, $X_3$ is aspartic acid. In some embodiments, $X_4$ is lysine. In some embodiments, $X_5$ is glutamine. In some embodiments, $X_6$ is tyrosine. In some embodiments, $X_7$ is serine. In

some embodiments, the prostate specific membrane antigen binding protein has a higher affinity towards a human prostate specific membrane antigen than that of a binding protein which has the sequence set forth as SEQ ID NO: 4. In some embodiments, $X_1$ is proline. In some embodiments, $X_5$ is glutamine. In some embodiments, $X_6$ is tyrosine. In some embodiments, $X_4$ is lysine, and $X_7$ is serine. In some embodiments, $X_2$ is histidine, $X_3$ is aspartic acid, $X_4$ is lysine, and $X_7$ is serine. In some embodiments, $X_1$ is proline, $X_2$ is histidine, $X_3$ is aspartic acid, and $X_7$ is serine. In some embodiments, $X_2$ is histidine, $X_3$ is aspartic acid, $X_5$ is glutamine, and $X_7$ is serine. In some embodiments, $X_2$ is histidine, $X_3$ is aspartic acid, $X_6$ is tyrosine, and $X_7$ is serine. In some embodiments, $X_2$ is histidine, and $X_7$ is serine. In some embodiments, $X_2$ is histidine, $X_3$ is aspartic acid, and $X_7$ is serine. In some embodiments, the prostate specific membrane antigen binding protein has a higher affinity towards a human prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4. In some embodiments, the prostate specific membrane antigen binding protein further has a higher affinity towards a cynomolgus prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4. In some embodiments, r1 comprises SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7. In some embodiments, r2 comprises SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14. In some embodiments,
r3 comprises SEQ ID NO: 15.

**[0006]** Another embodiment of the invention provides a prostate specific membrane antigen binding protein comprising CDR1, CDR2, and CDR3, comprising the sequence set forth as SEQ ID NO: 4 wherein one or more amino acid residues selected from amino acid positions 31, 33, 50, 55, 56, 62, and 97 are substituted. In some embodiments, the binding protein comprises one or more additional substitutions at amino acid positions other than positions 31, 33, 50, 55, 56, 62, and 97. In some embodiments, the binding protein comprises substitution at position 31. In some embodiments, the binding protein comprises substitution at position 33. In some embodiments, the binding protein comprises substitution at position 50. In some embodiments, the binding protein comprises substitution at position 55. In some embodiments, the binding protein comprises substitution at position 56. In some embodiments, the binding protein comprises substitution at position 62. In some embodiments, the binding protein comprises substitution at position 97. In some embodiments, the binding protein comprises substitutions at amino acid positions 55 and 97. In some embodiments, the prostate specific membrane antigen binding protein has a higher affinity towards human prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4. In some embodiments, the binding protein comprises substitutions at amino acid positions 33 and 97. In

some embodiments, the binding protein comprises substitutions at amino acid positions 33, 50, and 97. In some embodiments, the prostate specific membrane antigen binding protein has a higher affinity towards human prostate specific membrane antigen than that of a binding protein which has the sequence set forth as SEQ ID NO: 4. In some embodiments, the prostate specific membrane antigen binding protein has a higher affinity towards cynomolgus prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4. In some embodiments, the binding protein comprises substitutions at amino acid positions 31, 33, 50, and 97. In some embodiments, the binding protein comprises substitutions at amino acid positions 33, 50, 55, and 97. In some embodiments, the binding protein comprises substitutions in amino acid positions 33, 50, 56, and 97. In some embodiments, comprises substitutions at amino acid positions 33, 50, 62, and 97.

**[0007]** A further embodiment provides a prostate specific membrane antigen binding protein comprising a CDR1, CDR2 and CDR3, wherein CDR1 comprises the sequence as set forth is SEQ ID NO: 16. One embodiment provides a prostate specific membrane antigen binding protein comprising a CDR1, CDR2 and CDR3, wherein CDR2 comprises the sequence as set forth in SEQ ID NO: 17. An additional embodiment provides a prostate specific membrane antigen binding protein comprising a CDR1, CDR2 and CDR3, wherein CDR3 comprises the sequence as set forth in SEQ ID NO: 18. In one embodiment is provided a prostate specific membrane antigen binding protein comprising a sequence that is at least 80% identical to the sequence set forth in SEQ ID NO: 4. In one embodiment is provided a prostate specific membrane antigen binding protein comprising a CDR1, CDR2 and CDR3, wherein CDR1 has at least 80% identity to SEQ ID NO: 16, CDR2 has at least 85% identity to SEQ ID NO: 17, and CDR3 has at least 80% identity to SEQ ID NO: 18.

**[0008]** Another embodiment provides a prostate specific membrane antigen binding protein comprising a CDR1, CDR2 and CDR3, wherein CDR1 comprises the sequence set forth in SEQ ID NO: 16, CDR2 comprises the sequence set forth in SEQ ID NO: 17, and CDR3 comprises the sequence set forth in SEQ ID NO: 18. In some embodiments, the prostate specific membrane antigen binding protein binds to one or both of human prostate specific membrane antigen and cynomolgus prostate specific membrane antigen. In some embodiments, the binding protein binds to human prostate specific membrane antigen and cynomolgus prostate specific membrane antigen with comparable binding affinities. In some embodiments, the binding protein binds to human prostate specific membrane antigen with a higher binding affinity than cynomolgus prostate specific membrane antigen.

**[0009]** Another embodiment provides a polynucleotide encoding a PSMA binding protein according to the present disclosure. A further embodiment provides a vector comprising the

polynucleotide encoding a PSMA binding protein according to the present disclosure. In another embodiment is provided a host cell is transformed with the vector. In another embodiment is provided a pharmaceutical composition comprising (i) a PSMA binding protein according to the present disclosure, the polynucleotide according to the present disclosure, the vector according to the present disclosure or the host cell according to the present disclosure, and (ii) a pharmaceutically acceptable carrier. Another embodiment provides a process for the production of a PSMA binding protein according to the present disclosure, said process comprising culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding a PSMA albumin binding protein according to the present disclosure under conditions allowing the expression of the PSMA binding protein and recovering and purifying the produced protein from the culture.

In one embodiment is provided a method for the treatment or amelioration of a proliferative disease, a tumorous disease, an inflammatory disease, an immunological disorder, an autoimmune disease, an infectious disease, a viral disease, an allergic reaction, a parasitic reaction, a graft-versus-host disease or a host-versus-graft disease comprising the administration of the PSMA binding protein according to the present disclosure, to a subject in need thereof. In some embodiments, the subject is human. In some embodiments, the method further comprises administration of an agent in combination with the PSMA binding protein according to the present disclosure.

**[0010]** One embodiment provides a multispecific binding protein comprising the PSMA binding protein according to the present disclosure. A further embodiment provides an antibody comprising the PSMA binding protein according to the present disclosure. In one embodiment is provided a multispecific antibody, a bispecific antibody, an sdAb, a variable heavy domain, a peptide, or a ligand, comprising the PSMA binding protein according to the present disclosure. In one embodiment is provided an antibody comprising the PSMA binding protein according to the present disclosure, wherein said antibody is a single domain antibody. In some embodiments, the single domain antibody is derived from a heavy chain variable region of IgG. A further embodiment provides a multispecific binding protein or antibody comprising the PSMA binding protein according to the present disclosure. In one embodiment is provided a method for the treatment or amelioration of a proliferative disease, a tumorous disease, an inflammatory disease, an immunological disorder, an autoimmune disease, an infectious disease, a viral disease, an allergic reaction, a parasitic reaction, a graft-versus-host disease or a host-versus-graft disease comprising administration of the multispecific antibody according to the present disclosure, to a subject in need thereof. In a further embodiment is provided a method for the treatment or amelioration of a prostate condition comprising administration of the

multispecific antibody according to the present disclosure, to a subject in need thereof. Another embodiment provides a method for the treatment or amelioration of a prostate condition comprising administration of the PSMA binding protein according to any of the above embodiments, to a subject in need thereof. A further embodiment provides a method for the treatment or amelioration of a prostate condition comprising administration of the PSMA binding protein according to the present disclosure, to a subject in need thereof.

**[0011]** In some embodiments, the prostate specific membrane antigen binding protein comprises any combination of the following: (i) wherein $X_1$ is proline; (ii) wherein X2 is histidine; (iii) wherein X3 is aspartic acid; (iv) whereinX4 is lysine; (v) wherein X5 is glutamine; (vi) wherein X6 is tyrosine; and (vii) wherein X7 is serine. In some embodiments, the prostate specific membrane antigen binding protein of the above embodiment has a higher affinity towards a human prostate specific membrane antigen than that of a binding protein which has the sequence set forth as SEQ ID NO: 4. In some embodiments, the prostate specific membrane antigen binding comprises any combination of the following: (i) wherein $X_1$ is proline; wherein $X_5$ is glutamine; (ii) wherein $X_6$ is tyrosine; wherein $X_4$ is lysine and $X_7$ is serine; (iii) wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_4$ is lysine, and $X_7$ is serine; (iv) wherein $X_1$ is proline, $X_2$ is histidine, $X_3$ is aspartic acid, and $X_7$ is serine; (v) wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_5$ is glutamine, and $X_7$ is serine; (vi) wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_4$ is lysine, and $X_7$ is serine; (vii) wherein $X_1$ is proline, $X_2$ is histidine, $X_3$ is aspartic acid, and $X_7$ is serine; (viii) wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_5$ is glutamine, and $X_7$ is serine; (ix) wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_6$ is tyrosine, and $X_7$ is serine; and (x) wherein $X_2$ is histidine, $X_3$ is aspartic acid, and $X_7$ is serine. In some cases, the prostate specific membrane antigen binding protein of the above embodiment has a higher affinity towards a human prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4. In some cases, the prostate specific membrane antigen binding protein of the above embodiment further has a higher affinity towards a cynomolgus prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4. In some embodiments, the prostate specific membrane antigen binding protein comprises any combination of the following: (i) substitution at position 31; (ii) substitution at position 50; (iii) substitution at position 55; substitution at position 56; (iv) substitution at position 62; (v) substitution at position 97; (vi) substitutions at positions 55 and 97; (vii) substitutions at positions 33 and 97; (viii) substitutions at 33, 50, and 97; (ix) substitutions at positions 31, 33, 50, and 97; (x) substitutions at positions 33, 50, 55, and 97; (xi) substitutions at positions 33, 50, 56, and 97; and (xiii) substitutions at positions 33, 50, 62, and 97. In some cases, the prostate specific membrane antigen binding protein of the above

embodiment has a higher affinity towards human prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4. In some cases, the prostate specific membrane antigen binding protein of the above embodiment further has a higher affinity towards cynomolgus prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4.

**[0012]** One embodiment provides a method for the treatment or amelioration of prostate cancer, the method comprising administration of the PSMA binding protein comprising complementarity determining regions CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in $RFMISX_1YX_2MH$ (SEQ ID NO: 1); (b) the amino acid sequence of CDR2 is as set forth in $X_3INPAX_4X_5TDYAEX_6VKG$ (SEQ ID NO: 2); and (c) the amino acid sequence of CDR3 is as set forth in $DX_7YGY$ (SEQ ID NO: 3), to a subject in need thereof.

**[0013]** In some embodiments the PSMA binding protein is a single domain antibody. In some embodiments, said single domain antibody is part of a trispecific antibody.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0014]** The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

**[0015]** **Figure 1** is schematic representation of an exemplary PMSA targeting trispecific antigen-binding protein where the protein has an constant core element comprising an anti-CD3ε single chain variable fragment (scFv) and an anti-HSA variable heavy chain region; and a PMSA binding domain that can be a VH, scFv, a non-Ig binder, or ligand.

**[0016]** **Figures 2A-B** compare the ability of exemplary PSMA targeting trispecific proteins (PSMA targeting TriTAC molecules) with different affinities for CD3 to induce T cells to kill human prostate cancer cells. **Figure 2A** shows killing by different PMSA targeting TriTAC molecules in prostate cancer model LNCaP. **Figure 2B** shows killing by different PMSA targeting TriTAC molecules in prostate cancer model 22Rv1. **Figure 2C** shows EC50 values for PMSA targeting TriTAC in LNCaP and 22Rv1 prostate cancer models.

**[0017]** **Figure 3** shows the serum concentration of PSMA targeting TriTAC C236 in Cynomolgus monkeys after i.v. administration (100 μg/kg) over three weeks.

**[0018]** **Figure 4** shows the serum concentration of PSMA targeting TriTAC molecules with different CD3 affinities in Cynomolgus monkeys after i.v. administration (100 μg/kg) over three weeks.

**[0019]** **Figures 5A-C** show the ability of PSMA targeting TriTAC molecules with different affinities for PSMA to induce T cells to kill the human prostate cancer cell line LNCaP. **Figure 5A** shows the experiment performed in the absence of human serum albumin with a PSMA targeting BiTE as positive control. **Figure 5B** shows the experiment performed in the presence of human serum albumin with a PSMA targeting BiTE as positive control. **Figure 5C** shows EC50 values for PMSA targeting TriTAC in the presence or absence of HSA with a PSMA targeting BiTE as a positive control in LNCaP prostate cancer models.

**[0020]** **Figure 6** demonstrates the ability of PSMA targeting TriTAC molecules to inhibit tumor growth of human prostate cancer cells in a mouse xenograft experiment.

**[0021]** **Figures 7A-D** illustrates the specificity of TriTAC molecules in cell killing assays with target cell lines that do or do not express the target protein. **Figure 7A** shows EGFR and PSMA expression in LNCaP, KMS12BM, and OVCAR8 cell lines. **Figure 7B** shows killing of LNCaP tumor cells by PSMA, EGFR, and negative control TriTACs. **Figure 7C** shows killing of KMS12BM tumor cells by PSMA, EGFR, and negative control TriTACs. **Figure 7D** shows killing of OVCAR8 cells by PSMA, EGFR, and negative control TriTACs.

**[0022]** **Figures 8A-D** depict the impact of pre-incubation at 37˚C and freeze/thaw cycles on TriTAC activity. **Figure 8A** shows PSMA TriTAC C235 activity after pre-incubation at 37˚C or freeze/thaw cycles. **Figure 8B** shows PSMA TriTAC C359 activity after pre-incubation at 37˚C or freeze/thaw cycles. **Figure 8C** shows PSMA TriTAC C360 activity after pre-incubation at 37˚C or freeze/thaw cycles. **Figure 8D** shows PSMA TriTAC C361 activity after pre-incubation at 37˚C or freeze/thaw cycles.

**[0023]** **Figures 9A-B** depict the activity of a PSMA targeting TriTAC molecule of this disclosure in redirected T cell killing in T cell dependent cellular cytotoxicity assays (TDCC). **Figure 9A** shows the impact of the PSMA targeting TriTAC molecule in redirecting cynomolgus peripheral blood mononuclear cells (PBMCs), from cynomolgus monkey donor G322, in killing LNCaP cells. **Figure 9B** shows the impact of the PSMA targeting TriTAC molecule in redirecting cynomolgus PBMCs, from cynomolgus monkey donor D173, to kill MDAPCa2b cells.

**[0024]** **Figure 10** depicts the impact of a PSMA targeting TriTAC molecule of this disclosure on expression of T cell activation markers CD25 and CD69.

**[0025]** **Figure 11** depicts the ability of a PSMA targeting TriTAC molecule of this disclosure to stimulate T cell proliferation in the presence of PSMA expressing target cells.

**[0026]** **Figure 12** depicts redirected T cell killing of LnCaP cells by PSMA targeting TriTAC molecule PSMA Z2 TriTAC (SEQ ID NO: 156).

## DETAILED DESCRIPTION OF THE INVENTION

**[0027]** 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

**Certain definitions**

**[0028]** The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising."

**[0029]** The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *e.g.*, the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the given value. Where particular values are described in the application and claims, unless otherwise stated the term "about" should be assumed to mean an acceptable error range for the particular value.

**[0030]** The terms "individual," "patient," or "subject" are used interchangeably. None of the terms require or are limited to situation characterized by the supervision (*e.g.* constant or intermittent) of a health care worker (*e.g.* a doctor, a registered nurse, a nurse practitioner, a physician's assistant, an orderly, or a hospice worker).

**[0031]** The term "Framework" or "FR" residues (or regions) refer to variable domain residues other than the CDR or hypervariable region residues as herein defined. A "human consensus framework" is a framework which represents the most commonly occurring amino acid residue in a selection of human immunoglobulin VL or VH framework sequences.

**[0032]** As used herein, "Variable region" or "variable domain" refers to the fact that certain portions of the 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 complementarity-determining regions (CDRs) or 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 (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the βsheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs 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, Fifth Edition, National Institute 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 toxicity. "Variable domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or CDR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.*, residues 82a, 82b, and 82c, etc according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence. It is not intended that CDRs of the present disclosure necessarily correspond to the Kabat numbering convention.

**[0033]** As used herein, the term "Percent (%) amino acid sequence identity" with respect to a sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific 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 softwares such as EMBOSS MATCHER, EMBOSS WATER, EMBOSS STRETCHER, EMBOSS NEEDLE, EMBOSS LALIGN, BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

**[0034]** As used herein, "elimination half-time" is used in its ordinary sense, as is described in *Goodman and Gillman's The Pharmaceutical Basis of Therapeutics* 21-25 (Alfred Goodman Gilman, Louis S. Goodman, and Alfred Gilman, eds., 6th ed. 1980). Briefly, the term is meant to encompass a quantitative measure of the time course of drug elimination. The elimination of most drugs is exponential (i.e., follows first-order kinetics), since drug concentrations usually do not approach those required for saturation of the elimination process. The rate of an exponential process may be expressed by its rate constant, k, which expresses the fractional change per unit of time, or by its half-time, $t_{1/2}$ the time required for 50% completion of the process. The units of these two constants are $time^{-1}$ and time, respectively. A first-order rate constant and the half-time of the reaction are simply related ($k \times t_{1/2}=0.693$) and may be interchanged accordingly. Since first-order elimination kinetics dictates that a constant fraction of drug is lost per unit time, a plot of the log of drug concentration versus time is linear at all times following the initial distribution phase (i.e. after drug absorption and distribution are complete). The half-time for drug elimination can be accurately determined from such a graph.

**[0035]** As used herein, the term "binding affinity" refers to the affinity of the proteins described in the disclosure to their binding targets, and is expressed numerically using "Kd" values. If two or more proteins are indicated to have comparable binding affinities towards their binding targets, then the Kd values for binding of the respective proteins towards their binding targets, are within ±2-fold of each other. If two or more proteins are indicated to have comparable binding affinities towards single binding target, then the Kd values for binding of the respective proteins towards said single binding target, are within ±2-fold of each other. If a protein is indicated to bind two or more targets with comparable binding affinities, then the Kd values for binding of said protein to the two or more targets are within ±2-fold of each other. In general, a higher Kd value corresponds to a weaker binding. In some embodiments, the "Kd" is measured by a radiolabeled antigen binding assay (RIA) or surface plasmon resonance assays using a BIAcore™-2000 or a BIAcore™-3000 (BIAcore, Inc., Piscataway, N.J.). In certain embodiments, an "on-rate" or "rate of association" or "association rate" or "kon" and an "off-rate" or "rate of dissociation" or "dissociation rate" or "koff" are also determined with the surface plasmon resonance technique using a BIAcore™-2000 or a BIAcore™-3000 (BIAcore, Inc., Piscataway, N.J.). In additional embodiments, the "Kd", "kon", and "koff" are measured using the OCTET® Systems (Pall Life Sciences). In an exemplary method for measuring binding affinity using the OCTET® Systems, the ligand, *e.g.,* biotinylated human or cynomolgus PSMA, is immobilized on the OCTET® streptavidin capillary sensor tip surface which streptavidin tips are then activated according to manufacturer's instructions using about 20-50 μg/ml human or cynomolgus PSMA protein. A solution of PBS/Casein is also introduced as a blocking agent. For association kinetic measurements, PSMA binding protein variants are introduced at a concentration ranging from about 10 μg/ml to about 1000 μg/ml. Complete dissociation is observed in case of the negative control, assay

buffer without the binding proteins. The kinetic parameters of the binding reactions are then determined using an appropriate tool, *e.g.,* ForteBio software.

**[0036]** Described herein are PSMA binding proteins, pharmaceutical compositions as well as nucleic acids, recombinant expression vectors, and host cells for making such PSMA binding proteins. Also provided are methods of using the disclosed PSMA binding proteins in the prevention, and/or treatment of diseases, conditions and disorders. The PSMA binding proteins are capable specifically binding to PSMA. In some embodiments, the PSMA binding proteins include additional domains, such as a CD3 binding domain.

**Prostate Specific Membrane Antigen (PSMA) and its role in prostate conditions**

**[0037]** Contemplated herein are prostate specific membrane antigen binding proteins. Prostate-specific membrane antigen (PSMA), also known as glutamate carboxypeptidase II, N-acetyl-α-linked acidic dipeptidase I [Naaladase (NLD) I], or folate hydrolase, is a 750-residue type II transmembrane glycoprotein that has been found to be highly expressed in prostate cancer cells and in nonprostatic solid tumor neovasculature and expressed at lower levels in other tissues including healthy prostate, kidney, liver, small intestine, small bowel, salivary gland, duodenal mucosa, proximal renal tubules, and brain. PSMA is a member of a superfamily of zinc-dependent exopeptidases, which include carboxypeptidases with a mononuclear zinc active site (*e.g.,* carboxypeptidase A) and carboxy- and aminopeptidases with a binuclear zinc active site [*e.g.,* carboxypeptidase G2 (CPG2), peptidases T and V (PepT and PepV), *Streptomyces griseus* aminopeptidase (Sgap), and *Aeromonas proteolytica* aminopeptidase (AAP)]. In addition to a limited region of homology with these soluble single-domain (*e.g.,* AAP), or double-domain (*e.g.,* CPG2) zinc-dependent exopeptidases, the entire sequence of PSMA is homologous to at least four other human proteins: NLDL (expressed in ileum; 35% identity), NLD2 (expressed in ovary, testis, and brain; 67% identity), transferrin receptor (TfR) 1 (TfR1; expressed in most cell types; 26% identity), and TfR2 (expressed predominantly in liver; 28% identity).

**[0038]** The crystal structure of PSMA has been shown to comprise a symmetric dimer with each polypeptide chain containing three domains analogous to the three TfR1 domains: a protease domain, an apical domain, and a helical domain. A large cavity (≈1,100 Å2) at the interface between the three domains includes a binuclear zinc site and predominantly polar residues (66% of 70 residues). The observation of two zinc ions and conservation of many of the cavity-forming residues among PSMA orthologs and homologs identify the cavity as the probable substrate-binding site.

**[0039]** Typically, PSMA expression is found to increase with prostate disease progression and metastasis. The expression of PSMA is increased in prostate cancer, especially in poorly differentiated, metastatic, and hormone refractory carcinomas. PSMA is also expressed in

endothelial cells of capillary vessels in peritumoral and endotumoral areas of certain malignancies, including renal cell carcinomas, and colon carcinomas, but not in blood vessels from normal tissues. In addition, PSMA is reported to be related to tumor angiogenesis. PSMA has been demonstrated to be expressed in endothelial cells of tumor-associated neovasculature in carcinomas of the colon, breast, bladder, pancreas, kidney, and melanoma.

**[0040]** In addition to its role as a tumor marker, PSMA contains a binuclear zinc site and is active as a glutamate carboxypeptidase, catalyzing the hydrolytic cleavage of α- or γ-linked glutamates from peptides or small molecules. Its substrates include poly-γ-glutamated folates, which are essential nutrients, and the poly-γ-glutamated form of the anticancer drug methotrexate, in which case cleavage renders it less efficacious. The enzymatic activity of PSMA can be exploited for the design of prodrugs, in which an inactive glutamated form of the drug is selectively cleaved and thereby activated only at cells that express PSMA. PSMA also cleaves and inactivates the abundant neuropeptide N-acetyl-l-aspartyl-l-glutamate (α-NAAG), which is an inhibitor of the NMDA ionotropic receptor and an agonist of the type II metabotropic glutamate receptor subtype 3. A breakdown of the regulation of glutamatergic neurotransmission by α-NAAG is implicated in schizophrenia, seizure disorders, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis. Thus, inhibition of PSMA potentially confers neuroprotection both by reducing glutamate and increasing α-NAAG. For example, the subnanomolar inhibitor 2-(phosphonomethyl) pentanedioc acid has been shown to provide neuroprotection in cell culture and/or animal models of ischemia, diabetic neuropathy, drug abuse, chronic pain, and amyotrophic lateral sclerosis.

**[0041]** Prostate cancer is the most prevalent type of cancer and one of the leading causes of death from cancer in American men. The number of men diagnosed with prostate cancer has steadily increasing as a result of the increasing population of older men as well as a greater awareness of the disease leading to its earlier diagnosis. The life time risk for men developing prostate cancer is about 1 in 5 for Caucasians, 1 in 6 for African Americans. High risk groups are represented by those with a positive family history of prostate cancer or African Americans. Over a lifetime, more than two-thirds of the men diagnosed with prostate cancer die of the disease. Moreover, many patients who do not succumb to prostate cancer require continuous treatment to ameliorate symptoms such as pain, bleeding and urinary obstruction. Thus, prostate cancer also represents a major cause of suffering and increased health care expenditures. Where prostate cancer is localized and the patient's life expectancy is 10 years or more, radical prostatectomy offers the best chance for eradication of the disease. Historically, the drawback of this procedure is that most cancers had spread beyond the bounds of the operation by the time they were detected. Patients with bulky, high-grade tumors are less likely to be successfully

treated by radical prostatectomy. Radiation therapy has also been widely used as an alternative to radical prostatectomy. Patients generally treated by radiation therapy are those who are older and less healthy and those with higher-grade, more clinically advanced tumors. Particularly preferred procedures are external-beam therapy which involves three dimensional, confocal radiation therapy where the field of radiation is designed to conform to the volume of tissue treated; interstitial-radiation therapy where seeds of radioactive compounds are implanted using ultrasound guidance; and a combination of external-beam therapy and interstitial-radiation therapy. For treatment of patients with locally advanced disease, hormonal therapy before or following radical prostatectomy or radiation therapy has been utilized. Hormonal therapy is the main form of treating men with disseminated prostate cancer. Orchiectomy reduces serum testosterone concentrations, while estrogen treatment is similarly beneficial. Diethylstilbestrol from estrogen is another useful hormonal therapy which has a disadvantage of causing cardiovascular toxicity. When gonadotropin-releasing hormone agonists are administered testosterone concentrations are ultimately reduced. Flutamide and other nonsteroidal, anti-androgen agents block binding of testosterone to its intracellular receptors. As a result, it blocks the effect of testosterone, increasing serum testosterone concentrations and allows patients to remain potent—a significant problem after radical prostatectomy and radiation treatments. Cytotoxic chemotherapy is largely ineffective in treating prostate cancer. Its toxicity makes such therapy unsuitable for elderly patients. In addition, prostate cancer is relatively resistant to cytotoxic agents. Relapsed or more advanced disease is also treated with anti-androgen therapy. Unfortunately, almost all tumors become hormone-resistant and progress rapidly in the absence of any effective therapy. Accordingly, there is a need for effective therapeutics for prostate cancer which are not overwhelmingly toxic to normal tissues of a patient, and which are effective in selectively eliminating prostate cancer cells. The present disclosure provides, in certain embodiments, PSMA binding proteins that are useful in treating prostate cancer. In additional embodiments, the disclosure provides a method of treating prostate cancer by immunotherapy using the PSMA binding proteins described herein.

**[0042]** Prostate cancer is also difficult to diagnose because the prostate specific membrane antigen screening method is associated with many false positives. Accordingly, in some embodiments, the present disclosure provides an improved method of detecting prostate cancer using the PSMA binding proteins described herein.

**PSMA Binding Proteins**

**[0043]** Provided herein in certain embodiments are binding proteins, such as anti-PSMA antibodies or antibody variants, which bind to a PSMA protein. The PSMA protein, in some embodiments, is a multimer. A PSMA protein multimer, as used herein, is a protein complex of

at least two PSMA proteins or fragments thereof. The PSMA protein multimers can be composed of various combinations of full-length PSMA proteins (*e.g.,* SEQ ID NO: 20), recombinant soluble PSMA (rsPSMA, *e.g.,* amino acids 44-750 of SEQ ID NO: 20) and fragments of the foregoing that form multimers (*i.e.,* that retain the protein domain required for forming dimers and/or higher order multimers of PSMA). In some embodiments, at least one of the PSMA proteins forming the multimer is a recombinant, soluble PSMA (rsPSMA) polypeptide. In some embodiments, PSMA protein multimers are dimers, such as those formed from recombinant soluble PSMA protein. In some embodiments, rsPSMA is a homodimer. While not being bound by any particular theory, the PSMA protein multimers referred to herein are believed to assume a native conformation and preferably have such a conformation. The PSMA proteins in certain embodiments are noncovalently bound together to form the PSMA protein multimer. For example, it has been discovered that PSMA protein noncovalently associates to form dimers under non-denaturing conditions. The PSMA protein multimers can, and preferably do, retain the activities of PSMA. The activity of a PSMA protein is, in certain embodiments, an enzymatic activity, such as folate hydrolase activity, NAALADase activity, dipeptidyl peptidase IV activity and γ-glutamyl hydrolase activity. Methods for testing the PSMA activity of multimers are known in the field (*e.g.,* reviewed by O'Keefe et al. in: Prostate Cancer: Biology, Genetics, and the New Therapeutics, L. W. K. Chung, W. B. Isaacs and J. W. Simons (eds.) Humana Press, Totowa, N.J., 2000, pp. 307-326).

**[0044]** In some embodiments, the binding proteins of the present disclosure that bind a PSMA protein or a PSMA protein multimer modulate enzymatic activity of the PSMA protein or the PSMA protein multimer. In some embodiments, the PSMA binding protein inhibits at least one enzymatic activity such as NAALADase activity, folate hydrolase activity, dipeptidyl dipeptidase IV activity, γ-glutamyl hydrolase activity, or combinations thereof. In other embodiments, the PSMA binding protein enhances at least one enzymatic activity such as NAALADase activity, folate hydrolase activity, dipeptidyl dipeptidase IV activity, γ-glutamyl hydrolase activity, or combinations thereof.

**[0045]** As used herein, the term "antibody variants" refers to variants and derivatives of an antibody described herein. In certain embodiments, amino acid sequence variants of the anti-PSMA antibodies described herein are contemplated. For example, in certain embodiments amino acid sequence variants of anti-PSMA antibodies described herein are contemplated to improve the binding affinity and/or other biological properties of the antibodies. Exemplary method for preparing amino acid variants include, but are not limited to, introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such

modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody.

**[0046]** Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.,* antigen-binding. In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitution mutagenesis include the CDRs and framework regions. Examples of such substitutions are described below. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.,* retained/improved antigen binding, decreased immunogenicity, or improved antibody-dependent cell mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC). Both conservative and non-conservative amino acid substitutions are contemplated for preparing the antibody variants.

**[0047]** In another example of a substitution to create a variant anti-PSMA antibody, one or more hypervariable region residues of a parent antibody are substituted. In general, variants are then selected based on improvements in desired properties compared to a parent antibody, for example, increased affinity, reduced affinity, reduced immunogenicity, increased pH dependence of binding. For example, an affinity matured variant antibody can be generated, *e.g.,* using phage display-based affinity maturation techniques such as those described herein and known in the field.

**[0048]** Substitutions can be made in hypervariable regions (HVR) of a parent anti-PSMA antibody to generate variants and variants are then selected based on binding affinity, i.e., by affinity maturation. In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR- directed approaches, in which several HVR residues (*e.g.,* 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. Substitutions can be in one, two, three, four, or more sites within a parent antibody sequence.

