PROSTATE-SPECIFIC MEMBRANE ANTIGEN BINDING PROTEINS AND RELATED COMPOSITIONS AND METHODS

[001] This application claims priority to U.S. provisional patent application no. 61/478,449, filed April 22, 2011, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[002] The present invention relates to mono-specific and multi-specific protein therapeutics that specifically target cells expressing prostate-specific membrane antigen (PSMA) and are useful for the treatment of disorders characterized by overexpression of PSMA, such as, for example, prostate cancer (*e.g.*, castrate-resistant prostate cancer), tumor-related angiogenesis, or benign prostatic hyperplasia (BPH). In one embodiment, the multi-specific protein therapeutic binds both PSMA-expressing cells and the T-cell receptor complex on T cells to induce target-dependent T-cell cytotoxicity, activation and proliferation.

ACCOMPANYING SEQUENCE LISTING

[003] The contents of the text file (Name: "Sequence_Listing.txt", Size: 272,014 bytes; Date of Creation: April 20, 2012) submitted electronically herewith are incorporated herein by reference in their entirety.

BACKGROUND

[004] Prostate-specific Membrane Antigen (PSMA), also known as glutamate carboxypeptidase II and N-acetylated alpha-linked acidic dipeptidase 1, is a dimeric type II transmembrane glycoprotein belonging to the M28 peptidase family encoded by the gene FOLH1 (folate hydrolase 1). The protein acts as a glutamate carboxypeptidase on different alternative substrates, including the nutrient folate and the neuropeptide N-acetyl-l-aspartyl-l-glutamate and is expressed in a number of tissues such as the prostate, and to a lesser extent, the small intestine, central and peripheral nervous system and kidney. The gene encoding PSMA is alternatively spliced to produce at least three variants. A mutation in this gene may be associated with impaired intestinal absorption of dietary folates, resulting in low blood folate levels and consequent hyperhomocysteinemia. Expression of this protein in the

brain may be involved in a number of pathological conditions associated with glutamate excitotoxicity.

[005] PSMA is a well-established, highly restricted prostate-cancer-related cell membrane antigen. In prostate cancer cells, PSMA is expressed 1000-fold higher than on normal prostate epithelium (Su *et al.*, *Cancer Res.* 1995 44:1441-1443). Expression of PSMA increases with prostate cancer progression and is highest in metastatic disease, hormone refractory cases, and higher-grade lesions (Israeli *et al.*, *Cancer Res.* 1994, 54:1807-1811; Wright *et al.*, *Urologic Oncology: Seminars and Original Investigations* 1995 1:18-28; Wright *et al.*, *Urology* 1996 48:326-332; Sweat *et al.*, *Urology* 1998 52:637-640). Additionally, PSMA is abundantly expressed on the neovasculature of a variety of other solid tumors, including bladder, pancreas, melanoma, lung and kidney cancers, but not on normal neovasculature (Chang *et al.*, *Urology* 2001 57:801-805; Divgi *et al.*, *Clin. Cancer Res.* 1998 4:2729-3279).

[006] PSMA has been shown to be an important target for immunological approaches such as vaccines or directed therapy with monoclonal antibodies. Unlike other prostate-restricted molecules that are secretory proteins (PSA, prostatic acid phosphatase), PSMA is an integral cell–surface membrane protein that is not secreted, which makes it an ideal target for antibody therapy. PROSTASCINT® (capromab pendetide) is an ^{111}In-labelled anti-PSMA murine monoclonal antibody approved by the FDA for imaging and staging of newly diagnosed and recurrent prostate cancer patients (Hinkle *et al.*, *Cancer* 1998, 83:739-747). However, capromab binds to an intracellular epitope of PSMA, requiring internalization or exposure of the internal domain of PSMA, therefore preferentially binding apoptotic or necrosing cells (Troyer *et al.*, *Urologic Oncology: Seminars and Original Investigations* 1995 1:29-37; Troyer *et al.*, *Prostate* 1997 30:232-242). As a result, capromab may not be of therapeutic benefit (Liu *et al.*, *Cancer Res.* 1997 57:3629-3634).

[007] Other monoclonal antibodies which target the external domain of PSMA have been developed (e.g., J591, J415, J533, and E99) (Liu *et al.*, *Cancer Res.* 1997 57:3629-3634). Radiolabelled J591 is currently in clinical trials (Tagawa et al., Cancer 2010 116(S4):1075). However, evidence suggests that PSMA may act as a receptor mediating the internalization of a putative ligand. PSMA undergoes internalization constitutively, and PSMA-specific antibodies can induce and/or increase the rate of internalization, which then causes the antibodies to accumulate in the endosomes (Liu *et al.*, *Cancer Res.* 1998 58:4055-4060). While PSMA-specific internalizing antibodies may aid in the development of therapeutics to target the delivery of toxins, drugs, or radioisotopes to the interior of prostate cancer cells (Tagawa et al., Cancer 2010 116(S4):1075), PSMA-specific antibodies utilizing native or engineered effector mechanisms (*e.g.*, antibody-dependent cell-mediated cytotoxicity

(ADCC), complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated phagocytosis (ADCP), or re-directed T-cell cytotoxicity (RTCC)) are problematic since the PSMA-specific antibody may be internalized before it is recognized by effector cells.

SUMMARY

[008] In one embodiment, the present disclosure provides a prostate-specific membrane antigen (PSMA)-binding polypeptide comprising, in order from amino-terminus to carboxyl-terminus, (a) a PSMA-binding domain that specifically binds human PSMA, (b) a hinge region, and (c) an immunoglobulin constant region. In certain embodiments, suitable PSMA-binding domains include binding domains that compete for binding to human PSMA with a single chain Fv (scFv) having the amino acid sequence set forth in SEQ ID NO:21. In certain embodiments, the PSMA-binding polypeptide is capable of forming a dimer with a second, identical polypeptide chain through association between the respective immunoglobulin constant regions and/or hinge regions.

[008a] In an aspect, there is provided a prostate-specific membrane antigen (PSMA)-binding polypeptide comprising a humanized PSMA-binding domain wherein the PSMA binding domain comprises (i) an immunoglobulin light chain variable region comprising LCDR1, LCDR2, and LCDR3, and (ii) an immunoglobulin heavy chain variable region comprising HCDR1, HCDR2, and HCDR3, wherein the LCDR1, LCDR2 and LCDR3 has the amino acid sequences set forth in SEQ ID NO:15, 16 and 17, respectively, and the HCDR1, HCDR2, and HCDR3 has the amino acid sequences set forth in SEQ ID NO:9, 10 and 11, respectively.

[009] In certain embodiments, the PSMA-binding domain comprises (i) an immunoglobulin light chain variable region comprising CDRs LCDR1, LCDR2, and LCDR3, and/or (ii) an immunoglobulin heavy chain variable region comprising CDRs HCDR1, HCDR2, and HCDR3. In certain variations, LCDR3 has the amino acid sequence set forth in SEQ ID NO:17 and/or HCDR3 has the amino acid sequence set forth in SEQ ID NO:11; in some such embodiments, LCDR1 and LCDR2 have the amino acid sequences as set forth in SEQ ID NO:15 and SEQ ID NO:16, respectively, and/or HCDR1 and HCDR2 have the amino acid sequences as set forth in SEQ ID NO:9 and SEQ ID NO:10, respectively. In another variation, (i) the light chain variable region comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:5 or SEQ ID NO:23; and/or (ii) the heavy chain variable region comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:25, or SEQ ID NO:27. One or both of the light and heavy chain variable regions can be humanized.

[0010] In certain variations, the PSMA-binding domain is a single chain Fv (scFv) comprising the immunoglobulin light and heavy chain variable regions disclosed herein. In certain embodiments, PSMA-binding scFvs include, for example, scFvs comprising an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:35. In certain embodiments, the heavy chain variable region of the scFv is carboxyl-terminal to the light chain variable region (also referred to

herein as a "VL-VH orientation"). In some embodiments of an scFv having a VL-VH orientation, the scFv comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:21, SEQ ID NO:30, or SEQ ID NO:31. The light chain variable region and heavy chain variable region of the scFv can be joined by a peptide linker such as, for example, a peptide linker comprising an amino acid sequence $(Gly_4Ser)_n$, wherein n = 1-5 (SEQ ID NO:165).

[0011] In some embodiments of a PSMA-binding polypeptide disclosed herein, the hinge region is derived from an immunoglobulin hinge region, such as, for example, an immunoglobulin hinge region of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgD. Such an immunoglobulin hinge region can be either a wild-type or an altered immunoglobulin hinge region.

[0012] In further embodiments of a PSMA-binding polypeptide disclosed herein, the immunoglobulin constant region comprises immunoglobulin CH2 and CH3 domains, such as, for example, immunoglobulin CH2 and CH3 domains of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgD. In another embodiment, the immunoglobulin constant region comprises immunoglobulin CH2 and CH3 domains and the constant region does not comprise an immunoglobulin CH1 domain.

[0013] In certain variations, a PSMA-binding polypeptide disclosed herein includes at least one effector function selected from antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). In some embodiments, the hinge region is derived from an immunoglobulin hinge region and the immunoglobulin constant region comprises immunoglobulin CH2 and CH3 domains of IgG1, IgG2, IgG3, or IgG4. In another embodiment, the immunoglobulin hinge region is derived from the hinge region of IgG1 and the immunoglobulin constant region comprises immunoglobulin CH2 and CH3 domains of IgG1.

[0014] In some embodiments, a PSMA-binding polypeptide disclosed herein comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:70, or SEQ ID NO:72.

[0015] In still further embodiments, a PSMA-binding polypeptide disclosed herein further includes (d) a second hinge region carboxyl-terminal to the immunoglobulin constant region, and (e) a second binding domain carboxyl-terminal to the second hinge region. In some embodiments, second hinge regions include those derived from a stalk region of a type II C lectin or an immunoglobulin hinge region. In certain variations, the second hinge region has

an amino acid sequence as set forth in SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, or SEQ ID NO:66.

[0016] In another embodiment, the present disclosure provides a prostate-specific membrane antigen (PSMA)-binding polypeptide that specifically binds human PSMA and comprises a first binding domain comprising (i) an immunoglobulin light chain variable region comprising CDRs LCDR1, LCDR2, and LCDR3, and (ii) an immunoglobulin heavy chain variable region comprising CDRs HCDR1, HCDR2, and HCDR3; wherein LCDR3 has the amino acid sequence set forth in SEQ ID NO:17 and/or HCDR3 has the amino acid sequence set forth in SEQ ID NO:11. In some embodiments, LCDR1 and LCDR2 have the amino acid sequences as set forth in SEQ ID NO:15 and SEQ ID NO:16, respectively, and/or HCDR1 and HCDR2 have the amino acid sequences as set forth in SEQ ID NO:9 and SEQ ID NO:10, respectively. In some variations, LCDR1, LCDR2, and LCDR3 have the amino acid sequences as set forth in SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, respectively; and HCDR1, HCDR2, and HCDR3 have the amino acid sequences as set forth in SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:11, respectively. In some variations, (i) the light chain variable region comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:5 or SEQ ID NO:23; and/or (ii) the heavy chain variable region comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:25, or SEQ ID NO:27. In certain embodiments, the light chain variable region is encoded by a nucleic acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the nucleic acid sequence set forth in SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:22; and/or the heavy chain variable region is encoded by a nucleic acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:24, or SEQ ID NO:26. One or both of the light and heavy chain variable regions can be humanized. In some embodiments, the PSMA-binding polypeptide is capable of forming a dimer with a second, identical polypeptide chain.

[0017] In certain embodiments disclosed herein, the first binding domain is a single chain Fv (scFv) comprising the immunoglobulin light and heavy chain variable regions. In some embodiments, PSMA-binding scFvs include, for example, scFvs comprising an amino acid sequence that is at least 90%, at least 95% at least 99%, or 100% identical to the amino acid set forth in SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:35. In certain embodiments, the heavy chain variable region of the scFv is carboxyl-terminal to the light chain variable region (a "VL-VH orientation"). In some embodiments of an scFv having a VL-VH orientation, the scFv comprises an amino acid

sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:21, SEQ ID NO:30, or SEQ ID NO:31. The light chain variable region and heavy chain variable region of the scFv can be joined by a peptide linker such as, for example, a peptide linker comprising an amino acid sequence $(Gly_4Ser)_n$, wherein n = 1-5 (SEQ ID NO:165).

[0018] In certain embodiments, the PSMA-binding polypeptide further includes an immunoglobulin constant region. For example, in some variations, the immunoglobulin constant region comprises immunoglobulin CH2 and CH3 domains of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgD. In some variations, the PSMA-binding polypeptide further includes one or more hinge regions. In certain embodiments, the hinge region can be derived, for instance, from a stalk region of a type II C lectin or from an immunoglobulin hinge region.

[0019] In another embodiment, the PSMA binding polypeptide comprises, in order from amino to carboxyl-terminus, a first binding domain, a hinge region, and an immunoglobulin constant region. A PSMA-binding polypeptide in this format can also be referred to as a PSMA-specific SMIP molecule. General SMIP configurations are provided, for example, in US Patent Application Publication Nos. 2003/0133939, 2003/0118592, and 2005/0136049, which are incorporated herein in their entirety by reference.

[0020] In another embodiment, the orientation of the polypeptide is reversed such that the polypeptide comprises, in order from amino to carboxyl-terminus, an immunoglobulin constant region, a hinge region and a first binding domain. In this orientation, the polypeptide can also be referred to as a PSMA-specific PIMS molecule. General PIMS configurations are provided, for example, in US Patent Application Publication No. 2009/0148447, which is incorporated herein in its entirety by reference. In some embodiments, a PSMA-binding polypeptide having an immunoglobulin constant region and, optionally, a hinge region as disclosed herein is capable of forming a dimer with a second, identical polypeptide chain through association between the respective immunoglobulin constant regions and/or hinge regions.

[0021] In another embodiment, the PSMA-binding polypeptide includes a second binding domain, such as, *e.g.*, a single-chain Fv (scFv). For example, in some variations, the PSMA-binding polypeptide comprises, in order from amino-terminus to carboxyl-terminus or in order from carboxyl-terminus to amino-terminus, (a) a first binding domain, (b) a first hinge region, (c) an immunoglobulin constant region, (d) a second hinge region, and (e) a second binding domain.

[0022] In yet another embodiment, the present disclosure provides a PSMA-binding polypeptide as in other embodiments disclosed herein and comprising an additional binding

domain, *e.g.*, a second binding domain, wherein the second binding domain specifically binds a T cell. In certain embodiments, the second binding domain specifically binds a T cell receptor (TCR) complex or a component thereof. In some embodiments, the second binding domain includes those that specifically bind CD3, *e.g.*, CD3ε. In certain variations, the second binding domain competes for binding to CD3 with the CRIS-7 or HuM291 monoclonal antibody. In some such variations, the second binding domain comprises an immunoglobulin light chain variable region and an immunoglobulin heavy chain variable region derived from the CRIS-7 or HuM291 monoclonal antibody. For example, in certain embodiments, the light and heavy chain variable regions of the second binding domain are humanized variable regions comprising, respectively, the light and heavy chain CDRs of the CRIS-7 or HuM291 monoclonal antibody. In another embodiment, the light and heavy chain variable regions of the second binding domain are selected from (a) a light chain variable region comprising an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in residues 139-245 of SEQ ID NO:47 and a heavy chain variable region comprising an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in residues 1-121 of SEQ ID NO:47; and (b) a light chain variable region comprising an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in residues 634-740 of SEQ ID NO:78 and a heavy chain variable region comprising an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in residues 496-616 of SEQ ID NO:78.