**[0049]** In some embodiments, the PSMA binding protein described herein is a single domain antibody such as a heavy chain variable domain (VH), a variable domain (VHH) of camelid derived sdAb, peptide, ligand or small molecule entity specific for PSMA. In some embodiments, the PSMA binding domain of the PSMA binding protein described herein is any domain that binds to PSMA including but not limited to domains from a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody. In

certain embodiments, the PSMA binding protein is a single-domain antibody. In other embodiments, the PSMA binding protein is a peptide. In further embodiments, the PSMA binding protein is a small molecule.

**[0050]** Generally, it should be noted that the term single domain antibody as used herein in its broadest sense is not limited to a specific biological source or to a specific method of preparation. For example, in some embodiments, the single domain antibodies of the disclosure are obtained: (1) by isolating the VHH domain of a naturally occurring heavy chain antibody; (2) by expression of a nucleotide sequence encoding a naturally occurring VHH domain; (3) by "humanization" of a naturally occurring VHH domain or by expression of a nucleic acid encoding a such humanized VHH domain; (4) by "camelization" of a naturally occurring VH domain from any animal species, and in particular from a species of mammal, such as from a human being, or by expression of a nucleic acid encoding such a camelized VH domain; (5) by "camelisation" of a "domain antibody" or "Dab", or by expression of a nucleic acid encoding such a camelized VH domain; (6) by using synthetic or semi-synthetic techniques for preparing proteins, polypeptides or other amino acid sequences; (7) by preparing a nucleic acid encoding a single domain antibody using techniques for nucleic acid synthesis known in the field, followed by expression of the nucleic acid thus obtained; and/or (8) by any combination of one or more of the foregoing.

**[0051]** In one embodiment, a single domain antibody corresponds to the VHH domains of naturally occurring heavy chain antibodies directed against PSMA. As further described herein, such VHH sequences can generally be generated or obtained by suitably immunizing a species of Camelid with PSMA, (i.e., so as to raise an immune response and/or heavy chain antibodies directed against PSMA), by obtaining a suitable biological sample from said Camelid (such as a blood sample, serum sample or sample of B-cells), and by generating VHH sequences directed against PSMA, starting from said sample, using any suitable technique known in the field.

**[0052]** In another embodiment, such naturally occurring VHH domains against PSMA, are obtained from naïve libraries of Camelid VHH sequences, for example by screening such a library using PSMA, or at least one part, fragment, antigenic determinant or epitope thereof using one or more screening techniques known in the field. Such libraries and techniques are for example described in WO 99/37681, WO 01/90190, WO 03/025020 and WO 03/035694. Alternatively, improved synthetic or semi-synthetic libraries derived from naïve VHH libraries are used, such as VHH libraries obtained from naïve VHH libraries by techniques such as random mutagenesis and/or CDR shuffling, as for example described in WO 00/43507.

**[0053]** In a further embodiment, yet another technique for obtaining VHH sequences directed against PSMA, involves suitably immunizing a transgenic mammal that is capable of expressing

heavy chain antibodies (*i.e.*, so as to raise an immune response and/or heavy chain antibodies directed against PSMA), obtaining a suitable biological sample from said transgenic mammal (such as a blood sample, serum sample or sample of B-cells), and then generating VHH sequences directed against PSMA, starting from said sample, using any suitable technique known in the field. For example, for this purpose, the heavy chain antibody-expressing rats or mice and the further methods and techniques described in WO 02/085945 and in WO 04/049794 can be used.

**[0054]** In some embodiments, a single domain PSMA antibody, as described herein comprises single domain antibody with an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring VHH domain, but that has been "humanized", *i.e.*, by replacing one or more amino acid residues in the amino acid sequence of said naturally occurring VHH sequence (and in particular in the framework sequences) by one or more of the amino acid residues that occur at the corresponding position(s) in a VH domain from a conventional 4-chain antibody from a human being (*e.g.*, as indicated above). This can be performed in a manner known in the field, which will be clear to the skilled person, for example on the basis of the further description herein. Again, it should be noted that such humanized anti-PSMA single domain antibodies of the disclosure are obtained in any suitable manner known per se (*i.e.*, as indicated under points (1)-(8) above) and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring VHH domain as a starting material. In some additional embodiments, a single domain PSMA antibody, as described herein, comprises a single domain antibody with an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring VH domain, but that has been "camelized", *i.e.*, by replacing one or more amino acid residues in the amino acid sequence of a naturally occurring VH domain from a conventional 4-chain antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a VHH domain of a heavy chain antibody. Such "camelizing" substitutions are preferably inserted at amino acid positions that form and/or are present at the VH-VL interface, and/or at the so-called Camelidae hallmark residues (see for example WO 94/04678 and Davies and Riechmann (1994 and 1996)). Preferably, the VH sequence that is used as a starting material or starting point for generating or designing the camelized single domain is preferably a VH sequence from a mammal, more preferably the VH sequence of a human being, such as a VH3 sequence. However, it should be noted that such camelized anti-PSMA single domain antibodies of the disclosure, in certain embodiments, is obtained in any suitable manner known in the field (*i.e.*, as indicated under points (1)-(8) above) and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring VH domain as a starting material. For

example, as further described herein, both "humanization" and "camelization" is performed by providing a nucleotide sequence that encodes a naturally occurring VHH domain or VH domain, respectively, and then changing, one or more codons in said nucleotide sequence in such a way that the new nucleotide sequence encodes a "humanized" or "camelized" single domain antibody, respectively. This nucleic acid can then be expressed, so as to provide the desired anti-PSMA single domain antibody of the disclosure. Alternatively, in other embodiments, based on the amino acid sequence of a naturally occurring VHH domain or VH domain, respectively, the amino acid sequence of the desired humanized or camelized anti-PSMA single domain antibody of the disclosure, respectively, are designed and then synthesized de novo using known techniques for peptide synthesis. In some embodiments, based on the amino acid sequence or nucleotide sequence of a naturally occurring VHH domain or VH domain, respectively, a nucleotide sequence encoding the desired humanized or camelized anti-PSMA single domain antibody of the disclosure, respectively, is designed and then synthesized de novo using known techniques for nucleic acid synthesis, after which the nucleic acid thus obtained is expressed in using known expression techniques, so as to provide the desired anti-PSMA single domain antibody of the disclosure.

**[0055]** Other suitable methods and techniques for obtaining the anti-PSMA single domain antibody of the disclosure and/or nucleic acids encoding the same, starting from naturally occurring VH sequences or VHH sequences for example comprises combining one or more parts of one or more naturally occurring VH sequences (such as one or more framework (FR) sequences and/or complementarity determining region (CDR) sequences), one or more parts of one or more naturally occurring VHH sequences (such as one or more FR sequences or CDR sequences), and/or one or more synthetic or semi-synthetic sequences, in a suitable manner, so as to provide an anti-PSMA single domain antibody of the disclosure or a nucleotide sequence or nucleic acid encoding the same.

**[0056]** It is contemplated that in some embodiments the PSMA binding protein is fairly small and no more than 25 kD, no more than 20 kD, no more than 15 kD, or no more than 10 kD in some embodiments. In certain instances, the PSMA binding protein is 5 kD or less if it is a peptide or small molecule entity.

**[0057]** In some embodiments, the PSMA binding protein is an anti-PSMA specific antibody comprising a heavy chain variable complementarity determining regions (CDR), CDR1, a heavy chain variable CDR2, a heavy chain variable CDR3, a light chain variable CDR1, a light chain variable CDR2, and a light chain variable CDR3. In some embodiments, the PSMA binding protein comprises any domain that binds to PSMA including but not limited to domains from a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a

humanized antibody, or antigen binding fragments such as single domain antibodies (sdAb), Fab, Fab', F(ab)2, and Fv fragments, fragments comprised of one or more CDRs, single-chain antibodies (*e.g.*, single chain Fv fragments (scFv)), disulfide stabilized (dsFv) Fv fragments, heteroconjugate antibodies (*e.g.*, bispecific antibodies), pFv fragments, heavy chain monomers or dimers, light chain monomers or dimers, and dimers consisting of one heavy chain and one light chain. In some instances, it is beneficial for the PSMA binding domain to be derived from the same species in which the PSMA binding protein described herein will ultimately be used in. For example, for use in humans, it may be beneficial for the PSMA binding domain of the PSMA binding protein to comprise human or humanized residues from the antigen binding domain of an antibody or antibody fragment. In some embodiments, the PSMA binding protein is an anti-PSMA specific binding protein comprising a heavy chain variable CDR1, a heavy chain variable CDR2, and a heavy chain variable CDR3. In some embodiments, the PSMA binding protein is an anti-PSMA single domain antibody comprising a heavy chain variable CDR1, a heavy chain variable CDR2, and a heavy chain variable CDR3.

**[0058]** In some embodiments, the PSMA binding protein of the present disclosure is a polypeptide comprising an amino acid sequence that is comprised of four framework regions/sequences (f1-f4) interrupted by three complementarity determining regions/sequences, as represented by the formula: f1-r1-f2-r2-f3-r3-f4, wherein r1, r2, and r3 are complementarity determining regions CDR1, CDR2, and CDR3, respectively, and f1, f2, f3, and f4 are framework residues. The framework residues of the PSMA binding protein of the present disclosure comprise, for example, 75, 76, 77, 78, 79, 80, 81 amino acid residues, and the complementarity determining regions comprise, for example, 30, 31, 32, 33, 34, 35, 36 amino acid residues. In some embodiments, the PSMA binding protein comprises an amino acid sequence as set forth in SEQ ID NO: 4 comprising framework residues and CDR1, a CDR2, and a CDR3, wherein (a) the CDR1 comprises the amino acid sequence as set forth in SEQ ID NO: 16 or a variant having one, two, three, or four amino acid substitutions in SEQ ID NO: 16, (b) the CDR2 comprises a sequence as set forth in SEQ ID NO: 17 or a variant having one, two, three, or four amino acid substitutions in SEQ ID NO: 17, and (c) the CDR3 comprises a sequence as set forth in SEQ ID NO: 18 or a variant having one, two, three, or four amino acid substitutions in SEQ ID NO: 18.

**[0059]** In some embodiments, the PSMA binding protein comprises an amino acid sequence as set forth in SEQ ID NO: 19 comprising framework residues and CDR1, a CDR2, and a CDR3, wherein (a) the CDR1 comprises the amino acid sequence as set forth in SEQ ID NO: 16 or a variant having one, two, three, or four amino acid substitutions in SEQ ID NO: 16, (b) the CDR2 comprises a sequence as set forth in SEQ ID NO: 17 or a variant having one, two, three, or four amino acid substitutions in SEQ ID NO: 17, and (c) the CDR3 comprises a sequence as set forth

in SEQ ID NO: 18 or a variant having one, two, three, or four amino acid substitutions in SEQ ID NO: 18.

**[0060]** In embodiments wherein the CDR1 of the PSMA binding protein comprises the amino acid sequence as set forth in SEQ ID NO: 16 or a variant having one, two, three, or four amino acid substitutions in SEQ ID NO: 16, such substitutions include, for example, proline, histidine. In embodiments wherein the CDR2 of the PSMA binding protein comprises the amino acid sequence as set forth in SEQ ID NO: 17 or a variant having one, two, three, or four amino acid substitutions in SEQ ID NO: 17, such substitutions include, for example, aspartic acid, lysine, glutamine, tyrosine.

**[0061]** In embodiments wherein the CDR3 of the PSMA binding protein comprises the amino acid sequence as set forth in SEQ ID NO: 18 or a variant having one, two, three, or four amino acid substitutions in SEQ ID NO: 18, such substitutions include, for example, serine.

**[0062]** In some embodiments, the PSMA binding protein of the present disclosure comprises the following formula: f1-r1-f2-r2-f3-r3-f4, wherein r1, r2, and r3 are complementarity determining regions CDR1, CDR2, and CDR3, respectively, and f1, f2, f3, and f4 are framework residues, and wherein r1 comprises SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7, r2 comprises SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14, and r3 comprises SEQ ID NO: 15. In some embodiments, the PSMA binding protein of the present disclosure is a single domain antibody comprising the following formula: f1-r1-f2-r2-f3-r3-f4, wherein r1, r2, and r3 are complementarity determining regions CDR1, CDR2, and CDR3, respectively, and f1, f2, f3, and f4 are framework residues, and wherein r1 is SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7, r2 is SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14, and r3 is SEQ ID NO: 15.

**[0063]** In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in SEQ ID NO: 1 (RFMISX$_1$YX$_2$MH), (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 2 (X$_3$INPAX4X$_5$TDYAEX6VKG), and(c) the amino acid sequence of CDR3 is as set forth in SEQ ID NO: 3 (DX$_7$YGY). In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in SEQ ID NO: 1 (RFMISX$_1$YX$_2$MH), (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 17, and(c) the amino acid sequence of CDR3 is as set forth in SEQ ID NO: 18. In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in SEQ ID NO: 16, (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 2 (X$_3$INPAX$_4$X$_5$TDYAEX6VKG), and(c) the amino acid

sequence of CDR3 is as set forth in SEQ ID NO: 18. In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in SEQ ID NO: 16, (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 17, and (c) the amino acid sequence of CDR3 is as set forth in SEQ ID NO: 3 ($DX_7YGY$). In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in SEQ ID NO: 1 ($RFMISX_1YX_2MH$), (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 2 ($X_3INPAX_4X_5TDYAEX_6VKG$), and (c) the amino acid sequence of CDR3 is as set forth in SEQ ID NO: 18. In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in SEQ ID NO: 1 ($RFMISX_1YX_2MH$), (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 17, and (c) the amino acid sequence of CDR3 is as set forth in SEQ ID NO: 3 ($DX_7YGY$). In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in SEQ ID NO: 16, (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 2 ($X_3INPAX_4X_5TDYAEX_6VKG$), and(c) the amino acid sequence of CDR3 is as set forth in SEQ ID NO: 3 ($DX_7YGY$).

**[0064]** In some embodiments, the amino acid residues $X_1$, $X_2$, $X_3$, $X_4$, $X_5$, $X_6$, and $X_7$ are independently selected from glutamic acid, proline, serine, histidine, threonine, aspartic acid, glycine, lysine, threonine, glutamine, and tyrosine. In some embodiments, $X_1$ is proline. In some embodiments, $X_2$ is histidine. In some embodiments, $X_3$ is aspartic acid. In some embodiments, $X_4$ is lysine. In some embodiments, $X_5$ is glutamine. In some embodiments, $X_6$ is tyrosine. In some embodiments, $X_7$ is serine. The PSMA binding protein of the present disclosure may in some embodiments comprise CDR1, CDR2, and CDR3 sequences wherein $X_1$ is glutamic acid, $X_2$ is histidine, $X_3$ is aspartic acid, $X_4$ is glycine, $X_5$ is threonine, $X_6$ is serine, and $X_7$ is serine.

**[0065]** In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in SEQ ID NO: 1 ($RFMISX_1YX_2MH$), (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 2 ($X_3INPAX_4X_5TDYAEX_6VKG$), and (c) the amino acid sequence of CDR3 is as set forth in SEQ ID NO: 3 ($DX_7YGY$), wherein X1 is proline. In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in SEQ ID NO: 1 ($RFMISX_1YX_2MH$), (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 2 ($X_3INPAX_4X_5TDYAEX_6VKG$), and(c) the amino acid sequence of CDR3 is as set forth in SEQ ID NO: 3 ($DX_7YGY$), wherein X5 is glutamine. In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid

sequence of CDR1 is as set forth in SEQ ID NO: 1 ($RFMISX_1YX_2MH$), (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 2 ($X_3INPAX_4X_5TDYAEX_6VKG$), and(c) the amino acid sequence of CDR3 is as set forth in SEQ ID NO: 3 ($DX_7YGY$), wherein $X_6$ is tyrosine. In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in SEQ ID NO: 1 ($RFMISX_1YX_2MH$), (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 2 ($X_3INPAX_4X_5TDYAEX_6VKG$), and(c) the amino acid sequence of CDR3 is as set forth in SEQ ID NO: 3 ($DX_7YGY$), wherein $X_4$ is lysine, and $X_7$ is serine. In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in SEQ ID NO: 1 ($RFMISX_1YX_2MH$), (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 2 (X3$INPAX_4X_5TDYAEX_6VKG$), and(c) the amino acid sequence of CDR3 is as set forth in SEQ ID NO: 3 ($DX_7YGY$), wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_4$ is lysine, and $X_7$ is serine. In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in SEQ ID NO: 1 ($RFMISX_1YX_2MH$), (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 2 ($X_3INPAX_4X_5TDYAEX_6VKG$), and(c) the amino acid sequence of CDR3 is as set forth in SEQ ID NO: 3 ($DX_7YGY$), wherein $X_1$ is proline, $X_2$ is histidine, $X_3$ is aspartic acid, and $X_7$ is serine. In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in SEQ ID NO: 1 ($RFMISX_1YX_2MH$), (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 2 ($X_3INPAX_4X_5TDYAEX_6VKG$), and(c) the amino acid sequence of CDR3 is as set forth in SEQ ID NO: 3 ($DX_7YGY$), wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_5$ is glutamine, and $X_7$ is serine. In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in SEQ ID NO: 1 ($RFMISX_1YX_2MH$), (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 2 ($X_3INPAX_4X_5TDYAEX_6VKG$), and(c) the amino acid sequence of CDR3 is as set forth in SEQ ID NO: 3 ($DX_7YGY$), wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_6$ is tyrosine, and $X_7$ is serine. In some embodiments, the PSMA binding protein comprises a CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in SEQ ID NO: 1 ($RFMISX_1YX_2MH$), (b) the amino acid sequence of CDR2 is as set forth in SEQ ID NO: 2 ($X_3INPAX_4X_5TDYAEX_6VKG$), and(c) the amino acid sequence of CDR3 is as set forth in SEQ ID NO: 3 ($DX_7YGY$), wherein $X_2$ is histidine, $X_3$ is aspartic acid, and $X_7$ is serine.

**[0066]** The PSMA binding protein of the present disclosure may in some embodiments comprise CDR1, CDR2, and CDR3 sequences wherein $X_1$ is glutamic acid, $X_2$ is histidine, $X_3$ is threonine, $X_4$ is glycine, $X_5$ is threonine, $X_6$ is serine, and $X_7$ is serine. The PSMA binding

protein of the present disclosure may in some embodiments comprise CDR1, CDR2, and CDR3 sequences wherein $X_1$ is glutamic acid, $X_2$ is histidine, $X_3$ is threonine, $X_4$ is glycine, $X_5$ is threonine, $X_6$ is serine, and $X_7$ is serine. The PSMA binding protein of the present disclosure may in some embodiments comprise CDR1, CDR2, and CDR3 sequences wherein $X_1$ is glutamic acid, $X_2$ is serine, $X_3$ is threonine, $X_4$ is lysine, $X_5$ is threonine, $X_6$ is serine, and $X_7$ is serine. The PSMA binding protein of the present disclosure may in some embodiments comprise CDR1, CDR2, and CDR3 sequences wherein $X_1$ is proline, $X_2$ is serine, $X_3$ is threonine, $X_4$ is glycine, $X_5$ is threonine, X6 is serine, and X7 is glycine. The PSMA binding protein of the present disclosure may in some embodiments comprise CDR1, CDR2, and CDR3 sequences wherein $X_1$ is glutamic acid, $X_2$ is serine, $X_3$ is threonine, $X_4$ is glycine, $X_5$ is glutamine, $X_6$ is serine, and $X_7$ is glycine. The PSMA binding protein of the present disclosure may in some embodiments comprise CDR1, CDR2, and CDR3 sequences wherein $X_1$ is glutamic acid, $X_2$ is serine, $X_3$ is threonine, $X_4$ is glycine, $X_5$ is threonine, $X_6$ is tyrosine, and $X_7$ is glycine. The PSMA binding protein of the present disclosure may in some embodiments comprise CDR1, CDR2, and CDR3 sequences wherein $X_1$ is glutamic acid, $X_2$ is histidine, $X_3$ is aspartic acid, $X_4$ is lysine, $X_5$ is threonine, $X_6$ is serine, and $X_7$ is serine. The PSMA binding protein of the present disclosure may in some embodiments comprise CDR1, CDR2, and CDR3 sequences wherein $X_1$ is proline, $X_2$ is histidine, $X_3$ is aspartic acid, $X_4$ is glycine, $X_5$ is threonine, $X_6$ is serine, and $X_7$ is serine. The PSMA binding protein of the present disclosure may in some embodiments comprise CDR1, CDR2, and CDR3 sequences wherein $X_1$ is glutamic acid, $X_2$ is histidine, $X_3$ is aspartic acid, $X_4$ is glutamine, $X_5$ is threonine, $X_6$ is serine, and $X_7$ is serine. The PSMA binding protein of the present disclosure may in some embodiments comprise CDR1, CDR2, and CDR3 sequences wherein $X_1$ is glutamic acid, $X_2$ is histidine, $X_3$ is aspartic acid, $X_4$ is glycine, $X_5$ is threonine, $X_6$ is tyrosine, and $X_7$ is serine. The PSMA binding protein of the present disclosure may in some embodiments comprise CDR1, CDR2, and CDR3 sequences wherein $X_2$ is histidine, and $X_7$ is serine. Exemplary framework sequences are disclosed as SEQ ID NO: 165-168.

**[0067]** In some embodiments, the prostate specific membrane antigen binding protein comprises any combination of the following: (i) wherein $X_1$ is proline; (ii) wherein X2 is histidine; (iii) wherein X3 is aspartic acid; (iv) whereinX4 is lysine; (v) wherein X5 is glutamine; (vi) wherein X6 is tyrosine; and (vii) wherein X7 is serine. In some embodiments, the prostate specific membrane antigen binding protein of the above embodiment has a higher affinity towards a human prostate specific membrane antigen than that of a binding protein which has the sequence set forth as SEQ ID NO: 4. In some embodiments, the prostate specific membrane antigen binding comprises any combination of the following: (i) wherein $X_1$ is proline; wherein $X_5$ is

glutamine; (ii) wherein $X_6$ is tyrosine; wherein $X_4$ is lysine and $X_7$ is serine; (iii) wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_4$ is lysine, and $X_7$ is serine; (iv) wherein $X_1$ is proline, $X_2$ is histidine, $X_3$ is aspartic acid, and $X_7$ is serine; (v) wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_5$ is glutamine, and $X_7$ is serine; (vi) wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_4$ is lysine, and $X_7$ is serine; (vii) wherein $X_1$ is proline, $X_2$ is histidine, $X_3$ is aspartic acid, and $X_7$ is serine; (viii) wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_5$ is glutamine, and $X_7$ is serine; (ix) wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_6$ is tyrosine, and $X_7$ is serine; and (x) wherein $X_2$ is histidine, $X_3$ is aspartic acid, and $X_7$ is serine.

**[0068]** In some embodiments, the PSMA binding protein has an amino acid sequence as set forth in SEQ ID NO: 4. In some embodiments, the PSMA binding protein has an amino acid sequence as set forth in SEQ ID NO: 4 wherein one or more amino acid positions are substituted. In some embodiments, one or more of amino acid positions 19, 86, 87, and 106 of SEQ ID NO: 4 are substituted. Exemplary substitutions in amino acid positions 19, 86, 87, and 106, include but are not limited to T19R, K86R, P87A, and Q106L. In some embodiments, one or more of amino acid positions 31, 33, 50, 55, 56, 62, and 97 of SEQ ID NO: 4 are substituted. In some embodiments, amino acid position 31 of SEQ ID NO:4 is substituted as E31P. In some embodiments, amino acid position 33 of SEQ ID NO:4 is substituted as S33H. In some embodiments, amino acid position 50 of SEQ ID NO:4 is substituted as T50D. In some embodiments, amino acid position 55 of SEQ ID NO:4 is substituted as G55K. In some embodiments, amino acid position 56 of SEQ ID NO:4 is substituted as T56Q. In some embodiments, amino acid position 62 of SEQ ID NO:4 is substituted as S62Y. In some embodiments, amino acid position 97 of SEQ ID NO:4 is substituted as G97S. In some embodiments, amino acid positions 33 and of SEQ ID NO:4 is substituted as S33H. In some embodiments, the substitution of SEQ ID NO: 4 at position 31 is combined with substitutions at positions 50 and 97. In some embodiments, the amino acid positions 31, 50, and 97 of SEQ ID NO: 4 are respectively substituted as E31P, T50D, and G97S. In some embodiments, the substitution of SEQ ID NO: 4 at position 33 is combined with substitutions at position 97. In some embodiments, the amino acid positions 33 and 97 of SEQ ID NO: 4 are respectively substituted as S33H and G97S. In some embodiments, the substitution of SEQ ID NO: 4 at position 33 is combined with substitutions at positions 50 and 97. In some embodiments, the amino acid positions 33, 50, and 97 of SEQ ID NO: 4 are respectively substituted as S33H, T50D, and G97S. In some embodiments, the substitution of SEQ ID NO: 4 at position 33 is combined with substitutions at positions 50, 55 and 97. In some embodiments, the amino acid positions 33, 50, 55 and 97 of SEQ ID NO: 4 are respectively substituted as S33H, T50D, G55K, and G97S. In some embodiments, the substitution of SEQ ID NO: 4 at position 33 is combined

with substitutions at positions 31, 50, and 97. In some embodiments, the amino acid positions 31, 33, 50, and 97 of SEQ ID NO: 4 are respectively substituted as E31P, S33H, T50D, and G97S. In some embodiments, the substitution of SEQ ID NO: 4 at position 33 is combined with substitutions at positions 50, 56, and 97. In some embodiments, the amino acid positions 33, 50, 56, and 97 of SEQ ID NO: 4 are respectively substituted as S33H, T50D, T56Q, and G97S. In some embodiments, the substitution of SEQ ID NO: 4 at position 33 is combined with substitutions at positions 50, 62, and 97. In some embodiments, the amino acid positions 33, 50, 62, and 97 of SEQ ID NO: 4 are respectively substituted as S33H, T50D, S62Y, and G97S.

**[0069]** In some embodiments, the PSMA binding protein has an amino acid sequence as set forth in SEQ ID NO: 19 In some embodiments, the PSMA binding protein has an amino acid sequence as set forth in SEQ ID NO: 19 wherein one or more amino acid positions are substituted. In some embodiments, one or more of amino acid positions 31, 33, 50, 55, 56, 62, and 97 of SEQ ID NO: 19 are substituted. In some embodiments, amino acid position 31 of SEQ ID NO:4 is substituted as E31P. In some embodiments, amino acid position 33 of SEQ ID NO: 19 is substituted as S33H. In some embodiments, amino acid position 50 of SEQ ID NO: 19 is substituted as T50D. In some embodiments, amino acid position 55 of SEQ ID NO: 19 is substituted as G55K. In some embodiments, amino acid position 56 of SEQ ID NO: 19 is substituted as T56Q. In some embodiments, amino acid position 62 of SEQ ID NO: 19 is substituted as S62Y. In some embodiments, amino acid position 97 of SEQ ID NO: 19 is substituted as G97S. In some embodiments, amino acid positions 33 and of SEQ ID NO: 19 is substituted as S33H. In some embodiments, the substitution of SEQ ID NO: 19 at position 31 is combined with substitutions at positions 50 and 97. In some embodiments, the amino acid positions 31, 50, and 97 of SEQ ID NO: 19 are respectively substituted as E31P, T50D, and G97S. In some embodiments, the substitution of SEQ ID NO: 19 at position 33 is combined with substitutions at position 97. In some embodiments, the amino acid positions 33 and 97 of SEQ ID NO: 19 are respectively substituted as S33H and G97S. In some embodiments, the substitution of SEQ ID NO: 19 at position 33 is combined with substitutions at positions 50 and 97. In some embodiments, the amino acid positions 33, 50, and 97 of SEQ ID NO: 19 are respectively substituted as S33H, T50D, and G97S. In some embodiments, the substitution of SEQ ID NO: 19 at position 33 is combined with substitutions at positions 50, 55 and 97. In some embodiments, the amino acid positions 33, 50, 55 and 97 of SEQ ID NO: 19 are respectively substituted as S33H, T50D, G55K, and G97S. In some embodiments, the substitution of SEQ ID NO: 19 at position 33 is combined with substitutions at positions 31, 50, and 97. In some embodiments, the amino acid positions 31, 33, 50, and 97 of SEQ ID NO: 19 are respectively substituted as E31P, S33H, T50D, and G97S. In some embodiments, the

substitution of SEQ ID NO: 19 at position 33 is combined with substitutions at positions 50, 56, and 97. In some embodiments, the amino acid positions 33, 50, 56, and 97 of SEQ ID NO: 19 are respectively substituted as S33H, T50D, T56Q, and G97S. In some embodiments, the substitution of SEQ ID NO: 4 at position 33 is combined with substitutions at positions 50, 62, and 97. In some embodiments, the amino acid positions 33, 50, 62, and 97 of SEQ ID NO: 4 are respectively substituted as S33H, T50D, S62Y, and G97S.

**[0070]** In some embodiments, the prostate specific membrane antigen binding protein comprises any combination of the following: (i) substitution at position 31; (ii) substitution at position 50; (iii) substitution at position 55; substitution at position 56; (iv) substitution at position 62; (v) substitution at position 97; (vi) substitutions at positions 55 and 97; (vii) substitutions at positions 33 and 97; (viii) substitutions at 33, 50, and 97; (ix) substitutions at positions 31, 33, 50, and 97; (x) substitutions at positions 33, 50, 55, and 97; (xi) substitutions at positions 33, 50, 56, and 97; and (xiii) substitutions at positions 33, 50, 62, and 97.

**[0071]** In some embodiments, the PSMA binding protein is cross-reactive with human and cynomolgus PSMA. In some embodiments, the PSMA binding protein is specific for human PSMA. In various embodiments, the PSMA binding protein of the present disclosure is at least about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to the amino acid sequence set forth in SEQ ID NO: 4.

**[0072]** In various embodiments, the PSMA binding protein of the present disclosure is at least about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to the amino acid sequence set forth in SEQ ID NO: 19.

**[0073]** In various embodiments, a complementarity determining region of the PSMA binding protein of the present disclosure is at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to the amino acid sequence set forth in SEQ ID NO: 16.

**[0074]** In various embodiments, a complementarity determining region of the PSMA binding protein of the present disclosure is at least about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about

95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to the amino acid sequence set forth in SEQ ID NO: 17.

**[0075]** In various embodiments, a complementarity determining region of the PSMA binding protein PSMA binding protein of the present disclosure is at least about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to the amino acid sequence set forth in SEQ ID NO: 18.

**Humanization and Affinity Maturation**

**[0076]** In designing binding proteins for therapeutic applications, it is desirable to create proteins that, for example, modulate a functional activity of a target, and/or improved binding proteins such as binding proteins with higher specificity and/or affinity and/or and binding proteins that are more bioavailable, or stable or soluble in particular cellular or tissue environments.

**[0077]** The PSMA binding proteins described in the present disclosure exhibit improved the binding affinities towards the target binding domain, which is PSMA. The present disclosure identifies amino acid substitutions in the complementarity determining regions (CDRs) of the PSMA binding proteins described herein which lead to higher binding affinity towards one or both of human and cyno PSMA. In some embodiments, the PSMA binding protein is an antibody. In certain embodiments, the PSMA binding protein is a humanized antibody. Generally, a humanized antibody comprises one or more variable domains in which CDRs or portions of CDRs are derived from a non-human antibody, and framework regions or portions of framework regions are derived from human antibody sequences. Optionally, a humanized antibody also comprises at least a portion of a human constant region. In some embodiments, selected framework residues are substituted with corresponding residues from a non-human antibody (*e.g.,* the antibody from which the CDRs are derived), *e.g.,* to restore or improve antibody specificity, affinity, or pH dependence. Human framework regions that can be used for humanization include but are not limited to framework regions selected using a best- fit method (*e.g.,* Sims et al. J Immunol 151:2296, 1993); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (*e.g.,* Carter et al. Proc Natl Acad Sci USA, 89:4285, 1992; and Presta et al., J Immunol, 151:2623, 1993); human mature (somatically mutated) framework regions or human germline framework regions (*e.g.,* Almagro and Fransson, Front Biosci 13:1619-1633, 2008); and framework regions derived from screening framework libraries (*e.g.,* Baca et al., J Biol Chem 272:10678-10684, 1997; and Rosok et al., J Biol Chem 271:22611-22618, 1996)). Thus, in one aspect, the PSMA binding protein comprises a humanized or human antibody or an

antibody fragment. In one embodiment, the humanized or human anti-PSMA binding protein comprises one or more (*e.g.,* all three) light chain complementary determining region 1 (LC CDR1), light chain complementary determining region 2 (LC CDR2), and light chain complementary determining region 3 (LC CDR3) of a humanized or human anti- PSMA binding domain described herein, and/or one or more (*e.g.,* all three) heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of a humanized or human anti-PSMA binding domain described herein, *e.g.,* a humanized or human anti-PSMA binding domain comprising one or more, *e.g.,* all three, LC CDRs and one or more, *e.g.,* all three, HC CDRs. In some embodiments, the humanized or human anti-PSMA binding domain comprises a humanized or human light chain variable region specific to PSMA where the light chain variable region specific to PSMA comprises human or non-human light chain CDRs in a human light chain framework region. In certain instances, the light chain framework region is a λ (lambda) light chain framework. In other instances, the light chain framework region is a κ (kappa) light chain framework. In some embodiments, the humanized or human anti-PSMA binding domain comprises a humanized or human heavy chain variable region specific to PSMA where the heavy chain variable region specific to PSMA comprises human or non-human heavy chain CDRs in a human heavy chain framework region. In certain instances, the complementary determining regions of the heavy chain and/or the light chain are derived from known anti-PSMA antibodies, such as, for example, 7E11, EPR6253, 107.1A4, GCP-05, EP3253, BV9, SP29, human PSMA/FOLH1/NAALADase I antibody.

**[0078]** The PSMA binding proteins of the present disclosure is, in some embodiments, affinity matured to increase its binding affinity to the target binding domain. Where it is desired to improve the affinity of the PSMA binding proteins of the disclosure, such as anti-PSMA antibodies, containing one or more of the above-mentioned CDRs, such antibodies with improved affinity may be obtained by a number of affinity maturation protocols, including but not limited to maintaining the CDRs, chain shuffling, use of mutation strains of *E. coli*, DNA shuffling, phage display and sexual. Above exemplary methods of affinity maturation are discussed by Vaughan et al. (Nature Biotechnology, 16, 535-539, 1998). Thus, in addition to the PSMA binding protein variants discussed in the foregoing sections, the disclosure provides further sequence variants which improve the affinity of the binding protein towards its target, i.e., PSMA. In certain embodiments, such sequence variants comprise one or more semi-conservative or conservative substitutions within the PSMA binding protein sequences and such substitutions preferably do not significantly affect the desired activity of the binding protein. Substitutions may be naturally occurring or may be introduced for example using mutagenesis

(*e.g.*, Hutchinson et al., 1978, J. Biol. Chem. 253:6551). For example, the amino acids glycine, alanine, valine, leucine and isoleucine can often be substituted for one another (amino acids having aliphatic side chains). Of these possible substitutions, it is typically glycine and alanine are used to substitute for one another since they have relatively short side chains and valine, leucine, and isoleucine are used to substitute for one another since they have larger aliphatic side chains which are hydrophobic. Other amino acids which may often be substituted for one another include but are not limited to: phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains); lysine, arginine and histidine (amino acids having basic side chains); aspartate and glutamate (amino acids having acidic side chains); asparagine and glutamine (amino acids having amide side chains); and cysteine and methionine (amino acids having sulphur-containing side chains).

**[0079]** In some embodiments, the PSMA binding proteins are isolated by screening combinatorial libraries, for example, by generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Further, the binding affinity of the PSMA binding protein towards its binding target can be selected so as to target a specific elimination half-time in a particular PSMA albumin binding protein. Thus, in some embodiments, the PSMA binding protein has a high binding affinity towards its binding target. In other embodiments, the PSMA binding protein has a medium binding affinity towards its binding target. In yet other embodiments, the PSMA binding protein has a low or marginal binding affinity towards its binding target. Exemplary binding affinities include Kd of 10 nM or less (high), between 10 nM and 100 nM (medium), and greater than 100 nM (low). The affinity to bind to PSMA can be determined, for example, by the ability of binding protein itself or its PSMA binding domain to bind to PSMA coated on an assay plate; displayed on a microbial cell surface; in solution; etc. The binding activity of the protein of the present disclosure to PSMA can also be assayed by immobilizing the ligand (*e.g.*, PSMA) or said binding protein itself or its PSMA binding domain, to a bead, substrate, cell, etc. In some embodiments, binding between the PSMA binding protein itself, or its PSMA binding domain, and a target ligand (such as PSMA) is determined, for example, by a binding kinetics assay. The binding kinetics assay, in certain embodiments, is carried out using an OCTET® system. In such embodiments, a first step comprises immobilizing a ligand (*e.g.*, biotinylated PSMA) onto the surface of a biosensor (*e.g.*, a streptavidin biosensor) at an optimal loading density, followed by a wash with an assay buffer to remove unbound ligands, which is followed by association of the analyte, *i.e.*, the PSMA binding protein itself or its PSMA binding domain with the ligand, which is followed by exposing the biosensor to a buffer that does not contain the analyte, thereby resulting in dissociation of the PSMA binding protein itself or its PSMA binding domain from the ligand.

Suitable blocking agents, such as BSA, Casein, Tween-20, PEG, gelatin, are used to block the non-specific binding sites on the bio-sensor. The binding kinetics data is subsequently analyzed using an appropriate software (*e.g.,* ForteBio's Octet software) to determine the association and dissociation rate constants for binding interaction between the PSMA binding protein itself or its PSMA binding domain and a ligand.

**[0080]** In certain embodiments, the PSMA binding protein disclosed herein binds to human PSMA with a human Kd (hKd). In certain embodiments, the PSMA binding protein disclosed herein binds to cynomolgus PSMA with a cyno Kd (cKd). In certain embodiments, the PSMA binding protein disclosed herein binds to cynomolgus PSMA with a cyno Kd (cKd) and to human PSMA with a human Kd (hKd). In some embodiments, the hKd and the cKd range from about 0.1 nM to about 500 nM. In some embodiments, the hKd and the cKd range from about 0.1 nM to about 450 nM. In some embodiments, the hKd and the cKd range from about 0.1 nM to about 400 nM. In some embodiments, the hKd and the cKd range from about 0.1 nM to about 350 nM. In some embodiments, the hKd and the cKd range from about 0.1 nM to about 300 nM . In some embodiments, the hKd and the cKd range from about 0.1 nM to about 250 nM. In some embodiments, the hKd and the cKd range from about 0.1 nM to about 200 nM. In some embodiments, the hKd and the cKd range from about 0.1 nM to about 150 nM. In some embodiments, the hKd and the cKd range from about 0.1 nM to about 100 nM. In some embodiments, the hKd and the cKd range from about 0.1 nM to about 90 nM. In some embodiments, the hKd and the cKd range from about 0.2 nM to about 80 nM. In some embodiments, the hKd and the cKd range from about 0.3 nM to about 70 nM. In some embodiments, the hKd and the cKd range from about 0.4 nM to about 50 nM. In some embodiments, the hKd and the cKd range from about 0.5 nM to about 30 nM. In some embodiments, the hKd and the cKd range from about 0.6 nM to about 10 nM. In some embodiments, the hKd and the cKd range from about 0.7 nM to about 8 nM. In some embodiments, the hKd and the cKd range from about 0.8 nM to about 6 nM. In some embodiments, the hKd and the cKd range from about 0.9 nM to about 4 nM. In some embodiments, the hKd and the cKd range from about 1 nM to about 2 nM. In some embodiments, the PSMA binding protein binds to human and cynomolgus PSMA with comparable binding affinity (Kd).

**[0081]** In some embodiments, the PSMA binding protein of the present disclosure comprises the sequence as set forth in SEQ ID NO: 4 and has an hKd of about 10 nM to about 20 nM. In some embodiments, the PSMA binding protein of the present disclosure comprises a glutamic acid to proline mutation in amino acid position 31 of SEQ ID NO: 4 and has an hKd of about 5 nM to about 10 nM. In some embodiments, the PSMA binding protein of the present disclosure

comprises a threonine to glutamine mutation in amino acid position 56 of SEQ ID NO: 4 and has a hKd of about 1 nM to about 7 nM. In some embodiments, the PSMA binding protein of the present disclosure comprises a glycine to lysine mutation in amino acid position 55 of SEQ ID NO: 4 and has a hKd of about 0.5 nM to about 5 nM. In some embodiments, the PSMA binding protein of the present disclosure comprises a serine to histidine mutation in amino acid position 33, threonine to aspartic acid in amino acid position 50, and glycine to serine substitution in amino acid position 97 of SEQ ID NO: 4 and has an hKd of about 5 nM to about 10 nM. In some embodiments, the PSMA binding protein of the present disclosure comprises a serine to histidine mutation in amino acid position 33, and glycine to serine substitution in amino acid position 97 of SEQ ID NO: 4 and has an hKd of about 0.05 nM to about 2 nM. Thus, in various embodiments, the PSMA binding proteins comprising one or more substitutions compared to the sequence as set forth in SEQ ID NO: 4 have binding affinities towards human PSMA that are 1.5 times to about 300 times higher that of a protein comprising the sequence of SEQ ID NO: 4 without any substitutions. For example, the binding affinity is about 1.5 times to about 3 times higher when the substitution(s) of SEQ ID NO: 4 comprises E31P; about 2 times to about 15 times higher when the substitution(s) of SEQ ID NO: 4 comprises T56Q; about 3 times to about 30 times the substitution(s) of SEQ ID NO: 4 comprises G55K; about 2 times to about 3 times the substitution(s) of SEQ ID NO: 4 comprises S33H T50D G97S; and about 5 times to about 300 times the substitution(s) of SEQ ID NO: 4 comprises S33H G97S. In some embodiments, the one or more amino acid substitutions of SEQ ID NO: 4, as described above, leads to enhanced binding affinity towards both human and cynomolgus PSMA, for example, a PSMA binding protein of the present disclosure comprising amino acid substitutions S33H and G97S in SEQ ID NO: 4, shows increased affinity towards human and cynomolgus PSMA compared to a protein that comprises the sequence of SEQ ID NO: 4 without any substitutions. A further example of such dual affinity enhancement is seen in case of a PSMA binding protein comprising amino acid substitutions S33H, T50D, and G97S in SEQ ID NO: 4. In some embodiments, any of the foregoing PSMA binding proteins (*e.g.,* anti-PSMA single domain antibodies of SEQ ID Nos. 21-32) are affinity peptide tagged for ease of purification. In some embodiments, the affinity peptide tag is six consecutive histidine residues, also referred to as 6his (SEQ ID NO: 33).

**[0082]** The binding affinity of PSMA binding proteins, *e.g.*, an anti-PSMA single domain antibody, of the present disclosure may also be described in relative terms or as compared to the binding affinity of a second binding protein that also specifically binds to PSMA (*e.g.,* a second anti-PSMA single domain antibody that is PSMA-specific, which may be referred to herein as a "second PSMA-specific antibody". In some embodiments, the second PSMA-specific antibody

is any of the PSMA binding protein variants described herein, such as binding proteins defined by SEQ ID Nos. 21-32. Accordingly, certain embodiments of the present disclosure relate to an anti-PSMA single domain antibody that binds to human PSMA and/or cynomolgus PSMA with greater affinity than the binding protein of SEQ ID NO: 4, or with a Kd that is lower than the Kd of the binding protein of SEQ ID NO: 4. Further, additional embodiments of the present disclosure relate to an anti-PSMA single domain antibody that binds to human PSMA and/or cynomolgus PSMA with greater affinity than the binding protein of SEQ ID NO: 19, or with a Kd that is lower than the Kd of the binding protein of SEQ ID NO: 19.

**CD3 Binding Domain**

**[0083]** The specificity of the response of T cells is mediated by the recognition of antigen (displayed in context of a major histocompatibility complex, MHC) by the T cell receptor complex. As part of the T cell receptor complex, CD3 is a protein complex that includes a CD3γ (gamma) chain, a CD3δ (delta) chain, and two CD3ε (epsilon) chains which are present on the cell surface. CD3 associates with the α (alpha) and β (beta) chains of the T cell receptor (TCR) as well as and CD3 ζ (zeta) altogether to comprise the T cell receptor complex. Clustering of CD3 on T cells, such as by immobilized anti-CD3 antibodies leads to T cell activation similar to the engagement of the T cell receptor but independent of its clone-typical specificity.

**[0084]** In one aspect is described herein a multispecific protein comprising a PSMA binding protein according to the present disclosure. In some embodiments, the multispecific protein further comprises a domain which specifically binds to CD3. In some embodiments, the multispecific protein further comprises a domain which specifically binds to CD3γ. In some embodiments, the multispecific protein further comprises a domain which specifically binds to CD3δ. In some embodiments, the multispecific protein further comprises a domain which specifically binds to CD3ε.

**[0085]** In additional embodiments, the multispecific protein further comprises a domain which specifically binds to the T cell receptor (TCR). In some embodiments, the multispecific protein further comprises a domain which specifically binds the α chain of the TCR. In some embodiments, the multispecific protein further comprises a domain which specifically binds the β chain of the TCR.

**[0086]** In some embodiments, the multispecific protein further comprises a domain which specifically binds to a bulk serum protein, such as human serum albumin (HSA). In some embodiments, the HSA binding domain comprises a sequence selected from the group consisting of SEQ ID NO: 123-146.

**[0087]** In some embodiments, the multispecific protein is a PSMA targeting trispecific antigen-binding protein, also referred to herein as a PSMA targeting TriTAC molecule or PSMA trispecific molecule or trispecific molecule.

**[0088]** In certain embodiments, the CD3 binding domain of the multispecific protein comprising a PSMA binding protein described herein exhibits not only potent CD3 binding affinities with human CD3, but show also excellent crossreactivity with the respective cynomolgus monkey CD3 proteins. In some instances, the CD3 binding domain of the multispecific proteins are cross-reactive with CD3 from cynomolgus monkey.

**[0089]** In some embodiments, the CD3 binding domain of the multispecific protein comprising a PSMA binding protein described herein can be any domain that binds to CD3 including but not limited to domains from a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, or antigen binding fragments of the CD3 binding antibodies, such as single domain antibodies (sdAb), Fab, Fab′, F(ab)2, and Fv fragments, fragments comprised of one or more CDRs, single-chain antibodies (*e.g.*, single chain Fv fragments (scFv)), disulfide stabilized (dsFv) Fv fragments, heteroconjugate antibodies (*e.g.*, bispecific antibodies), pFv fragments, heavy chain monomers or dimers, light chain monomers or dimers, and dimers consisting of one heavy chain and one light chain. In some instances, it is beneficial for the CD3 binding domain to be derived from the same species in which the multispecific protein comprising a single PSMA binding protein described herein will ultimately be used in. For example, for use in humans, it may be beneficial for the CD3 binding domain of the multispecific protein comprising a PSMA binding protein described herein to comprise human or humanized residues from the PSMA binding domain of an antibody or antibody fragment.

**[0090]** Thus, in one aspect, the CD3 binding domain of the multispecific protein comprising a PSMA binding protein comprises a humanized or human antibody or an antibody fragment, or a murine antibody or antibody fragment. In one embodiment, the humanized or human anti-CD3 binding domain comprises one or more (*e.g.*, all three) light chain complementary determining regions, light chain complementary determining region 1 (LC CDR1), light chain complementary determining region 2 (LC CDR2), and light chain complementary determining region 3 (LC CDR3) of a humanized or human anti- CD3 binding domain described herein, and/or one or more (*e.g.*, all three) heavy chain complementary determining regions, heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of a humanized or human anti-CD3 binding domain described herein, *e.g.*, a humanized

or human anti-CD3 binding domain comprising one or more, *e.g.*, all three, LC CDRs and one or more, *e.g.*, all three, HC CDRs.

**[0091]** In some embodiments, the humanized or human anti-CD3 binding domain comprises a humanized or human light chain variable region specific to CD3 where the light chain variable region specific to CD3 comprises human or non-human light chain CDRs in a human light chain framework region. In certain instances, the light chain framework region is a λ (lambda) light chain framework. In other instances, the light chain framework region is a κ (kappa) light chain framework.

**[0092]** In some embodiments, the humanized or human anti-CD3 binding domain comprises a humanized or human heavy chain variable region specific to CD3 where the heavy chain variable region specific to CD3 comprises human or non-human heavy chain CDRs in a human heavy chain framework region.

**[0093]** In certain instances, the complementary determining regions of the heavy chain and/or the light chain are derived from known anti-CD3 antibodies, such as, for example, muromonab-CD3 (OKT3), otelixizumab (TRX4), teplizumab (MGA031), visilizumab (Nuvion), SP34, TR-66 or X35-3, VIT3, BMA030 (BW264/56), CLB-T3/3, CRIS7, YTH12.5, F111-409, CLB-T3.4.2, TR-66, WT32, SPv-T3b, 11D8, XIII-141, XIII-46, XIII-87, 12F6, T3/RW2-8C8, T3/RW2-4B6, OKT3D, M-T301, SMC2, F101.01, UCHT-1 and WT-31.

**[0094]** In one embodiment, the anti-CD3 binding domain is a single chain variable fragment (scFv) comprising a light chain and a heavy chain of an amino acid sequence provided herein. As used herein, "single chain variable fragment" or "scFv" refers to an antibody fragment comprising a variable region of a light chain and at least one antibody fragment comprising a variable region of a heavy chain, wherein the light and heavy chain variable regions are contiguously linked via a short flexible polypeptide linker, and capable of being expressed as a single polypeptide chain, and wherein the scFv retains the specificity of the intact antibody from which it is derived. In an embodiment, the anti-CD3 binding domain comprises: a light chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions) of an amino acid sequence of a light chain variable region provided herein, or a sequence with 95-99% identity with an amino acid sequence provided herein; and/or a heavy chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions) of an amino acid sequence of a heavy chain variable region provided herein, or a sequence with 95-99% identity to an amino acid sequence provided herein. In one embodiment, the humanized or human anti-CD3 binding domain is a scFv, and a light chain variable region

comprising an amino acid sequence described herein, is attached to a heavy chain variable region comprising an amino acid sequence described herein, via a scFv linker. The light chain variable region and heavy chain variable region of a scFv can be, e.g., in any of the following orientations: light chain variable region- scFv linker-heavy chain variable region or heavy chain variable region- scFv linker-light chain variable region.

**[0095]** In some instances, scFvs which bind to CD3 are prepared according to known methods. For example, scFv molecules can be produced by linking VH and VL regions together using flexible polypeptide linkers. The scFv molecules comprise a scFv linker (e.g., a Ser-Gly linker) with an optimized length and/or amino acid composition. Accordingly, in some embodiments, the length of the scFv linker is such that the VH or VL domain can associate intermolecularly with the other variable domain to form the CD3 binding site. In certain embodiments, such scFv linkers are "short", i.e. consist of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 amino acid residues. Thus, in certain instances, the scFv linkers consist of about 12 or less amino acid residues. In the case of 0 amino acid residues, the scFv linker is a peptide bond. In some embodiments, these scFv linkers consist of about 3 to about 15, for example 8, 9 or 10 contiguous amino acid residues. Regarding the amino acid composition of the scFv linkers, peptides are selected that confer flexibility, do not interfere with the variable domains as well as allow inter-chain folding to bring the two variable domains together to form a functional CD3 binding site. For example, scFv linkers comprising glycine and serine residues generally provide protease resistance. In some embodiments, linkers in a scFv comprise glycine and serine residues. The amino acid sequence of the scFv linkers can be optimized, for example, by phage-display methods to improve the CD3 binding and production yield of the scFv. Examples of peptide scFv linkers suitable for linking a variable light chain domain and a variable heavy chain domain in a scFv include but are not limited to $(GS)_n$ (SEQ ID NO: 157), $(GGS)_n$ (SEQ ID NO: 158), $(GGGS)_n$ (SEQ ID NO: 159), $(GGSG)_n$ (SEQ ID NO: 160), $(GGSGG)_n$ (SEQ ID NO: 161), or $(GGGGS)_n$ (SEQ ID NO: 162), wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In one embodiment, the scFv linker can be $(GGGGS)_4$ (SEQ ID NO: 163) or $(GGGGS)_3$ (SEQ ID NO: 164). Variation in the linker length may retain or enhance activity, giving rise to superior efficacy in activity studies.

**[0096]** In some embodiments, CD3 binding domain of PSMA trispecific antigen-binding protein has an affinity to CD3 on CD3 expressing cells with a $K_D$ of 1000 nM or less, 500 nM or less, 200 nM or less, 100 nM or less, 80 nM or less, 50 nM or less, 20 nM or less, 10 nM or less, 5 nM or less, 1 nM or less, or 0.5 nM or less. In some embodiments, the CD3 binding domain of PSMA trispecific antigen-binding protein has an affinity to CD3ε, γ, or δ with a $K_D$ of 1000 nM or less, 500 nM or less, 200 nM or less, 100 nM or less, 80 nM or less, 50 nM or less, 20 nM or less, 10 nM or less, 5 nM or less, 1 nM or less, or 0.5 nM or less. In further embodiments, CD3

binding domain of PSMA trispecific antigen-binding protein has low affinity to CD3, i.e., about 100 nM or greater.

**[0097]** The affinity to bind to CD3 can be determined, for example, by the ability of the PSMA trispecific antigen-binding protein itself or its CD3 binding domain to bind to CD3 coated on an assay plate; displayed on a microbial cell surface; in solution; etc. The binding activity of the PSMA trispecific antigen-binding protein itself or its CD3 binding domain of the present disclosure to CD3 can be assayed by immobilizing the ligand (e.g., CD3) or the PSMA trispecific antigen-binding protein itself or its CD3 binding domain, to a bead, substrate, cell, etc. Agents can be added in an appropriate buffer and the binding partners incubated for a period of time at a given temperature. After washes to remove unbound material, the bound protein can be released with, for example, SDS, buffers with a high pH, and the like and analyzed, for example, by Surface Plasmon Resonance (SPR).

**[0098]** In some embodiments, CD3 binding domains described herein comprise a polypeptide having a sequence described in Table 7 (SEQ ID NO: 34-88) and subsequences thereof. In some embodiments, the CD3 binding domain comprises a polypeptide having at least 70%-95% or more homology to a sequence described in Table 7 (SEQ ID NO: 34-122). In some embodiments, the CD3 binding domain comprises a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 95%, or more homology to a sequence described in Table 7 (SEQ ID NO: 34-122). In some embodiments, the CD3 binding domain has a sequence comprising at least a portion of a sequence described in Table 7 (SEQ ID NO: 34-122). In some embodiments, the CD3 binding domain comprises a polypeptide comprising one or more of the sequences described in Table 7 (SEQ ID NO: 34-122).

**[0099]** In certain embodiments, CD3 binding domain comprises an scFv with a heavy chain CDR1 comprising SEQ ID NO: 49, and 56-67. In certain embodiments, CD3 binding domain comprises an scFv with a heavy chain CDR2 comprising SEQ ID NO: 50, and 68-77. In certain embodiments, CD3 binding domain comprises an scFv with a heavy chain CDR3 comprising SEQ ID NO: 51, and 78-87. In certain embodiments, CD3 binding domain comprises an scFv with a light chain CDR1 comprising SEQ ID NO: 53, and 88-100. In certain embodiments, CD3 binding domain comprises an scFv with a light chain CDR2 comprising SEQ ID NO: 54, and 101-113. In certain embodiments, CD3 binding domain comprises an scFv with a light chain CDR3 comprising SEQ ID NO: 55, and 114-120.

**[00100]** The affinity to bind to CD3 can be determined, for example, by the ability of the multispecific protein comprising a PSMA binding protein itself or its CD3 binding domain to bind to CD3 coated on an assay plate; displayed on a microbial cell surface; in solution; etc. The binding activity of multispecific protein comprising a PSMA binding protein itself or its

CD3 binding domain according to the present disclosure to CD3 can be assayed by immobilizing the ligand (*e.g.*, CD3) or said multispecific protein itself or its CD3 binding domain, to a bead, substrate, cell, etc. The binding activity of the multispecific protein comprising a PSMA binding protein itself or its CD3 binding domain to bind to CD3 can be determined by immobilizing the ligand (*e.g.,* CD3) or said multispecific protein itself or its PSMA binding domain, to a bead, substrate, cell, etc. In some embodiments, binding between the multispecific protein comprising a PSMA binding protein, and a target ligand (such as CD3) is determined, for example, by a binding kinetics assay. The binding kinetics assay, in certain embodiments, is carried out using an OCTET® system. In such embodiments, a first step comprises immobilizing a ligand (*e.g.,* biotinylated CD3) onto the surface of a biosensor (*e.g.,* a streptavidin biosensor) at an optimal loading density, followed by a wash with an assay buffer to remove unbound ligands; which is followed by association of the analyte, *e.g.,* the the multispecific protein comprising a PSMA binding protein with the ligand; which is followed by exposing the biosensor to a buffer that does not contain the analyte, thereby resulting in dissociation of the the multispecific protein comprising a PSMA binding protein from the ligand. Suitable blocking agents, such as BSA, Casein, Tween-20, PEG, gelatin, are used to block the non-specific binding sites on the bio-sensor, during the kinetic assay. The binding kinetics data is subsequently analyzed using an appropriate software (*e.g.,* ForteBio's Octet software) to determine the association and dissociation rate constants for binding interaction between the the multispecific protein comprising a PSMA binding protein and a ligand.

**[00101]** In one aspect, the PSMA targeting trispecific proteins comprise a domain (A) which specifically binds to CD3, a domain (B) which specifically binds to human serum albumin (HSA), and a domain (C) which specifically binds to PSMA. The three domains in PSMA targeting trispecific proteins are arranged in any order. Thus, it is contemplated that the domain order of the PSMA targeting trispecific proteins are:

$H_2N$-(A)-(B)-(C)-COOH,

$H_2N$-(A)-(C)-(B)-COOH,

$H_2N$-(B)-(A)-(C)-COOH,

$H_2N$-(B)-(C)-(A)-COOH,

$H_2N$-(C)-(B)-(A)-COOH, or

$H_2N$-(C)-(A)-(B)-COOH.

**[00102]** In some embodiments, the PSMA targeting trispecific proteins have a domain order of $H_2N$-(A)-(B)-(C)-COOH. In some embodiments, the PSMA targeting trispecific proteins have a domain order of $H_2N$-(A)-(C)-(B)-COOH. In some embodiments, the PSMA targeting trispecific proteins have a domain order of $H_2N$-(B)-(A)-(C)-COOH. In some embodiments, the

PSMA targeting trispecific proteins have a domain order of $H_2N$-(B)-(C)-(A)-COOH. In some embodiments, the PSMA targeting trispecific proteins have a domain order of $H_2N$-(C)-(B)-(A)-COOH. In some embodiments, the PSMA targeting trispecific proteins have a domain order of $H_2N$-(C)-(A)-(B)-COOH.

**[00103]** In some embodiments, the PSMA targeting trispecific proteins have the HSA binding domain as the middle domain, such that the domain order is $H_2N$-(A)-(B)-(C)-COOH or $H_2N$-(C)-(B)-(A)-COOH. It is contemplated that in such embodiments where the HSA binding domain as the middle domain, the CD3 and PSMA binding domains are afforded additional flexibility to bind to their respective targets.

**[00104]** In some embodiments, the PSMA targeting trispecific proteins described herein comprise a polypeptide having a sequence described in Table 10 (SEQ ID NO: 147-156) and subsequences thereof. In some embodiments, the trispecific antigen binding protein comprises a polypeptide having at least 70%-95% or more homology to a sequence described in Table 10 (SEQ ID NO: 147-156). In some embodiments, the trispecific antigen binding protein comprises a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 95%, or more homology to a sequence described in Table 10 (SEQ ID NO: 1470-156). In some embodiments, the trispecific antigen binding protein has a sequence comprising at least a portion of a sequence described in Table 10 (SEQ ID NO: 147-156). In some embodiments, the PSMA trispecific antigen-binding protein comprises a polypeptide comprising one or more of the sequences described in Table 10 (SEQ ID NO: 147-156). In further embodiments, the PSMA trispecific antigen-binding protein comprises one or more CDRs as described in the sequences in Table 10 (SEQ ID NO: 147-156).

**[00105]** The PSMA targeting trispecific proteins described herein are designed to allow specific targeting of cells expressing PSMA by recruiting cytotoxic T cells. This improves efficacy compared to ADCC (antibody dependent cell-mediated cytotoxicity) , which is using full length antibodies directed to a sole antigen and is not capable of directly recruiting cytotoxic T cells. In contrast, by engaging CD3 molecules expressed specifically on these cells, the PSMA targeting trispecific proteins can crosslink cytotoxic T cells with cells expressing PSMA in a highly specific fashion, thereby directing the cytotoxic potential of the T cell towards the target cell. The PSMA targeting trispecific proteins described herein engage cytotoxic T cells via binding to the surface-expressed CD3 proteins, which form part of the TCR. Simultaneous binding of several PSMA trispecific antigen-binding protein to CD3 and to PSMA expressed on the surface of particular cells causes T cell activation and mediates the subsequent lysis of the particular PSMA expressing cell. Thus, PSMA targeting trispecific proteins are contemplated to display strong, specific and efficient target cell killing. In some embodiments, the PSMA

targeting trispecific proteins described herein stimulate target cell killing by cytotoxic T cells to eliminate pathogenic cells (e.g., tumor cells expressing PSMA). In some of such embodiments, cells are eliminated selectively, thereby reducing the potential for toxic side effects.

**[00106]** The PSMA targeting trispecific proteins described herein confer further therapeutic advantages over traditional monoclonal antibodies and other smaller bispecific molecules. Generally, the effectiveness of recombinant protein pharmaceuticals depends heavily on the intrinsic pharmacokinetics of the protein itself. One such benefit here is that the PSMA targeting trispecific proteins described herein have extended pharmacokinetic elimination half-time due to having a half-life extension domain such as a domain specific to HSA. In this respect, the PSMA targeting trispecific proteins described herein have an extended serum elimination half-time of about two, three, about five, about seven, about 10, about 12, or about 14 days in some embodiments. This contrasts to other binding proteins such as BiTE or DART molecules which have relatively much shorter elimination half-times. For example, the BiTE CD19×CD3 bispecific scFv-scFv fusion molecule requires continuous intravenous infusion (i.v.) drug delivery due to its short elimination half-time. The longer intrinsic half-times of the PSMA targeting trispecific proteins solve this issue thereby allowing for increased therapeutic potential such as low-dose pharmaceutical formulations, decreased periodic administration and/or novel pharmaceutical compositions.

**[00107]** The PSMA targeting trispecific proteins described herein also have an optimal size for enhanced tissue penetration and tissue distribution. Larger sizes limit or prevent penetration or distribution of the protein in the target tissues. The PSMA targeting trispecific proteins described herein avoid this by having a small size that allows enhanced tissue penetration and distribution. Accordingly, the PSMA targeting trispecific proteins described herein, in some embodiments have a size of about 50 kD to about 80 kD, about 50 kD to about 75 kD, about 50 kD to about 70 kD, or about 50 kD to about 65 kD. Thus, the size of the PSMA targeting trispecific proteins is advantageous over IgG antibodies which are about 150 kD and the BiTE and DART diabody molecules which are about 55 kD but are not half-life extended and therefore cleared quickly through the kidney.