[0023] In certain embodiments of a PSMA-binding polypeptide comprising a second binding domain, the second binding domain is a single-chain Fv (scFv). For example, in some embodiments of a second binding domain comprising light and heavy chain variable regions derived from the CRIS-7 monoclonal antibody, the second binding domain is a scFv comprising an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from (i) the amino acid sequence set forth in residues 1-245 of SEQ ID NO:47, and (ii) the amino acid sequence set forth in residues 496-742 of SEQ ID NO:78. In some such embodiments, the PSMA-binding polypeptide comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:162, or SEQ ID NO:164.

[0024] In one aspect, there is provided a dimeric PSMA-binding protein comprising first and second polypeptide chains, wherein each of said polypeptide chains is the PSMA-binding polypeptide as defined herein.

[0025] In another embodiment, the present disclosure provides a PSMA-binding polypeptide comprising, in order from amino-terminus to carboxyl-terminus, (a) a binding domain that specifically binds human PSMA, (b) a hinge region, (c) an immunoglobulin constant region, and (d) an immunoglobulin heterodimerization domain. The heterodimerization domain can comprise, for example, an immunoglobulin CH1 domain or an immunoglobulin CL domain. In certain embodiments, the PSMA-binding domain competes for binding to human PSMA with a single chain Fv (scFv) having the amino acid sequence set forth in SEQ ID NO:21. In certain embodiments, the PSMA-binding domains include, *e.g.*, the PSMA-binding domains disclosed above.

[0026] In some embodiments, the hinge region is derived from an immunoglobulin hinge region, such as, for example, an immunoglobulin hinge region of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgD. Such an immunoglobulin hinge region can be either a wild-type or an altered immunoglobulin hinge region. In further embodiments, the immunoglobulin constant region comprises immunoglobulin CH2 and CH3 domains, such as, for example, immunoglobulin CH2 and CH3 domains of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, or any combination thereof; an immunoglobulin CH3 domain of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, IgM or any combination thereof; or immunoglobulin CH3 and CH4 domains of IgE, IgM or a combination thereof.

[0027] In certain embodiments, a PSMA-binding polypeptide includes at least one effector function selected from antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). In some such embodiments, the hinge region is derived from an immunoglobulin hinge region and the immunoglobulin constant region comprises immunoglobulin CH2 and CH3 domains of IgG1, IgG2, IgG3, or IgG4. In more specific variations, the immunoglobulin hinge region is derived from the hinge region of IgG1 and the immunoglobulin constant region comprises immunoglobulin CH2 and CH3 domains of IgG1.

[0028] In certain embodiments, a PSMA-binding polypeptide comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100%% identical to the amino acid sequence set forth in SEQ ID NO:46, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, or SEQ ID NO:61.

[0029] In another embodiment, the present disclosure provides a PSMA-binding protein comprising two, non-identical polypeptide chains that associate by way of heterodimerization

domains (*e.g.*, immunoglobulin heterodimerization domains) to form a heterodimer. In some embodiments, the heterodimeric PSMA binding protein comprises a first polypeptide chain comprising, in order from amino-terminus to carboxyl-terminus, (a) a first binding domain that specifically binds PSMA, (b) a first hinge region, (c) a first immunoglobulin constant region, and (d) a first immunoglobulin heterodimerization domain; and a second single chain polypeptide comprising, in order from amino-terminus to carboxyl-terminus, (a') a second hinge region, (b') a second immunoglobulin sub-region, and (c') a second immunoglobulin heterodimerization domain that is different from the first immunoglobulin heterodimerization domain of the first polypeptide chain, wherein the first and second immunoglobulin heterodimerization domains associate with each other to form a heterodimer. In certain embodiments, the PSMA-binding domain competes for binding to human PSMA with a single chain Fv (scFv) having the amino acid sequence set forth in SEQ ID NO:21. In certain embodiments, the PSMA-binding domains include, *e.g.*, the PSMA-binding domains disclosed above.

[0030] In certain embodiments, heterodimerization domains include domains comprising either an immunoglobulin CH1 domain or an immunoglobulin CL domain. In some such embodiments, the first immunoglobulin heterodimerization domain comprises a first immunoglobulin CH1 domain and the second immunoglobulin heterodimerization domain comprises a first immunoglobulin CL domain. Alternatively, in other embodiments, the first immunoglobulin heterodimerization domain comprises a first immunoglobulin CL domain and the second immunoglobulin heterodimerization domain comprises a first immunoglobulin CH1 domain.

[0031] In some embodiments, at least one of the first and second hinge regions is derived from an immunoglobulin hinge region, such as, for example, an immunoglobulin hinge region of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgD. Such an immunoglobulin hinge region can be either a wild-type or an altered immunoglobulin hinge region. In further embodiments, at least one of the first and second immunoglobulin constant regions comprises immunoglobulin CH2 and CH3 domains, such as, for example, immunoglobulin CH2 and CH3 domains of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, or any combination thereof; an immunoglobulin CH3 domain of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, IgM or any combination thereof; or immunoglobulin CH3 and CH4 domains of IgE, IgM or a combination thereof.

[0032] In certain variations of a heterodimeric PSMA-binding protein as disclosed herein, one or both of the first and second polypeptide chains include at least one effector function selected from antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). In some such embodiments, each of the first and second

hinge regions is derived from an immunoglobulin hinge region and each of the first and second immunoglobulin constant regions comprises immunoglobulin CH2 and CH3 domains of IgG1, IgG2, IgG3, or IgG4. In certain embodiments, each of the the first and second hinge regions is derived from the hinge region of IgG1 and each of the the first and second immunoglobulin constant region comprises immunoglobulin CH2 and CH3 domains of IgG1.

[0033] In some embodiments of a heterodimeric PSMA-binding protein as disclosed herein, the second polypeptide chain further includes a second binding domain. For example, the second polypeptide chain can further comprise a second binding domain amino-terminal to the second hinge region.

[0034] In certain variations, a heterodimeric PSMA-binding protein as disclosed herein can be monospecific (*i.e.*, monospecific for PSMA). Alternatively, in other embodiments, the heterodimeric PSMA-binding protein is multispecific. For instance, each polypeptide chain of the heterodimer can comprise different binding domains, *e.g.*, the first polypeptide chain comprising the PSMA-binding domain and the second polypeptide chain comprising a second binding (*e.g.*, amino-terminal to the second hinge region) that is specific for a second target antigen that is different from PSMA.

[0035] In some embodiments of a multispecific, heterodimeric PSMA-binding protein, the second binding domain specifically binds a T-cell. In certain embodiments, T-cell-binding domains include, *e.g.*, the additional binding domains and second binding domains disclosed above. In certain embodiments of a heterodimeric PSMA-binding protein comprising a second binding domain that specifically binds a T-cell, for example, (a) the first polypeptide chain comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 46 and the second polypeptide chain comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 47; (b) the first polypeptide chain comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 58 and the second polypeptide chain comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 57; (c) the first polypeptide chain comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 59 and the second polypeptide chain comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 57; (d) the first polypeptide chain comprises an amino acid sequence that is at least 99%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 60 and the second polypeptide chain comprises an amino

acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 47; or (e) the first polypeptide chain comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 61 and the second polypeptide chain comprises an amino acid sequence that is at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 47.

[0036] In certain embodiments of a dimeric or heterodimeric PSMA-binding protein as disclosed herein, the PSMA-binding protein exhibits increased serum half-life, reduced internalization by a cell expressing PSMA, and/or increased time of persistence on the surface of the cell expressing PSMA as compared to the murine monoclonal antibody 107-1A4.

[0037] In one aspect, there is provided an isolated nucleic acid encoding the PSMA-binding polypeptide as defined herein. For example, in certain variations, the nucleic acid comprises the nucleotide sequence set forth in SEQ ID NO NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:53, SEQ ID: NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:157, SEQ ID NO:159, SEQ ID NO:161, or SEQ ID NO:163.

[0038] In another embodiment, the present disclosure provides an expression vector for expressing a PSMA-binding polypeptide or protein as disclosed herein in a recombinant host cell. In some embodiments, the expression vector comprises a nucleic acid segment encoding the PSMA-binding polypeptide, wherein the nucleic acid segment is operably linked to regulatory sequences suitable for expression of the nucleic acid segment in a host cell. In some embodiments, the nucleic acid segment comprises the nucleotide sequence set forth in SEQ ID NO NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:53, SEQ ID: NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:157, SEQ ID NO:159, SEQ ID NO:161, or SEQ ID NO:163. In other embodiments, the expression vector comprises first and second expression units, wherein the first and second expression units respectively comprise first and second nucleic acid segments encoding the first and second polypeptide chains of a heterodimeric PSMA-binding protein as in certain embodiments disclosed herein, and

wherein the first and second nucleic acid segments are operably linked to regulatory sequences suitable for expression of the nucleic acid segments in a host cell. In certain variations, (a) the first nucleic acid segment comprises the nucleotide sequence set forth in SEQ ID NO:44 and the second nucleic acid segment comprises the nucleotide sequence set forth in SEQ ID NO:45; (b) the first nucleic acid segment comprises the nucleotide sequence set forth in SEQ ID NO:53 and the second nucleic acid segment comprises the nucleotide sequence set forth in SEQ ID NO:52; (c) the first nucleic acid segment comprises the nucleotide sequence set forth in SEQ ID NO:54 and the second nucleic acid segment comprises the nucleotide sequence set forth in SEQ ID NO:52; (d) the first nucleic acid segment comprises the nucleotide sequence set forth in SEQ ID NO:55 and the second nucleic acid segment comprises the nucleotide sequence set forth in SEQ ID NO:45; or (e) the first nucleic acid segment comprises the nucleotide sequence set forth in SEQ ID NO:56 and the second nucleic acid segment comprises the nucleotide sequence set forth in SEQ ID NO:45.

[0038a] In one aspect, there is provided a recombinant host cell comprising the nucleic acid as defined above.

[0039] In another embodiment, the present disclosure provides a recombinant host cell comprising an expression vector disclosed herein.

[0040] In another embodiment, the present disclosure provides a method for producing a PSMA-binding polypeptide or protein. For example, in some embodiments, the method is for producing a PSMA-binding polypeptide as disclosed herein. In certain embodiments, the method generally includes culturing a recombinant host cell comprising an expression vector, wherein the expression vector comprises a nucleic acid segment that encodes the PSMA-binding polypeptide and is operably linked to regulatory sequences suitable for expression of the nucleic acid segment in the host cell, and wherein the culturing is under conditions whereby the nucleic acid segment is expressed, thereby producing the PSMA-binding polypeptide. In certain variations, the nucleic acid segment comprises the nucleotide sequence set forth in SEQ ID NO NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO.28, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:53, SEQ ID: NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:157, SEQ ID NO:159, SEQ ID NO:161, or SEQ ID NO:163. In certain embodiments, the method further includes recovering the PSMA-binding polypeptide.

[0041] In some embodiments, the method is for producing a dimeric PSMA-binding protein as disclosed herein. In certain variations, the nucleic acid segment of the expression vector encodes the PSMA-binding polypeptide as disclosed herein, and the culturing is under

conditions whereby the nucleic acid segment is expressed and the encoded PSMA-binding polypeptide is produced as a dimeric PSMA-binding protein. The method can further include recovering the dimeric PSMA-binding protein.

[0042] In other embodiments, the method is for producing a heterodimeric PSMA-binding protein disclosed herein. In certain embodiments, the method generally includes culturing a recombinant host cell comprising first and second expression units, wherein the first and second expression units respectively comprise first and second nucleic acid segments encoding the first and second polypeptide chains of a heterodimeric PSMA-binding protein as set forth herein, wherein the first and second nucleic acid segments are operably linked to regulatory sequences suitable for expression of the nucleic acid segments in a host cell, and wherein the culturing is under conditions whereby the first and second nucleic acid segments are expressed and the encoded polypeptide chains are produced as the heterodimeric PSMA-binding protein. In some embodiments, the method further includes recovering the heterodimeric PSMA-binding protein.

[0042a] In one aspect, there is provided a composition comprising the PSMA-binding protein as defined above; and a pharmaceutically acceptable carrier, diluent, or excipient.

[0043] In another embodiment, the present disclosure provides a composition comprising any of the PSMA-binding polypeptides or proteins as set forth herein and a pharmaceutically acceptable carrier, diluent, or excipient.

[0044] In another embodiment, the present disclosure provides a method for inducing antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) against a cell expressing PSMA. For example, in some embodiments, a method for inducing ADCC or CDC against the cell expressing PSMA includes contacting the PSMA-expressing cell with a dimeric PSMA-binding protein comprising first and second polypeptide chains, wherein each of the polypeptide chains is a PSMA-binding polypeptide as disclosed herein, and wherein the contacting is under conditions whereby ADCC or CDC against the PSMA-expressing cell is induced. In other embodiments, a method for inducing ADCC or CDC against the PSMA-expressing cell includes contacting the cell with a heterodimeric PSMA-binding protein as in paragraph [0031], wherein the contacting is under conditions whereby ADCC or CDC against the PSMA-expressing cell is induced.

[0044a] In one aspect, there is provided an ex vivo method for inducing redirected T-cell cytotoxicity (RTCC) against a cell expressing prostate-specific membrane antigen (PSMA), the method comprising contacting said PSMA-expressing cell with the PSMA-binding protein of any as defined above, wherein said contacting is under conditions whereby RTCC against the PSMA-expressing cell is induced.

[0045] In another embodiment, the present disclosure provides a method for inducing redirected T-cell cytotoxicity (RTCC) against a cell expressing PSMA. In some variations, a method for inducing RTCC against the cell expressing PSMA includes contacting the PSMA-expressing cell with a dimeric PSMA-binding protein comprising first and second polypeptide chains, wherein each of said polypeptide chains is a PSMA-binding polypeptide disclosed herein, and wherein the contacting is under conditions whereby RTCC against the PSMA-expressing cell is induced. In other embodiments, a method for inducing RTCC against the

PSMA-expressing cell includes contacting the cell with a heterodimeric PSMA-binding protein as disclosed herein, wherein the contacting is under conditions whereby RTCC against the PSMA-expressing cell is induced.

[0046] In another embodiment, the present disclosure provides a method for treating a disorder in a subject, wherein the disorder is characterized by overexpression of PSMA. In some embodiments, the method includes administering to the subject a therapeutically effective amount of a dimeric PSMA-binding protein disclosed above. In some such embodiments, the first and second polypeptide chains of the dimeric PSMA-binding protein is a PSMA-binding polypeptide, *e.g.*, as disclosed above, and the dimeric PSMA-binding protein induces redirected T-cell cytotoxicity (RTCC) in the subject. In other variations, the method includes administering to the subject a therapeutically effective amount of a heterodimeric PSMA-binding protein, *e.g.*, as disclosed above. In some variations, the heterodimeric PSMA-binding protein is a protein as disclosed above, and the heterodimeric PSMA-binding protein induces RTCC in the subject. In certain embodiments of the disclosed methods, the disorder is a cancer such as, for example, prostate cancer (*e.g.*, castrate-resistant prostate cancer), colorectal cancer, gastric cancer, clear cell renal carcinoma, bladder cancer, or lung cancer. In some embodiments, the disorder is a prostate disorder such as, *e.g.*, prostate cancer or benign prostatic hyperplasia. In other variations, the disorder is an neovascular disorder. The neovascular disorder to be treated can be, for example, a cancer characterized by solid tumor growth such as, *e.g.*, clear cell renal carcinoma, colorectal cancer, bladder cancer, and lung cancer.