**[00108]** In further embodiments, the PSMA targeting trispecific proteins described herein have an optimal size for enhanced tissue penetration and distribution. In these embodiments, the PSMA targeting trispecific proteins are constructed to be as small as possible, while retaining specificity toward its targets. Accordingly, in these embodiments, the PSMA targeting trispecific proteins described herein have a size of about 20 kD to about 40 kD or about 25 kD to about 35 kD to about 40 kD, to about 45 kD, to about 50 kD, to about 55 kD, to about 60 kD, to about 65 kD. In some embodiments, the PSMA targeting trispecific proteins described herein

have a size of about 50kD, 49, kD, 48 kD, 47 kD, 46 kD, 45 kD, 44 kD, 43 kD, 42 kD, 41 kD, 40 kD, about 39 kD, about 38 kD, about 37 kD, about 36 kD, about 35 kD, about 34 kD, about 33 kD, about 32 kD, about 31 kD, about 30 kD, about 29 kD, about 28 kD, about 27 kD, about 26 kD, about 25 kD, about 24 kD, about 23 kD, about 22 kD, about 21 kD, or about 20 kD. An exemplary approach to the small size is through the use of single domain antibody (sdAb) fragments for each of the domains. For example, a particular PSMA trispecific antigen-binding protein has an anti-CD3 sdAb, anti-HSA sdAb and an sdAb for PSMA. This reduces the size of the exemplary PSMA trispecific antigen-binding protein to under 40 kD. Thus in some embodiments, the domains of the PSMA targeting trispecific proteins are all single domain antibody (sdAb) fragments. In other embodiments, the PSMA targeting trispecific proteins described herein comprise small molecule entity (SME) binders for HSA and/or the PSMA. SME binders are small molecules averaging about 500 to 2000 Da in size and are attached to the PSMA targeting trispecific proteins by known methods, such as sortase ligation or conjugation. In these instances, one of the domains of PSMA trispecific antigen-binding protein is a sortase recognition sequence, e.g., LPETG (SEQ ID NO: 57). To attach a SME binder to PSMA trispecific antigen-binding protein with a sortase recognition sequence, the protein is incubated with a sortase and a SME binder whereby the sortase attaches the SME binder to the recognition sequence. Known SME binders include MIP-1072 and MIP-1095 which bind to prostate-specific membrane antigen (PSMA). In yet other embodiments, the domain which binds to PSMA of PSMA targeting trispecific proteins described herein comprise a knottin peptide for binding PSMA. Knottins are disufide-stabilized peptides with a cysteine knot scaffold and have average sizes about 3.5 kD. Knottins have been contemplated for binding to certain tumor molecules such as PSMA. In further embodiments, domain which binds to PSMA of PSMA targeting trispecific proteins described herein comprise a natural PSMA ligand.

**[00109]** Another feature of the PSMA targeting trispecific proteins described herein is that they are of a single-polypeptide design with flexible linkage of their domains. This allows for facile production and manufacturing of the PSMA targeting trispecific proteins as they can be encoded by single cDNA molecule to be easily incorporated into a vector. Further, because the PSMA targeting trispecific proteins described herein are a monomeric single polypeptide chain, there are no chain pairing issues or a requirement for dimerization. It is contemplated that the PSMA targeting trispecific proteins described herein have a reduced tendency to aggregate unlike other reported molecules such as bispecific proteins with Fc-gamma immunoglobulin domains.

**[00110]** In the PSMA targeting trispecific proteins described herein, the domains are linked by internal linkers L1 and L2, where L1 links the first and second domain of the PSMA targeting trispecific proteins and L2 links the second and third domains of the PSMA targeting trispecific

proteins. Linkers L1 and L2 have an optimized length and/or amino acid composition. In some embodiments, linkers L1 and L2 are the same length and amino acid composition. In other embodiments, L1 and L2 are different. In certain embodiments, internal linkers L1 and/or L2 are "short", *i.e.*, consist of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 amino acid residues. Thus, in certain instances, the internal linkers consist of about 12 or less amino acid residues. In the case of 0 amino acid residues, the internal linker is a peptide bond. In certain embodiments, internal linkers L1 and/or L2 are "long", *i.e.*, consist of 15, 20 or 25 amino acid residues. In some embodiments, these internal linkers consist of about 3 to about 15, for example 8, 9 or 10 contiguous amino acid residues. Regarding the amino acid composition of the internal linkers L1 and L2, peptides are selected with properties that confer flexibility to the PSMA targeting trispecific proteins, do not interfere with the binding domains as well as resist cleavage from proteases. For example, glycine and serine residues generally provide protease resistance. Examples of internal linkers suitable for linking the domains in the PSMA targeting trispecific proteins include but are not limited to $(GS)_n$ (SEQ ID NO: 157), $(GGS)_n$ (SEQ ID NO: 158), $(GGGS)_n$ (SEQ ID NO: 159), $(GGSG)_n$ (SEQ ID NO: 160), $(GGSGG)_n$ (SEQ ID NO: 161), or $(GGGGS)_n$ (SEQ ID NO: 162), wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In one embodiment, internal linker L1 and/or L2 is $(GGGGS)_4$ (SEQ ID NO: 163) or $(GGGGS)_3$ (SEQ ID NO: 164).

**PSMA Binding Protein Modifications**

**[00111]** The PSMA binding proteins described herein encompass derivatives or analogs in which (i) an amino acid is substituted with an amino acid residue that is not one encoded by the genetic code, (ii) the mature polypeptide is fused with another compound such as polyethylene glycol, or (iii) additional amino acids are fused to the protein, such as a leader or secretory sequence or a sequence to block an immunogenic domain and/or for purification of the protein.

**[00112]** Typical modifications include, but are not limited to, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

**[00113]** Modifications are made anywhere in PSMA binding proteins described herein, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini. Certain common peptide modifications that are useful for modification of PSMA binding

proteins include glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation, blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, and ADP-ribosylation.

**Polynucleotides Encoding PSMA Binding Proteins**

**[00114]** Also provided, in some embodiments, are polynucleotide molecules encoding a PSMA binding protein as described herein. In some embodiments, the polynucleotide molecules are provided as a DNA construct. In other embodiments, the polynucleotide molecules are provided as a messenger RNA transcript.

**[00115]** The polynucleotide molecules are constructed by known methods such as by combining the genes encoding the anti-PSMA binding protein, operably linked to a suitable promoter, and optionally a suitable transcription terminator, and expressing it in bacteria or other appropriate expression system such as, for example CHO cells.

**[00116]** In some embodiments, the polynucleotide is inserted into a vector, preferably an expression vector, which represents a further embodiment. This recombinant vector can be constructed according to known methods. Vectors of particular interest include plasmids, phagemids, phage derivatives, virii (*e.g.*, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, lentiviruses, and the like), and cosmids.

**[00117]** A variety of expression vector/host systems may be utilized to contain and express the polynucleotide encoding the polypeptide of the described PSMA binding protein. Examples of expression vectors for expression in E.coli are pSKK (Le Gall et al., J Immunol Methods. (2004) 285(1):111-27), pcDNA5 (Invitrogen) for expression in mammalian cells, PICHIAPINK™ Yeast Expression Systems (Invitrogen), BACUVANCE™ Baculovirus Expression System (GenScript).

**[00118]** Thus, the PSMA albumin binding proteins as described herein, in some embodiments, are produced by introducing a vector encoding the protein as described above into a host cell and culturing said host cell under conditions whereby the protein domains are expressed, may be isolated and, optionally, further purified.

**Production of PSMA Binding Proteins**

**[00119]** Disclosed herein, in some embodiments, is a process for the production of a PSMA binding protein. In some embodiments, the process comprises culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding a PSMA binding protein under conditions allowing the expression of the PSMA binding protein and recovering and purifying the produced protein from the culture.

**[00120]** In an additional embodiment is provided a process directed to improving one or more properties, *e.g.*, affinity, stability, heat tolerance, cross-reactivity, etc., of the PSMA binding

proteins and/or the multispecific binding proteins comprising a PSMA binding protein described herein, compared to a reference binding compound. In some embodiments, a plurality of single-substitution libraries is provided each corresponding to a different domain, or amino acid segment of the PSMA binding protein or reference binding compound such that each member of the single-substitution library encodes only a single amino acid change in its corresponding domain, or amino acid segment. Typically, this allows all of the potential substitutions in a large protein or protein binding site to be probed with a few small libraries. In some embodiments, the plurality of domains forms or covers a contiguous sequence of amino acids of the PSMA binding protein or a reference binding compound. Nucleotide sequences of different single-substitution libraries overlap with the nucleotide sequences of at least one other single-substitution library. In some embodiments, a plurality of single-substitution libraries are designed so that every member overlaps every member of each single-substitution library encoding an adjacent domain.

**[00121]** Binding compounds expressed from such single-substitution libraries are separately selected to obtain a subset of variants in each library which has properties at least as good as those of the reference binding compound and whose resultant library is reduced in size. Generally, the number of nucleic acids encoding the selected set of binding compounds is smaller than the number of nucleic acids encoding members of the original single-substitution library. Such properties include, but are not limited to, affinity to a target compound, stability with respect to various conditions such as heat, high or low pH, enzymatic degradation, cross-reactivity to other proteins and the like. The selected compounds from each single-substitution library are referred to herein interchangeably as “pre-candidate compounds,” or “pre-candidate proteins.” Nucleic acid sequences encoding the pre-candidate compounds from the separate single-substitution libraries are then shuffled in a PCR to generate a shuffled library, using PCR-based gene shuffling techniques.

**[00122]** An exemplary work flow of the screening process is described herein. Libraries of pre-candidate compounds are generated from single substitution libraries and selected for binding to the target protein(s), after which the pre-candidate libraries are shuffled to produce a library of nucleic acids encoding candidate compounds which, in turn, are cloned into a convenient expression vector, such as a phagemid expression system. Phage expressing candidate compounds then undergo one or more rounds of selection for improvements in desired properties, such as binding affinity to a target molecule. Target molecules may be adsorbed or otherwise attached to a surface of a well or other reaction container, or target molecules may be derivatized with a binding moiety, such as biotin, which after incubation with candidate binding compounds may be captured with a complementary moiety, such as streptavidin, bound to

beads, such as magnetic beads, for washing. In exemplary selection regimens, the candidate binding compounds undergo a wash step so that only candidate compounds with very low dissociation rates from a target molecule are selected. Exemplary wash times for such embodiments are about 10 minutes, about 15 minutes, about 20 minutes, about 20 minutes, about 30 minutes, about 35 minutes, about 40 minutes, about 45 minutes, about 50 minutes, about 55 mins, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours; or in other embodiments, about 24 hours; or in other embodiments, about 48 hours; or in other embodiments, about 72 hours. Isolated clones after selection are amplified and subjected to an additional cycle of selection or analyzed, for example by sequencing and by making comparative measurements of binding affinity, for example, by ELISA, surface plasmon resonance (SPR), bio-layer interferometry (*e.g.,* OCTET® system, Pall Life Sciences, ForteBio, Menlo Park, CA) or the like.

**[00123]** In some embodiments, the above process is implemented to identify one or more PSMA binding proteins with improved binding affinity, improved cross reactivity to a selected set of binding targets compared to that of a reference PSMA binding protein. In some embodiments, the reference binding protein is a protein having the amino acid sequence as set forth in SEQ ID NO: 4. In some embodiments, the reference binding protein is a protein having the amino acid sequence as set forth in SEQ ID NO: 19. In certain embodiments, single substitution libraries are prepared by varying codons in the VH region of the reference PSMA binding protein, including codons in framework regions and in the CDRs. In another embodiment, the locations where codons are varied comprise the CDRs of the heavy chain of the reference PSMA binding protein, or a subset of such CDRs, such as solely CDR1, solely CDR2, solely CDR3, or pairs thereof. In another embodiment, locations where codons are varied occur solely in framework regions. In some embodiments, a library comprises single codon changes solely from a reference PSMA binding protein solely in framework regions of VH numbering in the range of from 10 to 111. In another embodiment, the locations where codons are varied comprise the CDR3s of the heavy chain of the reference PSMA binding protein, or a subset of such CDR3s. In another embodiment, the number of locations where codons of VH encoding regions are varied are in the range of from 10 to 111, such that up to 80 locations are in framework region. After preparation of the single substitution library, as outlined above, the following steps are carried out: (a) expressing separately each member of each single substitution library as a pre-candidate protein; (b) selecting members of each single substitution library which encode pre-candidate proteins which bind to a binding partner that may or may not differ from the original binding target [*e.g.,* a desired cross-reaction target(s)]; (c) shuffling members of the selected libraries in a PCR to produce a combinatorial shuffled

library; (d) expressing members of the shuffled library as candidate PSMA binding proteins; and (e) selecting members of the shuffled library one or more times for candidate PSMA binding proteins which bind the original binding partner and potentially (f) further selecting the candidate proteins for binding to the desired cross-reactive target(s) thereby providing a nucleic acid encoded PSMA binding protein with increased cross reactivity for the one or more substances with respect to the reference PSMA binding protein without loss of affinity for the original ligand. In additional embodiments, the method may be implemented for obtaining a PSMA binding protein with decreased reactivity to a selected cross-reactive substance(s) or compound(s) or epitope(s) by substituting step (f) with the following step: depleting candidate binding compounds one or more times from the subset of candidate PSMA binding protein which bind to the undesired cross-reactive compound.

**Pharmaceutical Compositions**

**[00124]** Also provided, in some embodiments, are pharmaceutical compositions comprising a PSMA binding protein described herein, a vector comprising the polynucleotide encoding the polypeptide of the PSMA binding proteins or a host cell transformed by this vector and at least one pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" includes, but is not limited to, any carrier that does not interfere with the effectiveness of the biological activity of the ingredients and that is not toxic to the patient to whom it is administered. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Such carriers can be formulated by conventional methods and can be administered to the subject at a suitable dose. Preferably, the compositions are sterile. These compositions may also contain adjuvants such as preservative, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents.

**[00125]** In some embodiments of the pharmaceutical compositions, the PSMA binding protein described herein is encapsulated in nanoparticles. In some embodiments, the nanoparticles are fullerenes, liquid crystals, liposome, quantum dots, superparamagnetic nanoparticles, dendrimers, or nanorods. In other embodiments of the pharmaceutical compositions, the PSMA binding protein is attached to liposomes. In some instances, the PSMA binding protein is conjugated to the surface of liposomes. In some instances, the PSMA binding protein is encapsulated within the shell of a liposome. In some instances, the liposome is a cationic liposome.

**[00126]** The PSMA binding proteins described herein are contemplated for use as a medicament. Administration is effected by different ways, *e.g.,* by intravenous, intraperitoneal,

subcutaneous, intramuscular, topical or intradermal administration. In some embodiments, the route of administration depends on the kind of therapy and the kind of compound contained in the pharmaceutical composition. The dosage regimen will be determined by the attending physician and other clinical factors. Dosages for any one patient depends on many factors, including the patient´s size, body surface area, age, sex, the particular compound to be administered, time and route of administration, the kind of therapy, general health and other drugs being administered concurrently. An "effective dose" refers to amounts of the active ingredient that are sufficient to affect the course and the severity of the disease, leading to the reduction or remission of such pathology and may be determined using known methods.

**Methods of Treatment**

**[00127]** Also provided herein, in some embodiments, are methods and uses for stimulating the immune system of an individual in need thereof comprising administration of a PSMA binding protein or a multispecific binding protein comprising the PSMA binding protein described herein. In some instances, the administration of a PSMA binding protein described herein induces and/or sustains cytotoxicity towards a cell expressing a target antigen. In some instances, the cell expressing a target antigen is a cancer or tumor cell, a virally infected cell, a bacterially infected cell, an autoreactive T or B cell, damaged red blood cells, arterial plaques, or fibrotic tissue.

**[00128]** Also provided herein are methods and uses for a treatment of a disease, disorder or condition associated with a target antigen comprising administering to an individual in need thereof a PSMA binding protein or a multispecific binding protein comprising the PSMA binding protein described herein. Diseases, disorders or conditions associated with a target antigen include, but are not limited to, viral infection, bacterial infection, auto-immune disease, transplant rejection, atherosclerosis, or fibrosis. In other embodiments, the disease, disorder or condition associated with a target antigen is a proliferative disease, a tumorous disease, an inflammatory disease, an immunological disorder, an autoimmune disease, an infectious disease, a viral disease, an allergic reaction, a parasitic reaction, a graft-versus-host disease or a host-versus-graft disease. In one embodiment, the disease, disorder or condition associated with a target antigen is cancer. In one instance, the cancer is a hematological cancer. In another instance, the cancer is a prostate cancer.

**[00129]** In some embodiments, the prostate cancer is an advanced stage prostate cancer. In some embodiments, the prostate cancer is drug resistant. In some embodiments, the prostate cancer is anti-androgen drug resistant. In some embodiments, the prostate cancer is metastatic. In some embodiments, the prostate cancer is metastatic and drug resistant (*e.g.*, anti-androgen drug resistant). In some embodiments, the prostate cancer is castration resistant. In some

embodiments, the prostate cancer is metastatic and castration resistant. In some embodiments, the prostate cancer is enzalutamide resistant. In some embodiments, the prostate cancer is enzalutamide and arbiraterone resistant. In some embodiments, the prostate cancer is enzalutamide, arbiraterone, and bicalutamide resistant. In some embodiments, the prostate cancer is docetaxel resistant. In some of these embodiments, the prostate cancer is enzalutamide, arbiraterone, bicalutamide, and docetaxel resistant.

**[00130]** In some embodiments, administering an anti-PSMA single domain antibody described herein or a PSMA targeting trispecific protein described herein inhibits prostate cancer cell growth; inhibits prostate cancer cell migration; inhibits prostate cancer cell invasion; ameliorates the symptoms of prostate cancer; reduces the size of a prostate cancer tumor; reduces the number of prostate cancer tumors; reduces the number of prostate cancer cells; induces prostate cancer cell necrosis, pyroptosis, oncosis, apoptosis, autophagy, or other cell death; or enhances the therapeutic effects of a compound selected from the group consisting of enzalutamide, abiraterone, docetaxel, bicalutamide, and any combinations thereof.

**[00131]** In some embodiments, the method comprises inhibiting prostate cancer cell growth by administering an anti-PSMA single domain antibody described herein or a PSMA targeting trispecific protein described herein. In some embodiments, the method comprises inhibiting prostate cancer cell migration by administering an anti-PSMA single domain antibody described herein or a PSMA targeting trispecific protein described herein. In some embodiments, the method comprises inhibiting prostate cancer cell invasion by administering an anti-PSMA single domain antibody described herein or a PSMA targeting trispecific protein described herein. In some embodiments, the method comprises ameliorating the symptoms of prostate cancer by administering an anti-PSMA single domain antibody described herein or a PSMA targeting trispecific protein described herein. In some embodiments, the method comprises reducing the size of a prostate cancer tumor by administering an anti-PSMA single domain antibody described herein or a PSMA targeting trispecific protein described herein. In some embodiments, the method comprises reducing the number of prostate cancer tumors by administering an anti-PSMA single domain antibody described herein or a PSMA targeting trispecific protein described herein. In some embodiments, the method comprises reducing the number of prostate cancer cells by administering an anti-PSMA single domain antibody described herein or a PSMA targeting trispecific protein described herein. In some embodiments, the method comprises inducing prostate cancer cell necrosis, pyroptosis, oncosis, apoptosis, autophagy, or other cell death by administering a PSMA targeting trispecific protein described herein.

**[00132]** As used herein, in some embodiments, "treatment" or "treating" or "treated" refers to therapeutic treatment wherein the object is to slow (lessen) an undesired physiological condition, disorder or disease, or to obtain beneficial or desired clinical results. For the purposes described herein, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of the extent of the condition, disorder or disease; stabilization (*i.e.*, not worsening) of the state of the condition, disorder or disease; delay in onset or slowing of the progression of the condition, disorder or disease; amelioration of the condition, disorder or disease state; and remission (whether partial or total), whether detectable or undetectable, or enhancement or improvement of the condition, disorder or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment. In other embodiments, "treatment" or "treating" or "treated" refers to prophylactic measures, wherein the object is to delay onset of or reduce severity of an undesired physiological condition, disorder or disease, such as, for example is a person who is predisposed to a disease (*e.g.*, an individual who carries a genetic marker for a disease such as breast cancer).

**[00133]** In some embodiments of the methods described herein, the PSMA binding proteins or a multispecific binding protein comprising the PSMA binding protein described herein are administered in combination with an agent for treatment of the particular disease, disorder or condition. Agents include but are not limited to, therapies involving antibodies, small molecules (*e.g.*, chemotherapeutics), hormones (steroidal, peptide, and the like), radiotherapies (γ-rays, X-rays, and/or the directed delivery of radioisotopes, microwaves, UV radiation and the like), gene therapies (*e.g.*, antisense, retroviral therapy and the like) and other immunotherapies. In some embodiments, the PSMA binding protein or a multispecific binding protein comprising the PSMA binding protein described herein are administered in combination with anti-diarrheal agents, anti-emetic agents, analgesics, opioids and/or non-steroidal anti-inflammatory agents. In some embodiments, the PSMA binding proteins or a multispecific binding protein comprising a PSMA binding protein as described herein are administered before, during, or after surgery. According to another embodiment of the invention, kits for detecting prostate cancer for diagnosis, prognosis or monitoring are provided. The kits include the foregoing PSMA binding proteins (*e.g.*, labeled anti-PSMA single domain antibodies or antigen binding fragments thereof), and one or more compounds for detecting the label. In some embodiments, the label is selected from the group consisting of a fluorescent label, an enzyme label, a radioactive label, a nuclear magnetic resonance active label, a luminescent label, and a chromophore label.

**[00134]** A further embodiment provides one or more of the above described binding proteins, such as anti-PSMA single domain antibodies or antigen-binding fragments thereof packaged in

lyophilized form, or packaged in an aqueous medium. In another aspect of the disclosure, methods for detecting the presence of PSMA, or a cell expressing PSMA, in a sample are provided. Such methods include contacting the sample with any of the foregoing PSMA binding proteins (such as anti-PSMA single domain antibodies or antigen-binding fragments thereof) which specifically bind to an extracellular domain of PSMA, for a time sufficient to allow the formation of a complex between the antibody or antigen-binding fragment thereof and PSMA, and detecting the PSMA-antibody complex or PSMA-antigen-binding fragment complex. In some embodiments, the presence of a complex in the sample is indicative of the presence in the sample of PSMA or a cell expressing PSMA. In another aspect, the disclosure provides other methods for diagnosing a PSMA-mediated disease in a subject. Such methods include administering to a subject suspected of having or previously diagnosed with PSMA-mediated disease an amount of any of the foregoing PSMA binding proteins (such as anti-PSMA single domain antibodies or antigen-binding fragments thereof) which specifically bind to an extracellular domain of prostate specific membrane antigen. The method also includes allowing the formation of a complex between the antibody or antigen-binding fragment thereof and PSMA, and detecting the formation of the PSMA-antibody complex or PSMA-antigen-binding fragment antibody complex to the target epitope. The presence of a complex in the subject suspected of having or previously diagnosed with prostate cancer is indicative of the presence of a PSMA-mediated disease.

**[00135]** In certain embodiments of the methods, the PSMA-mediated disease is prostate cancer. In other embodiments, the PSMA-mediated disease is a non-prostate cancer, such as those selected from the group consisting of bladder cancer including transitional cell carcinoma; pancreatic cancer including pancreatic duct carcinoma; lung cancer including non-small cell lung carcinoma; kidney cancer including conventional renal cell carcinoma; sarcoma including soft tissue sarcoma; breast cancer including breast carcinoma; brain cancer including glioblastoma multiforme; neuroendocrine carcinoma; colon cancer including colonic carcinoma; testicular cancer including testicular embryonal carcinoma; and melanoma including malignant melanoma.

**[00136]** In some embodiments of the foregoing methods, the PSMA binding proteins (such as anti-PSMA single domain antibodies or antigen-binding fragments thereof is labeled. In other embodiments of the foregoing methods, a second antibody is administered to detect the first antibody or antigen-binding fragment thereof. In a further aspect of the disclosure, methods for assessing the prognosis of a subject with a PSMA-mediated disease are provided. Such methods include administering to a subject suspected of having or previously diagnosed with PSMA-mediated disease an effective amount of any of the foregoing PSMA binding proteins (such as

anti-PSMA single domain antibodies or antigen-binding fragments thereof, allowing the formation of a complex between the antibody or antigen-binding fragment thereof and PSMA, and detecting the formation of the complex to the target epitope. The amount of the complex in the subject suspected of having or previously diagnosed with PSMA-mediated disease is indicative of the prognosis.

**[00137]** In another aspect of the disclosure, methods for assessing the effectiveness of a treatment of a subject with a PSMA-mediated disease are provided. Such methods include administering to a subject of having or previously diagnosed with PSMA-mediated disease an effective amount of any of the foregoing PSMA binding proteins, such as anti-PSMA single domain antibodies or antigen-binding fragments thereof, allowing the formation of a complex between the antibody or antigen-binding fragment thereof and PSMA, and detecting the formation of the complex to the target epitope. The amount of the complex in the subject suspected of having or previously diagnosed with PSMA-mediated disease is indicative of the effectiveness of the treatment. In certain embodiments, the PSMA-mediated disease is prostate cancer. In other embodiments, the PSMA-mediated disease is a non-prostate cancer. In those embodiments, the non-prostate cancer preferably is selected from the group consisting of bladder cancer including transitional cell carcinoma; pancreatic cancer including pancreatic duct carcinoma; lung cancer including non-small cell lung carcinoma; kidney cancer including conventional renal cell carcinoma; sarcoma including soft tissue sarcoma; breast cancer including breast carcinoma; brain cancer including glioblastoma multiforme; neuroendocrine carcinoma; colon cancer including colonic carcinoma; testicular cancer including testicular embryonal carcinoma; and melanoma including malignant melanoma. In still other embodiments, the antibody or antigen-binding fragment thereof is labeled. In further embodiments, a second antibody is administered to detect the first antibody or antigen-binding fragment thereof.

**[00138]** According to yet another aspect of the disclosure, methods for inhibiting the growth of a cell expressing PSMA are provided. Such methods include contacting a cell expressing PSMA with an amount of at least one of the foregoing antibodies or antigen-binding fragments thereof which specifically binds to an extracellular domain of PSMA effective to inhibit the growth of the cell expressing PSMA.

## EXAMPLES

**[00139]** The examples below further illustrate the described embodiments without limiting the scope of the invention.

**Example 1: Generation of Anti-PSMA Single Domain Antibody Variants with Equivalent or Improved Binding Properties to A Parental Anti-PSMA Single Domain Antibody**

*Characterization of Parental anti-PSMA Phage*

**[00140]** Specific binding of the parental anti-PSMA phage to an PSMA antigen was determined, (**Table 1**)

*Single Substitution PSMA sdAb Phage Libraries selected on cyno PSMA*

**[00141]** A single substitution library was provided for each of the three CDR domains. Single substitution libraries were bound to cynomolgus PSMA and then washed in buffer for 30 minutes. Phages bound at 0 and 30 minutes were rescued and counted. Phages selected using a 30 minute wash in the buffer were used to create two independent combinatorial phage libraries.

*Combinatorial Anti-PSMA Libraries*

**[00142]** Cynomolgus PSMA was used as the selection target for three rounds of selection. Wells were washed for 2 to 4 hours after combinatorial phage binding from two independent libraries for three rounds of selection. Inserts PCRed from the third round of selection were subcloned into the p34 expression vector. 96 clones were picked, DNA was purified, sequenced, and transfected into Expi293 cells.

*Single Substitution PSMA sdAb Phage Libraries selected on huPSMA*

**[00143]** A single substitution library was provided for each of the three CDR domains. Single substitution libraries were bound to human PSMA and then washed in buffer containing 30 µg/ml h PSMA-Fc for 24 hours. Phages bound at 0 and 24 hours were rescued and counted. Phages selected using the 24 hour competitive wash were used to create a combinatorial phage library.

*Combinatorial Anti-PSMA Libraries*

**[00144]** Human PSMA was used as the selection target for three rounds of selection. Wells were washed in buffer containing 30 µg/ml – 850 µg/ml human PSMA-Fc for 24 – 96 hours after combinatorial phage binding for three rounds of selection. Inserts PCRed from the third round of selection were subcloned into the p34 expression vector. 96 clones were picked, DNA was purified, sequenced, and transfected into Expi293 cells.

*Binding affinity measurement*

**[00145]** Supernatants were used to estimate Kd, kon, and koff (or kdis) to human and cynomolgus PSMA using the OCTET® system. Several clones were selected for further characterization (**Table 1**), based on their binding affinities, and association and dissociation

rate constants for interaction with human PSMA, compared to the parental sdAb as well as robust production, aggregation and stability profiles. The parental sdAb is listed as Anti-PSMA wt sdAb.6his in Table 1.

**Table 1: Binding Affinity (Kd) of several PSMA binding proteins to human PSMA**

| | Kd (hFc.flag.hPSMA) | kon(1/Ms) | kdis(1/s) |
|---|---|---|---|
| Anti-PSMA wt sdAb.6his | 15.0 nM | 8.77E+05 | 1.32E-02 |
| anti-PSMA E31P sdAb.6his | 9.5 nM | 3.83E+05 | 3.66E-03 |
| anti-PSMA T56Q sdAb.6his | 5.6 nM | 8.22E+05 | 4.61E-03 |
| anti-PSMA G55K sdAb.6his | 4.5 nM | 5.56E+05 | 2.48E-03 |
| anti-PSMA S33H T50D G97SsdAb.6his | 6.7 nM | 8.00E+05 | 5.38E-03 |
| anti-PSMA S33H G97SsdAb.6his | 0.21 nM | 9.36E+05 | 1.97E-05 |

## Example 2: Methods to assess binding and cytotoxic activities of exemplary PSMA targeting trispecific antigen-binding molecules

**[00146]** *Protein Production*

**[00147]** Sequences of trispecific molecules were cloned into mammalian expression vector pcDNA 3.4 (Invitrogen) preceded by a leader sequence and followed by a 6x Histidine Tag (SEQ ID NO: 33). Expi293F cells (Life Technologies A14527) were maintained in suspension in Optimum Growth Flasks (Thomson) between 0.2 to 8 x 1e6 cells/ml in Expi293 media. Purified plasmid DNA was transfected into Expi293 cells in accordance with Expi293 Expression System Kit (Life Technologies, A14635) protocols, and maintained for 4-6 days post transfection. Conditioned media was partially purified by affinity and desalting chromatography. Trispecific proteins were subsequently polished by ion exchange or, alternatively, concentrated with Amicon Ultra centrifugal filtration units (EMD Millipore), applied to Superdex 200 size exclusion media (GE Healthcare) and resolved in a neutral buffer containing excipients. Fraction pooling and final purity were assessed by SDS-PAGE and analytical SEC.

**[00148]** *Affinity Measurements*

**[00149]** The affinities of the all binding domains molecules were measured by biolayer inferometry using an Octet instrument.

**[00150]** PSMA affinities were measured by loading human PSMA-Fc protein (100 nM) onto anti-human IgG Fc biosensors for 120 seconds, followed by a 60 second baseline, after which associations were measured by incubating the sensor tip in a dilution series of the trispecific molecules for 180 seconds, followed by dissociation for 50 seconds. EGFR and CD3 affinities were measured by loading human EGFR-Fc protein or human CD3-Flag-Fc protein,

respectively, (100 nM) onto anti-human IgG Fc biosensors for 120 seconds, followed by a 60 second baseline, after which associations were measured by incubating the sensor tip in a dilution series of the trispecific molecules for 180 seconds, followed by dissociation for 300 seconds. Affinities to human serum albumin (HSA) were measured by loading biotinylated albumin onto streptavidin biosensors, then following the same kinetic parameters as for CD3 affinity measurements. All steps were performed at 30$^0$C in 0.25% casein in phosphate-buffered saline.

**[00151]** *Cytotoxicity assays*

**[00152]** A human T-cell dependent cellular cytotoxicity (TDCC) assay was used to measure the ability of T cell engagers, including trispecific molecules, to direct T cells to kill tumor cells (Nazarian et al. 2015. J Biomol Screen. 20:519-27). In this assay, T cells and target cancer cell line cells are mixed together at a 10:1 ratio in a 384 wells plate, and varying amounts of T cell engager are added. After 48 hours, the T cells are washed away leaving attached to the plate target cells that were not killed by the T cells. To quantitate the remaining viable cells, CellTiter-Glo® Luminescent Cell Viability Assay (Promega) is used. In some cases, the target cells are engineered to express luciferase. In these cases, viability of the target cells is assessed by performing a luminescent luciferase assay with STEADYGLO® reagent (Promega), where viability is directly proportional to the amount of luciferase activity.

**[00153]** *Stability assays*

**[00154]** The stability of the trispecific binding proteins was assessed at low concentrations in the presence of non-human primate serum. TriTACs were diluted to 33 µg/ml in Cynomolgus serum (BioReclamationIVT) and either incubated for 2 d at 37˚C or subjected to five freeze/thaw cycles. Following the treatment, the samples were assessed in cytotoxicity (TDCC) assays and their remaining activity was compared to untreated stock solutions.

**[00155]** *Xenograft assays*

**[00156]** The in vivo efficacy of trispecific binding proteins was assessed in xenograft experiments (Crown Bioscience, Taicang). NOD/SCID mice deficient in the common gamma chain (NCG, Model Animal Research Center of Nanjing University) were inoculated on day 0 with a mixture of 5e6 22Rv1 human prostate cancer cells and 5e6 resting, human T cells that were isolated from a healthy, human donor. The mice were randomized into three groups, and treated with vehicle, 0.5 mg/kg PSMA TriTAC C324 or 0.5 mg/kg PSMA BiTE. Treatments were administered daily for 10 days via i.v. bolus injection. Animals were checked daily for morbidity and mortality. Tumor volumes were determined twice weekly with a caliper. The study was terminated after 30 days.

**[00157]** *PK assays*

**[00158]** The purpose of this study was to evaluate the single dose pharmacokinetics of trispecific binding proteins following intravenous injection. 2 experimentally naïve cynomolgus monkeys per group (1 male and 1 female) were given compound via a slow IV bolus injection administered over approximately 1 minute. Following dose administration, cage side observations were performed once daily and body weights were recorded weekly. Blood samples were collected and processed to serum for pharmacokinetic analysis through 21 days post dose administration.

**[00159]** Concentrations of test articles were determined from monkey serum with an electroluminescent readout (Meso Scale Diagnostics, Rockville). 96 well plates with immobilized, recombinant CD3 were used to capture the analyte. Detection was performed with sulfo-tagged, recombinant PSMA on a MSD reader according to the manufacturer's instructions.

**Example 3: Assessing the impact of CD3 affinity on the properties of exemplary PSMA targeting trispecific molecules**

**[00160]** PSMA targeting trispecific molecules with distinct CD3 binding domains were studied to demonstrate the effects of altering CD3 affinity. An exemplary PSMA targeting trispecific molecule is illustrated in Figure 1. Table 2 lists the affinity of each molecule for the three binding partners (PSMA, CD3, HSA). Affinities were measured by biolayer interferometry using an Octet instrument (Pall Forté Bio). Reduced CD3 affinity leads to a loss in potency in terms of T cell mediated cellular toxicity (Figures 2A-C). The pharmacokinetic properties of these trispecific molecules were assessed in cynomolgus monkeys. Molecules with high affinity for CD3 like TriTAC C236 have a terminal half-life of approx. 90 h (Figure 3). Despite the altered ability to bind CD3 on T cells, the terminal half-life of two molecules with different CD3 affinities shown in Figure 4 is very similar. However, the reduced CD3 affinity appears to lead to a larger volume of distribution, which is consistent with reduced sequestration of trispecific molecule by T cells. There were no adverse clinical observations or body weight changes noted during the study period.

Table 2: Binding Affinities for Human and Cynomolgus Antigens

| | anti-PSMA KD value (nM) | | | anti-Albumin KD value (nM) | | | anti-CD3e $K_D$ value (nM) | | |
|---|---|---|---|---|---|---|---|---|---|
| | human | cyno | ratio cyno/ hum | pHSA | CSA | ratio cyno/ hum | human | cyno | ratio cyno/ hum |
| Tool TriTAC high aff. - C236 | 16.3 | 0 | 0 | 22.7 | 25.4 | 1.1 | 6.0 | 4.7 | 0.8 |
| TriTAC CD3 high aff. - C324 | 17.9 | 0 | 0 | 9.8 | 9.7 | 1 | 7.4 | 5.8 | 0.8 |
| TriTAC CD3 med aff. - C339 | 13.6 | 0 | 0 | 8.8 | 8.3 | 0.9 | 40.6 | 33.6 | 0.8 |
| TriTAC CD3 low aff - C325 | 15.3 | 0 | 0 | 10.1 | 9.7 | 1 | 217 | 160 | 0.7 |

**Example 4: Assessing the impact of PSMA affinity on the properties of exemplary PSMA targeting trispecific molecules**

**[00161]** PSMA targeting trispecific molecules with distinct PSMA binding domains were studied to demonstrate the effects of altering PSMA affinity. Table 3 lists the affinity of each molecule for the three binding partners (PSMA, CD3, HSA). Reduced PSMA affinity leads to a loss in potency in terms of T cell mediated cellular toxicity (Figures 5A-C).

Table 3: Binding Affinities for Human and Cynomolgus Antigens

| | anti-PSMA KD value (nM) | | | anti-Albumin KD value (nM) | | | anti-CD3e $K_D$ value (nM) | | |
|---|---|---|---|---|---|---|---|---|---|
| | human | cyno | ratio cyno/ hum | pHSA | CSA | ratio cyno/ hum | human | cyno | ratio cyno/ hum |
| PSMA-TriTAC (p8)-C362 | 22.0 | 0 | n/a | 6.6 | 6.6 | 1.0 | 8.3 | 4.3 | 0.52 |
| PSMA TriTAC (HDS) – C363 | 3.7 | 540 | 146 | 7.6 | 8.4 | 1.1 | 8.0 | 5.2 | 0.65 |
| PSMA TriTAC (HTS)- C364 | 0.15 | 663 | 4423 | 8.4 | 8.6 | 1.0 | 7.7 | 3.8 | 0.49 |

**Example 5: *In vivo* efficacy of exemplary PSMA targeting trispecific molecules**

**[00162]** The exemplary PSMA targeting trispecific molecule C324 was assessed for its ability to inhibit the growth of tumors in mice. For this experiment, immunocompromised mice reconstituted with human T cells were subcutaneously inoculated with PSMA expressing human prostate tumor cells (22Rv1) and treated daily for 10 days with 0.5 mg/kg i.v. of either PSMA targeting BiTE or TriTAC molecules. Tumor growth was measured for 30. Over the course of the experiment, the trispecific molecule was able to inhibit tumor growth with an efficacy comparable to a BiTE molecule (Figure 6).

**Example 6: Specificity of exemplary PSMA targeting trispecific molecules**

**[00163]** In order to assess the specificity of PSMA targeting TriTAC molecules, their ability to induce T cells to kill tumor cells was tested with tumor cells that are negative for PSMA (Figure 7A). An EGFR targeting TriTAC molecule served as positive control, a GFP targeting TriTAC molecule as negative control. All three TriTACs with distinct PSMA binding domains showed the expected activity against the PSMA positive cell line LNCaP (Figure 7B), but did not reach EC50s in the PSMA negative tumor cell lines KMS12BM and OVCAR8 (Figures 7C and 7D). The EC50s are summarized in Table 4. At very high TriTAC concentrations (> 1 nM), some limited off-target cell killing could be observed for TriTACs C362 and C363, while C364 did not show significant cell killing under any of the tested conditions.

Table 4: Cell killing activity of TriTAC molecules in with antigen positive and negative tumor cell lines (EC50 [pM])

| TriTAC | LNCaP | KMS12BM | OVCAR8 |
|---|---|---|---|
| PSMA p8 TriTAC C362 | 13.0 | >10,000 | >10,000 |
| PSMA HDS TriTAC C363 | 6.2 | >10,000 | >10,000 |
| PSMA HTS TriTAC C364 | 0.8 | >10,000 | >10,000 |
| EGFR TriTAC C131 | 9.4 | >10,000 | 6 |
| GFP TriTAC C | >10,000 | >10,000 | >10,000 |

**Example 7: Stress tests and protein stability**

**[00164]** Four PSMA targeting trispecific molecules were either incubated for 48 h in Cynomolgus serum at low concentrations (33.3 µg/ml) or subjected to five freeze thaw cycles in Cynomolgus serum. After the treatment, the bio-activity of the TriTAC molecules was assessed in cell killing assays and compared to unstressed samples ("positive control", Figure 8A-D). All molecules maintained the majority of their cell killing activity. TriTAC C362 was the most stress resistant and did not appear to lose any activity under the conditions tested here.

**Example 8: Xenograft Tumor Model**

**[00165]** An exemplary PSMA targeting trispecific protein is evaluated in a xenograft model.

**[00166]** Male immune-deficient NCG mice are subcutaneously inoculated with 5 x$10^6$ 22Rv1 cells into their right dorsal flank. When tumors reach 100 to 200 $mm^3$, animals are allocated into 3 treatment groups. Groups 2 and 3 (8 animals each) are intraperitoneally injected with 1.5x$10^7$ activated human T-cells. Three days later, animals from Group 3 are subsequently treated with a total of 9 intravenous doses of 50 µg of the exemplary PSMA trispecific antigen-binding protein of this disclosure (qdx9d). Groups 1 and 2 are only treated with vehicle. Body weight and tumor volume are determined for 30 days. It is expected that tumor growth in mice treated with the PSMA trispecific antigen-binding protein is significantly reduced in comparison to the tumor growth in respective vehicle-treated control group.

**Example 9: Proof-of-Concept Clinical Trial Protocol for Administration of an exemplary PSMA Trispecific Antigen-Binding Protein to Prostate Cancer Patients**

**[00167]** This is a Phase I/II clinical trial for studying the PSMA trispecific antigen-binding protein of Example 1 as a treatment for Prostate Cancer.

**[00168]** Study Outcomes:

**[00169]** *Primary*: Maximum tolerated dose of PSMA targeting trispecific proteins of the previous examples

**[00170]** *Secondary*: To determine whether in vitro response of PSMA targeting trispecific proteins of is the previous examples are associated with clinical response

**[00171] Phase I**

**[00172]** The maximum tolerated dose (MTD) will be determined in the phase I section of the trial.

1.1 The maximum tolerated dose (MTD) will be determined in the phase I section of the trial.

1.2 Patients who fulfill eligibility criteria will be entered into the trial to PSMA targeting trispecific proteins of the previous examples.

1.3 The goal is to identify the highest dose of PSMA targeting trispecific proteins of the previous examples that can be administered safely without severe or unmanageable side effects in participants. The dose given will depend on the number of participants who have been enrolled in the study prior and how well the dose was tolerated. Not all participants will receive the same dose.

**[00173] Phase II**

2.1 A subsequent phase II section will be treated at the MTD with a goal of determining if therapy with therapy of PSMA targeting trispecific proteins of the previous examples results in at least a 20% response rate.

Primary Outcome for the Phase II ---To determine if therapy of PSMA targeting trispecific proteins of the previous examples results in at least 20% of patients achieving a clinical response (blast response, minor response, partial response, or complete response)

**[00174] Eligibility:**

Histologically confirmed newly diagnosed aggressive prostate cancer according to the current World Health Organisation Classification, from 2001 to 2007

Any stage of disease.

Treatment with docetaxel and prednisone (+/- surgery).

Age $\geq$ 18 years

Karnofsky performance status $\geq$ 50% or ECOG performance status 0-2

Life expectancy $\geq$ 6 weeks

**Example 10: Activity of an exemplary PSMA antigen-binding protein (PSMA targeting TriTAC molecule) in redirected T cell killing assays using a panel of PSMA expressing cell lines and T cells from different donors**

**[00175]** This study was carried out to demonstrate that the activity of the exemplary PSMA trispecific antigen-binding protein is not limited to LNCaP cells or a single cell donor.

**[00176]** Redirected T cell killing assays were performed using T cells from four different donors and the human PSMA-expressing prostate cancer cell lines VCaP, LNCaP, MDAPCa2b, and 22Rv1. With one exception, the PSMA trispecific antigen-binding protein was able to direct killing of these cancer cell lines using T cells from all donors with $EC_{50}$ values of 0.2 to 1.5 pM, as shown in Table 5. With the prostate cancer cell line 22 Rv1 and Donor 24, little to no killing was observed (data not shown). Donor 24 also only resulted approximately 50% killing of the MDAPCa2b cell line whereas T cells from the other 3 donors resulted in almost complete killing of this cell line (data not shown). Control assays demonstrated that killing by the PSMA trispecific antigen-binding protein was PSMA specific. No killing was observed when PSMA-expressing cells were treated with a control trispecific protein targeting green fluorescent protein (GFP) instead of PSMA (data not shown). Similarly, the PSMA trispecific antigen-binding protein was inactive with cell lines that lack PSMA expression, NCI-1563 and HCT116, also shown in Table 5.

Table 5: $EC_{50}$ Values from TDCC Assays with Six Human Cancer Cell Lines and Four Different T Cell Donors

| Cell Line | TDCC $EC_{50}$ Values (M) | | | |
|---|---|---|---|---|
| | **Donor 24** | **Donor 8144** | **Donor 72** | **Donor 41** |
| **LNCaP** | 1.5E-12 | 2.2E-13 | 3.6E-13 | 4.3E-13 |
| **MDAPCa2b** | 4.8E-12 | 4.1E-13 | 4.9E-13 | 6.5E-13 |
| **VCaP** | 6.4E-13 | 1.6E-13 | 2.0E-13 | 3.5E-13 |
| **22Rv1** | n/a | 7.2E-13 | 1.4E-12 | 1.3E-12 |
| **HCT116** | >1.0E-8 | >1.0E-8 | >1.0E-8 | >1.0E-8 |
| **NCI-1563** | >1.0E-8 | >1.0E-8 | >1.0E-8 | >1.0E-8 |

## Example 11: Stimulation of cytokine expression in by an exemplary PSMA trispecific antigen-binding protein (PSMA targeting TriTAC molecule) in redirected T cell killing assays

**[00177]** This study was carried out to demonstrate activation of T cells by the exemplary PSMA trispecific antigen-binding protein during redirected T cell killing assays by measuring secretion of cytokine into the assay medium by activated T cells.

**[00178]** Conditioned media collected from redirected T cell killing assays, as described above in Example 9, were analyzed for expression of the cytokines TNFα and IFNγ. Cytokines were measured using AlphaLISA assays (Perkin-Elmer). Adding a titration of the PSMA antigen-binding protein to T cells from four different donors and four PSMA-expressing cell lines,

LNCaP, VCaP, MDAPCa2b, and 22Rv1 resulted in increased levels of TNFα. The results for TNFα expression and IFN γ expression levels in the conditioned media are shown in Tables 6 and 7, respectively. The $EC_{50}$ values for the PSMA antigen-binding protein induced expression of these cytokines ranged from 3 to 15 pM. Increased cytokine levels were not observed with a control trispecific protein targeting GFP. Similarly, when assays were performed with two cell lines that lack PSMA expression, HCT116 and NCI-H1563, PSMA HTS TriTAC also did not increase TNFα or IFNγ expression.

Table 6: $EC_{50}$ Values for TNFα Expression in Media from PSMA Trispecific Antigen-Binding Protein TDCC Assays with Six Human Cancer Cell Lines and T Cells from Four Different Donors

| Cell Line | Donor 24 | Donor 8144 | Donor 41 | Donor72 |
|---|---|---|---|---|
| LNCaP | 4.9E-12 | 2.8E-12 | 4.0E-12 | 3.2E-12 |
| VCaP | 3.2E-12 | 2.9E-12 | 2.9E-12 | 2.9E-12 |
| MDAPCa2b | 2.1E-11 | 4.0E-12 | 5.5E-12 | 3.6E-12 |
| 22Rv1 | 8.9E-12 | 2.5E-12 | 4.0E-12 | 3.3E-12 |
| HCT116 | >1E-8 | >1E-8 | >1E-8 | >1E-8 |
| NCI-H1563 | >1E-8 | >1E-8 | >1E-8 | >1E-8 |

Table 7: $EC_{50}$ Values for IFNγ Expression in Media from PSMA Trispecific Antigen-Binding Protein TDCC Assays with Six Human Cancer Cell Lines and T Cells from Four Different Donors

| Cell Line | Donor 24 | Donor 8144 | Donor 41 | Donor72 |
|---|---|---|---|---|
| LNCaP | 4.2E-12 | 4.2E-12 | 4.2E-12 | 2.8E-12 |
| VCaP | 5.1E-12 | 1.5E-11 | 3.4E-12 | 4.9E-12 |
| MDAPCa2b | 1.5E-11 | 5.8E-12 | 9.7E-12 | 3.5E-12 |
| 22Rv1 | 7.8E-12 | 3.0E-12 | 9.1E-12 | 3.0E-12 |
| HCT116 | >1E-8 | >1E-8 | >1E-8 | >1E-8 |
| NCI-H1563 | >1E-8 | >1E-8 | >1E-8 | >1E-8 |

**Example 12: Activity of an exemplary PSMA trispecific antigen-binding protein (PSMA targeting TriTAC) in redirected T cell killing assay (TDCC) using T cells from cynomolgus monkeys**

**[00179]** This study was carried out to test the ability of the exemplary PSMA trispecific antigen-binding protein to direct T cells from cynomolgus monkeys to kill PSMA-expressing cell lines.

**[00180]** TDCC assays were set up using peripheral blood mononuclear cells (PBMCs) from cynomolgus monkeys. Cyno PBMCs were added to LNCaP cells at a 10:1 ratio. It was

observed that the PSMA trispecific antigen-binding protein redirected killing of LNCaP by the cyno PBMCs with an $EC_{50}$ value of 11 pM. The result is shown in Figure 9A. To confirm these results, a second cell line was used, MDAPCa2b, and PBMCs from a second cynomolgus monkey donor were tested. Redirected killing of the target cells was observed with an $EC_{50}$ value of 2.2 pM. The result is shown in Figure 9B. Killing was specific to the anti-PMSA arm of the PSMA trispecific antigen-binding protein as killing was not observed with a negative control trispecific protein targeting GFP. These data demonstrate that the PSMA antigen-binding trispecific protein can direct cynomolgus T cells to kill target cells expressing human PSMA.

**Example 13: Expression of markers of T cell activation in redirect T cell killing assays with an exemplary PSMA trispecific antigen-binding protein (PSMA targeting TriTAC molecule)**

**[00181]** This study was performed to assess whether T cells were activated when the exemplary PSMA trispecific antigen-binding protein directed the T cells to kill target cells.

**[00182]** The assays were set up using conditions for the redirected T cell killings assays described in the above example. T cell activation was assessed by measuring expression of CD25 and CD69 on the surface of the T cells using flow cytometry. The PSMA trispecific antigen-binding protein was added to a 10:1 mixture of purified human T cells and the prostate cancer cell line VCaP. Upon addition of increasing amounts of the PSMA trispecific antigen-binding protein, increased CD69 expression and CD25 expression was observed, as shown in Figure 10. $EC_{50}$ value was 0.3 pM for CD69 and 0.2 pM for CD25. A trispecific protein targeting GFP was included in these assays as negative control, and little to no increase in CD69 or CD25 expression is observed with the GFP targeting trispecific protein, also shown in Figure 10.

**Example 14: Stimulation of T cell proliferation by an exemplary PSMA trispecific antigen-binding protein (PSMA targeting TriTAC molecule) in the presence of PSMA expressing target cells**

**[00183]** This study was used as an additional method to demonstrate that the exemplary PSMA trispecific antigen-binding protein was able to activate T cells when it redirects them to kill target cells.

**[00184]** T cell proliferation assays were set up using the conditions of the T cell redirected killing assay using LNCaP target cells, as described above, and measuring the number of T cells present at 72 hours. The exemplary PSMA trispecific antigen-binding protein stimulated proliferation with an $EC_{50}$ value of 0.5 pM. As negative control, a trispecific protein targeting

GFP was included in the assay, and no increased proliferation was observed with this protein. The results for the T cell proliferation assay are illustrated in Figure 11.

**Example 15: Redirected T cell killing of LNCaP cells by an exemplary PSMA trispecific antigen-binding proteins (PSMA targeting TriTAC molecule Z2)**

**[00185]** This study was carried out to test the ability of an exemplary PSMA trispecific antigen-binding protein, having the sequence as set forth in SEQ ID Nos: 156, to redirect T cells to kill the LNCaP cell line.

**[00186]** In TDCC assays, set up as described in above examples, the PSMA Z2 TriTAC (SEQ ID NO: 156) protein directed killing with an $EC_{50}$ value of 0.8 pM, as shown in Figure 12.

Table 8

| SEQ ID Nos. | Sequence |
|---|---|
| SEQ ID NO: 1 | RFMISX$_1$YX$_2$MH |
| SEQ ID NO: 2 | X$_3$INPAX$_4$X$_5$TDYAEX$_6$VKG |
| SEQ ID NO: 3 | DX$_7$YGY |
| SEQ ID NO: 4 | EVQLVESGGGLVQPGGSLTLSCAASRFMISEYSMHWVRQAPGKGLEWVS TINPAGTTDYAESVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCDGY GYRGQGTQVTVSS |
| SEQ ID NO: 5 | RFMISEYHMH |
| SEQ ID NO: 6 | RFMISPYSMH |
| SEQ ID NO: 7 | RFMISPYHMH |
| SEQ ID NO: 8 | DINPAGTTDYAESVKG |
| SEQ ID NO: 9 | TINPAKTTDYAESVKG |
| SEQ ID NO: 10 | TINPAGQTDYAESVKG |
| SEQ ID NO: 11 | TINPAGTTDYAEYVKG |
| SEQ ID NO: 12 | DINPAKTTDYAESVKG |
| SEQ ID NO: 13 | DINPAGQTDYAESVKG |
| SEQ ID NO: 14 | DINPAGTTDYAEYVKG |
| SEQ ID NO: 15 | DSYGY |
| SEQ ID NO: 16 | RFMISEYSMH |
| SEQ ID NO: 17 | TINPAGTTDYAESVKG |
| SEQ ID NO: 18 | DGYGY |
| SEQ ID NO: 19 | EVQLVESGGGLVQPGGSLRLSCAASRFMISEYSMHWVRQAPGKGLEWVS TINPAGTTDYAESVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCDGY GYRGQGTLVTVSS |
| SEQ ID NO: 20 | MWNLLHETDSAVATARRPRWLCAGALVLAGGFFLLGFLFGWFIKSSNEA |

| | |
|---|---|
| | TNITPKHNMKAFLDELKAENIKKFLYNFTQIPHLAGTEQNFQLAKQIQSQ WKEFGLDSVELAHYDVLLSYPNKTHPNYISIINEDGNEIFNTSLFEPPPPGY ENVSDIVPPFSAFSPQGMPEGDLVYVNYARTEDFFKLERDMKINCSGKIVI ARYGKVFRGNKVKNAQLAGAKGVILYSDPADYFAPGVKSYPDGWNLPG GGVQRGNILNLNGAGDPLTPGYPANEYAYRRGIAEAVGLPSIPVHPIGYY DAQKLLEKMGGSAPPDSSWRGSLKVPYNVGPGFTGNFSTQKVKMHIHST NEVTRIYNVIGTLRGAVEPDRYVILGGHRDSWVFGGIDPQSGAAVVHEIV RSFGTLKKEGWRPRRTILFASWDAEEFGLLGSTEWAEENSRLLQERGVAY INADSSIEGNYTLRVDCTPLMYSLVHNLTKELKSPDEGFEGKSLYESWTK KSPSPEFSGMPRISKLGSGNDFEVFFQRLGIASGRARYTKNWETNKFSGYP LYHSVYETYELVEKFYDPMFKYHLTVAQVRGGMVFELANSIVLPFDCRD YAVVLRKYADKIYSISMKHPQEMKTYSVSFDSLFSAVKNFTEIASKFSERL QDFDKSNPIVLRMMNDQLMFLERAFIDPLGLPDRPFYRHVIYAPSSHNKY AGESFPGIYDALFDIESKVDPSKAWGEVKRQIYVAAFTVQAAAETLSEVA |
| SEQ ID NO: 21 | EVQLVESGGGLVQPGGSLRLSCAASRFMISEYHMHWVRQAPGKGLEWV SDINPAGTTDYAESVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCDS YGYRGQGTLVTVSS |
| SEQ ID NO: 22 | EVQLVESGGGLVQPGGSLRLSCAASRFMISEYHMHWVRQAPGKGLEWV STINPAGTTDYAESVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCDSY GYRGQGTLVTVSS |
| SEQ ID NO: 23 | EVQLVESGGGLVQPGGSLRLSCAASRFMISEYSMHWVRQAPGKGLEWVS TINPAKTTDYAESVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCDSY GYRGQGTLVTVSS |
| SEQ ID NO: 24 | EVQLVESGGGLVQPGGSLRLSCAASRFMISPYSMHWVRQAPGKGLEWVS TINPAGTTDYAESVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCDGY GYRGQGTLVTVSS |
| SEQ ID NO: 25 | EVQLVESGGGLVQPGGSLRLSCAASRFMISEYSMHWVRQAPGKGLEWVS TINPAGQTDYAESVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCDGY GYRGQGTLVTVSS |
| SEQ ID NO: 26 | EVQLVESGGGLVQPGGSLRLSCAASRFMISEYSMHWVRQAPGKGLEWVS TINPAGTTDYAEYVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCDGY GYRGQGTLVTVSS |
| SEQ ID NO: 27 | EVQLVESGGGLVQPGGSLRLSCAASRFMISEYHMHWVRQAPGKGLEWV SDINPAKTTDYAESVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCDS YGYRGQGTLVTVSS |
| SEQ ID NO: 28 | EVQLVESGGGLVQPGGSLRLSCAASRFMISPYHMHWVRQAPGKGLEWV SDINPAGTTDYAESVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCDS YGYRGQGTLVTVSS |

| | |
|---|---|
| SEQ ID NO: 29 | EVQLVESGGGLVQPGGSLRLSCAASRFMISEYHMHWVRQAPGKGLEWV SDINPAGQTDYAESVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCDS YGYRGQGTLVTVSS |
| SEQ ID NO: 30 | EVQLVESGGGLVQPGGSLRLSCAASRFMISEYHMHWVRQAPGKGLEWV SDINPAGTTDYAEYVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCDS YGYRGQGTLVTVSS |
| SEQ ID NO: 31 | EVQLVESGGGLVQPGGSLTLSCAASRFMISEYHMHWVRQAPGKGLEWV SDINPAGTTDYAESVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCDSY GYRGQGTQVTVSS |
| SEQ ID NO: 32 | EVQLVESGGGLVQPGGSLTLSCAASRFMISEYHMHWVRQAPGKGLEWV STINPAGTTDYAESVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCDSY GYRGQGTQVTVSS |

Table 9: CD3 Binding Domain Sequences

| **SEQ ID NO:** | **Description** | **AA Sequence** |
|---|---|---|
| 34 | Anti-CD3, clone 2B2 | EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAINWVRQAPGKG LEWVARIRSKYNNYATYYADQVKDRFTISRDDSKNTAYLQMNN LKTEDTAVYYCVRHANFGNSYISYWAYWGQGTLVTVSSGGGGS GGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCASSTGAVTSGNY PNWVQQKPGQAPRGLIGGTKFLVPGTPARFSGSLLGGKAALTLS GVQPEDEAEYYCTLWYSNRWVFGGGTKLTVL |
| 35 | Anti-CD3, clone 9F2 | EVQLVESGGGLVQPGGSLKLSCAASGFEFNKYAMNWVRQAPGK GLEWVARIRSKYNKYATYYADSVKDRFTISRDDSKNTAYLQMN NLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSFGAVTSGNY PNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLS GVQPEDEAEYYCVLWYDNRWVFGGGTKLTVL |
| 36 | Anti-CD3, clone 5A2 | EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGK GLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMN NLKTEDTAVYYCVRHGNFGNSHISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGYVTSGN YPNWVQQKPGQAPRGLIGGTSFLAPGTPARFSGSLLGGKAALTLS GVQPEDEAEYYCVLWYSNRWIFGGGTKLTVL |
| 37 | Anti-CD3, clone 6A2 | EVQLVESGGGLVQPGGSLKLSCAASGFMFNKYAMNWVRQAPGK GLEWVARIRSKSNNYATYYADSVKDRFTISRDDSKNTAYLQMNN LKTEDTAVYYCVRHGNFGNSYISYWATWGQGTLVTVSSGGGGS GGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSFGAVTSGNYP NWVQQKPGQAPRGLIGGTKLLAPGTPARFSGSLLGGKAALTLSG VQPEDEAEYYCVLWYSNSWVFGGGTKLTVL |

| SEQ ID NO: | Description | AA Sequence |
|---|---|---|
| | | |
| 38 | Anti-CD3, clone 2D2 | EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGK GLEWVARIRSKYNNYATYYKDSVKDRFTISRDDSKNTAYLQMN NLKTEDTAVYYCVRHGNFGNSPISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVVSGN YPNWVQQKPGQAPRGLIGGTEFLAPGTPARFSGSLLGGKAALTLS GVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL |
| 39 | Anti-CD3, clone 3F2 | EVQLVESGGGLVQPGGSLKLSCAASGFTYNKYAMNWVRQAPGK GLEWVARIRSKYNNYATYYADEVKDRFTISRDDSKNTAYLQMN NLKTEDTAVYYCVRHGNFGNSPISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSKGAVTSGN YPNWVQQKPGQAPRGLIGGTKELAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCTLWYSNRWVFGGGTKLTVL |
| 40 | Anti-CD3, clone 1A2 | EVQLVESGGGLVQPGGSLKLSCAASGNTFNKYAMNWVRQAPGK GLEWVARIRSKYNNYETYYADSVKDRFTISRDDSKNTAYLQMN NLKTEDTAVYYCVRHTNFGNSYISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGY YPNWVQQKPGQAPRGLIGGTYFLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL |
| 41 | Anti-CD3, clone 1C2 | EVQLVESGGGLVQPGGSLKLSCAASGFTFNNYAMNWVRQAPGK GLEWVARIRSKYNNYATYYADAVKDRFTISRDDSKNTAYLQMN NLKTEDTAVYYCVRHGNFGNSQISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTDGN YPNWVQQKPGQAPRGLIGGIKFLAPGTPARFSGSLLGGKAALTLS GVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL |
| 42 | Anti-CD3, clone 2E4 | EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAVNWVRQAPGK GLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMN NLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGESTGAVTSGN YPNWVQQKPGQAPRGLIGGTKILAPGTPARFSGSLLGGKAALTLS GVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL |
| 43 | Anti-CD3, clone 10E4 | EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYPMNWVRQAPGK GLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMN NLKNEDTAVYYCVRHGNFNNSYISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTKGN YPNWVQQKPGQAPRGLIGGTKMLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCALWYSNRWVFGGGTKLTVL |
| 44 | Anti-CD3, clone 2H2 | EVQLVESGGGLVQPGGSLKLSCAASGFTFNGYAMNWVRQAPGK |