[0046a] In one aspect, there is provided the use of the dimeric PSMA-binding protein as defined above in the manufacture of a medicament for treating a disorder in a subject characterized by overexpression of prostate-specific membrane antigen (PSMA), wherein preferably, the disorder is a cancer, a prostate disorder, a neovascular disorder, a prostate cancer, colorectal cancer, gastric cancer, castrate-resistant prostate cancer, benign prostatic hyperplasia, solid tumor growth, clear cell renal carcinoma, colorectal cancer, bladder cancer, or lung cancer.

[0047] These and other embodiments and/or other aspects of the disclosure will become evident upon reference to the following detailed description of the disclosure and the attached drawings.

DESCRIPTION OF THE FIGURES

[0048] Figure 1 is a graph illustrating the results of a binding study used to compare the parent 107-1A4 murine antibody (TSC045) with TSC085, TSC092 and TSC122 in PSMA(+) (LNCaP) and PSMA(-) (DU-145) prostate cancer cell lines.

[0049] Figure 2A is a graph illustrating the results of a binding study used to compare humanized TSC188 and TSC189 in PSMA(+) (C4-2) and PSMA(-) (DU-145) prostate cancer cell lines.

[0050] Figure 2B is a graph illustrating the results of a binding study used to compare binding of humanized SCORPION molecules TSC194 and TSC199 to that of parent

humanized SMIP molecules TSC188 and TSC189 and chimeric Interceptor molecule TSC122 in PSMA(+) (C4-2) and PSMA(-) (DU-145) prostate cancer cell lines.

[0051] Figure 3 is a graph illustrating the results of internalization experiments comparing the parent 107-1A4 murine antibody to PSMA-binding proteins built on Interceptor and SMIP scaffolds.

[0052] Figure 4 is a graph illustrating potent target-dependent cytotoxic activity over 24 hours observed with the chimeric TSC122 Interceptor molecule at decreasing concentrations (300, 100, 30, 10 and 0 pM) in the presence of T cells from human blood from two different donors (labeled as AG and VV).

[0053] Figure 5 is a graph illustrating cytotoxicity activity of TSC200, TSC202, TSC204 alongside the parent chimeric Interceptor molecule TSC122.

[0054] Figure 6 is a graph illustrating T-cell cytotoxicity mediated by humanized 107-1A4 SCORPION molecules (TSC194, TSC199, TSC212, TSC213) compared to the chimeric Interceptor molecule TSC122.

[0055] Figures 7A and 7B are graphs illustrating target-dependent proliferation of CD4+ T-cells (Figure 7A) and CD8+ T-cells (Figure 7B) induced by anti-PSMA bispecific molecules (TSC194, TSC199, TSC202 and TSC122) reacting with C4-2 cells.

[0056] Figures 8A-8C are graphs illustrating competitive binding studies of mAbs J591 and J415 versus 107-1A4 mAb and chimeric and humanized 107-1A4 SMIP molecules to PSMA on C4-2 cells. Specifically, Figure 8A shows the results of a competitive binding assay to determine if the humanized J591 antibody (Hu591) competes with the binding of 107-1A4, J591 or J415 murine antibodies to PSMA on C4-2 cells; Figure 8B shows the results of a competitive binding assay to determine if the three murine antibodies compete with the binding of the chimeric 107-1A4 SMIP molecule (TSC085) to PSMA on C4-2 cells; and Figure 8C shows the results of a competitive binding assay to determine if the three murine antibodies compete with the binding of the humanized 107-1A4 SMIP molecule (TSC189) to PSMA on C4-2 cells.

DETAILED DESCRIPTION OF THE INVENTION

I. General Description

[0057] The invention provides PSMA-binding polypeptides and proteins that specifically bind prostate-specific membrane antigen (PSMA). Administration of a therapeutically effective amount of a PSMA-binding polypeptide or protein of the invention to a patient in

need thereof is useful for treatment of certain disorders associated with the over-expression of PSMA, including certain cancers and prostate disorders. In one embodiment, the PSMA-binding polypeptide or protein simultaneously bind a target cell over-expressing PSMA and a T-cell, thereby "cross-linking" the target cell over-expressing PSMA and the T-cell. The binding of both domains to their targets elicits potent target-dependent redirected T-cell cytotoxicity (RTCC) (*e.g.*, induces target-dependent T-cell cytotoxicity, T-cell activation and T-cell proliferation).

[0058] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited herein, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose. In the event that one or more of the incorporated documents or portions of documents define a term that contradicts that term's definition in the application, the definition that appears in this application controls.

[0059] In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. As used herein, "about" means ± 20% of the indicated range, value, or structure, unless otherwise indicated. It should be understood that the terms "a" and "an" as used herein refer to "one or more" of the enumerated components unless otherwise indicated. The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the terms "include" and "comprise" are used synonymously. In addition, it should be understood that the polypeptides comprising the various combinations of the components (*e.g.*, domains or regions) and substituents described herein, are disclosed by the present application to the same extent as if each polypeptide was set forth individually. Thus, selection of particular components of individual polypeptides is within the scope of the present disclosure.

II. Definitions

[0060] As used herein, the term "binding domain" or "binding region" refers to the domain, region, portion, or site of a protein, polypeptide, oligopeptide, or peptide that possesses the ability to specifically recognize and bind to a target molecule, such as an antigen, ligand, receptor, substrate, or inhibitor (*e.g.*, CD3, PSMA). Exemplary binding domains include single-chain antibody variable regions (*e.g.*, domain antibodies, sFv, scFv, scFab), receptor ectodomains, and ligands (*e.g.*, cytokines, chemokines). In certain embodiments, the

binding domain comprises or consists of an antigen binding site (*e.g.*, comprising a variable heavy chain sequence and variable light chain sequence or three light chain complementary determining regions (CDRs) and three heavy chain CDRs from an antibody placed into alternative framework regions (FRs) (*e.g.*, human FRs optionally comprising one or more amino acid substitutions). A variety of assays are known for identifying binding domains of the present disclosure that specifically bind a particular target, including Western blot, ELISA, phage display library screening, and BIACORE® interaction analysis. As used herein, a PSMA-binding polypeptide can have a "first binding domain" and, optionally, a "second binding domain." In certain embodiments, the "first binding domain" is a PSMA-binding domain and, depending on the particular polypeptide format (*e.g.*, SMIP or PIMS), can be located at either the amino- or carboxyl-terminus. In certain embodiments comprising both the first and second binding domains, the second binding domain is a T cell binding domain such as a scFv derived from a mouse monoclonal antibody (*e.g.*, CRIS-7) that binds to a T cell surface antigen (*e.g.*, CD3). In other embodiments, the second binding domain is a second PSMA-binding domain. In yet other embodiments, the second binding domain is a binding domain other than a PSMA-binding domain or a T cell binding domain.

[0061] A binding domain "specifically binds" a target if it binds the target with an affinity or K_a (*i.e.*, an equilibrium association constant of a particular binding interaction with units of 1/M) equal to or greater than 10^5 M^{-1}, while not significantly binding other components present in a test sample. Binding domains can be classified as "high affinity" binding domains and "low affinity" binding domains. "High affinity" binding domains refer to those binding domains with a K_a of at least 10^7 M^{-1}, at least 10^8 M^{-1}, at least 10^9 M^{-1}, at least 10^{10} M^{-1}, at least 10^{11} M^{-1}, at least 10^{12} M^{-1}, or at least 10^{13} M^{-1}. "Low affinity" binding domains refer to those binding domains with a K_a of up to 10^7 M^{-1}, up to 10^6 M^{-1}, up to 10^5 M^{-1}. Alternatively, affinity can be defined as an equilibrium dissociation constant (K_d) of a particular binding interaction with units of M (*e.g.*, 10^{-5} M to 10^{-13} M). Affinities of binding domain polypeptides and single chain polypeptides according to the present disclosure can be readily determined using conventional techniques (*see, e.g.*, Scatchard et al. (1949) Ann. N.Y. Acad. Sci. 51:660; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent).

[0062] "CD3" is known in the art as a multi-protein complex of six chains (*see, e.g.*, Abbas and Lichtman, 2003; Janeway *et al.*, p. 172 and 178, 1999), which are subunits of the T cell receptor complex. In mammals, the CD3 subunits of the T cell receptor complex are a CD3γ chain, a CD3δ chain, two CD3ε chains, and a homodimer of CD3ζ chains. The CD3γ, CD3δ, and CD3ε chains are highly related cell surface proteins of the immunoglobulin superfamily containing a single immunoglobulin domain. The transmembrane regions of the CD3γ, CD3δ, and CD3ε chains are negatively charged, which is a characteristic that allows

these chains to associate with the positively charged T cell receptor chains. The intracellular tails of the CD3γ, CD3δ, and CD3ε chains each contain a single conserved motif known as an immunoreceptor tyrosine-based activation motif or ITAM, whereas each CD3ζ chain has three. It is believed the ITAMs are important for the signaling capacity of a TCR complex. CD3 as used in the present disclosure can be from various animal species, including human, monkey, mouse, rat, or other mammals.

[0063] As used herein, a "conservative substitution" is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are well-known in the art (*see, e.g.*, WO 97/09433, page 10, published March 13, 1997; Lehninger, Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp.71-77; Lewin, Genes IV, Oxford University Press, NY and Cell Press, Cambridge, MA (1990), p. 8). In certain embodiments, a conservative substitution includes a leucine to serine substitution.

[0064] As used herein, the term "derivative" refers to a modification of one or more amino acid residues of a peptide by chemical or biological means, either with or without an enzyme, *e.g.*, by glycosylation, alkylation, acylation, ester formation, or amide formation.

[0065] As used herein, a polypeptide or amino acid sequence "derived from" a designated polypeptide or protein refers to the origin of the polypeptide. In certain embodiments, the polypeptide or amino acid sequence which is derived from a particular sequence (sometimes referred to as the "starting" or "parent" or "parental" sequence) has an amino acid sequence that is essentially identical to the starting sequence or a portion thereof, wherein the portion consists of at least 10-20 amino acids, at least 20-30 amino acids, or at least 30-50 amino acids, or at least 50-150 amino acids, or which is otherwise identifiable to one of ordinary skill in the art as having its origin in the starting sequence.

[0066] Polypeptides derived from another polypeptide can have one or more mutations relative to the starting polypeptide, *e.g.*, one or more amino acid residues which have been substituted with another amino acid residue or which has one or more amino acid residue insertions or deletions. The polypeptide can comprise an amino acid sequence which is not naturally occurring. Such variations necessarily have less than 100% sequence identity or similarity with the starting polypeptide. In one embodiment, the variant will have an amino acid sequence from about 60% to less than 100% amino acid sequence identity or similarity with the amino acid sequence of the starting polypeptide. In another embodiment, the variant will have an amino acid sequence from about 75% to less thant 100%, from about 80% to less than 100%, from about 85% to less than 100%, from about 90% to less than

100%, from about 95% to less than 100% amino acid sequence identity or similarity with the amino acid sequence of the starting polypeptide.

[0067] As used herein, unless otherwise provided, a position of an amino acid residue in a variable region of an immunoglobulin molecule is numbered according to the Kabat numbering convention (Kabat, Sequences of Proteins of Immunological Interest, 5th ed. Bethesda, MD: Public Health Service, National Institutes of Health (1991)), and a position of an amino acid residue in a constant region of an immunoglobulin molecule is numbered according to EU nomenclature (Ward *et al.*, 1995 *Therap. Immunol.* 2:77-94).

[0068] As used herein, the term "dimer" refers to a biological entity that consists of two subunits associated with each other via one or more forms of intramolecular forces, including covalent bonds (*e.g.*, disulfide bonds) and other interactions (*e.g.*, electrostatic interactions, salt bridges, hydrogen bonding, and hydrophobic interactions), and is stable under appropriate conditions (*e.g.*, under physiological conditions, in an aqueous solution suitable for expressing, purifying, and/or storing recombinant proteins, or under conditions for non-denaturing and/or non-reducing electrophoresis). A "heterodimer" or "heterodimeric protein," as used herein, refers to a dimer formed from two different polypeptides. A heterodimer does not include an antibody formed from four polypeptides (*i.e.*, two light chains and two heavy chains). A "homodimer" or "homodimeric protein," as used herein, refers to a dimer formed from two identical polypeptides.

[0069] As used herein, a "hinge region" or a "hinge" refers to a polypeptide derived from (a) an interdomain region of a transmembrane protein (*e.g.*, a type I transmembrane protein); or (b) a stalk region of a type II C-lectin. For example, a hinge region can be derived from an interdomain region of an immunoglobulin superfamily member; suitable hinge regions within this particular class include (i) immunoglobulin hinge regions (made up of, for example, upper and/or core region(s)) or functional variants thereof, including wild-type and altered immunoglobulin hinges, and (ii) regions (or functional variants thereof) that connect immunoglobulin V-like or immunoglobulin C-like domains.

[0070] A "wild-type immunoglobulin hinge region" refers to a naturally occurring upper and middle hinge amino acid sequences interposed between and connecting the CH1 and CH2 domains (for IgG, IgA, and IgD) or interposed between and connecting the CH1 and CH3 domains (for IgE and IgM) found in the heavy chain of an antibody. In certain embodiments, a wild type immunoglobulin hinge region sequence is human, and can comprise a human IgG hinge region.

[0071] An "altered wild-type immunoglobulin hinge region" or "altered immunoglobulin hinge region" refers to (a) a wild type immunoglobulin hinge region with up to 30% amino

acid changes (*e.g.*, up to 25%, 20%, 15%, 10%, or 5% amino acid substitutions or deletions), or (b) a portion of a wild type immunoglobulin hinge region that has a length of about 5 amino acids (*e.g.*, about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids) up to about 120 amino acids (for instance, having a length of about 10 to about 40 amino acids or about 15 to about 30 amino acids or about 15 to about 20 amino acids or about 20 to about 25 amino acids), has up to about 30% amino acid changes (*e.g.*, up to about 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% amino acid substitutions or deletions or a combination thereof), and has an IgG core hinge region as disclosed in PCT/US2010/62436 and PCT/US2010/62404.