| SEQ ID NO: | Description | AA Sequence |
|---|---|---|
| | | GLEWVARIRSKYNNYATYYADEVKDRFTISRDDSKNTAYLQMN NLKTEDTAVYYCVRHGNFGNSPISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVVSGN YPNWVQQKPGQAPRGLIGGTEFLAPGTPARFSGSLLGGKAALTLS GVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL |
| 45 | Anti-CD3, clone 2A4 | EVQLVESGGGLVQPGGSLKLSCAASGNTFNKYAMNWVRQAPGK GLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMN NLKTEDTAVYYCVRHGNFGDSYISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTHGN YPNWVQQKPGQAPRGLIGGTKVLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL |
| 46 | Anti-CD3, clone 10B2 | EVQLVESGGGLVQPGGSLKLSCAASGFTFNNYAMNWVRQAPGK GLEWVARIRSGYNNYATYYADSVKDRFTISRDDSKNTAYLQMN NLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSYTGAVTSGN YPNWVQQKPGQAPRGLIGGTKFNAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYANRWVFGGGTKLTVL |
| 47 | Anti-CD3, clone 1G4 | EVQLVESGGGLVQPGGSLKLSCAASGFEFNKYAMNWVRQAPGK GLEWVARIRSKYNNYETYYADSVKDRFTISRDDSKNTAYLQMN NLKTEDTAVYYCVRHGNFGNSLISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSSGAVTSGNY PNWVQQKPGQAPRGLIGGTKFGAPGTPARFSGSLLGGKAALTLS GVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL |
| 48 | wt anti-CD3 | EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGK GLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMN NLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGN YPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL |
| 49 | wt anti-CD3 HC CDR1 | GFTFNKYAMN |
| 50 | wt anti-CD3 HC CDR2 | RIRSKYNNYATYYADSVK |
| 51 | wt anti-CD3 HC CDR3 | HGNFGNSYISYWAY |
| 53 | wt anti-CD3 LC CDR1 | GSSTGAVTSGNYPN |
| 54 | wt anti-CD3 LC CDR2 | GTKFLAP |
| 55 | wt anti-CD3 LC CDR3 | VLWYSNRWV |
| 56 | HC CDR1 variant 1 | GNTFNKYAMN |
| 57 | HC CDR1 variant 2 | GFEFNKYAMN |
| 58 | HC CDR1 variant 3 | GFMFNKYAMN |
| 59 | HC CDR1 variant 4 | GFTYNKYAMN |

| SEQ ID NO: | Description | AA Sequence |
|---|---|---|
| 60 | HC CDR1 variant 5 | GFTFNNYAMN |
| 61 | HC CDR1 variant 6 | GFTFNGYAMN |
| 62 | HC CDR1 variant 7 | GFTFNTYAMN |
| 63 | HC CDR1 variant 8 | GFTFNEYAMN |
| 64 | HC CDR1 variant 9 | GFTFNKYPMN |
| 65 | HC CDR1 variant 10 | GFTFNKYAVN |
| 66 | HC CDR1 variant 11 | GFTFNKYAIN |
| 67 | HC CDR1 variant 12 | GFTFNKYALN |
| 68 | HC CDR2 variant 1 | RIRSGYNNYATYYADSVK |
| 69 | HC CDR2 variant 2 | RIRSKSNNYATYYADSVK |
| 70 | HC CDR2 variant 3 | RIRSKYNKYATYYADSVK |
| 71 | HC CDR2 variant 4 | RIRSKYNNYETYYADSVK |
| 72 | HC CDR2 variant 5 | RIRSKYNNYATEYADSVK |
| 73 | HC CDR2 variant 6 | RIRSKYNNYATYYKDSVK |
| 74 | HC CDR2 variant 7 | RIRSKYNNYATYYADEVK |
| 75 | HC CDR2 variant 8 | RIRSKYNNYATYYADAVK |
| 76 | HC CDR2 variant 9 | RIRSKYNNYATYYADQVK |
| 77 | HC CDR2 variant 10 | RIRSKYNNYATYYADDVK |
| 78 | HC CDR3 variant 1 | HANFGNSYISYWAY |
| 79 | HC CDR3 variant 2 | HTNFGNSYISYWAY |
| 80 | HC CDR3 variant 3 | HGNFNNSYISYWAY |
| 81 | HC CDR3 variant 4 | HGNFGDSYISYWAY |
| 82 | HC CDR3 variant 5 | HGNFGNSHISYWAY |
| 83 | HC CDR3 variant 6 | HGNFGNSPISYWAY |
| 84 | HC CDR3 variant 7 | HGNFGNSQISYWAY |
| 85 | HC CDR3 variant 8 | HGNFGNSLISYWAY |
| 86 | HC CDR3 variant 9 | HGNFGNSGISYWAY |
| 87 | HC CDR3 variant 10 | HGNFGNSYISYWAT |
| 88 | LC CDR1 variant 1 | ASSTGAVTSGNYPN |
| 89 | LC CDR1 variant 2 | GESTGAVTSGNYPN |
| 90 | LC CDR1 variant 3 | GSYTGAVTSGNYPN |
| 91 | LC CDR1 variant 4 | GSSFGAVTSGNYPN |
| 92 | LC CDR1 variant 5 | GSSKGAVTSGNYPN |
| 93 | LC CDR1 variant 6 | GSSSGAVTSGNYPN |
| 94 | LC CDR1 variant 7 | GSSTGYVTSGNYPN |
| 95 | LC CDR1 variant 8 | GSSTGAVVSGNYPN |
| 96 | LC CDR1 variant 9 | GSSTGAVTDGNYPN |

| SEQ ID NO: | Description | AA Sequence |
|---|---|---|
| 97 | LC CDR1 variant 10 | GSSTGAVTKGNYPN |
| 98 | LC CDR1 variant 11 | GSSTGAVTHGNYPN |
| 99 | LC CDR1 variant 12 | GSSTGAVTVGNYPN |
| 100 | LC CDR1 variant 13 | GSSTGAVTSGYYPN |
| 101 | LC CDR2 variant 1 | GIKFLAP |
| 102 | LC CDR2 variant 2 | GTEFLAP |
| 103 | LC CDR2 variant 3 | GTYFLAP |
| 104 | LC CDR2 variant 4 | GTSFLAP |
| 105 | LC CDR2 variant 5 | GTNFLAP |
| 106 | LC CDR2 variant 6 | GTKLLAP |
| 107 | LC CDR2 variant 7 | GTKELAP |
| 108 | LC CDR2 variant 8 | GTKILAP |
| 109 | LC CDR2 variant 9 | GTKMLAP |
| 110 | LC CDR2 variant 10 | GTKVLAP |
| 111 | LC CDR2 variant 11 | GTKFNAP |
| 112 | LC CDR2 variant 12 | GTKFGAP |
| 113 | LC CDR2 variant 13 | GTKFLVP |
| 114 | LC CDR3 variant 1 | TLWYSNRWV |
| 115 | LC CDR3 variant 2 | ALWYSNRWV |
| 116 | LC CDR3 variant 3 | VLWYDNRWV |
| 117 | LC CDR3 variant 4 | VLWYANRWV |
| 118 | LC CDR3 variant 5 | VLWYSNSWV |
| 119 | LC CDR3 variant 6 | VLWYSNRWI |
| 120 | LC CDR3 variant 7 | VLWYSNRWA |
| 121 | Anti-CD3, clone 2G5 | EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYALNWVRQAPGK GLEWVARIRSKYNNYATEYADSVKDRFTISRDDSKNTAYLQMN NLKTEDTAVYYCVRHGNFGNSPISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGN YPNWVQQKPGQAPRGLIGGTNFLAPGTPERFSGSLLGGKAALTLS GVQPEDEAEYYCVLWYSNRWAFGGGTKLTVL |
| 122 | Anti-CD3, clone 8A5 | EVQLVESGGGLVQPGGSLKLSCAASGFTFNEYAMNWVRQAPGK GLEWVARIRSKYNNYATYYADDVKDRFTISRDDSKNTAYLQMN NLKTEDTAVYYCVRHGNFGNSGISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTVGN YPNWVQQKPGQAPRGLIGGTEFLAPGTPARFSGSLLGGKAALTLS GVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL |

Table 10: HSA Binding Domain Sequences

| SEQ ID NO: | Description | AA Sequence |
|---|---|---|
| 123 | Anti-HSA sdAb clone 6C | EVQLVESGGGLVQPGNSLRLSCAASGFTFSRFGMSWVRQAPGKGL EWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTA VYYCTIGGSLSRSSQGTLVTVSS |
| 124 | Anti-HSA sdAb clone 7A | EVQLVESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGL EWVSSISGSGADTLYADSLKGRFTISRDNAKTTLYLQMNSLRPEDT AVYYCTIGGSLSKSSQGTLVTVSS |
| 125 | Anti-HSA sdAb clone 7G | EVQLVESGGGLVQPGNSLRLSCAASGFTYSSFGMSWVRQAPGKGL EWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTA VYYCTIGGSLSKSSQGTLVTVSS |
| 126 | Anti-HSA sdAb clone 8H | EVQLVESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGL EWVSSISGSGTDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDT AVYYCTIGGSLSRSSQGTLVTVSS |
| 127 | Anti-HSA sdAb clone 9A | EVQLVESGGGLVQPGNSLRLSCAASGFTFSRFGMSWVRQAPGKGL EWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTA VYYCTIGGSLSKSSQGTLVTVSS |
| 128 | Anti-HSA sdAb clone 10G | EVQLVESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGL EWVSSISGSGRDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDT AVYYCTIGGSLSVSSQGTLVTVSS |
| 129 | wt anti-HSA | EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTA VYYCTIGGSLSRSSQGTLVTVSS |
| 130 | wt anti-HSA CDR1 | GFTFSSFGMS |
| 131 | wt anti-HSA CDR2 | SISGSGSDTLYADSVK |
| 132 | wt anti-HSACDR3 | GGSLSR |
| 133 | CDR1 variant 1 | GFTFSRFGMS |
| 134 | CDR1 variant 2 | GFTFSKFGMS |
| 135 | CDR1 variant 3 | GFTYSSFGMS |
| 136 | CDR2 variant 1 | SISGSGADTLYADSLK |
| 137 | CDR2 variant 2 | SISGSGTDTLYADSVK |
| 138 | CDR2 variant 3 | SISGSGRDTLYADSVK |
| 139 | CDR2 variant 4 | SISGSGSDTLYAESVK |
| 140 | CDR2 variant 5 | SISGSGTDTLYAESVK |
| 141 | CDR2 variant 6 | SISGSGRDTLYAESVK |
| 142 | CDR3 variant 1 | GGSLSK |
| 143 | CDR3 variant 2 | GGSLSV |
| 144 | Anti-HSA sdAb clone 6CE | EVQLVESGGGLVQPGNSLRLSCAASGFTFSRFGMSWVRQAPGKGL EWVSSISGSGSDTLYAESVKGRFTISRDNAKTTLYLQMNSLRPEDTA VYYCTIGGSLSRSSQGTLVTVSS |

| SEQ ID NO: | Description | AA Sequence |
|---|---|---|
| 145 | Anti-HSA sdAb clone 8HE | EVQLVESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGL EWVSSISGSGTDTLYAESVKGRFTISRDNAKTTLYLQMNSLRPEDTA VYYCTIGGSLSRSSQGTLVTVSS |
| 146 | Anti-HSA sdAb clone 10GE | EVQLVESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGL EWVSSISGSGRDTLYAESVKGRFTISRDNAKTTLYLQMNSLRPEDTA VYYCTIGGSLSVSSQGTLVTVSS |

Table 11: PSMA Targeting Trispecific Protein Sequences

| SEQ ID NO: | C-Number | Construct | Sequence |
|---|---|---|---|
| 147 | C00324 | PSMA TriTAC CD3 high aff. | EVQLVESGGGLVQPGGSLTLSCAASRFMISEYSMHWVRQAPG KGLEWVSTINPAGTTDYAESVKGRFTISRDNAKNTLYLQMNS LKPEDTAVYYCDGYGYRGQGTQVTVSSGGGGSGGGSEVQLV ESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGLE WVSSISGSGRDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPE DTAVYYCTIGGSLSVSSQGTLVTVSSGGGGSGGGSEVQLVES GGGLVQPGGSLKLSCAASGFTFNKYAINWVRQAPGKGLEWV ARIRSKYNNYATYYADQVKDRFTISRDDSKNTAYLQMNNLK TEDTAVYYCVRHANFGNSYISYWAYWGQGTLVTVSSGGGGS GGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCASSTGAVTSG NYPNWVQQKPGQAPRGLIGGTKFLVPGTPARFSGSLLGGKAA LTLSGVQPEDEAEYYCTLWYSNRWVFGGGTKLTVLHHHHHH |
| 148 | C00339 | PSMA TriTAC CD3 med. aff. | EVQLVESGGGLVQPGGSLTLSCAASRFMISEYSMHWVRQAPG KGLEWVSTINPAGTTDYAESVKGRFTISRDNAKNTLYLQMNS LKPEDTAVYYCDGYGYRGQGTQVTVSSGGGGSGGGSEVQLV ESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGLE WVSSISGSGRDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPE DTAVYYCTIGGSLSVSSQGTLVTVSSGGGGSGGGSEVQLVES GGGLVQPGGSLKLSCAASGFTFNNYAMNWVRQAPGKGLEW VARIRSGYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNL KTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSYTGAVTS GNYPNWVQQKPGQAPRGLIGGTKFNAPGTPARFSGSLLGGKA ALTLSGVQPEDEAEYYCVLWYANRWVFGGGTKLTVLHHHH HH |
| 149 | C00325 | PSMA TriTAC CD3 low aff. | EVQLVESGGGLVQPGGSLTLSCAASRFMISEYSMHWVRQAPG KGLEWVSTINPAGTTDYAESVKGRFTISRDNAKNTLYLQMNS LKPEDTAVYYCDGYGYRGQGTQVTVSSGGGGSGGGSEVQLV ESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGLE WVSSISGSGRDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPE DTAVYYCTIGGSLSVSSQGTLVTVSSGGGGSGGGSEVQLVES GGGLVQPGGSLKLSCAASGFEFNKYAMNWVRQAPGKGLEW VARIRSKYNNYETYYADSVKDRFTISRDDSKNTAYLQMNNLK TEDTAVYYCVRHGNFGNSLISYWAYWGQGTLVTVSSGGGGS GGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSSGAVTSG NYPNWVQQKPGQAPRGLIGGTKFGAPGTPARFSGSLLGGKAA LTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLHHHHHH |

| SEQ ID NO: | C-Number | Construct | Sequence |
|---|---|---|---|
| 150 | C00236 | Tool PSMA TriTAC | EVQLVESGGGLVQPGGSLTLSCAASRFMISEYSMHWVRQAPG KGLEWVSTINPAGTTDYAESVKGRFTISRDNAKNTLYLQMNS LKPEDTAVYYCDGYGYRGQGTQVTVSSGGGGSGGGSEVQLV ESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEW VSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPED TAVYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGG GLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVA RIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTE DTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGGGGSG GGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGN YPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAAL TLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLHHHHHH |
| 151 | C00362 | PSMA p8 TriTAC | EVQLVESGGGLVQPGGSLRLSCAASRFMISEYSMHWVRQAPG KGLEWVSTINPAGTTDYAESVKGRFTISRDNAKNTLYLQMNS LRAEDTAVYYCDGYGYRGQGTLVTVSSGGGGSGGGSEVQLV ESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGLE WVSSISGSGRDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPE DTAVYYCTIGGSLSVSSQGTLVTVSSGGGGSGGGSEVQLVES GGGLVQPGGSLKLSCAASGFTFNKYAINWVRQAPGKGLEWV ARIRSKYNNYATYYADQVKDRFTISRDDSKNTAYLQMNNLK TEDTAVYYCVRHANFGNSYISYWAYWGQGTLVTVSSGGGGS GGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCASSTGAVTSG NYPNWVQQKPGQAPRGLIGGTKFLVPGTPARFSGSLLGGKAA LTLSGVQPEDEAEYYCTLWYSNRWVFGGGTKLTVLHHHHHH |
| 152 | C00363 | PSMA HDS TriTAC C363 | EVQLVESGGGLVQPGGSLTLSCAASRFMISEYHMHWVRQAP GKGLEWVSDINPAGTTDYAESVKGRFTISRDNAKNTLYLQMN SLKPEDTAVYYCDSYGYRGQGTQVTVSSGGGGSGGGSEVQL VESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGLE WVSSISGSGRDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPE DTAVYYCTIGGSLSVSSQGTLVTVSSGGGGSGGGSEVQLVES GGGLVQPGGSLKLSCAASGFTFNKYAINWVRQAPGKGLEWV ARIRSKYNNYATYYADQVKDRFTISRDDSKNTAYLQMNNLK TEDTAVYYCVRHANFGNSYISYWAYWGQGTLVTVSSGGGGS GGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCASSTGAVTSG NYPNWVQQKPGQAPRGLIGGTKFLVPGTPARFSGSLLGGKAA LTLSGVQPEDEAEYYCTLWYSNRWVFGGGTKLTVLHHHHHH |
| 153 | C00364 | PSMA HTS TriTAC C364 | EVQLVESGGGLVQPGGSLTLSCAASRFMISEYHMHWVRQAP GKGLEWVSTINPAGTTDYAESVKGRFTISRDNAKNTLYLQMN SLKPEDTAVYYCDSYGYRGQGTQVTVSSGGGGSGGGSEVQL VESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGLE WVSSISGSGRDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPE DTAVYYCTIGGSLSVSSQGTLVTVSSGGGGSGGGSEVQLVES GGGLVQPGGSLKLSCAASGFTFNKYAINWVRQAPGKGLEWV ARIRSKYNNYATYYADQVKDRFTISRDDSKNTAYLQMNNLK TEDTAVYYCVRHANFGNSYISYWAYWGQGTLVTVSSGGGGS GGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCASSTGAVTSG NYPNWVQQKPGQAPRGLIGGTKFLVPGTPARFSGSLLGGKAA LTLSGVQPEDEAEYYCTLWYSNRWVFGGGTKLTVLHHHHHH |

| SEQ ID NO: | C-Number | Construct | Sequence |
|---|---|---|---|
| 154 | C00298 | PSMA BiTE | QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVRQAP GKGLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSLYLQMN SLKAEDTAVYYCARGFPLLRHGAMDYWGQGTLVTVSSGGG GSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCKASQNVD TNVAWYQQKPGQAPKSLIYSASYRYSDVPSRFSGSASGTDFTL TISSVQSEDFATYYCQQYDSYPYTFGGGTKLEIKSGGGGSEVQ LVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKG LEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQM NNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSG GGGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGA VTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLG GKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLHH HHHH |
| 155 | C00131 | EGFR TriTAC | QVKLEESGGGSVQTGGSLRLTCAASGRTSRSYGMGWFRQAP GKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQM NSLKPEDTAIYYCAAAAGSAWYGTLYEYDYWGQGTQVTVSS GGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNA KTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSSGGG GSGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMN WVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDD SKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWG QGTLVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTVSPGGTV TLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGT PARFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFG GGTKLTVLHHHHHH |
| 156 | C00410 | PSMA Z2 TriTAC | EVQLVESGGGLVQPGGSLTLSCAASRFMISEYHMHWVRQAP GKGLEWVSTINPAGTTDYAESVKGRFTISRDNAKNTLYLQMN SLRAEDTAVYYCDSYGYRGQGTLVTVSSGGGGSGGGSEVQL VESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGLE WVSSISGSGRDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPE DTAVYYCTIGGSLSVSSQGTLVTVSSGGGGSGGGSEVQLVES GGGLVQPGGSLKLSCAASGFTFNKYAINWVRQAPGKGLEWV ARIRSKYNNYATYYADQVKDRFTISRDDSKNTAYLQMNNLK TEDTAVYYCVRHANFGNSYISYWAYWGQGTLVTVSSGGGGS GGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCASSTGAVTSG NYPNWVQQKPGQAPRGLIGGTKFLVPGTPARFSGSLLGGKAA LTLSGVQPEDEAEYYCTLWYSNRWVFGGGTKLTVLHHHHHH |

Table 12: Exemplary Framework Sequences

| SEQ ID NO: | Description | Sequence |
|---|---|---|
| 165 | Framework (f1) | EVQLVESGGGLVQPGGSLTLSCAAS |
| 166 | Framework (f2) | WVRQAPGKGLEWVS |
| 167 | Framework (f3) | RFTISRDNAKNTLYLQMNSLRAEDTAVYYC |
| 168 | Framework (f4) | DGYGYRGQGTLVTVSS |

## CLAIMS

WHAT IS CLAIMED IS:

1. A prostate specific membrane antigen binding protein, comprising complementarity determining regions CDR1, CDR2, and CDR3, wherein

(a) the amino acid sequence of CDR1 is as set forth in RFMIS$X_1$Y$X_2$MH (SEQ ID NO: 1);

(b) the amino acid sequence of CDR2 is as set forth in $X_3$INPA$X_4$$X_5$TDYAE$X_6$VKG (SEQ ID NO: 2); and

(c) the amino acid sequence of CDR3 is as set forth in D$X_7$YGY (SEQ ID NO: 3).

2. The prostate specific membrane antigen binding protein of claim 1, wherein said protein comprises the following formula:

f1-r1-f2-r2-f3-r3-f4

wherein, r1 is SEQ ID NO: 1; r2 is SEQ ID NO: 2; and r3 is SEQ ID NO: 3; and wherein $f_1$, $f_2$, $f_3$ and $f_4$ are framework residues selected so that said protein is at least eighty percent identical to the amino acid sequence set forth in SEQ ID NO: 4.

3. The prostate specific membrane antigen binding protein of claim 2, wherein $X_1$ is proline.

4. The prostate specific membrane antigen binding protein of claim 2, wherein $X_2$ is histidine.

5. The prostate specific membrane antigen binding protein of claim 2, wherein $X_3$ is aspartic acid.

6. The prostate specific membrane antigen binding protein of claim 2, wherein $X_4$ is lysine.

7. The prostate specific membrane antigen binding protein of claim 2, wherein $X_5$ is glutamine.

8. The prostate specific membrane antigen binding protein of claim 2, wherein $X_6$ is tyrosine.

9. The prostate specific membrane antigen binding protein of claim 2, wherein $X_7$ is serine.

10. The prostate specific membrane antigen binding protein of any one of claims 1-9, wherein the binding protein has a higher affinity towards a human prostate specific membrane antigen than that of a binding protein which has the sequence set forth as SEQ ID NO: 4.

11. The prostate specific membrane antigen binding protein of any one of claims 1-9, wherein $X_1$ is proline.

12. The prostate specific membrane antigen binding protein of any one of claims 1-9, wherein $X_5$ is glutamine.

13. The prostate specific membrane antigen binding protein of any one of claims 1-9, wherein $X_6$ is tyrosine

14. The prostate specific membrane antigen binding protein of any one of claims 1-9, wherein $X_4$ is lysine, and $X_7$ is serine.

15. The prostate specific membrane antigen binding protein of any one of claim 1-9, wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_4$ is lysine, and $X_7$ is serine.

16. The prostate specific membrane antigen binding protein of any one of claims 1-9, wherein $X_1$ is proline, $X_2$ is histidine, $X_3$ is aspartic acid, and $X_7$ is serine.

17. The prostate specific membrane antigen binding protein of any one claims 1-9, wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_5$ is glutamine, and $X_7$ is serine.

18. The prostate specific membrane antigen binding protein of any one claims 1-9, wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_6$ is tyrosine, and $X_7$ is serine.

19. The prostate specific membrane antigen binding protein of any one of claims 1-9, wherein $X_2$ is histidine, and $X_7$ is serine.

20. The prostate specific membrane antigen binding protein of any one of claims 1-9, wherein $X_2$ is histidine, $X_3$ is aspartic acid, and $X_7$ is serine.

21. The prostate specific membrane antigen binding protein of any one of claims 11-20, wherein the binding protein has a higher affinity towards a human prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4.

22. The prostate specific membrane antigen binding protein of any one of claims 19-21, wherein the binding protein further has a higher affinity towards a cynomolgus prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4.

23. The prostate specific membrane antigen binding protein of any one of claims 1-22, wherein r1 comprises SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7.

24. The prostate specific membrane antigen binding protein of any one of claims 1-23, wherein r2 comprises SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14.

25. The prostate specific membrane antigen binding protein of any one of claims 1-24, wherein r3 comprises SEQ ID NO: 15.

26. A prostate specific membrane antigen binding protein comprising CDR1, CDR2, and CDR3, comprising the sequence set forth as SEQ ID NO: 4 wherein one or more amino acid residues selected from amino acid positions 31, 33, 50, 55, 56, 62, and 97 are substituted.

27. The prostate specific membrane antigen binding protein of claim 26, comprising one or more additional substitutions at amino acid positions other than positions 31, 33, 50, 55, 56, 62, and 97.

28. The prostate specific membrane antigen binding protein of claim 26 or 27, comprising substitution at position 31.

29. The prostate specific membrane antigen binding protein of claim 26 or 27, comprising substitution at position 33.

30. The prostate specific membrane antigen binding protein of claim 26 or 27, comprising substitution at position 50.

31. The prostate specific membrane antigen binding protein of claim 26 or 27, comprising substitution at position 55.

32. The prostate specific membrane antigen binding protein of claim 26 or 27, comprising substitution at position 56.

33. The prostate specific membrane antigen binding protein of claim 26 or 27, comprising substitution at position 62.

34. The prostate specific membrane antigen binding protein of claim 26 or 27, comprising substitution at position 97.

35. The prostate specific membrane antigen binding protein of claim 26 or 27, comprising substitutions at amino acid positions 55 and 97.

36. The prostate specific membrane antigen binding protein of any one of claims 28-35, wherein the binding protein has a higher affinity towards human prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4.

37. The specific membrane antigen binding protein of claim 26 or 27, comprising substitutions at amino acid positions 33 and 97.

38. The specific membrane antigen binding protein of claim 26 or 27, comprising substitutions at amino acid positions 33, 50, and 97.

39. The prostate specific membrane antigen binding protein of claim 37 or 38, wherein the binding protein has a higher affinity towards human prostate specific membrane antigen than that of a binding protein which has the sequence set forth as SEQ ID NO: 4.

40. The prostate specific membrane antigen binding protein of claim 37 or 38, wherein the binding protein further has a higher affinity towards cynomolgus prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4.

41. The prostate specific membrane antigen binding protein of claim 26 or 27, comprising substitutions at amino acid positions 31, 33, 50, and 97.

42. The prostate specific membrane antigen binding protein of claim 26 or 27, comprising substitutions at amino acid positions 33, 50, 55, and 97.

43. The prostate specific membrane antigen binding protein of claim 26 or 27, comprising substitutions in amino acid positions 33, 50, 56, and 97.

44. The prostate specific membrane antigen binding protein of claim 26 or 27, comprising substitutions at amino acid positions 33, 50, 62, and 97.

45. A prostate specific membrane antigen binding protein comprising a CDR1, CDR2 and CDR3, wherein CDR1 comprises the sequence as set forth is SEQ ID NO: 16.

46. A prostate specific membrane antigen binding protein comprising a CDR1, CDR2 and CDR3, wherein CDR2 comprises the sequence as set forth in SEQ ID NO: 17.

47. A prostate specific membrane antigen binding protein comprising a CDR1, CDR2 and CDR3, wherein CDR3 comprises the sequence as set forth in SEQ ID NO: 18.

48. A prostate specific membrane antigen binding protein comprising a sequence that is at least 80% identical to the sequence set forth in SEQ ID NO: 4.

49. A prostate specific membrane antigen binding protein comprising a CDR1, CDR2 and CDR3, wherein CDR1 has at least 80% identity to SEQ ID NO: 16, CDR2 has at least 85% identity to SEQ ID NO: 17, and CDR3 has at least 80% identity to SEQ ID NO: 18.

50. A prostate specific membrane antigen binding protein comprising a CDR1, CDR2 and CDR3, wherein CDR1 comprises the sequence set forth in SEQ ID NO: 16, CDR2 comprises the sequence set forth in SEQ ID NO: 17, and CDR3 comprises the sequence set forth in SEQ ID NO: 18.

51. The prostate specific membrane antigen binding protein of any one of claims 1-50, wherein said binding protein binds to one or both of human prostate specific membrane antigen and cynomolgus prostate specific membrane antigen.

52. The prostate specific membrane antigen binding protein of any one of claims 1-51, wherein said binding protein binds to human prostate specific membrane antigen and cynomolgus prostate specific membrane antigen with comparable binding affinities.

53. The prostate specific membrane antigen binding protein of any one of claims 1-51, wherein said binding protein binds to human prostate specific membrane antigen with a higher binding affinity than cynomolgus prostate specific membrane antigen.

54. A polynucleotide encoding a PSMA binding protein according to any one of claims 1-53.

55. A vector comprising the polynucleotide of claim 54.

56. A host cell transformed with the vector according to claim 55.

57. A pharmaceutical composition comprising (i) a PSMA binding protein according to any one of claims 1-53, the polynucleotide according to claim 54, the vector according to claim 55 or the host cell according to claim 56, and (ii) a pharmaceutically acceptable carrier.

58. A process for the production of a PSMA binding protein according to any one of claims 1-53, said process comprising culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding a PSMA albumin binding protein according to any one of claims 1-53 under conditions allowing the expression of the PSMA binding protein and recovering and purifying the produced protein from the culture.

59. A method for the treatment or amelioration of a proliferative disease, a tumorous disease, an inflammatory disease, an immunological disorder, an autoimmune disease, an infectious disease, a viral disease, an allergic reaction, a parasitic reaction, a graft-versus-host disease or a host-versus-graft disease comprising the administration of the PSMA binding protein according to any one of claims 1-53, to a subject in need thereof.

60. The method of claim 59, wherein the subject is human.

61. The method of claim 60, wherein the method further comprises administration of an agent in combination with the PSMA binding protein according to any one of claims 1-53.

62. A multispecific binding protein comprising the PSMA binding protein according to any one of claim 1, claim 26, or claims 45-50.

63. An antibody comprising the PSMA binding protein according to claim 1, claim 26, or claims 45-50.

64. A multispecific antibody, a bispecific antibody, an sdAb, a variable heavy domain, a peptide, or a ligand, comprising the PSMA binding protein according to claim 1, claim 26, or claims 45-50.

65. An antibody comprising the PSMA binding protein according to claim 1, claim 26, or claims 45-50, wherein said antibody is a single domain antibody.

66. The single domain antibody of claim 64, wherein said antibody is derived from a heavy chain variable region of IgG.

67. A multispecific binding protein or antibody comprising the PSMA binding protein according to any one of claim 1, claim 26, and claims 45-50 and a CD3 binding domain.

68. A method for the treatment or amelioration of a proliferative disease, a tumorous disease, an inflammatory disease, an immunological disorder, an autoimmune disease, an infectious disease, a viral disease, an allergic reaction, a parasitic reaction, a graft-versus-host disease or a host-versus-graft disease comprising administration of the multispecific antibody according to any one of claims 64-68, to a subject in need thereof.

69. A method for the treatment or amelioration of a prostate condition comprising administration of the multispecific antibody according to any one of claims 64-68, to a subject in need thereof.

70. A method for the treatment or amelioration of a prostate condition comprising administration of the PSMA binding protein according to any one of claims 1-53, to a subject in need thereof.

71. The prostate specific membrane antigen binding protein of claim 1, comprising any combination of the following:

(i) wherein $X_1$ is proline;

(ii) wherein X2 is histidine;

(iii) wherein X3 is aspartic acid;

(iv) whereinX4 is lysine;

(v) wherein X5 is glutamine;

(vi) wherein X6 is tyrosine; and

(vii) wherein X7 is serine.

72. The prostate specific membrane antigen binding protein of claim 71, wherein the binding protein has a higher affinity towards a human prostate specific membrane antigen than that of a binding protein which has the sequence set forth as SEQ ID NO: 4.

73. The prostate specific membrane antigen binding protein of claim 1, comprising any combination of the following:

(i) wherein $X_1$ is proline; wherein $X_5$ is glutamine;

(ii) wherein $X_6$ is tyrosine; wherein $X_4$ is lysine and $X_7$ is serine;

(iii) wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_4$ is lysine, and $X_7$ is serine;

(iv) wherein $X_1$ is proline, $X_2$ is histidine, $X_3$ is aspartic acid, and $X_7$ is serine;

(v) wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_5$ is glutamine, and $X_7$ is serine;

(vi) wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_4$ is lysine, and $X_7$ is serine;

(vii) wherein $X_1$ is proline, $X_2$ is histidine, $X_3$ is aspartic acid, and $X_7$ is serine;

(viii) wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_5$ is glutamine, and $X_7$ is serine;

(ix) wherein $X_2$ is histidine, $X_3$ is aspartic acid, $X_6$ is tyrosine, and $X_7$ is serine; and

(x) wherein $X_2$ is histidine, $X_3$ is aspartic acid, and $X_7$ is serine.