[0072] As used herein, the term "humanized" refers to a process of making an antibody or immunoglobulin binding proteins and polypeptides derived from a non-human species (*e.g.*, mouse or rat) less immunogenic to humans, while still retaining antigen-binding properties of the original antibody, using genetic engineering techniques. In some embodiments, the binding domain(s) of an antibody or immunoglobulin binding proteins and polypeptides (*e.g.*, light and heavy chain variable regions, Fab, scFv) are humanized. Non-human binding domains can be humanized using techniques known as CDR grafting (Jones *et al.*, *Nature* 321:522 (1986)) and variants thereof, including "reshaping" (Verhoeyen, *et al.*, 1988 *Science* 239:1534-1536; Riechmann, *et al.*, 1988 *Nature* 332:323-337; Tempest, *et al.*, *Bio/Technol* 1991 9:266-271), "hyperchimerization" (Queen, *et al.*, 1989 *Proc Natl Acad Sci USA* 86:10029-10033; Co, *et al.*, 1991 *Proc Natl Acad Sci USA* 88:2869-2873; Co, *et al.*, 1992 *J Immunol* 148:1149-1154), and "veneering" (Mark, *et al.*, "Derivation of therapeutically active humanized and veneered anti-CD18 antibodies. In: Metcalf BW, Dalton BJ, eds. Cellular adhesion: molecular definition to therapeutic potential. New York: Plenum Press, 1994: 291-312). If derived from a non-human source, other regions of the antibody or immunoglobulin binding proteins and polypeptides, such as the hinge region and constant region domains, can also be humanized.

[0073] An "immunoglobulin dimerization domain" or "immunoglobulin heterodimerization domain", as used herein, refers to an immunoglobulin domain of a polypeptide chain that preferentially interacts or associates with a different immunoglobulin domain of a second polypeptide chain, wherein the interaction of the different immunoglobulin heterodimerization domains substantially contributes to or efficiently promotes heterodimerization of the first and second polypeptide chains (*i.e.*, the formation of a dimer between two different polypeptide chains, which is also referred to as a "heterodimer"). The interactions between immunoglobulin heterodimerization domains "substantially contributes to or efficiently promotes" the heterodimerization of first and second polypeptide chains if there is a statistically significant reduction in the dimerization between the first and second polypeptide

chains in the absence of the immunoglobulin heterodimerization domain of the first polypeptide chain and/or the immunoglobulin heterodimerization domain of the second polypeptide chain. In certain embodiments, when the first and second polypeptide chains are co-expressed, at least 60%, at least about 60% to about 70%, at least about 70% to about 80%, at least 80% to about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the first and second polypetpide chains form heterodimers with each other. Representative immunoglobulin heterodimerization domains include an immunoglobulin CH1 domain, an immunoglobulin CL domain (*e.g.*, Cκ or Cλ isotypes), or derivatives thereof, including wild type immunoglobulin CH1 and CL domains and altered (or mutated) immunoglobulin CH1 and CL domains, as provided therein.

[0074] An "immunoglobulin constant region" or "constant region" is a term defined herein to refer to a peptide or polypeptide sequence that corresponds to or is derived from part or all of one or more constant region domains. In certain embodiments, the immunoglobulin constant region corresponds to or is derived from part or all of one or more constant region domains, but not all constant region domains of a source antibody. In certain embodiments, the constant region comprises IgG CH2 and CH3 domains, e.g., IgG1 CH2 and CH3 domains. In certain embodiments, the constant region does not comprise a CH1 domain. In certain embodiments, the constant region domains making up the constant region are human. In some embodiments (for example, in certain variations of a PSMA-binding polypeptide or protein comprising a second binding domain that specifically binds CD3 or another T cell surface antigen), the constant region domains of a fusion protein of this disclosure lack or have minimal effector functions of antibody-dependent cell-mediated cytotoxicity (ADCC) and complement activation and complement-dependent cytotoxicity (CDC), while retaining the ability to bind some F_C receptors (such as F_CRn, the neonatal Fc receptor) and retaining a relatively long half life *in vivo*. In other variations, a fusion protein of this disclosure includes constant domains that retain such effector function of one or both of ADCC and CDC. In certain embodiments, a binding domain of this disclosure is fused to a human IgG1 constant region, wherein the IgG1 constant region has one or more of the following amino acids mutated: leucine at position 234 (L234), leucine at position 235 (L235), glycine at position 237 (G237), glutamate at position 318 (E318), lysine at position 320 (K320), lysine at position 322 (K322), or any combination thereof (numbering according to EU). For example, any one or more of these amino acids can be changed to alanine. In a further embodiment, an IgG1 Fc domain has each of L234, L235, G237, E318, K320, and K322 (according to EU numbering) mutated to an alanine (*i.e.*, L234A, L235A, G237A, E318A, K320A, and K322A, respectively), and optionally an N297A mutation as well (*i.e.*, essentially eliminating glycosylation of the CH2 domain).

[0075] "Fc region" or "Fc domain" refers to a polypeptide sequence corresponding to or derived from the portion of a source antibody that is responsible for binding to antibody receptors on cells and the C1q component of complement. Fc stands for "fragment crystalline," the fragment of an antibody that will readily form a protein crystal. Distinct protein fragments, which were originally described by proteolytic digestion, can define the overall general structure of an immunoglobulin protein. As originally defined in the literature, the Fc fragment consists of the disulfide-linked heavy chain hinge regions, CH2, and CH3 domains. However, more recently the term has been applied to a single chain consisting of CH3, CH2, and at least a portion of the hinge sufficient to form a disulfide-linked dimer with a second such chain. For a review of immunoglobulin structure and function, see Putnam, *The Plasma Proteins*, Vol. V (Academic Press, Inc., 1987), pp. 49-140; and Padlan, *Mol. Immunol.* 31:169-217, 1994. As used herein, the term Fc includes variants of naturally occuring sequences.

[0076] As used here the term "SMIP" is used to refer to protein scaffold as generally disclosed in, for example, in US Patent Application Publication Nos. 2003/0133939, 2003/0118592, and 2005/0136049, which are incorporated herein by reference in their entirety. The "PSMA-specific SMIP molecules" or "SMIP molecules" described in the Examples and throughout the disclosure herein should be understood to be PSMA-binding proteins comprising SMIP scaffolding, *e.g.*, in order from amino to carboxyl-terminus, a first binding domain, a hinge region, and an immunoglobulin constant constant region.

[0077] As used here the term "PIMS" is used to refer to protein scaffold as generally disclosed in, for example, in US Patent Application Publication No. 2009/0148447, which is incorporated herein in its entirety by reference. The "PSMA-specific PIMS molecules" or "PIMS molecules" described in the Examples and throughout the disclosure herein should be understood to be PSMA-binding proteins comprising PIMS scaffolding, *e.g.*, in order from amino to carboxyl-terminus, an immunoglobulin constant region, a hinge region and a first binding domain.

[0078] As used herein, the term "Interceptor" is used to refer to a monospecific or multispecific heterodimeric protein scaffold as generally disclosed in PCT applications PCT/US2010/62436 and PCT/US2010/62404, which are incorporated herein in their entirety. The "PSMA-specific Interceptor molecules" or "Interceptor molecules" described in the Examples and throughout the disclosure herein should be understood to be PSMA-binding proteins comprising Interceptor scaffolding, *e.g.*, two non-identical polypeptide chains, each polypeptide chain comprising an immunoglobulin heterodimerization domain. The interfacing immunoglobulin heterodimerization domains are different. In one embodiment, the immunoglobulin heterodimerization domain comprises a CH1 domain or a derivative

thereof. In another embodiment, the immunoglobulin heterodimerization domain comprises a CL domain or a derivative thereof. In one embodiment, the CL domain is a Cκ or Cλ isotype or a derivative thereof.

[0079] As used herein, "SCORPION", is a term used to refer to a multi-specific binding protein scaffold. SCORPION™ is a trademark of Emergent Product Development Seattle, LLC. Multi-specific binding proteins and polypeptides are disclosed, for instance, in PCT Application Publication No. WO 2007/146968, U.S. Patent Application Publication No. 2006/0051844, PCT Application Publication No. WO 2010/040105, PCT Application Publication No. WO 2010/003108, and U.S. Patent No. 7,166,707, which are incorporated herein by reference in their entirety. A SCORPION polypeptide comprises two binding domains (the domains can be designed to specifically bind the same or different targets), two hinge regions, and an immunoglobulin constant region. SCORPION proteins are homodimeric proteins comprising two identical, disulfide-bonded SCORPION polypeptides. The "PSMA-specific SCORPION molecules" or " SCORPION molecules" described in the Examples and throughout the disclosure herein should be understood to be PSMA-binding proteins comprising SCORPION scaffolding, *e.g.*, two binding domains (the domains can be designed to specifically bind the same or different targets), two hinge regions, and an immunoglobulin constant region.

[0080] As used herein, the "stalk region" of a type II C-lectin refers to the portion of the extracellular domain of the type II C-lectin that is located between the C-type lectin-like domain (CTLD; *e.g.*, similar to CTLD of natural killer cell receptors) and the transmembrane domain. For example, in the human CD94 molecule (GenBank Accession No. AAC50291.1, PRI November 30, 1995), the extracellular domain corresponds to amino acid residues 34-179, whereas the CTLD corresponds to amino acid residues 61-176. Accordingly, the stalk region of the human CD94 molecule includes amino acid residues 34-60, which is found between the membrane and the CTLD (*see* Boyington *et al.*, *Immunity* 10:75, 1999; for descriptions of other stalk regions, *see also* Beavil *et al.*, *Proc. Nat'l. Acad. Sci. USA* 89:753, 1992; and Figdor *et al.*, *Nature Rev. Immunol.* 2:77, 2002). These type II C-lectins can also have from six to 10 junction amino acids between the stalk region and the transmembrane region or the CTLD. In another example, the 233 amino acid human NKG2A protein (GenBank Accession No. P26715.1, PRI June 15, 2010) has a transmembrane domain ranging from amino acids 71-93 and an extracellular domain ranging from amino acids 94-233. The CTLD is comprised of amino acids 119-231, and the stalk region comprises amino acids 99-116, which is flanked by junctions of five and two amino acids. Other type II C-lectins, as well as their extracellular ligand-bind domains, interdomain or stalk regions, and CTLDs are known in the art (*see, e.g.*, GenBank Accession Nos. NP_001993.2;

AAH07037.1, PRI July 15, 2006; NP_001773.1, PRI June 20, 1010; AAL65234.1, PRI January 17, 2002, and CAA04925.1, PRI November 14, 2006, for the sequences of human CD23, CD69, CD72, NKG2A and NKG2D and their descriptions, respectively).

[0081] As used herein, the "interdomain region" of a transmembrane protein (*e.g.*, a type I transmembrane protein) refers to a portion of the extracellular domain of the transmembrane protein that is located between two adjacent domains. Examples of interdomain regions include regions linking adjacent Ig domains of immunoglobulin superfamily members (*e.g.*, an immunoglobulin hinge region from IgG, IgA, IgD, or IgE; the region linking the IgV and IgC2 domains of CD2; or the region linking the IgV and IgC domains of CD80 or CD86). Another example of an interdomain region is the region linking the non-Ig and IgC2 domain of CD22, a type I sialic acid-binding Ig-like lectin.

[0082] A polypeptide region "derived from" a stalk region of a type II C-lectin, or "derived from" a transmembrane protein interdomain region (*e.g.*, an immunoglobulin hinge region), refers to an about five to about 150 amino acid sequence, an about 5 to about 100 amino acid sequence, an about 5 to about 50 amino acid sequence, an about 5 to about 40 amino acid sequence, an about 5 to about 30 amino acid sequence, an about 5 to about 25 amino acid sequence, an about 5 to about 20 amino acid sequence, an about 10 to about 25 amino acid sequence, an about 10 to about 20 amino acid sequence, about 8 to about 20 amino acid sequence, about 9 to about 20 amino acid sequence, about 10 to about 20 amino acid sequence, about 11 to about 20 amino acid sequence, about 12 to about 20 amino acid sequence, about 13 to about 20 amino acid sequence, about 14 to about 20 amino acid sequence, about 15 to about 20 amino acid sequence, or an about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid sequence, wherein all or at least a portion of which includes (i) a wild-type stalk region or interdomain region sequence; (ii) a fragment of the wild-type stalk region or interdomain region sequence; (iii) a polypeptide having at least 80%, 85%, 90%, or 95% amino acid sequence identity with either (i) or (ii); or (iv) either (i) or (ii) in which one, two, three, four, or five amino acids have a deletion, insertion, substitution, or any combination thereof, for instance, the one or more changes are substitutions or the one or more mutations include only one deletion. In some embodiments, a derivative of a stalk region is more resistant to proteolytic cleavage as compared to the wild-type stalk region sequence, such as those derived from about eight to about 20 amino acids of NKG2A, NKG2D, CD23, CD64, CD72, or CD94.

[0083] As used herein, the term "junction amino acids" or "junction amino acid residues" refers to one or more (*e.g.*, about 2-10) amino acid residues between two adjacent regions or domains of a polypeptide, such as between a hinge and an adjacent immunoglobulin constant region or between a hinge and an adjacent binding domain or between a peptide

linker that links two immunoglobulin variable domains and an adjacent immunoglobulin variable domain. Junction amino acids can result from the construct design of a polypeptide (*e.g.*, amino acid residues resulting from the use of a restriction enzyme site during the construction of a nucleic acid molecule encoding a polypeptide).

[0084] As used herein, the phrase a "linker between CH3 and CH1 or CL" refers to one or more (*e.g.*, about 2-12, about 2-10, about 4-10, about 5-10, about 6-10, about 7-10, about 8-10, about 9-10, about 8-12, about 9-12, or about 10-12) amino acid residues between the C-terminus of a CH3 domain (*e.g.*, a wild type CH3 or a mutated CH3) and the N-terminus of a CH1 domain or CL domain (*e.g.*, Ck).

[0085] As used herein, the term "patient in need" refers to a patient at risk of, or suffering from, a disease, disorder or condition that is amenable to treatment or amelioration with a PSMA-binding protein or polypeptide or a composition thereof provided herein.

[0086] As used herein, the term "peptide linker" refers to an amino acid sequence that connects a heavy chain variable region to a light chain variable region and provides a spacer function compatible with interaction of the two sub-binding domains so that the resulting polypeptide retains a specific binding affinity to the same target molecule as an antibody that comprises the same light and heavy chain variable regions. In certain embodiments, a linker is comprised of five to about 35 amino acids, for instance, about 15 to about 25 amino acids.

[0087] As used herein, the term "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce allergic or other serious adverse reactions when administered using routes well known in the art. Molecular entities and compositions approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans are considered to be "pharmaceutically acceptable."

[0088] As used herein, the term "promoter" refers to a region of DNA involved in binding RNA polymerase to initiate transcription.

[0089] As used herein, the terms "nucleic acid," "nucleic acid molecule," or "polynucleotide" refer to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the terms encompass nucleic acids containing analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions) and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions can be achieved by generating sequences in

which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.* (1991) *Nucleic Acid Res.* 19:5081; Ohtsuka *et al.* (1985) *J. Biol. Chem.* 260:2605-2608; Cassol *et al.* (1992); Rossolini *et al.* (1994) *Mol. Cell. Probes* 8:91-98). The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene. As used herein, the terms "nucleic acid," "nucleic acid molecule," or "polynucleotide" are intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof.

[0090] The term "expression" refers to the biosynthesis of a product encoded by a nucleic acid. For example, in the case of nucleic acid segment encoding a polypeptide of interest, expression involves transcription of the nucleic acid segment into mRNA and the translation of mRNA into one or more polypeptides.

[0091] The terms "expression unit" and "expression cassette" are used interchangeably herein and denote a nucleic acid segment encoding a polypeptide of interest and capable of providing expression of the nucleic acid segment in a host cell. An expression unit typically comprises a transcription promoter, an open reading frame encoding the polypeptide of interest, and a transcription terminator, all in operable configuration. In addition to a transcriptional promoter and terminator, an expression unit can further include other nucleic acid segments such as, *e.g.*, an enhancer or a polyadenylation signal.

[0092] The term "expression vector," as used herein, refers to a nucleic acid molecule, linear or circular, comprising one or more expression units. In addition to one or more expression units, an expression vector can also include additional nucleic acid segments such as, for example, one or more origins of replication or one or more selectable markers. Expression vectors are generally derived from plasmid or viral DNA, or can contain elements of both.

[0093] As used herein, the term "sequence identity" refers to a relationship between two or more polynucleotide sequences or between two or more polypeptide sequences. When a position in one sequence is occupied by the same nucleic acid base or amino acid residue in the corresponding position of the comparator sequence, the sequences are said to be "identical" at that position. The percentage "sequence identity" is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of "identical" positions. The number of "identical" positions is then divided by the total number of positions in the comparison window and multiplied by 100 to yield the percentage of "sequence identity." Percentage of "sequence identity" is determined by comparing two optimally aligned sequences over a comparison

window. The comparison window for nucleic acid sequences can be, for instance, at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 or more nucleic acids in length. The comparison windon for polypeptide sequences can be, for instance, at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300 or more amino acids in length. In order to optimally align sequences for comparison, the portion of a polynucleotide or polypeptide sequence in the comparison window can comprise additions or deletions termed gaps while the reference sequence is kept constant. An optimal alignment is that alignment which, even with gaps, produces the greatest possible number of "identical" positions between the reference and comparator sequences. Percentage "sequence identity" between two sequences can be determined using the version of the program "BLAST 2 Sequences" which was available from the National Center for Biotechnology Information as of September 1, 2004, which program incorporates the programs BLASTN (for nucleotide sequence comparison) and BLASTP (for polypeptide sequence comparison), which programs are based on the algorithm of Karlin and Altschul (Proc. Natl. Acad. Sci. USA 90(12):5873-5877, 1993). When utilizing "BLAST 2 Sequences," parameters that were default parameters as of September 1, 2004, can be used for word size (3), open gap penalty (11), extension gap penalty (1), gap dropoff (50), expect value (10) and any other required parameter including but not limited to matrix option. Two nucleotide or amino acid sequences are considered to have "substantially similar sequence identity" or "substantial sequence identity" if the two sequences have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity relative to each other.

[0094] As used herein, a "polypeptide" or "polypeptide chain" is a single, linear and contiguous arrangement of covalently linked amino acids. It does not include two polypeptide chains that link together in a non-linear fashion, such as via an interchain disulfide bond (*e.g.*, a half immunoglobulin molecule in which a light chain links with a heavy chain via a disulfide bond). Polypeptides can have or form one or more intrachain disulfide bonds. With regard to polypeptides as described herein, reference to amino acid residues corresponding to those specified by SEQ ID NO includes post-translational modifications of such residues.

[0095] A "protein" is a macromolecule comprising one or more polypeptide chains. A protein can also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents can be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein

in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

[0096] As used herein, "small modular immunopharmaceutical proteins" or SMIP refers to a protein scaffold generally disclosed, for instance, U.S. Patent Publication Nos. 2003/0133939, 2003/0118592, and 2005/0136049. SMIP™ is a trademark of Emergent Product Development Seattle LLC. A SMIP protein can comprise a polypeptide chain having a binding domain, a hinge region and an immunoglobulin constant region.

[0097] The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl-terminus of the reference sequence, but is not necessarily at the carboxyl-terminus of the complete polypeptide.

[0098] "T cell receptor" (TCR) is a molecule found on the surface of T cells that, along with CD3, is generally responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules. It consists of a disulfide-linked heterodimer of the highly variable α and β chains in most T cells. In other T cells, an alternative receptor made up of variable γ and δ chains is expressed. Each chain of the TCR is a member of the immunoglobulin superfamily and possesses one N-terminal immunoglobulin variable domain, one immunoglobulin constant domain, a transmembrane region, and a short cytoplasmic tail at the C-terminal end (*see* Abbas and Lichtman, *Cellular and Molecular Immunology* (5th Ed.), Editor: Saunders, Philadelphia, 2003; Janeway *et al.*, *Immunobiology: The Immune System in Health and Disease*, 4th Ed., Current Biology Publications, p148, 149, and 172, 1999). TCR as used in the present disclosure can be from various animal species, including human, mouse, rat, or other mammals.

[0099] "TCR complex," as used herein, refers to a complex formed by the association of CD3 chains with other TCR chains. For example, a TCR complex can be composed of a CD3γ chain, a CD3δ chain, two CD3ε chains, a homodimer of CD3ζ chains, a TCRα chain, and a TCRβ chain. Alternatively, a TCR complex can be composed of a CD3γ chain, a CD3δ chain, two CD3ε chains, a homodimer of CD3ζ chains, a TCRγ chain, and a TCRδ chain.

[0100] "A component of a TCR complex," as used herein, refers to a TCR chain (*i.e.*, TCRα, TCRβ, TCRγ or TCRδ), a CD3 chain (*i.e.*, CD3γ, CD3δ, CD3ε or CD3ζ), or a complex formed by two or more TCR chains or CD3 chains (*e.g.*, a complex of TCRα and TCRβ, a

complex of TCRγ and TCRδ, a complex of CD3ε and CD3δ, a complex of CD3γ and CD3ε, or a sub-TCR complex of TCRα, TCRβ, CD3γ, CD3δ, and two CD3ε chains).

[0101] "Antibody-dependent cell-mediated cytotoxicity" and "ADCC," as used herein, refer to a cell-mediated process in which nonspecific cytotoxic cells that express FcγRs (e.g., monocytic cells such as Natural Killer (NK) cells and macrophages) recognize bound antibody (or other protein capable of binding FcγRs) on a target cell and subsequently cause lysis of the target cell. In principle, any effector cell with an activating FcγR can be triggered to mediate ADCC. The primary cells for mediating ADCC are NK cells, which express only FcγRIII, whereas monocytes, depending on their state of activation, localization, or differentiation, can express FcγRI, FcγRII, and FcγRIII. For a review of FcγR expression on hematopoietic cells, see, e.g., Ravetch et al., 1991, Annu. Rev. Immunol., 9:457-92.

[0102] The term "having ADCC activity," as used herein in reference to a polypeptide or protein, means that the polypeptide or protein (for example, one comprising an immunoglobulin hinge region and an immunoglobulin constant region having CH2 and CH3 domains, such as derived from IgG (e.g., IgG1)), is capable of mediating antibody-dependent cell-mediated cytotoxicity (ADCC) through binding of a cytolytic Fc receptor (e.g., FcγRIII) on a cytolytic immune effector cell expressing the Fc receptor (e.g., an NK cell).

[0103] "Complement-dependent cytotoxicity" and "CDC," as used herein, refer to a process in which components in normal serum ("complement"), together with an antibody or other C1q-complement-binding protein bound to a target antigen, exhibit lysis of a target cell expressing the target antigen. Complement consists of a group of serum proteins that act in concert and in an orderly sequence to exert their effect.

[0104] The terms "classical complement pathway" and "classical complement system," as used herein, are synonymous and refer to a particular pathway for the activation of complement. The classical pathway requires antigen-antibody complexes for initiation and involves the activation, in an orderly fashion, of nine major protein components designated C1 through C9. For several steps in the activation process, the product is an enzyme that catalyzes the subsequent step. This cascade provides amplification and activation of large amounts of complement by a relatively small initial signal.

[0105] The term "having CDC activity," as used herein in reference to a polypeptide or protein, means that the polypeptide or protein (for example, one comprising an immunoglobulin hinge region and an immunoglobulin constant region having CH2 and CH3 domains, such as derived from IgG (e.g., IgG1)) is capable of mediating complement-dependent cytotoxicity (CDC) through binding of C1q complement protein and activation of the classical complement system.

[0106] "Redirected T-cell cytotoxicity" and "RTCC," as used herein, refer to a T-cell-mediated process in which a cytotoxic T-cell is recruited to a target cell using a multi-specific protein that is capable of specifically binding both the cytotoxic T-cell and the target cell, and whereby a target-dependent cytotoxic T-cell response is elicited against the target cell.

[0107] The terms "neovascularization" and "angiogenesis" are used interchangeably herein. Neovascularization and angiogenesis refer to the generation of new blood vessels into cells, tissue, or organs. The control of angiogenesis is typically altered in certain disease states and, in many case, the pathological damage associated with the disease is related to altered or unregulated angiogenesis. Persistant, unregulated angiogenesis occurs in a variety of disease states, including those characterized by the abnormal growth by endothelial cells, and supports the pathological damage seen in these conditions including leakage and permeability of blood vessels.

[0108] The term "neovascular disorder" are used herein refers to any disease or disorder having a pathology that is mediated, at least in part, by increased or unregulated angiogenesis activity. Examples of such diseases or disorders include various cancers comprising solid tumors. Such diseases or disorders comprising a vasculature characterized by PSMA overexpression (e.g., certain cancers comprising solid tumors, such as clear cell renal carcinoma, colorectal cancer, bladder cancer, and lung cancer) are particularly amenable to certain treatment methods for inhibition angiogenesis, as described further herein.

[0109] As used herein, the term "treatment," "treating," or "ameliorating" refers to either a therapeutic treatment or prophylactic/preventative treatment. A treatment is therapeutic if at least one symptom of disease in an individual receiving treatment improves or a treatment can delay worsening of a progressive disease in an individual, or prevent onset of additional associated diseases.

[0110] As used herein, the term "therapeutically effective amount (or dose)" or "effective amount (or dose)" of a specific binding molecule or compound refers to that amount of the compound sufficient to result in amelioration of one or more symptoms of the disease being treated in a statistically significant manner. When referring to an individual active ingredient, administered alone, a therapeutically effective dose refers to that ingredient alone. When referring to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered serially or simultaneously (in the same formuation or concurrently in separate formulations).

[0111] As used herein, the term "transformation," "transfection," and "transduction" refer to the transfer of nucleic acid (i.e., a nucleotide polymer) into a cell. As used herein, the

term "genetic transformation" refers to the transfer and incorporation of DNA, especially recombinant DNA, into a cell. The transferred nucleic acid can be introduced into a cell via an expression vector.

[0112] As used herein, the term "variant" or "variants" refers to a nucleic acid or polypeptide differing from a reference nucleic acid or polypeptide, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the reference nucleic acid or polypeptide. For instance, a variant may exhibit at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% sequence identity compared to the active portion or full length reference nucleic acid or polypeptide.

[0113] The terms "light chain variable region" (also referred to as "light chain variable domain" or "VL") and "heavy chain variable region" (also referred to as "heavy chain variable domain" or "VH") refer to the variable binding region from an antibody light and heavy chain, respectively. The variable binding regions are made up of discrete, well-defined sub-regions known as "complementarity determining regions" (CDRs) and "framework regions" (FRs). In one embodiment, the FRs are humanized. The term "CL" refers to an "immunoglobulin light chain constant region" or a "light chain constant region," i.e., a constant region from an antibody light chain. The term "CH" refers to an "immunoglobulin heavy chain constant region" or a "heavy chain constant region," which is further divisible, depending on the antibody isotype into CH1, CH2, and CH3 (IgA, IgD, IgG), or CH1, CH2, CH3, and CH4 domains (IgE, IgM). A "Fab" (fragment antigen binding) is the part of an antibody that binds to antigens and includes the variable region and CH1 domain of the heavy chain linked to the light chain via an inter-chain disulfide bond.

III. PSMA-binding Polypeptides, Proteins, and Components Thereof

[0114] The present disclosure provides polypeptides and proteins comprising binding domains, in particular, a first binding domain that specifically binds PSMA. The polypeptides and proteins comprising binding domains of this disclosure can further comprise immunoglobulin constant regions, linker peptides, hinge regions, immunoglobulin dimerization/heterodimerization domains, junctional amino acids, tags, *etc.* These components of the disclosed polypeptides and proteins are described in further detail below.

[0115] Additionally, the PSMA-binding polypeptides and proteins disclosed herein can be in the form of an antibody or a fusion protein of any of a variety of different formats (*e.g.*, the fusion protein can be in the form of a SMIP molecule, a PIMS molecule, a SCORPION molecule or an Interceptor molecule).

[0116] A PSMA-binding protein in accordance with the present invention generally includes at least one PSMA-binding polypeptide chain comprising (a) a PSMA-binding domain as set forth herein. In certain variations, the PSMA-binding polypeptide further includes (b) a hinge region carboxyl-terminal to the PSMA-binding domain, and (c) an immunoglobulin constant region (*e.g.*, a SMIP molecule). In further variations, the PSMA-binding polypeptide further includes (d) a second hinge region carboxyl-terminal to the immunoglobulin constant region, and (e) a second binding domain carboxyl-terminal to the second hinge region (*e.g.*, a SCORPION polypeptide).

[0117] In yet other variations, the PSMA-binding polypeptide comprises (b) a hinge region amino-terminal to the PSMA-binding domain, and (c) an immunoglobulin sub-region amino-terminal to the hinge region (*e.g.*, a PIMS polypeptide).

[0118] Typically, PSMA-binding polypeptides of the above formats (SMIP, SCORPION, or PIMS) are capable of homodimerization, typically through disulfide bonding, via the immunoglobulin constant region and/or hinge region (*e.g.*, via an immunoglobulin constant region comprising IgG CH2 and CH3 domains and an IgG hinge region). Thus, in certain embodiments of the present invention, two identical PSMA-binding polypeptides homodimerize to form a dimeric PSMA-binding protein.

[0119] In other embodiments, a PSMA-binding polypeptide further includes a heterodimerization domain that is capable of heterodimerization with a different heterodimerization domain in a second, non-identical polypeptide chain. In certain variations, the second polypeptide chain for heterodimerization includes a second binding domain. Accordingly, in certain embodiments of the present invention, two non-identical polypeptide chains, one comprising the PSMA-binding domain and the second optionally comprising a second binding domain, dimerize to form a heterodimeric PSMA-binding protein.

[0120] PSMA-binding polypeptides, proteins, and their various components are further described herein below.

A. Binding Domains

[0121] As indicated above, an immunoglobulin binding polypeptide of the present disclosure comprises a binding domain that specifically binds PSMA. In some variations, the PSMA-binding domain is capable of competing for binding to PSMA with an antibody having V_L and V_H regions having amino acid sequences as shown in SEQ ID NO:5 and SEQ ID NO:2, respectively (*e.g.*, mAb 107-1A4), or with a single-chain Fv (scFv) having an amino

acid sequence as shown in SEQ ID NO:21. In certain embodiments, the PSMA-binding domain comprises (i) an immunoglobulin light chain variable region (V_L) comprising CDRs LCDR1, LCDR2, and LCDR3, and (ii) an immunoglobulin heavy chain variable region (V_H) comprising CDRs HCDR1, HCDR2, and HCDR3. Suitable PSMA-binding domains include those having V_L and V_H regions derived from mAb 107-1A4. In some such embodiments, LCDR3 has the amino acid sequence set forth in SEQ ID NO:17 and/or HCDR3 has the amino acid sequence set forth in SEQ ID NO:11; and LCDR1 and LCDR2 optionally have the amino acid sequences as set forth in SEQ ID NO:15 and SEQ ID NO:16, respectively, and HCDR1 and HCDR2 optionally have the amino acid sequences as set forth in SEQ ID NO:9 and SEQ ID NO:10, respectively. In some embodiments, for example, LCDR1, LCDR2, and LCDR3 have the amino acid sequences respectively shown in SEQ ID NOs:15, 16, and 17; and/or HCDR1, HCDR2, and HCDR3 have the amino acid sequences as respectively shown in SEQ ID NOs:9, 10, and 11.