74. The prostate specific membrane antigen binding protein of claim 73, wherein the binding protein has a higher affinity towards a human prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4.

75. The prostate specific membrane antigen binding protein of claim 74, wherein the binding protein further has a higher affinity towards a cynomolgus prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4.

76. The prostate specific membrane antigen binding protein of claim 26, comprising any combination of the following:

(i) substitution at position 31;

(ii) substitution at position 50;

(iii) substitution at position 55; substitution at position 56;

(iv) substitution at position 62;

(v) substitution at position 97;

(vi) substitutions at positions 55 and 97;

(vii) substitutions at positions 33 and 97;

(viii) substitutions at 33, 50, and 97;

(ix) substitutions at positions 31, 33, 50, and 97;

(x) substitutions at positions 33, 50, 55, and 97;

(xi) substitutions at positions 33, 50, 56, and 97; and

(xiii) substitutions at positions 33, 50, 62, and 97.

77. The prostate specific membrane antigen binding protein of claim 76, wherein the binding protein has a higher affinity towards human prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4.

78. The prostate specific membrane antigen binding protein of claim 77, wherein the binding protein further has a higher affinity towards cynomolgus prostate specific membrane antigen than that of a binding protein which has the sequence set forth in SEQ ID NO: 4.

79. A method for the treatment or amelioration of prostate cancer, the method comprising administration of the PSMA binding protein comprising complementarity determining regions CDR1, CDR2, and CDR3, wherein

(a) the amino acid sequence of CDR1 is as set forth in RFMISX$_1$YX$_2$MH (SEQ ID NO: 1);

(b) the amino acid sequence of CDR2 is as set forth in X$_3$INPAX$_4$X$_5$TDYAEX$_6$VKG (SEQ ID NO: 2); and

(c) the amino acid sequence of CDR3 is as set forth in DX$_7$YGY (SEQ ID NO: 3), to a subject in need thereof.

80. An antibody comprising the PSMA binding protein according to claim 1, wherein said antibody is a single domain antibody.

81. The antibody comprising the PSMA binding protein according to claim 80, wherein said single domain antibody is part of a trispecific antibody.

82. An antibody comprising the PSMA binding protein according to claim 26, wherein said antibody is a single domain antibody.

83. The antibody comprising the PSMA binding protein according to claim 82, wherein said single domain antibody is part of a trispecific antibody.

84. An antibody comprising the PSMA binding protein according to claim 49, wherein said antibody is a single domain antibody.

85. The antibody comprising the PSMA binding protein according to claim 84, wherein said single domain antibody is part of a trispecific antibody.

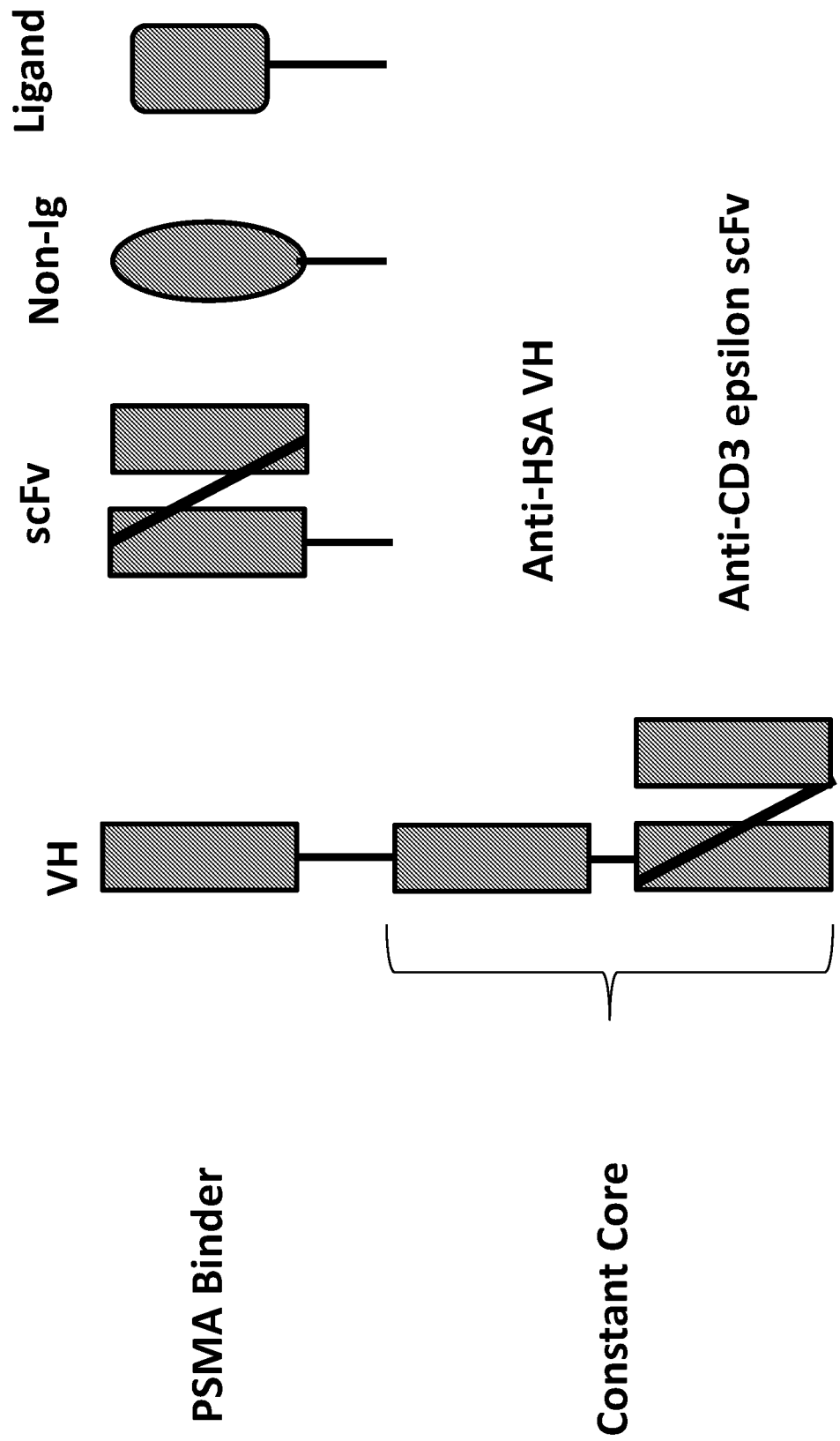

Ligand
Non-Ig
scFv
Anti-HSA VH
Anti-CD3 epsilon scFv
VH
PSMA Binder
Constant Core

Figure 1

**Figure 2A**

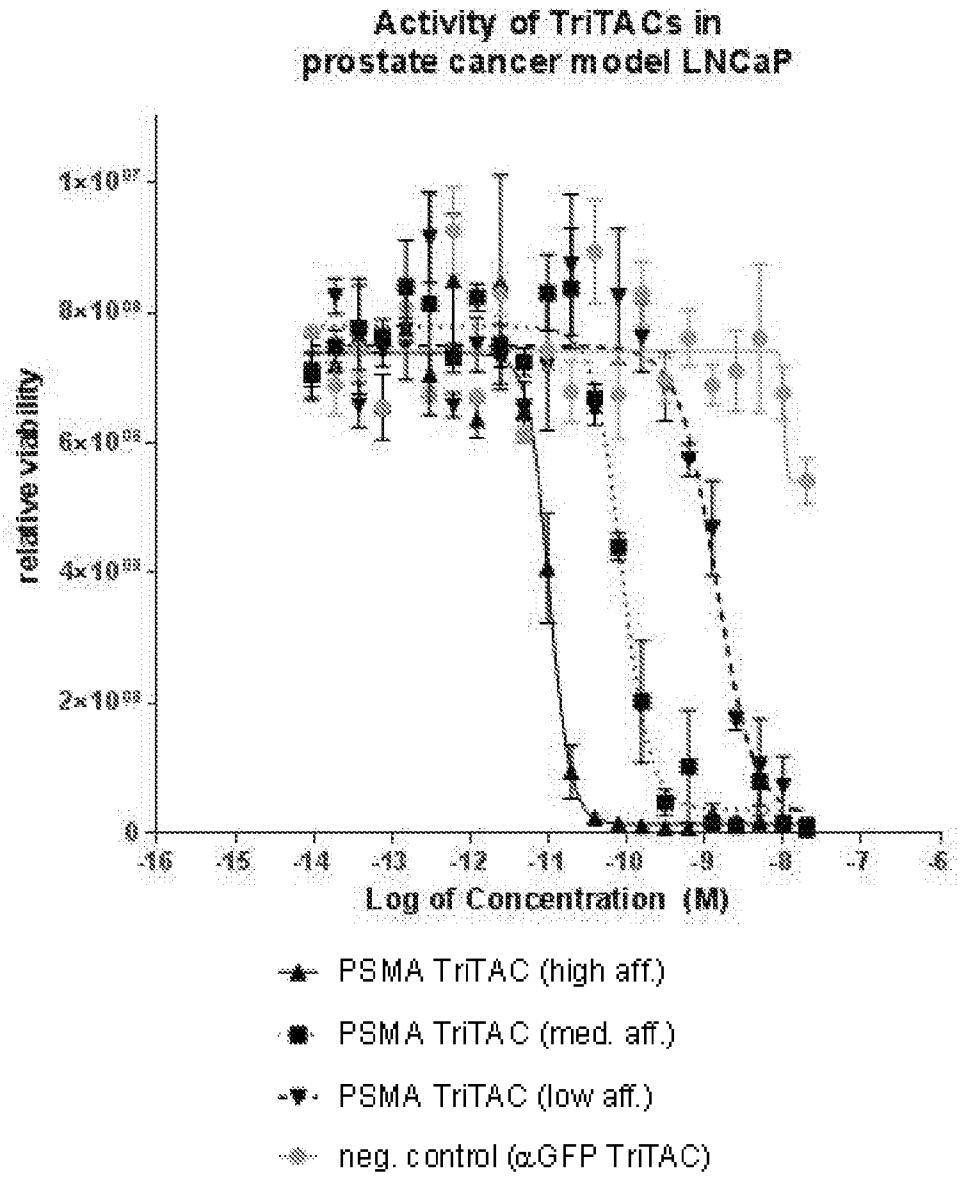

Activity of TriTACs in prostate cancer model LNCaP
relative viability
1x10^07
8x10^06
6x10^06
4x10^06
2x10^06
0
-16
-15
-14
-13
-12
-11
-10
-9
-8
-7
-6
Log of Concentration (M)
PSMA TriTAC (high aff.)
PSMA TriTAC (med. aff.)
PSMA TriTAC (low aff.)
neg. control (αGFP TriTAC)

**Figure 2B**

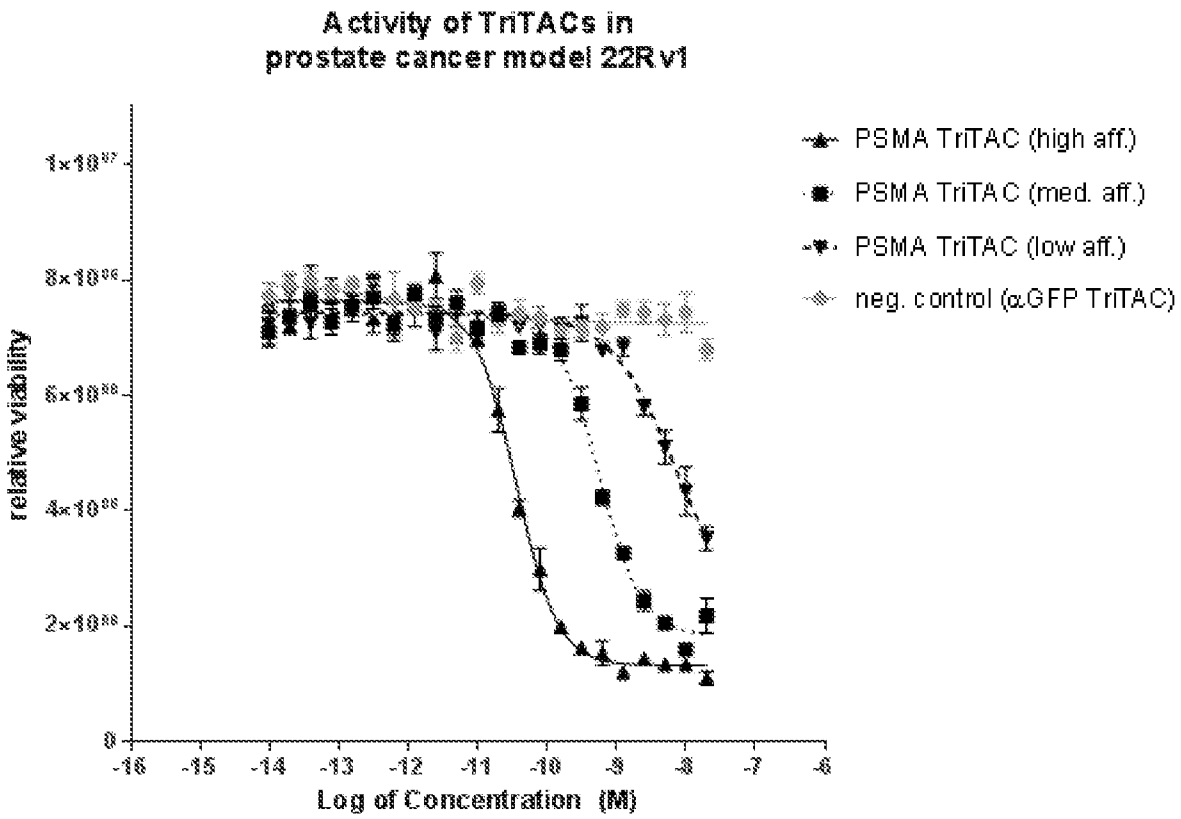
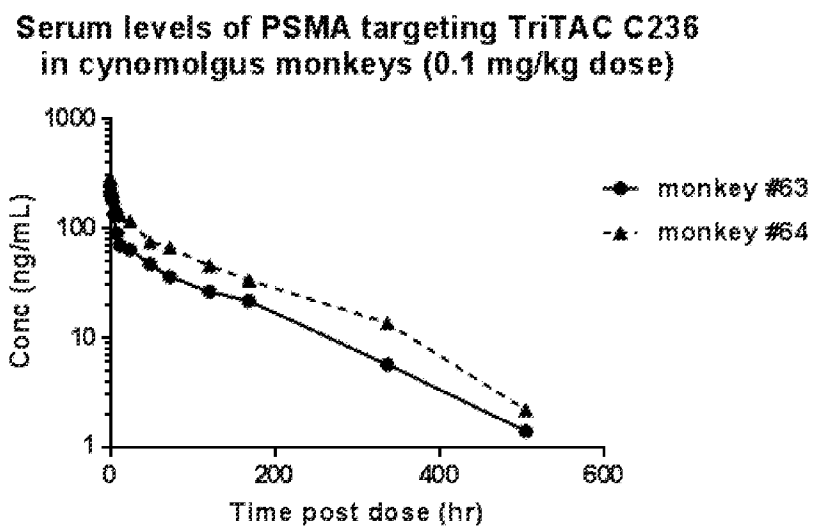



**Figure 2C**

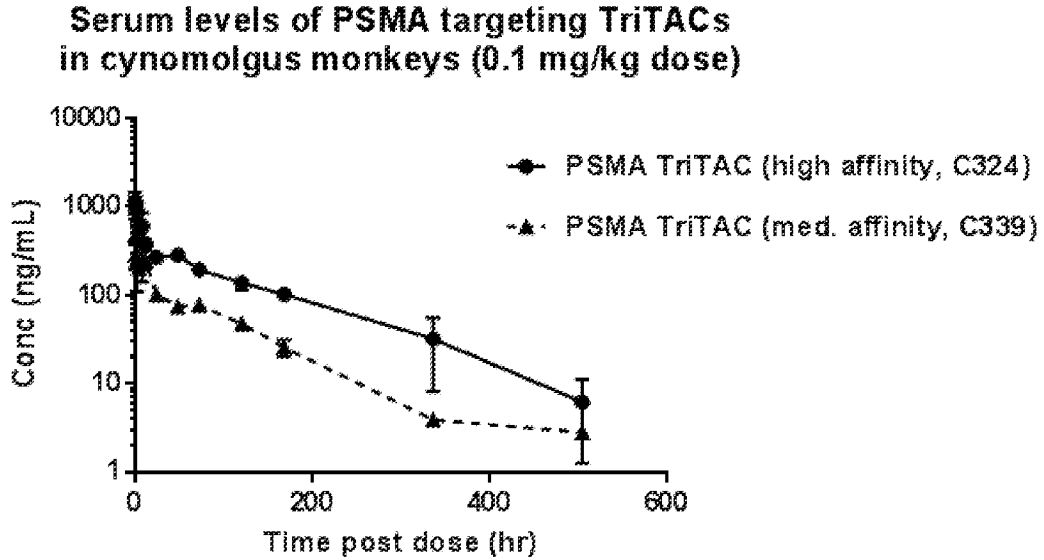
| EC50 [pM] | LNCaP | 22Rv1 |
|---|---|---|
| TriTAC CD3 high aff. – C324 | 10 | 35 |
| TriTAC CD3 med. aff. – C339 | 87 | 561 |
| TriTAC CD3 low aff. - C325 | 1,389 | 7,460 |

**Figure 3**




| Dose Level | Animal ID | No points lambda_z | Terminal t1/2 (hr) | Cmax (ng/mL) | C0 (ng/mL) | AUC, 0-last (hr*ng/mL) | AUC, 0-inf (hr*ng/mL) | AUC %Extrapolated (%) | Clearance (mL/hr/kg) | Vss (L/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.1 mg/kg | 63 | 6 | 91.6 | 245 | 253 | 10100 | 10300 | 1.8 | 9.68 | 1.15 |
| | 64 | 6 | 93.7 | 287 | 298 | 17500 | 17800 | 1.7 | 5.61 | 0.71 |
| | **Mean** | **6** | **92.6** | **266** | **276** | **13800** | **14100** | **1.8** | **7.64** | **0.93** |

**Figure 4**




| Dose Level | Animal ID | # points lambda_z | Terminal t1/2 (hr) | Cmax (ng/mL) | C0 (ng/mL) | AUC, 0-last (hr*ng/mL) | AUC, 0-inf (hr*ng/mL) | AUC %Extrapolated (%) | Clearance (mL/hr/kg) | Vss (L/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| C324 0.1 mg/kg | 2389M | 5 | 70.3 | 1360 | 1390 | 47800 | 48100 | 0.568 | 2.08 | 0.192 |
| | 71F | 5 | 101 | 918 | 941 | 56100 | 57500 | 2.46 | 1.74 | 0.244 |
| | **Mean** | **5** | **85.8** | **1140** | **1170** | **51900** | **52800** | **1.52** | **1.91** | **0.218** |
| C339 0.1 mg/kg | 2390M | 6 | 85.3 | 497 | 533 | 17800 | 18100 | 1.79 | 5.53 | 0.530 |
| | 70F | 6 | 86.5 | 456 | 523 | 15600 | 16000 | 2.32 | 6.25 | 0.621 |
| | **Mean** | **6** | **85.9** | **477** | **528** | **16700** | **17000** | **2.05** | **5.89** | **0.575** |

**Figure 5A**

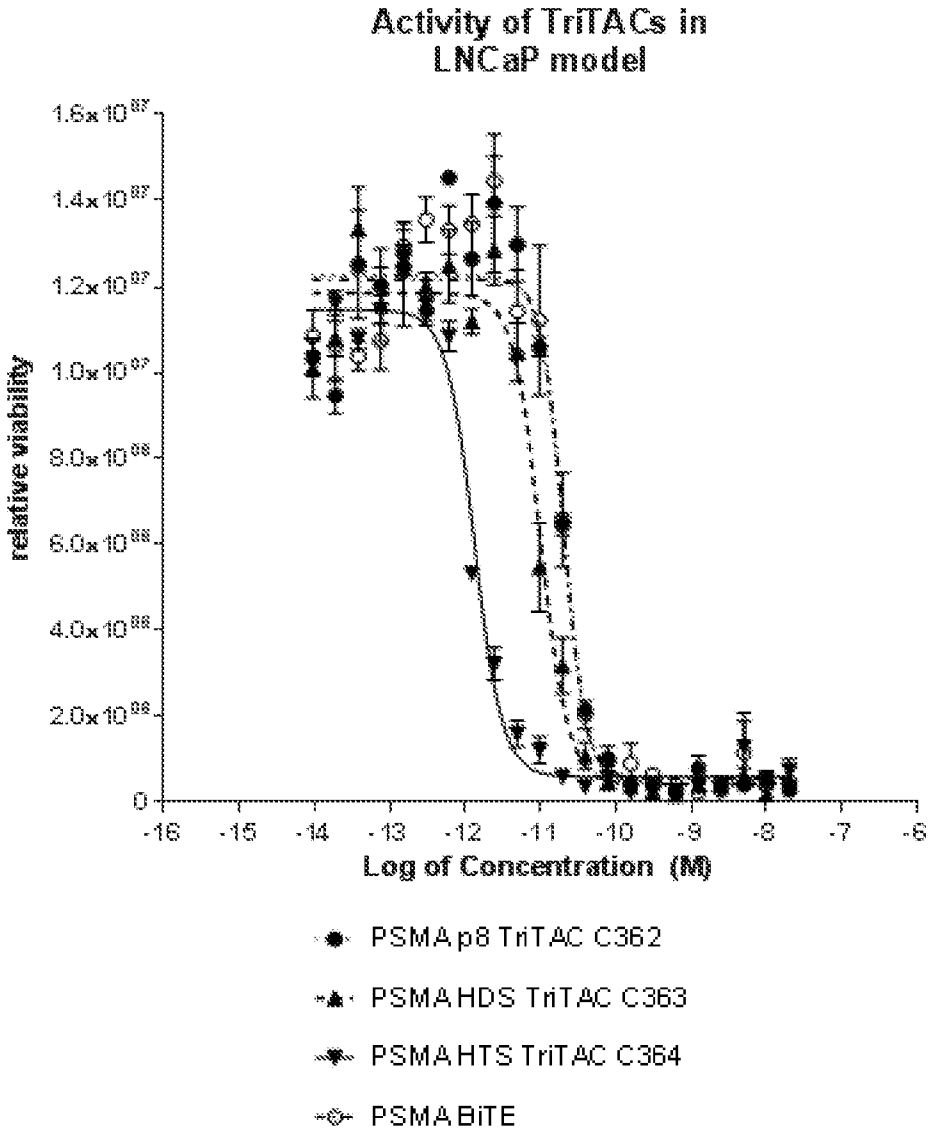

Activity of TriTACs in LNCaP model
relative viability
1.6x10^07
1.4x10^07
1.2x10^07
1.0x10^07
8.0x10^06
6.0x10^06
4.0x10^06
2.0x10^06
0
-16
-15
-14
-13
-12
-11
-10
-9
-8
-7
-6
Log of Concentration (M)
PSMA p8 TriTAC C362
PSMA HDS TriTAC C363
PSMA HTS TriTAC C364
PSMA BiTE

**Figure 5B**

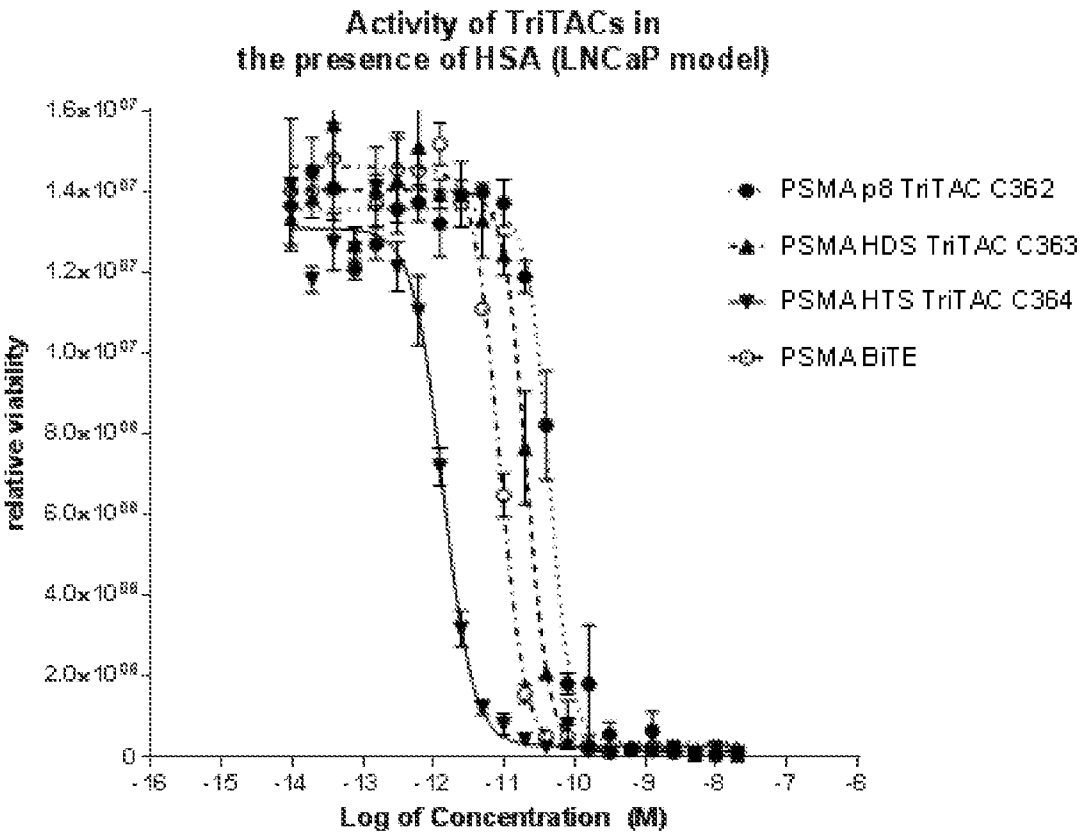
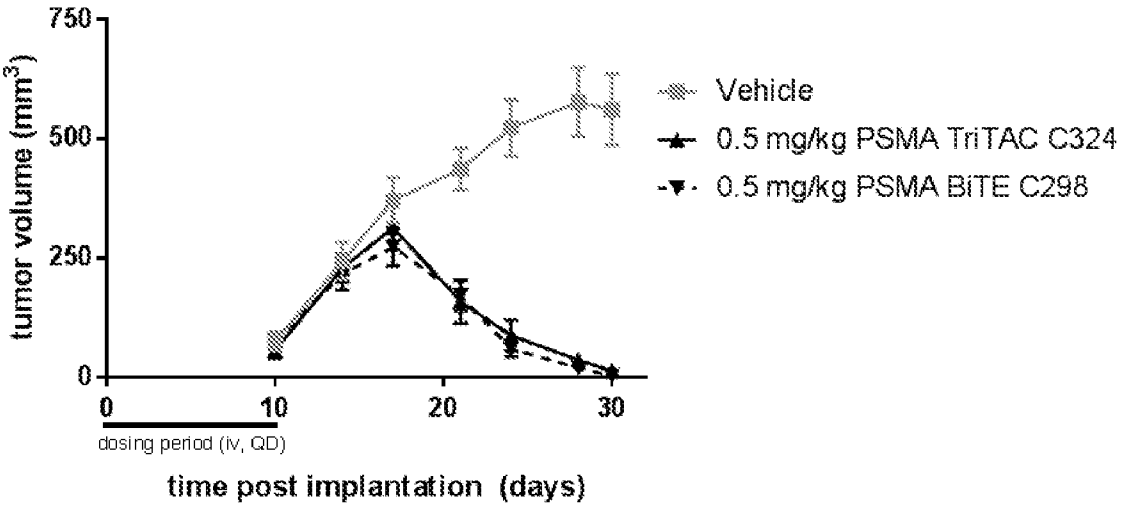



**Figure 5C**

| EC50 [pM] | LNCaP | LNCaP with HSA | HSA shift |
|---|---|---|---|
| PSMA p8 TriTAC C362 | 20 | 43 | 2x |
| PSMA HDS TriTAC C363 | 10 | 21 | 2x |
| PSMA HTS TriTAC C364 | 1.3 | 1.3 | 1x |
| PSMA BiTE | 20 | 9 | 0.5x |

**Figure 6**




* 22Rv1 human prostate cancer xenograft study in NOD/SCID/gamma mice reconstituted with resting, primary human T cells mixed at 1:1 ratio with cancer cells

**Figure 7A**

| Cell line | EGFR expression | PSMA expression |
|---|---|---|
| LNCaP | Positive | Positive |
| KMS12BM | Negative | Negative |
| OVCAR8 | Positive | Negative |