[0122] In certain embodiments, a PSMA-binding protein can comprise one or more additional binding domains (*e.g.*, second binding domain) that bind a target other than PSMA. These other target molecules can comprise, for example, a particular cytokine or a molecule that targets the binding domain polypeptide to a particular cell type, a toxin, an additional cell receptor, an antibody, *etc.*

[0123] In certain embodiments, a binding domain, for instance, as part of an Interceptor or SCORPION molecule, can comprise a TCR binding domain for recruitment of T cells to target cells expressing PSMA. In certain embodiments, a polypeptide heterodimer as described herein can comprise a binding domain that specifically binds a TCR complex or a component thereof (*e.g.*, TCRα, TCRβ, CD3γ, CD3δ, and CD3ε) and another binding domain that specifically binds to PSMA.

[0124] Exemplary anti-CD3 antibodies from which the binding domain of this disclosure can be derived include CRIS-7 monoclonal antibody (Reinherz, E. L. *et al.* (eds.), *Leukocyte typing II.*, Springer Verlag, New York, (1986); V_L and V_H amino acid sequences respectively shown in SEQ ID NO:153 (QVVLTQSPAIMSAFPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDSS KLASGVPARFSGSGSGTSYSLTISSMETEDAATYYCQQWSRNPPTFGGGTKLQITR) and SEQ ID NO:154 (QVQLQQSGAELARPGASVKMSCKASGYTFTRSTMHWVKQRPGQGLEWIGYINP SSAYTNYNQKFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCASPQVHYDYNGFPYWGQ GTLVTVSA)); HuM291 (Chau *et al.* (2001) *Transplantion* 71:941-950; V_L and V_H amino acid sequences respectively shown in SEQ ID NO:86 (DIQMTQSPSSLSASVGDRVTITCSASSSV

SYMNWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ WSSNPPTFGGGTKVEIK) and SEQ ID NO:87 (QVQLVQSGAEVKKPGASVKVSCKASGYTFISY TMHWVRQAPGQGLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDT AVYYCARSAYYDYDGFAYWGQGTLVTVSS)); BC3 monoclonal antibody (Anasetti *et al.* (1990) *J. Exp. Med.* 172:1691); OKT3 monoclonal antibody (Ortho multicenter Transplant Study Group (1985) *N. Engl. J. Med.* 313:337) and derivatives thereof such as OKT3 ala-ala (also referred to as OKT3 AA-FL or OKT3 FL), a humanized, Fc variant with alanine substitutions at positions 234 and 235 (Herold *et al.* (2003) *J. Clin. Invest.* 11:409); visilizumab (Carpenter *et al.* (2002) *Blood* 99:2712), G19-4 monoclonal antibody (Ledbetter *et al.*, 1986, *J. Immunol.* 136:3945) and 145-2C11 monoclonal antibody (Hirsch *et al.* (1988) *J. Immunol.* 140: 3766). An exemplary anti-TCR antibody is the BMA031 monoclonal antibody (Borst *et al.* (1990) *Human Immunology* 29:175-188).

[0125] In some embodiments, a binding domain is a single-chain Fv fragment (scFv) that comprises V_H and V_L regions specific for a target of interest. In certain embodiments, the V_H and V_L regions are human.

[0126] In certain embodiments, a PSMA-binding domain comprises or is a scFv that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to an amino acid sequence of a scFv of SEQ ID NO: 19, 21, 30, 31, 34 or 35.

[0127] In related embodiments, a PSMA-binding domain comprises or is a sequence that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to an amino acid sequence of a light chain variable region (V_L) (*e.g.*, SEQ ID NO:23) or to a heavy chain variable region (V_H) (*e.g.*, SEQ ID NO:25 or SEQ ID NO:27), or both.

[0128] In further embodiments, each CDR comprises no more than one, two, or three substitutions, insertions or deletions, as compared to that from a monoclonal antibody or fragment or derivative thereof that specifically binds to a target of interest (*e.g.*, PSMA).

[0129] In some embodiments of a PSMA-binding protein comprising a second binding domain that specifically binds CD3ε, the second binding domain competes for binding to CD3ε with the CRIS-7 or HuM291 monoclonal antibody. In certain variations, the CD3-binding domain comprises an immunoglobulin light chain variable region (V_L) and an immunoglobulin heavy chain variable region (V_H) derived from the CRIS-7 or HuM291

monoclonal antibody (*e.g.*, the V_L and V_H of the second binding domain can be humanized variable regions comprising, respectively, the light chain CDRs and the heavy chain CDRs of the monoclonal antibody). For example, the V_L and V_H regions derived from CRIS-7 can be selected from (a) a V_L region comprising an amino acid sequence that is at least 95% identical or 100% to the amino acid sequence set forth in residues 139-245 of SEQ ID NO:47 and a V_H region comprising an amino acid sequence that is at least 95% identical or 100% to the amino acid sequence set forth in residues 1-122 of SEQ ID NO:47; and (b) a V_L region comprising an amino acid sequence that is at least 95% identical or 100% identical to the amino acid sequence set forth in residues 634-740 of SEQ ID NO:78 and a V_H region comprising an amino acid sequence that is at least 95% or 100% identical to the amino acid sequence set forth in residues 496-616 of SEQ ID NO:78.

[0130] In certain embodiments, a binding domain V_L and/or V_H region of the present disclosure is derived from a V_L and/or V_H of a known monoclonal antibody (*e.g.*,107-1A4, CRIS-7, or HuM291) and contains about one or more (*e.g.*, about 2, 3, 4, 5, 6, 7, 8, 9, 10) insertions, about one or more (*e.g.*, about 2, 3, 4, 5, 6, 7, 8, 9, 10) deletions, about one or more (*e.g.*, about 2, 3, 4, 5, 6, 7, 8, 9, 10) amino acid substitutions (*e.g.*, conservative amino acid substitutions or non-conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_L and/or V_H of a known monoclonal antibody. The insertion(s), deletion(s) or substitution(s) can be anywhere in the V_L and/or V_H region, including at the amino- or carboxyl-terminus or both ends of this region, provided that each CDR comprises zero changes or at most one, two, or three changes and provided a binding domain containing the modified V_L and/or V_H region can still specifically bind its target with an affinity similar to the wild type binding domain.

[0131] In some variations, the binding domain is a single-chain Fv (scFv) comprising immunoglobulin V_L and V_H regions joined by a peptide linker. The use of peptide linkers for joining V_L and V_H regions is well-known in the art, and a large number of publications exist within this particular field. A widely used peptide linker is a 15mer consisting of three repeats of a Gly-Gly-Gly-Gly-Ser amino acid sequence ($(Gly_4Ser)_3$) (SEQ ID NO:152). Other linkers have been used, and phage display technology, as well as selective infective phage technology, has been used to diversify and select appropriate linker sequences (Tang *et al.*, *J. Biol. Chem.* 271, 15682-15686, 1996; Hennecke *et al.*, *Protein Eng.* 11, 405-410, 1998). In certain embodiments, the V_L and V_H regions are joined by a peptide linker having an amino acid sequence comprising the formula $(Gly_4Ser)_n$, wherein n = 1-5 (SEQ ID NO:165). Other suitable linkers can be obtained by optimizing a simple linker (*e.g.*, $(Gly_4Ser)_n$) through random mutagenesis.

[0132] In certain embodiments, a binding domain comprises humanized immunoglobulin V_L and/or V_H regions. Techniques for humanizing immunoglobulin V_L and V_H regions are known in the art and are discussed, for example, in United States Patent Application Publication No. 2006/0153837.

[0133] "Humanization" is expected to result in an antibody that is less immunogenic, with complete retention of the antigen-binding properties of the original molecule. In order to retain all of the antigen-binding properties of the original antibody, the structure of its antigen binding site should be reproduced in the "humanized" version. This can be achieved by grafting only the nonhuman CDRs onto human variable framework domains and constant regions, with or without retention of critical framework residues (Jones *et al.*, *Nature* 321:522 (1986); Verhoeyen *et al.*, *Science* 239:1539 (1988)) or by recombining the entire nonhuman variable domains (to preserve ligand-binding properties), but "cloaking" them with a human-like surface through judicious replacement of exposed residues (to reduce antigenicity) (Padlan, *Molec. Immunol.* 28:489 (1991)).

[0134] Essentially, humanization by CDR grafting involves recombining only the CDRs of a non-human antibody onto a human variable region framework and a human constant region. Theoretically, this should substantially reduce or eliminate immunogenicity (except if allotypic or idiotypic differences exist). However, it has been reported that some framework residues of the original antibody also may need to be preserved (Reichmann *et al.*, *Nature*, 332:323 (1988); Queen *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:10,029 (1989)).

[0135] The framework residues that need to be preserved are amenable to identification through computer modeling. Alternatively, critical framework residues can potentially be identified by comparing known antigen-binding site structures (Padlan, *Molec. Immunol.*, 31(3):169-217 (1994), incorporated herein by reference).

[0136] The residues that potentially affect antigen binding fall into several groups. The first group comprises residues that are contiguous with the antigen site surface, which could therefore make direct contact with antigens. These residues include the amino-terminal residues and those adjacent to the CDRs. The second group includes residues that could alter the structure or relative alignment of the CDRs, either by contacting the CDRs or another peptide chain in the antibody. The third group comprises amino acids with buried side chains that could influence the structural integrity of the variable domains. The residues in these groups are usually found in the same positions (Padlan, 1994, *supra*) although their positions as identified may differ depending on the numbering system (*see* Kabat *et al.*, "Sequences of proteins of immunological interest, 5th ed., Pub. No. 91-3242, U.S. Dept. Health & Human Services, NIH, Bethesda, Md., 1991).

[0137] Although the embodiments described herein involve the humanization of SMIP, SCORPION, and Interceptor molecules, and not antibodies, knowledge about humanized antibodies in the art is applicable to the polypeptides according to the invention.

B. Hinge Region

[0138] In certain embodiments, a hinge is a wild-type human immunoglobulin hinge region. In certain other embodiments, one or more amino acid residues can be added at the amino- or carboxyl-terminus of a wild type immunoglobulin hinge region as part of a fusion protein construct design. For example, additional junction amino acid residues at the hinge amino-terminus can be "RT," "RSS," "TG," or "T," or at the hinge carboxyl-terminus can be "SG", or a hinge deletion can be combined with an addition, such as ΔP with "SG" added at the carboxyl-terminus.

[0139] In certain embodiments, a hinge is an altered immunoglobulin hinge in which one or more cysteine residues in a wild type immunoglobulin hinge region is substituted with one or more other amino acid residues (*e.g.*, serine or alanine).

[0140] Exemplary altered immunoglobulin hinges include an immunoglobulin human IgG1 hinge region having one, two or three cysteine residues found in a wild type human IgG1 hinge substituted by one, two or three different amino acid residues (*e.g.*, serine or alanine). An altered immunoglobulin hinge can additionally have a proline substituted with another amino acid (*e.g.*, serine or alanine). For example, the above-described altered human IgG1 hinge can additionally have a proline located carboxyl-terminal to the three cysteines of wild type human IgG1 hinge region substituted by another amino acid residue (*e.g.*, serine, alanine). In one embodiment, the prolines of the core hinge region are not substituted.

[0141] In certain embodiments, a hinge polypeptide comprises or is a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a wild type immunoglobulin hinge region, such as a wild type human IgG1 hinge, a wild type human IgG2 hinge, or a wild type human IgG4 hinge.

[0142] In further embodiments, a hinge present in a PSMA-binding polypeptide can be a hinge that is not based on or derived from an immunoglobulin hinge (*i.e.*, not a wild-type immunoglobulin hinge or an altered immunoglobulin hinge). Examples for such hinges include peptides of about five to about 150 amino acids derived from an interdomain region

of a transmembrane protein or stalk region of a type II C-lectin, for instance, peptides of about eight to 25 amino acids and peptides of about seven to 18 amino acids.

[0143] In certain embodiments, interdomain or stalk region hinges have seven to 18 amino acids and can form an α-helical coiled coil structure. In certain embodiments, interdomain or stalk region hinges contain 0, 1, 2, 3, or 4 cysteines. Exemplary interdomain or stalk region hinges are peptide fragments of the interdomain or stalk regions, such as ten to 150 amino acid fragments from the stalk regions of CD69, CD72, CD94, NKG2A and NKG2D.

[0144] In certain embodiments, hinge sequences have about 5 to 150 amino acids, 5 to 10 amino acids, 10 to 20 amino acids, 20 to 30 amino acids, 30 to 40 amino acids, 40 to 50 amino acids, 50 to 60 amino acids, 5 to 60 amino acids, 5 to 40 amino acids, 8 to 20 amino acids, or 10 to 15 amino acids. The hinge can be primarily flexible, but can also provide more rigid characteristics or can contain primarily α-helical structure with minimal β-sheet structure. The lengths or the sequences of the hinges can affect the binding affinities of the binding domains to which the hinges are directly or indirectly (via another region or domain, such as an heterodimerization domain) connected as well as one or more activities of the Fc region portions to which the hinges are directly or indirectly connected.

[0145] In certain embodiments, hinge sequences are stable in plasma and serum and are resistant to proteolytic cleavage. The first lysine in the IgG1 upper hinge region can be mutated to minimize proteolytic cleavage, for instance, the lysine can be substituted with methionine, threonine, alanine or glycine, or is deleted.

[0146] In some embodiments of the invention, the PSMA-binding polypeptide is capable of forming a heterodimer with a second polypeptide chain and comprises a hinge region (a) immediately amino-terminal to an immunoglobulin constant region (*e.g.*, amino-terminal to a CH2 domain wherein the immungobloubolin constant region includes CH2 and CH3 domains, or amino-terminal to a CH3 domain wherein the immunoglobulin sub-regions includes CH3 and CH4 domains), (b) interposed between and connecting a binding domain (*e.g.*, scFv) and a immunoglobulin heterodimerization domain, (c) interposed between and connecting a immunoglobulin heterodimerization domain and an immunoglobulin constant region (*e.g.*, wherein the immunoglobulin constant region includes CH2 and CH3 domains or CH3 and CH4 domains), (d) interposed between and connecting an immunoglobulin constant region and a binding domain, (e) at the amino-terminus of a polypeptide chain, or (f) at the carboxyl-terminus of a polypeptide chain. A polypeptide chain comprising a hinge region as described herein will be capable of associating with a different polypeptide chain to form a heterodimeric protein provided herein, and the heterodimer formed will contain a binding domain that retains its target specificity or its specific target binding affinity.

[0147] In certain embodiments, a hinge present in a polypeptide that forms a heterodimer with another polypeptide chain can be an immunoglobulin hinge, such as a wild-type immunoglobulin hinge region or an altered immunoglobulin hinge region thereof. In certain embodiments, a hinge of one polypeptide chain of a heterodimeric protein is identical to a corresponding hinge of the other polypeptide chain of the heterodimer. In certain other embodiments, a hinge of one chain is different from that of the other chain (in their length or sequence). The different hinges in the different chains allow different manipulation of the binding affinities of the binding domains to which the hinges are connected, so that the heterodimer is able to preferentially bind to the target of one binding domain over the target of the other binding domain. For example, in certain embodiments, a heterodimeric protein has a CD3- or TCR-binding domain in one chain and a PSMA-binding domain in another chain. Having two different hinges in the two chains may allow the heterodimer to bind to the PSMA first, and then to a CD3 or other TCR component second. Thus, the heterodimer may recruit $CD3^+$ T cells to PSMA-expressing cells (*e.g.*, PSMA-expressing tumor cells), which in turn may damage or destroy the PSMA-expressing cells.

[0148] Exemplary hinge regions suitable for use in accordance with the present invention are shown in the Tables 1 and 2 below. Additional exemplary hinge regions are set forth in SEQ ID NOs: 241-244, 601, 78, 763-791, 228, 379-434, 618-749 of WO2011/090762 (said sequences incorporated by reference herein).

Table 1: Exemplary hinge regions

Hinge Region	Amino Acid Sequence	SEQ ID NO
sss(s)-hIgG1 hinge	EPKSSDKTHTSPPSS	SEQ ID NO:88
csc(s)-hIgG1 hinge	EPKSCDKTHTSPPCS	SEQ ID NO:89
ssc(s)-hIgG1 hinge	EPKSSDKTHTSPPCS	SEQ ID NO:90
scc(s)-hIgG1 hinge	EPKSSDKTHTCPPCS	SEQ ID NO:91
css(s)-hIgG1 hinge	EPKSCDKTHTSPPSS	SEQ ID NO:92
scs(s)-hIgG1 hinge	EPKSSDKTHTCPPSS	SEQ ID NO:93
ccc(s)-hIgG1 hinge	EPKSCDKTHTSPPCS	SEQ ID NO:94
ccc(p)-hIgG1 hinge	EPKSCDKTHTSPPCP	SEQ ID NO:95
sss(p)-hIgG1 hinge	EPKSSDKTHTSPPSP	SEQ ID NO:96
csc(p)-hIgG1 hinge	EPKSCDKTHTSPPCP	SEQ ID NO:97
ssc(p)-hIgG1 hinge	EPKSSDKTHTSPPCP	SEQ ID NO:98
scc(p)-hIgG1 hinge	EPKSSDKTHTCPPCP	SEQ ID NO:99
css(p)-hIgG1 hinge	EPKSCDKTHTSPPSP	SEQ ID NO:100
scs(p)-hIgG1 hinge	EPKSSDKTHTCPPSP	SEQ ID NO:101
Scppcp	SCPPCP	SEQ ID NO:102
STD1	NYGGGGSGGGGSGGGGSGNS	SEQ ID NO:103

Hinge Region	Amino Acid Sequence	SEQ ID NO
STD2	NYGGGGSGGGGSGGGGSGNY GGGGSGGGGSGGGGSGNS	SEQ ID NO:104
H1	NS	SEQ ID NO:105
H2	GGGGSGNS	SEQ ID NO:106
H3	NYGGGGSGNS	SEQ ID NO:107
H4	GGGGSGGGGSGNS	SEQ ID NO:108
H5	NYGGGGSGGGGSGNS	SEQ ID NO:109
H6	GGGGSGGGGSGGGGSGNS	SEQ ID NO:110
H7	GCPPCPNS	SEQ ID NO:62
$(G_4S)_3$	GGGGSGGGGSGGGGS	SEQ ID NO:111
H105	SGGGGSGGGGSGGGGS	SEQ ID NO:155
$(G_4S)_4$	GGGGSGGGGSGGGGSGGGGS	SEQ ID NO:112
H75 (NKG2A quadruple mutant)	QRHNNSSLNTGTQMAGHSPNS	SEQ ID NO:63
H83 (NKG2A derived)	SSLNTGTQMAGHSPNS	SEQ ID NO:65
H106 (NKG2A derived)	QRHNNSSLNTGTQMAGHS	SEQ ID NO:156
H81 (NKG2D derived)	EVQIPLTESYSPNS	SEQ ID NO:64
H91 (NKG2D derived)	NSLANQEVQIPLTESYSPNS	SEQ ID NO:66
H94	SGGGGSGGGGSGGGGSPNS	SEQ ID NO:67

Table 2: Exemplary hinge regions (derived from H7 hinge, stalk region of a type II C-lectin, or interdomain region of a type I transmembrane protein)

Hinge Region	Amino Acid Sequence	Molecule and/or hinge from which derived	SEQ ID NO:
H16	LSVKADFLTPSIGNS	CD80	SEQ ID NO:113
H17	LSVKADFLTPSISCPPCPNS	CD80 + H7	SEQ ID NO:114
H18	LSVLANFSQPEIGNS	CD86	SEQ ID NO:115
H19	LSVLANFSQPEISCPPCPNS	CD86 + H7	SEQ ID NO:116
H20	LKIQERVSKPKISNS	CD2	SEQ ID NO:117
H21	LKIQERVSKPKISCPPCPNS	CD2 + H7	SEQ ID NO:118
H22	LNVSERPFPPHIQNS	CD22	SEQ ID NO:119
H23	LDVSERPFPPHIQSCPPCPNS	CD22 + H7	SEQ ID NO:120
H24	REQLAEVTLSLKANS	CD80	SEQ ID NO:121
H25	REQLAEVTLSLKACPPCPNS	CD80 + H7	SEQ ID NO:122
H26	RIHQMNSELSVLANS	CD86	SEQ ID NO:123
H27	RIHQMNSELSVLACPPCPNS	CD86 + H7	SEQ ID NO:124
H28	DTKGKNVLEKIFSNS	CD2	SEQ ID NO:125

Hinge Region	Amino Acid Sequence	Molecule and/or hinge from which derived	SEQ ID NO:
H30	LPPETQESQEVTLNS	CD22	SEQ ID NO:126
H32	RIHLNVSERPFPPNS	CD22	SEQ ID NO:127
H33	RIHLNVSERPFPPCPPCPNS	CD22 + H7	SEQ ID NO:128
H36	GCPPCPGGGGSNS	H7	SEQ ID NO:129
H40	GCPPCPANS	H7	SEQ ID NO:130
H41	GCPPCPANS	H7	SEQ ID NO:131
H42	GCPPCPNS	H7	SEQ ID NO:132
H44	GGGASCPPCPGNS	H7	SEQ ID NO:133
H45	GGGASCPPCAGNS	H7	SEQ ID NO:134
H46	GGGASCPPCANS	H7	SEQ ID NO:135
H47	LSVKADFLTPSIGNS	CD80	SEQ ID NO:136
H48	ADFLTPSIGNS	CD80	SEQ ID NO:137
H50	LSVLANFSQPEIGNS	CD86	SEQ ID NO:138
H51	LSVLANFSQPEIGNS	CD86	SEQ ID NO:139
H52	SQPEIVPISNS	CD86	SEQ ID NO:140
H53	SQPEIVPISCPPCPNS	CD86 + H7	SEQ ID NO:141
H54	SVLANFSQPEISCPPCPNS	CD86 + H7	SEQ ID NO:142
H55	RIHQMNSELSVLANS	CD86	SEQ ID NO:143
H56	QMNSELSVLANS	CD86	SEQ ID NO:144
H57	VSERPFPPNS	CD22	SEQ ID NO:145
H58	KPFFTCGSADTCPNS	CD72	SEQ ID NO:146
H59	KPFFTCGSADTCPNS	CD72	SEQ ID NO:147
H60	QYNCPGQYTFSMPNS	CD69	SEQ ID NO:148
H61	EPAFTPGPNIELQKDSDCPNS	CD94	SEQ ID NO:149
H62	QRHNNSSLNTRTQKARHCPNS	NKG2A	SEQ ID NO:150
H63	NSLFNQEVQIPLTESYCPNS	NKG2D	SEQ ID NO:151

C. Immunoglobulin Heterodimerization Domains

[0149] In certain embodiments, a PSMA-binding polypeptide or protein of the invention can comprise an "immunoglobulin dimerization domain" or "immunoglobulin heterodimerization domain."

[0150] An "immunoglobulin dimerization domain" or "immunoglobulin heterodimerization domain," as used herein, refers to an immunoglobulin domain of a polypeptide chain that preferentially interacts or associates with a different immunoglobulin domain of another

polypeptide chain, wherein the interaction of the different immunoglobulin heterodimerization domains substantially contributes to or efficiently promotes heterodimerization of the first and second polypeptide chains (*i.e.*, the formation of a dimer between two different polypeptide chains, which is also referred to as a "heterodimer" or "heterodimeric protein"). The interactions between immunoglobulin heterodimerization domains "substantially contributes to or efficiently promotes" the heterodimerization of first and second polypeptide chains if there is a statistically significant reduction in the dimerization between the first and second polypeptide chains in the absence of the immunoglobulin heterodimerization domain of the first polypeptide chain and/or the immunoglobulin heterodimerization domain of the second polypeptide chain. In certain embodiments, when the first and second polypeptide chains are co-expressed, at least 60%, at least about 60% to about 70%, at least about 70% to about 80%, at least 80% to about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the first and second polypetpide chains form heterodimers with each other. Representative immunoglobulin heterodimerization domains include an immunoglobulin CH1 domain, an immunoglobulin CL1 domain (*e.g.*, Cκ or Cλ isotypes), or derivatives thereof, including wild-type immunoglobulin CH1 and CL domains and altered (or mutated) immunoglobulin CH1 and CL domains, such as provided herein.

[0151] Dimerization/heterodimerization domains can be used where it is desired to form heterodimers from two non-identical polypeptide chains, where one or both polypeptide chains comprises a binding domain. In certain embodiments, one polypeptide chain member of certain heterodimers described herein does not contain a binding domain. As indicated above, a heterodimeric protein of the present disclosure comprises an immunoglobulin heterodimerization domain in each polypeptide chain. The immunoglobulin heterodimerization domains in the polypeptide chains of a heterodimer are different from each other and thus can be differentially modified to facilitate heterodimerization of both chains and to minimize homodimerization of either chain. As shown in the examples, immunoglobulin heterodimerization domains provided herein allow for efficient heterodimerization between different polypeptides and facilitate purification of the resulting heterodimeric protein.

[0152] As provided herein, immunoglobulin heterodimerization domains useful for promoting heterodimerization of two different single chain polypeptides (*e.g.*, one short and one long) according to the present disclosure include immunoglobulin CH1 and CL domains, for instance, human CH1 and CL domains. In certain embodiments, an immunoglobulin heterodimerization domain is a wild-type CH1 domain, such as a wild type IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM CH1 domain. In further embodiments, an immunoglobulin heterodimerization domain is a wild-type human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD,

IgE, or IgM CH1 domain as set forth in SEQ ID NOS:114, 186-192 and 194, respectively, of PCT Publication No. WO2011/090762 (said sequences incorporated by reference herein). In certain embodiments, an immunoglobulin heterodimerization domain is a wild-type human IgG1 CH1 domain as set forth in SEQ ID NO:114 of WO2011/090762 (said sequence incorporated by reference herein).

[0153] In further embodiments, an immunoglobulin heterodimerization domain is an altered immunoglobulin CH1 domain, such as an altered IgG1, IgG2, IgG3, IgG4, IgA1, IgA2 IgD, IgE, or IgM CH1 domain. In certain embodiments, an immunoglobulin heterodimerization domain is an altered human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM CH1 domain. In still further embodiments, a cysteine residue of a wild-type CH1 domain (*e.g.*, a human CH1) involved in forming a disulfide bond with a wild type immunoglobulin CL domain (*e.g.*, a human CL) is deleted or substituted in the altered immunoglobulin CH1 domain such that a disulfide bond is not formed between the altered CH1 domain and the wild-type CL domain.

[0154] In certain embodiments, an immunoglobulin heterodimerization domain is a wild-type CL domain, such as a wild type Cκ domain or a wild type Cλ domain. In certain embodiments, an immunoglobulin heterodimerization domain is a wild type human Cκ or human Cλ domain as set forth in SEQ ID NOS:112 and 113, respectively, of WO2011/090762 (said sequences incorporated by reference herein). In further embodiments, an immunoglobulin heterodimerization domain is an altered immunoglobulin CL domain, such as an altered Cκ or Cλ domain, for instance, an altered human Cκ or human Cλ domain.

[0155] In certain embodiments, a cysteine residue of a wild-type CL domain (*e.g.*, a human CL) involved in forming a disulfide bond with a wild type immunoglobulin CH1 domain (*e.g.*, a human CH1) is deleted or substituted in the altered immunoglobulin CL domain. Such altered CL domains can further comprise an amino acid deletion at their amino-termini. An exemplary Cκ domain is set forth in SEQ ID NO:141 of WO2011/090762 (said sequence incorporated by reference herein), in which the first arginine and the last cysteine of the wild type human Ck domain are both deleted. In certain embodiments, only the last cysteine of the wild type human Ck domain is deleted in the altered Ck domain because the first arginine deleted from the wild type human Ck domain can be provided by a linker that has an arginine at its carboxyl-terminus and links the amino-terminus of the altered Ck domain with another domain (*e.g.*, an immunoglobulin sug-region, such as a sub-region comprising immunoglobulin CH2 and CH3 domains). An exemplary Cλ domain is set forth in SEQ ID NO:140 of WO2011/090762 (said sequence incorporated by reference herein), in which the

first arginine of a wild type human Cλ domain is deleted and the cysteine involved in forming a disulfide bond with a cysteine in a CH1 domain is substituted by a serine.

[0156] In further embodiments, an immunoglobulin heterodimerization domain is an altered Cκ domain that contains one or more amino acid substitutions, as compared to a wild type Cκ domain, at positions that may be involved in forming the interchain-hydrogen bond network at a Cκ-Cκ interface. For example, in certain embodiments, an immunoglobulin heterodimerization domain is an altered human Cκ domain having one or more amino acids at positions N29, N30, Q52, V55, T56, S68 or T70 that are substituted with a different amino acid. The numbering of the amino acids is based on their positions in the altered human Cκ sequence as set forth in SEQ ID NO:141 of WO2011/090762 (said sequence incorporated by reference herein). In certain embodiments, an immunoglobulin heterodimerization domain is an altered human Cκ domain having one, two, three or four amino acid substitutions at positions N29, N30, V55, or T70. The amino acid used as a substitute at the above-noted positions can be an alanine, or an amino acid residue with a bulk side chain moiety such as arginine, tryptophan, tyrosine, glutamate, glutamine, or lysine. Additional amino acid residues that can be used to substitute amino acid residues of the wild type human Ck sequence at the above noted positions (*e.g.*, N30) include aspartate, methionine, serine and phenyalanine. Exemplary altered human Cκ domains are set forth in SEQ ID NOS:142-178 of WO2011/090762 (said sequences incorporated by reference herein). Altered human Cκ domains are those that facilitate heterodimerization with a CH1 domain, but minimize homodimerization with another Cκ domain. Representative altered human Cκ domains are set forth in SEQ ID NOS:160 (N29W V55A T70A), 161 (N29Y V55A T70A), 202 (T70E N29A N30A V55A), 167 (N30R V55A T70A), 168 (N30K V55A T70A), 170 (N30E V55A T70A), 172 (V55R N29A N30A), 175 (N29W N30Y V55A T70E), 176 (N29Y N30Y V55A T70E), 177 (N30E V55A T70E), 178 (N30Y V55A T70E), 838 (N30D V55A T70E), 839 (N30M V55A T70E), 840 (N30S V55A T70E), and 841 (N30F V55A T70E) of WO2011/090762 (said sequences incorporated by reference herein).