**Figure 7B**

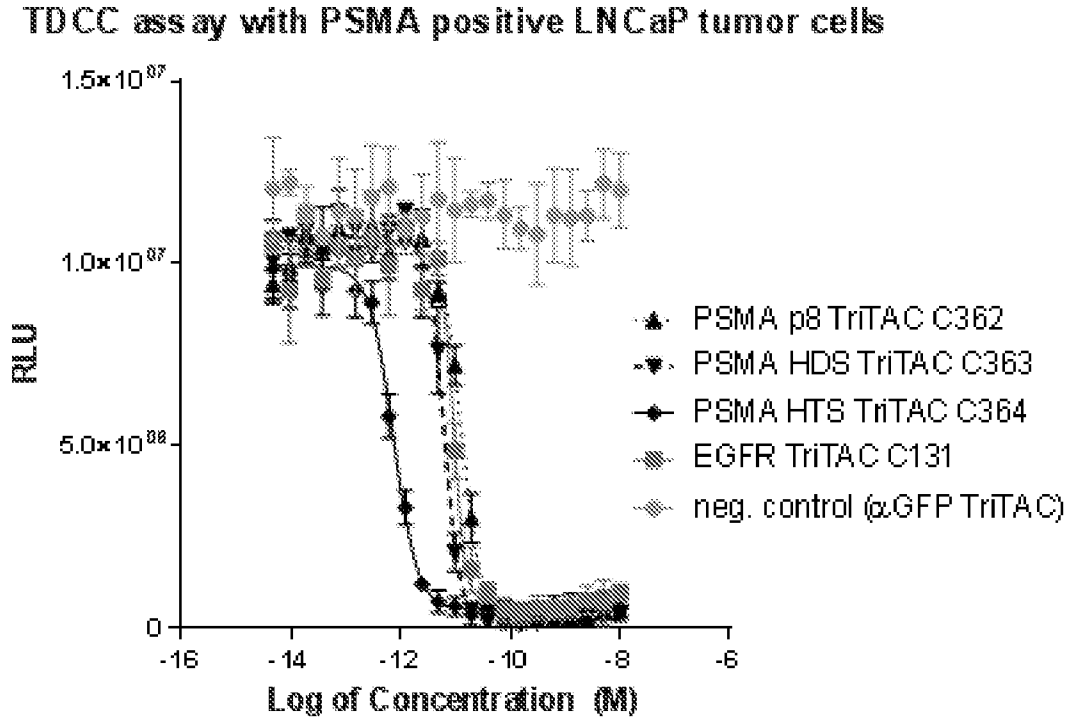


**Figure 7C**

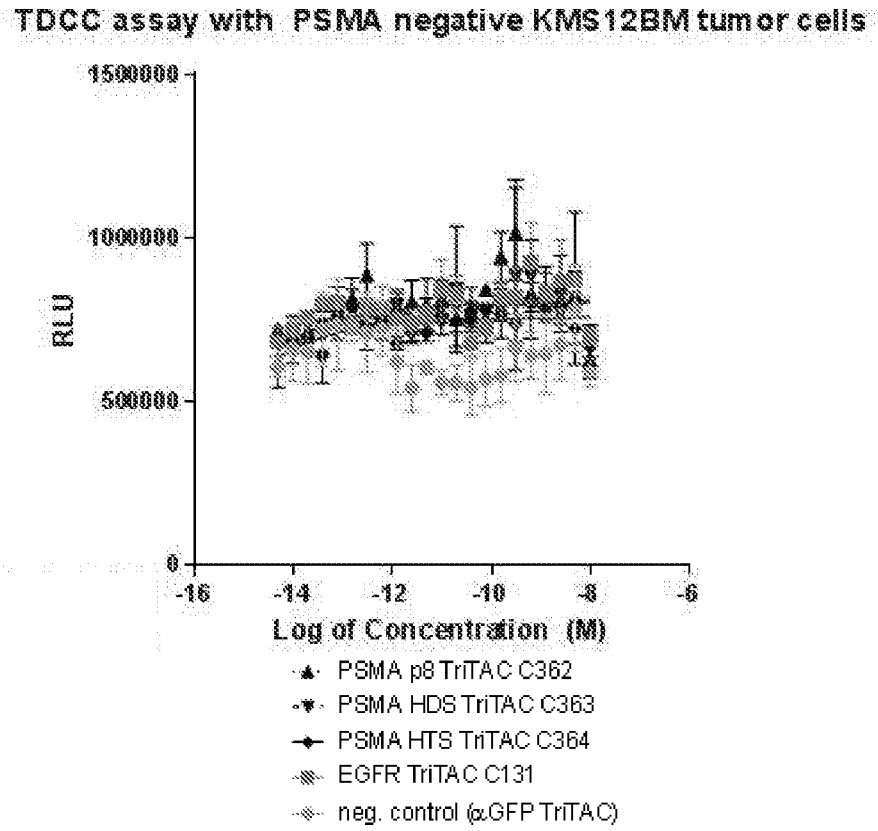



**Figure 7D**

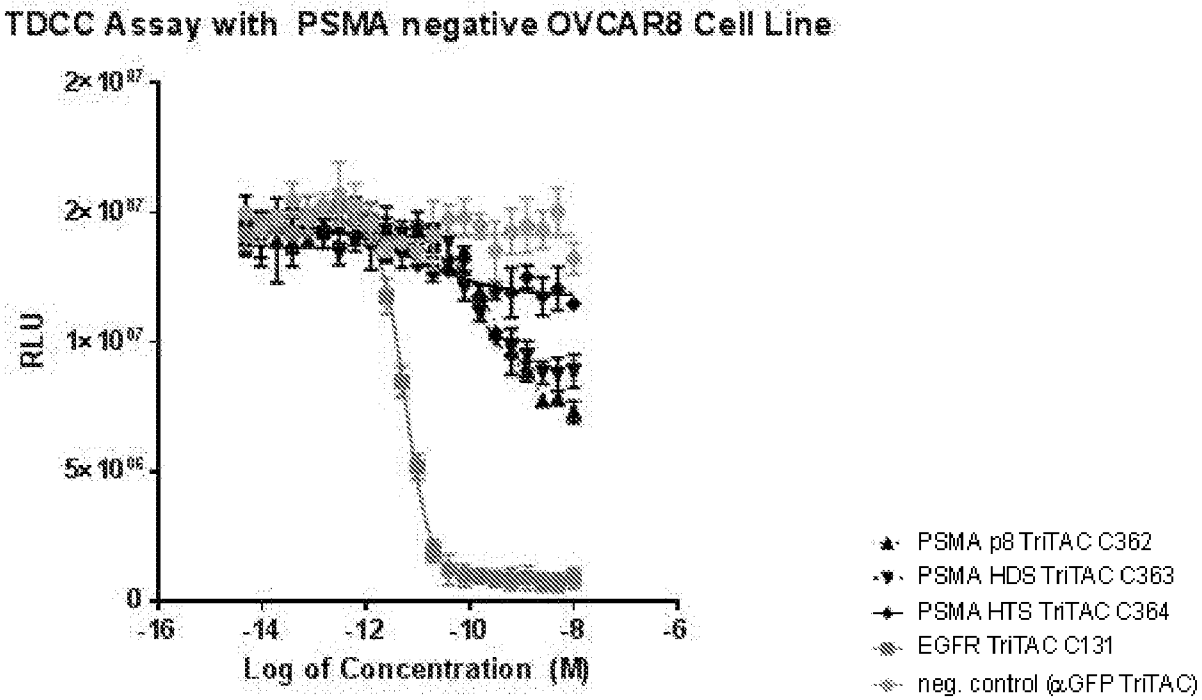


**Figure 8A**

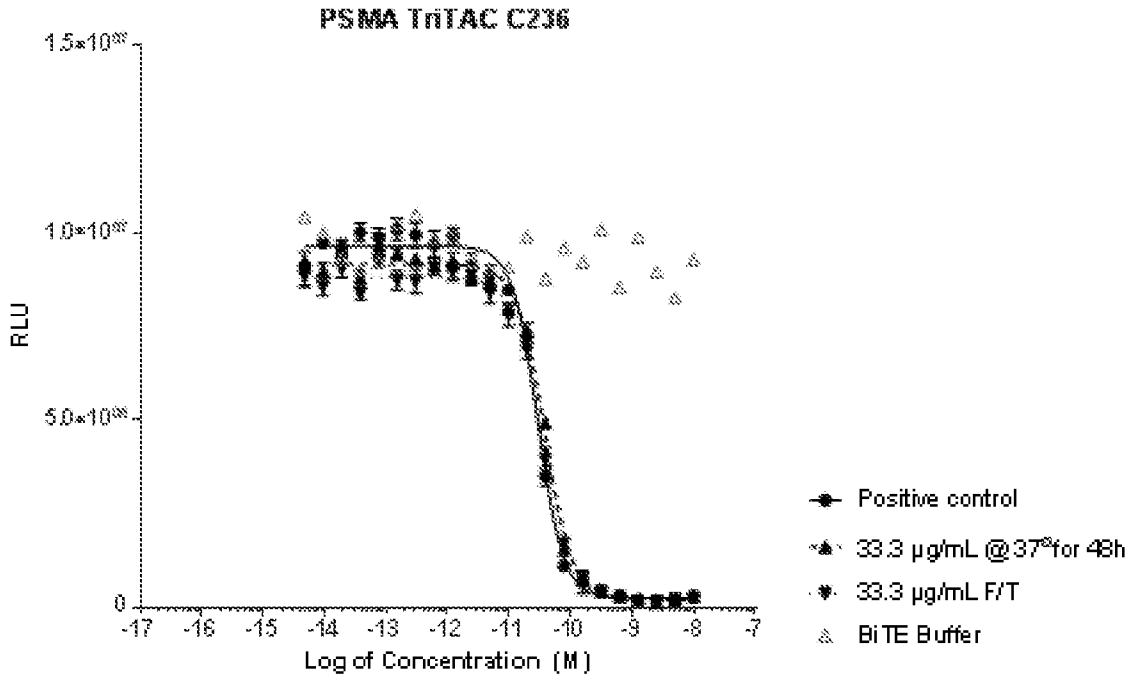



**Figure 8B**

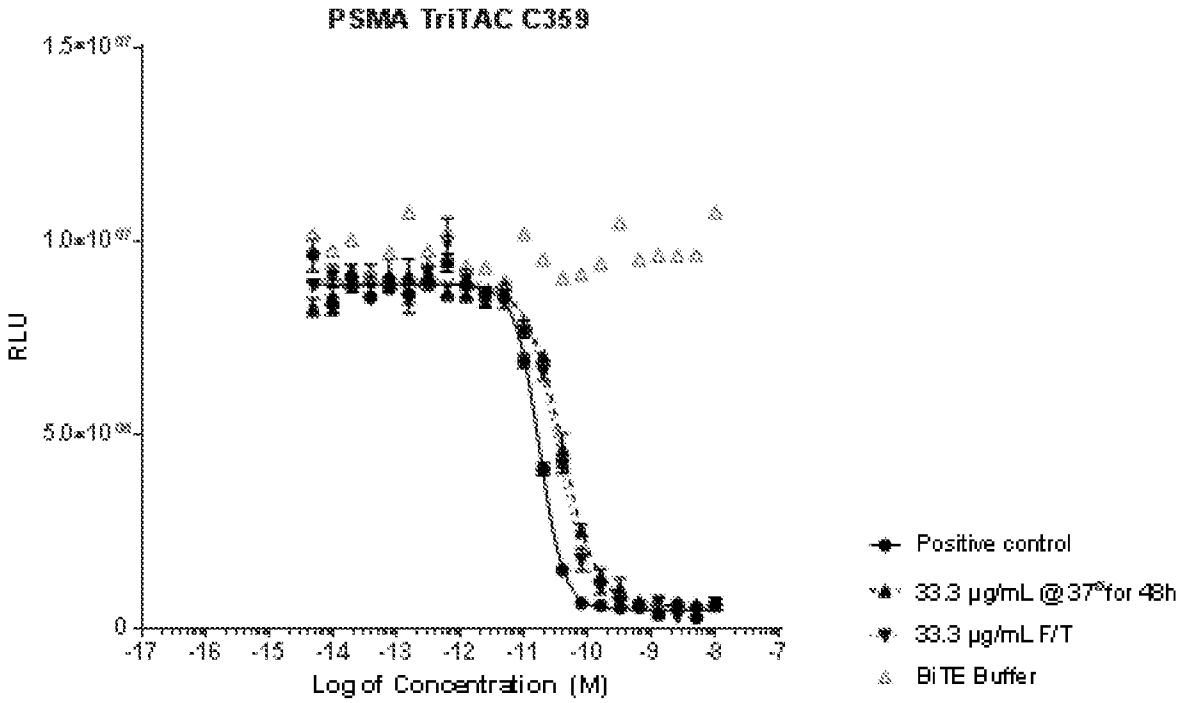


**Figure 8C**

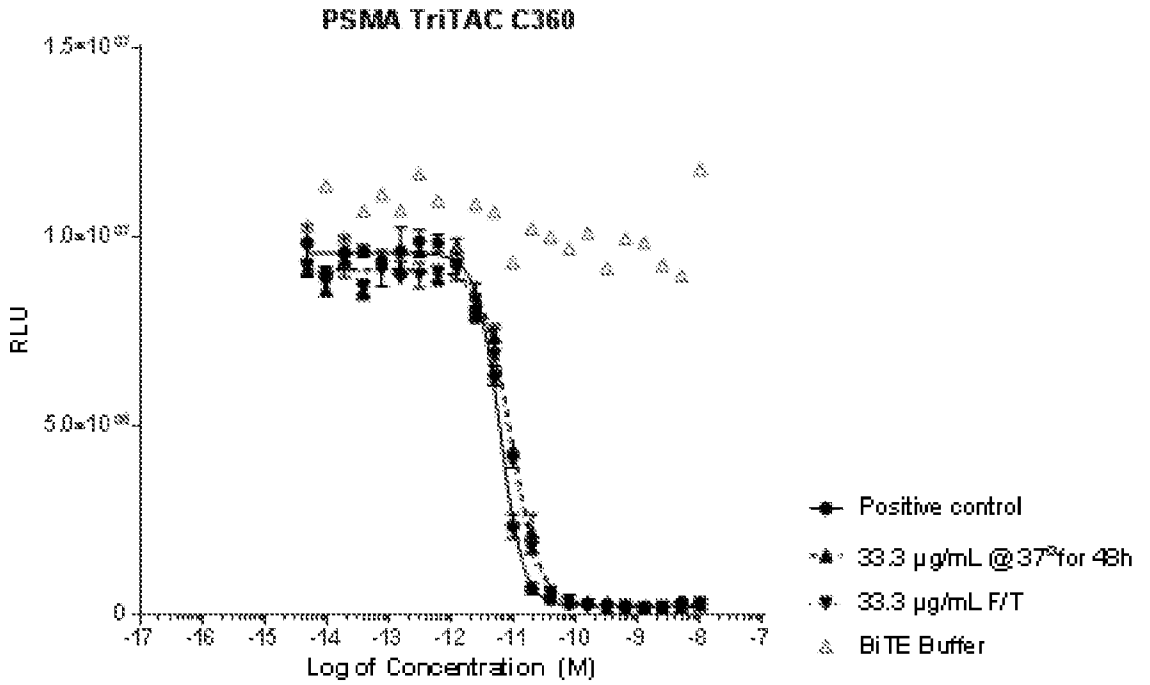



**Figure 8D**

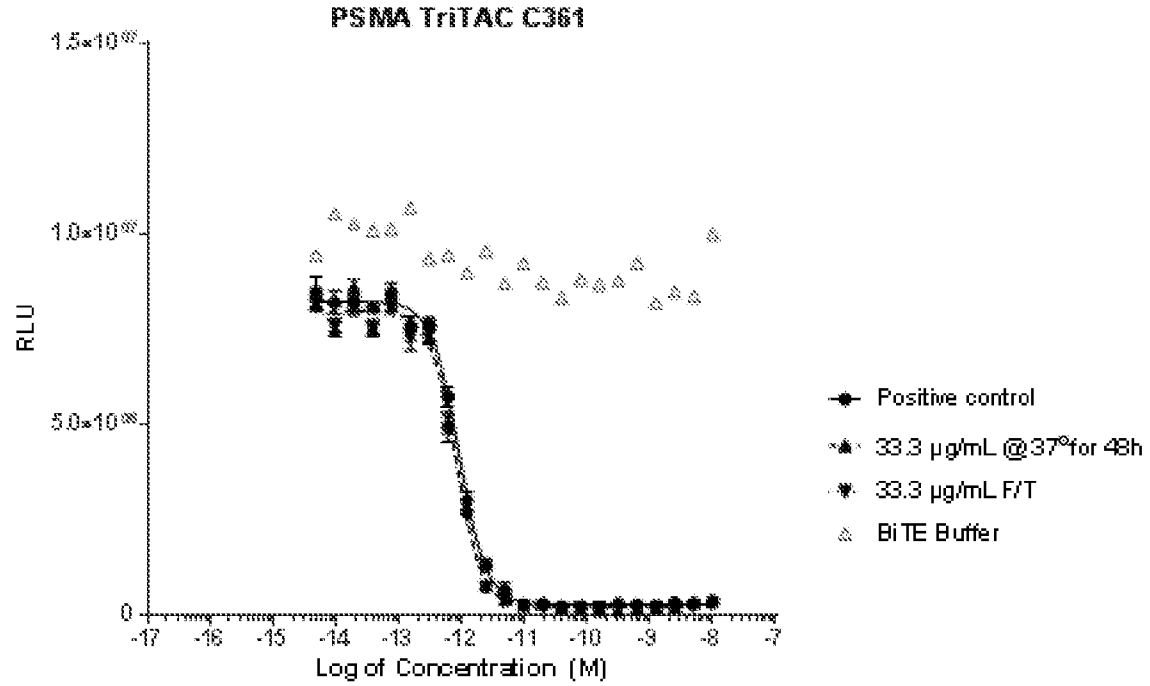


**Figure 9A**

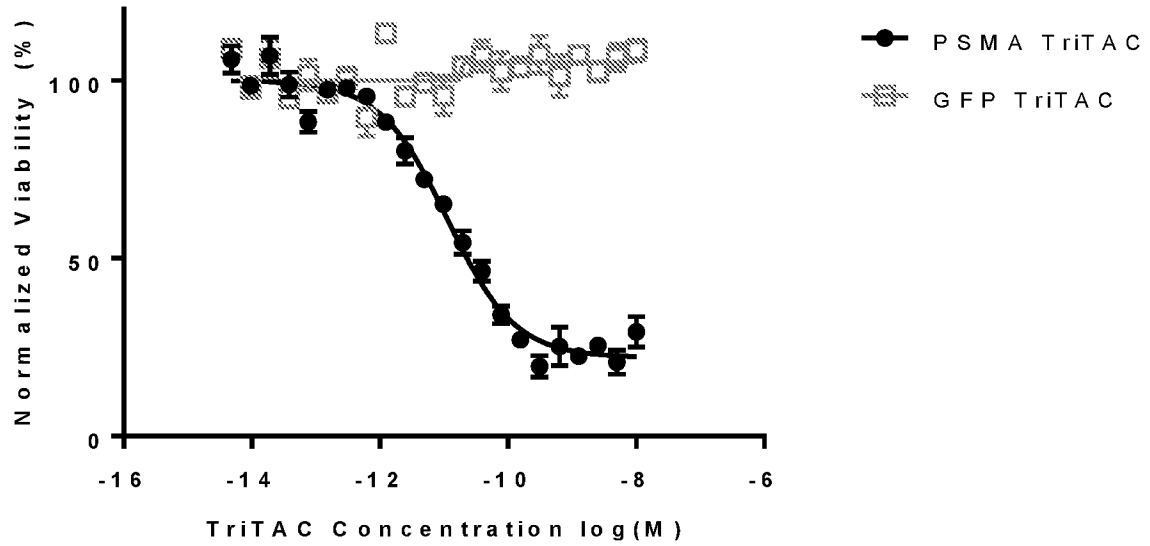



**Figure 9B**

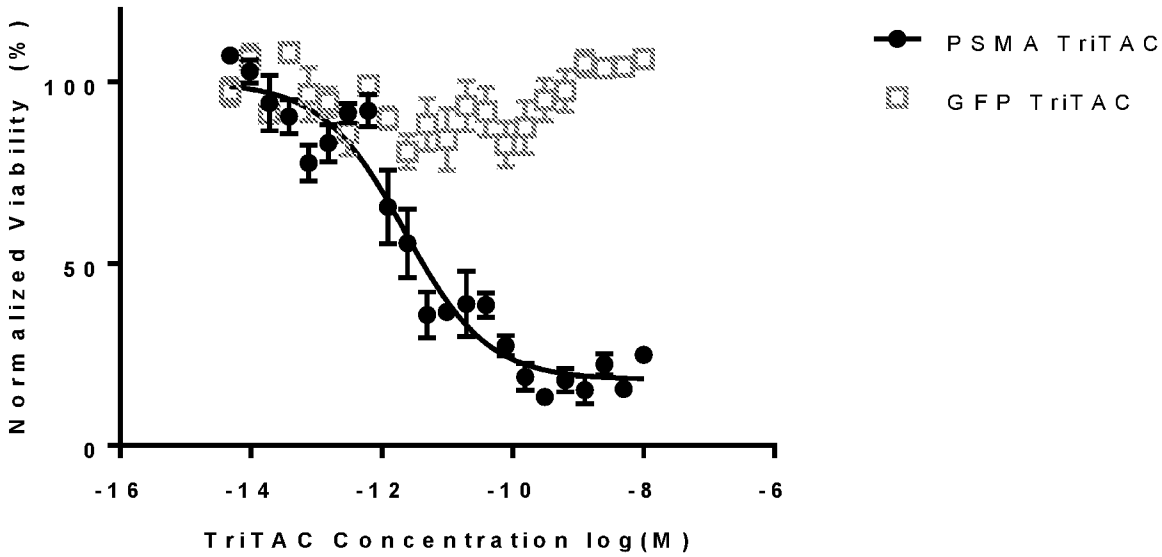


**Figure 10**

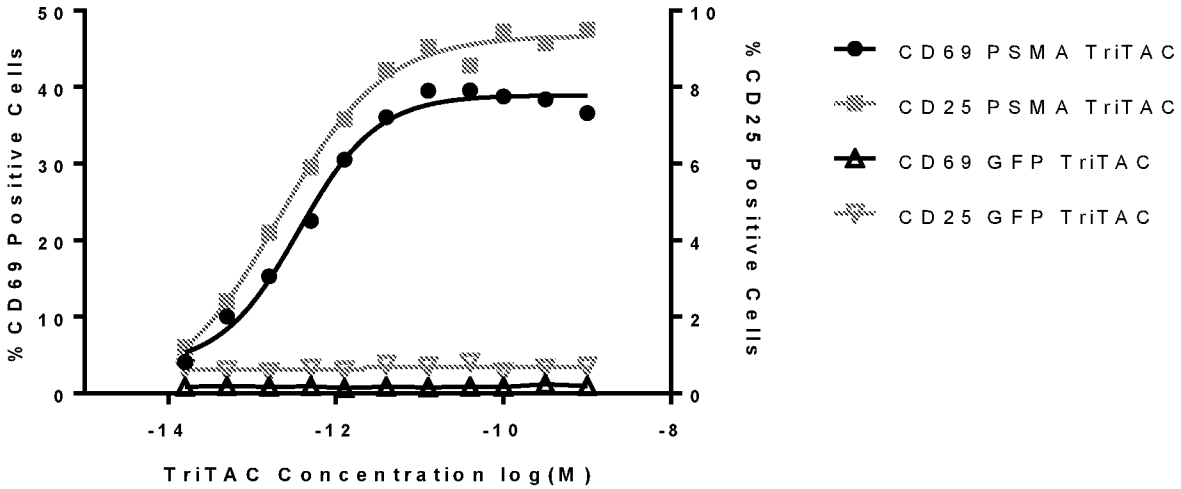



**Figure 11**

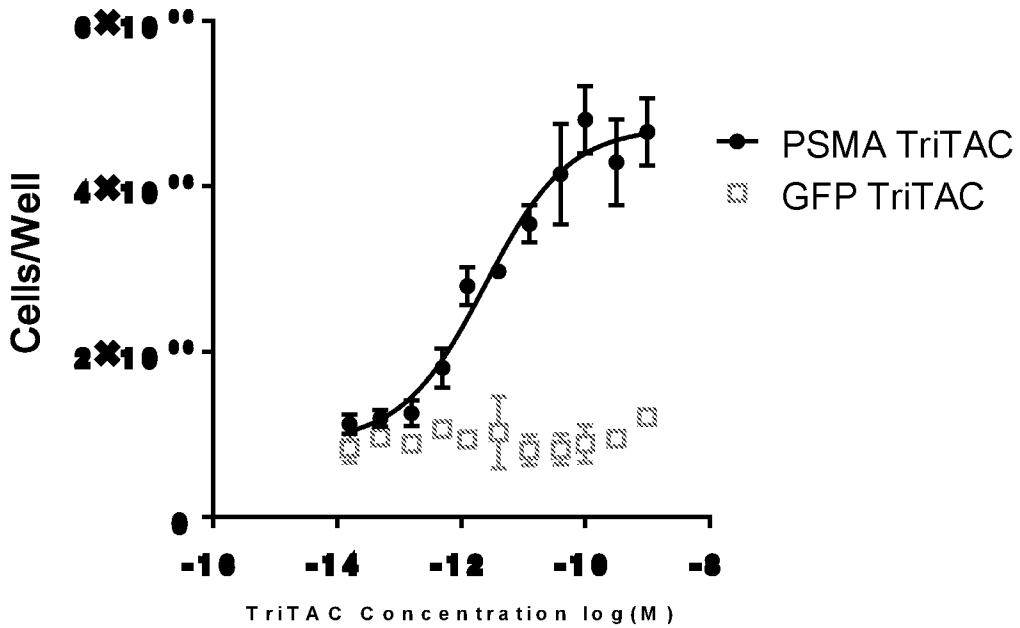
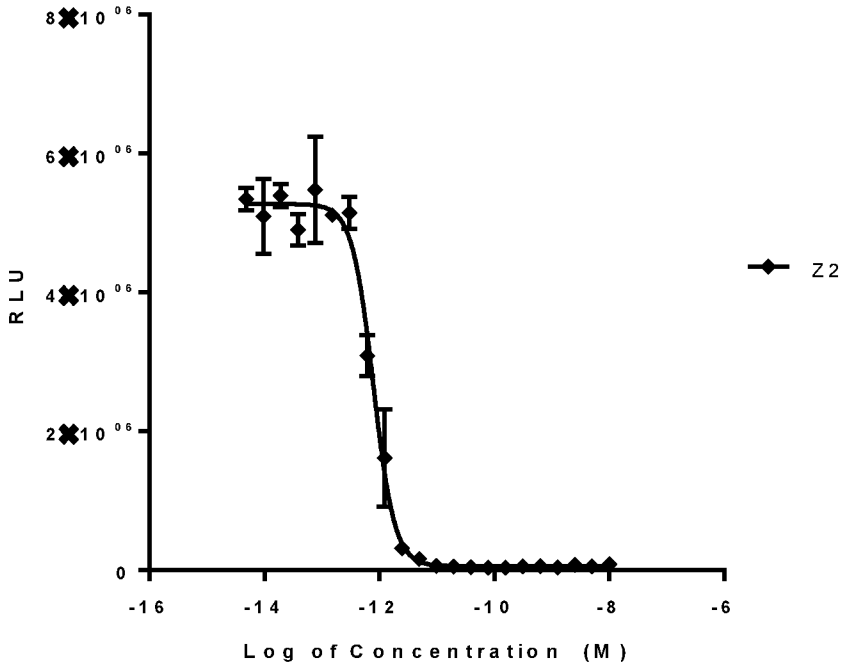


**Figure 12**


8 10 06
6 10 06
4 10 06
2 10 06
0
RLU
-16
-14
-12
-10
-8
-6
Log of Concentration (M)
Z2

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/63121

A. CLASSIFICATION OF SUBJECT MATTER
IPC - C07K 16/18, 16/28, 16/30, 16/46 (2018.01)
CPC - C07K 16/18, 16/2809, 16/3069, 16/468

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 2010/003118 A1 (TRUBION PHARMACEUTICALS, INC) 7 January 2010. paragraphs [0062], [00109], [00110], [00111], [00112] | 1-20, 26-35, 37, 38, 48, 62-67/1, 62-67/26, 71-85 |
| A | US 2012/0231024 A1 (ELSASSER-BEILE, U et al.) 13 September 2012; paragraph [0028] page 4, line 4, page 71, line 10, page 72; line 25 | 1-20, 26-35, 37, 38, 48, 62-67/1, 62-67/26, 71-85 |
| A | US 2013/0330335 A1 (BREMEL, RD et al.) 12 December 2013. paragraph [0267], Tables 14A and B | 1-20, 62-67/1, 71-75, 79-81 |
| A | WO 2016/171999 A2 (AGBIOME, INC) 27 October 2016; page 4, line 4, page 71, line 10, page 72; line 25 | 1-20, 62-67/1, 71-75, 79-81 |
| A | US 2016/0319040 A1 (ARGEN-X, BV) 3 November 2016; page 205 | 25-35, 37, 38, 41-44, 48, 62-67/26, 76-78, 82-83 |
| A | US 2011/0165621 (DREIER, T et al.) 7 July 2011; paragraph [0142], figure 2 | 25-35, 37, 38, 41-44, 48, 62-67/26, 76-78, 82-83 |
| P, X | US 2016/0340444 A1 (HARPOON THERAPEUTICS, INC.) 24 November 2016; entire document | 1-20, 26-35, 37, 38, 48, 62-67/1, 62-67/26, 71-85 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 23 February 2018 (23.02.2018) | 26 MAR 2018 |
| Name and mailing address of the ISA/<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300 | Authorized officer<br>Shane Thomas<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/210 (second sheet) (January 2015)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/63121

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/63121

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 21-25, 36-40, 51-61, 63-65, 68-70
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

-***-Please see supplemental page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Claims 1-20 (in-part), 26-35, 37, 38, 41-44, 48, 62 (in-part), 66-67 (in-part), 71-83 (in-part); SEQ ID NO: 1, wherein X1 is P and X2 is H (CDR1); SEQ ID NO: 2, wherein X3 is D, X4 is K, X5 is Q and X6 is Y (CDR2); SEQ ID NO: 3, wherein X7 is S (CDR3) and SEQ ID NO: 4 (Variable region sequence)

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-20, 26-35, 37, 38, 40-50, 62-67, 71-85 and SEQ ID NO: 1, wherein X1 is P and X2 is H (CDR1); SEQ ID NO: 2, wherein X3 is D, X4 is K, X5 is Q and X6 is Y (CDR2); SEQ ID NO: 3, wherein X7 is S (CDR3) and SEQ ID NO: 4 (Variable region sequence) are directed toward a prostate specific membrane antigen binding protein; multispecific antigen binding proteins and antibodies comprising the antigen binding protein; and a method for the treatment of prostate cancer therewith.

The antigen binding proteins, multispecific binding proteins and methods will be searched to the extent that they comprise a binding protein encompassing SEQ ID NO: 1, wherein X1 is P and X2 is H (CDR1); SEQ ID NO: 2, wherein X3 is D, X4 is K, X5 is Q and X6 is Y (CDR2); SEQ ID NO: 3, wherein X7 is S (CDR3) and SEQ ID NO: 4 (Variable region sequence). Applicant is invited to elect additional fully specified variable region sequence(s), with associated fully specified CDRs (i.e. with specified SEQ ID NO: for each CDR, or with specified substitution(s) at specified site(s) of a SEQ ID NO:), to be searched. Additional variable region sequence(s) and associated CDR sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 2 (in-part), 3 (in-part), 4 (in-part), 5 (in-part), 6 (in-part), 7 (in-part), 8 (in-part), 9 (in-part), 10 (in-part), 11 (in-part), 12 (in-part), 13 (in-part), 14 (in-part), 15 (in-part), 16 (in-part), 17 (in-part), 18 (in-part) , 19 (in-part), 20 (in-part), 26-35, 37, 38, 41-44, 48, 62 (in-part), 63 (in-part), 64 (in-part), 65 (in-part), 66 (in-part), 67 (in-part), 71 (in-part), 72 (in-part), 73 (in-part), 74 (in-part), 75 (in-part), 76 (in-part), 77 (in-part), 78 (in-part), 79 (in-part), 80 (in-part), 81 (in-part), 82 (in-part), and 83 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1, wherein X1 is P and X2 is H (CDR1); SEQ ID NO: 2, wherein X3 is D, X4 is K, X5 is Q and X6 is Y (CDR2); SEQ ID NO: 3, wherein X7 is S (CDR3) and SEQ ID NO: 4 (Variable region sequence). Applicants must specify the claims that encompass any additionally elected variable region sequence(s) and associated CDR sequence (s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a variable region encompassing SEQ ID NO: 4 (variable region sequence), with CDR1 SEQ ID NO: 1, wherein X1 is E and X2 is S (CDR1); CDR2 SEQ ID NO: 2, wherein X3 is T, X4 is G, X5 is T and X6 is S (CDR2); and CDR3 SEQ ID NO: 3, wherein X7 is G (CDR3).

No technical features are shared between the antibody CDR sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features including: a prostate specific membrane antigen binding protein, comprising complementarity determining regions CDR1, CDR2, and CDR3; a method for the treatment or amelioration of prostate cancer, the method comprising administration of the PSMA binding protein to a subject in need thereof; a multispecific antibody, a bispecific antibody, an sdAb, a variable heavy domain, a peptide, or a ligand, comprising the PSMA binding protein; an antibody comprising the PSMA binding protein, wherein said antibody is a single domain antibody; a multispecific binding protein or antibody comprising the PSMA binding protein and a CD3 binding domain; these shared technical features are previously disclosed by US 2012/0231024 A1 to Elsasser-Beile et al. (hereinafter 'Elsasser-Beile').

Elsasser-Beile discloses a prostate specific membrane antigen binding protein (a prostate specific membrane antigen binding protein; abstract), comprising complementarity determining regions CDR1, CDR2, and CDR3 (comprising complementarity determining regions CDR1, CDR2, and CDR3; paragraph [0028]); a method for the treatment or amelioration of prostate cancer (a method for the treatment or amelioration of prostate cancer; paragraph [0042], Claims 1, 15, 16), the method comprising administration of the PSMA binding protein to a subject in need thereof (the method comprising administration of the PSMA binding protein to a subject in need thereof; paragraph [0042], Claims 1, 15, 16); a multispecific antibody comprising the PSMA binding protein (a bispecific (multispecific) antibody comprising the PSMA binding protein; abstract); an antibody comprising the PSMA binding protein (an antibody comprising the PSMA binding protein; abstract, paragraph [0030]), wherein said antibody is a single domain antibody (wherein said antibody is a fab fragment or dAb fragment (single domain antibody); paragraph [0030]); and a multispecific binding protein or antibody comprising the PSMA binding protein and a CD3 binding domain (a bispecific (multispecific) binding protein or antibody comprising the PSMA binding protein and a CD3 binding domain; paragraph [0156]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Elsasser-Beile reference, unity of invention is lacking.