[0157] In certain embodiments, in addition to or alternative to the mutations in Ck domains described herein, both the immunoglobulin heterodimerization domains (*i.e.*, immunoglobulin CH1 and CL domains) of a polypeptide heterodimer have mutations so that the resulting immunoglobulin heterodimerization domains form salt bridges (*i.e.*, ionic interactions) between the amino acid residues at the mutated sites. For example, the immunoglobulin heterodimerization domains of a polypeptide heterodimer can be a mutated CH1 domain in combination with a mutated Ck domain. In the mutated CH1 domain, valine at position 68 (V68) of the wild type human CH1 domain is substituted by an amino acid residue having a negative charge (*e.g.*, aspartate or glutamate), whereas leucine at position 29 (L29) of a

mutated human Ck domain in which the first arginine and the last cysteine have been deleted is substituted by an amino acid residue having a positive charge (*e.g.*, lysine, arginine or histidine). The charge-charge interaction between the amino acid residue having a negative charge of the resulting mutated CH1 domain and the amino acid residue having a positive charge of the resulting mutated Ck domain forms a salt bridge, which stabilizes the heterodimeric interface between the mutated CH1 and Ck domains. Alternatively, V68 of the wild type CH1 can be substituted by an amino acid residue having a positive charge, whereas L29 of a mutated human Ck domain in which the first arginine and the last cysteine have been deleted can be substituted by an amino acid residue having a negative charge. Exemplary mutated CH1 sequences in which V68 is substituted by an amino acid with either a negative or positive charge are set forth in SEQ ID NOS:844 and 845 of WO2011/090762 (said sequences incorporated by reference herein). Exemplary mutated Ck sequences in which L29 is substituted by an amino acid with either a negative or positive charge are set forth in SEQ ID NOS:842 and 843 of WO2011/090762 (said sequences incorporated by reference herein).

[0158] Positions other than V68 of human CH1 domain and L29 of human Ck domain can be substituted with amino acids having opposite charges to produce ionic interactions between the amino acids in addition or alternative to the mutations in V68 of CH1 domain and L29 of Ck domain. Such positions can be identified by any suitable method, including random mutagenesis, analysis of the crystal structure of the CH1-Ck pair to identify amino acid residues at the CH1-Ck interface, and further identifying suitable positions among the amino acid residues at the CH1-Ck interface using a set of criteria (*e.g.*, propensity to engage in ionic interactions, proximity to a potential partner residue, etc.).

[0159] In certain embodiments, polypeptide heterodimers of the present disclosure contain only one pair of immunoglobulin heterodimerization domains. For example, a first chain of a polypeptide heterodimer can comprise a CH1 domain as an immunoglobulin heterodimerization domain, while a second chain can comprise a CL domain (*e.g.*, a Cκ or Cλ) as an immunoglobulin heterodimerization domain. Alternatively, a first chain can comprise a CL domain (*e.g.*, a Cκ or Cλ) as an immunoglobulin heterodimerization domain, while a second chain can comprise a CH1 domain as an immunoglobulin heterodimerization domain. As set forth herein, the immunoglobulin heterodimerization domains of the first and second chains are capable of associating to form a heterodimeric protein of this disclosure.

[0160] In certain other embodiments, heterodimeric proteins of the present disclosure can have two pairs of immunoglobulin heterodimerization domains. For example, a first chain of a heterodimer can comprise two CH1 domains, while a second chain can have two CL domains that associate with the two CH1 domains in the first chain. Alternatively, a first

chain can comprise two CL domains, while a second chain can have two CH1 domains that associate with the two CL domains in the first chain. In certain embodiments, a first polypeptide chain comprises a CH1 domain and a CL domain, while a second polypeptide chain comprises a CL domain and a CH1 domain that associate with the CH1 domain and the CL domain, respectively, of the first polypeptide chain.

[0161] In the embodiments where a heterodimeric protein comprises only one heterodimerization pair (*i.e.*, one immunoglobulin heterodimerization domain in each chain), the immunoglobulin heterodimerization domain of each chain can be located amino-terminal to the immunoglobulin constant region of that chain. Alternatively, the immunoglobulin heterodimerization domain in each chain can be located carboxyl-terminal to the immunoglobulin constant region of that chain.

[0162] In the embodiments where a heterodimeric protein comprises two heterodimerization pairs (*i.e.*, two immunoglobulin heterodimerization domains in each chain), both immunoglobulin heterodimerization domains in each chain can be located amino-terminal to the immunoglobulin constant region of that chain. Alternatively, both immunoglobulin heterodimerization domains in each chain can be located carboxyl-terminal to the immunoglobulin constant region of that chain. In further embodiments, one immunoglobulin heterodimerization domain in each chain can be located amino-terminal to the immunoglobulin constant region of that chain, while the other immunoglobulin heterodimerization domain of each chain can be located carboxyl-terminal to the immunoglobulin constant region of that chain. In other words, in those embodiments, the immunoglobulin constant region is interposed between the two immunoglobulin heterodimerization domains of each chain.

D. Immunoglobulin Constant regions

[0163] As indicated herein, in certain embodiments, PSMA-binding polypeptides of the present disclosure (*e.g.*, SMIP, PIMS, SCORPION, and Interceptor molecules) comprise an immunoglobulin constant region (also referred to as an constant region) in each polypeptide chain. The inclusion of an immunoglobulin constant region slows clearance of the homodimeric and heterodimeric proteins formed from two PSMA-binding polypeptide chains from circulation after administration to a subject. By mutations or other alterations, an immunoglobulin constant region further enables relatively easy modulation of dimeric polypeptide effector functions (*e.g.*, ADCC, ADCP, CDC, complement fixation, and binding to Fc receptors), which can either be increased or decreased depending on the disease being treated, as known in the art and described herein. In certain embodiments, an

immunoglobulin constant region of one or both of the polypeptide chains of the polypeptide homodimers and heterodimers of the present disclosure will be capable of mediating one or more of these effector functions In other embodiments, one or more of these effector functions are reduced or absent in an immunoglobulin constant region of one or both of the polypeptide chains of the polypeptide homodimers and heterodimers of the present disclosure, as compared to a corresponding wild-type immunoglobulin constant region. For example, for dimeric PSMA-binding polypeptides designed to elicit RTCC, such as, *e.g.*, via the inclusion of a CD3-binding domain, an immunoglobulin constant region preferably has reduced or no effector function relative to a corresponding wild-type immunoglobulin constant region.

[0164] An immunoglobulin constant region present in PSMA binding polypeptides of the present disclosure can comprise of or is derived from part or all of: a CH2 domain, a CH3 domain, a CH4 domain, or any combination thereof. For example, an immunoglobulin constant region can comprise a CH2 domain, a CH3 domain, both CH2 and CH3 domains, both CH3 and CH4 domains, two CH3 domains, a CH4 domain, two CH4 domains, and a CH2 domain and part of a CH3 domain.

[0165] A CH2 domain that can form an immunoglobulin constant region of a PSMA-binding polypeptide of the present disclosure can be a wild type immunoglobulin CH2 domain or an altered immunoglobulin CH2 domain thereof from certain immunoglobulin classes or subclasses (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgD) and from various species (including human, mouse, rat, and other mammals).

[0166] In certain embodiments, a CH2 domain is a wild type human immunoglobulin CH2 domain, such as wild type CH2 domains of human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgD, as set forth in SEQ ID NOS:115, 199-201 and 195-197, respectively, of PCT Publication WO2011/090762 (said sequences incorporated by reference herein). In certain embodiments, the CH2 domain is a wild type human IgG1 CH2 domain as set forth in SEQ ID NO:115 of WO2011/090762 (said sequence incorporated by reference herein).

[0167] In certain embodiments, a CH2 domain is an altered immunoglobulin CH2 region (*e.g.*, an altered human IgG1 CH2 domain) that comprises an amino acid substitution at the asparagine of position 297 (*e.g.*, asparagine to alanine). Such an amino acid substitution reduces or eliminates glycosylation at this site and abrogates efficient Fc binding to FcγR and C1q. The sequence of an altered human IgG1 CH2 domain with an Asn to Ala substitution at position 297 is set forth in SEQ ID NO:324 of WO2011/090762 said (sequence incorporated by reference herein).

[0168] In certain embodiments, a CH2 domain is an altered immunoglobulin CH2 region (*e.g.*, an altered human IgG1 CH2 domain) that comprises at least one substitution or deletion at positions 234 to 238. For example, an immunoglobulin CH2 region can comprise a substitution at position 234, 235, 236, 237 or 238, positions 234 and 235, positions 234 and 236, positions 234 and 237, positions 234 and 238, positions 234-236, positions 234, 235 and 237, positions 234, 236 and 238, positions 234, 235, 237, and 238, positions 236-238, or any other combination of two, three, four, or five amino acids at positions 234-238. In addition or alternatively, an altered CH2 region can comprise one or more (*e.g.*, two, three, four or five) amino acid deletions at positions 234-238, for instance, at one of position 236 or position 237 while the other position is substituted. The above-noted mutation(s) decrease or eliminate the antibody-dependent cell-mediated cytotoxicity (ADCC) activity or Fc receptor-binding capability of a polypeptide heterodimer that comprises the altered CH2 domain. In certain embodiments, the amino acid residues at one or more of positions 234-238 has been replaced with one or more alanine residues. In further embodiments, only one of the amino acid residues at positions 234-238 have been deleted while one or more of the remaining amino acids at positions 234-238 can be substituted with another amino acid (*e.g.*, alanine or serine).

[0169] In certain other embodiments, a CH2 domain is an altered immunoglobulin CH2 region (*e.g.*, an altered human IgG1 CH2 domain) that comprises one or more amino acid substitutions at positions 253, 310, 318, 320, 322, and 331. For example, an immunoglobulin CH2 region can comprise a substitution at position 253, 310, 318, 320, 322, or 331, positions 318 and 320, positions 318 and 322, positions 318, 320 and 322, or any other combination of two, three, four, five or six amino acids at positions 253, 310, 318, 320, 322, and 331. The above-noted mutation(s) decrease or eliminate the complement-dependent cytotoxicity (CDC) of a polypeptide heterodimer that comprises the altered CH2 domain.

[0170] In certain other embodiments, in addition to the amino acid substitution at position 297, an altered CH2 region (*e.g.*, an altered human IgG1 CH2 domain) can further comprise one or more (*e.g.*, two, three, four, or five) additional substitutions at positions 234-238. For example, an immunoglobulin CH2 region can comprise a substitution at positions 234 and 297, positions 234, 235, and 297, positions 234, 236 and 297, positions 234-236 and 297, positions 234, 235, 237 and 297, positions 234, 236, 238 and 297, positions 234, 235, 237, 238 and 297, positions 236-238 and 297, or any combination of two, three, four, or five amino acids at positions 234-238 in addition to position 297. In addition or alternatively, an altered CH2 region can comprise one or more (*e.g.*, two, three, four or five) amino acid deletions at positions 234-238, such as at position 236 or position 237. The additional

mutation(s) decreases or eliminates the antibody-dependent cell-mediated cytotoxicity (ADCC) activity or Fc receptor-binding capability of a polypeptide heterodimer that comprises the altered CH2 domain. In certain embodiments, the amino acid residues at one or more of positions 234-238 have been replaced with one or more alanine residues. In further embodiments, only one of the amino acid residues at positions 234-238 has been deleted while one or more of the remaining amino acids at positions 234-238 can be substituted with another amino acid (*e.g.*, alanine or serine).

[0171] In certain embodiments, in addition to one or more (*e.g.*, 2, 3, 4, or 5) amino acid substitutions at positions 234-238, a mutated CH2 region (*e.g.*, an altered human IgG1 CH2 domain) in a fusion protein of the present disclosure can contain one or more (*e.g.*, 2, 3, 4, 5, or 6) additional amino acid substitutions (*e.g.*, substituted with alanine) at one or more positions involved in complement fixation (*e.g.*, at positions I253, H310, E318, K320, K322, or P331). Examples of mutated immunoglobulin CH2 regions include human IgG1, IgG2, IgG4 and mouse IgG2a CH2 regions with alanine substitutions at positions 234, 235, 237 (if present), 318, 320 and 322. An exemplary mutated immunoglobulin CH2 region is mouse IGHG2c CH2 region with alanine substitutions at L234, L235, G237, E318, K320, and K322.

[0172] In still further embodiments, in addition to the amino acid substitution at position 297 and the additional deletion(s) or substitution(s) at positions 234-238, an altered CH2 region (*e.g.*, an altered human IgG1 CH2 domain) can further comprise one or more (*e.g.*, two, three, four, five, or six) additional substitutions at positions 253, 310, 318, 320, 322, and 331. For example, an immunoglobulin CH2 region can comprise a (1) substitution at position 297, (2) one or more substitutions or deletions or a combination thereof at positions 234-238, and one or more (*e.g.*, 2, 3, 4, 5, or 6) amino acid substitutions at positions I253, H310, E318, K320, K322, and P331, such as one, two, three substitutions at positions E318, K320 and K322. The amino acids at the above-noted positions can be substituted by alanine or serine.

[0173] In certain embodiments, an immunoglobulin CH2 region polypeptide comprises: (i) an amino acid substitution at the asparagines of position 297 and one amino acid substitution at position 234, 235, 236 or 237; (ii) an amino acid substitution at the asparagine of position 297 and amino acid substitutions at two of positions 234-237; (iii) an amino acid substitution at the asparagine of position 297 and amino acid substitutions at three of positions 234-237; (iv) an amino acid substitution at the asparagine of position 297, amino acid substitutions at positions 234, 235 and 237, and an amino acid deletion at position 236; (v) amino acid substitutions at three of positions 234-237 and amino acid substitutions at positions 318, 320 and 322; or (vi) amino acid substitutions at three of positions 234-237, an

amino acid deletion at position 236, and amino acid substitutions at positions 318, 320 and 322.

[0174] Exemplary altered immunoglobulin CH2 regions with amino acid substitutions at the asparagine of position 297 include: human IgG1 CH2 region with alanine substitutions at L234, L235, G237 and N297 and a deletion at G236 (SEQ ID NO:325 of WO2011/090762, said sequence incorporated by reference herein), human IgG2 CH2 region with alanine substitutions at V234, G236, and N297 (SEQ ID NO:326 of WO2011/090762, said sequence incorporated by reference herein), human IgG4 CH2 region with alanine substitutions at F234, L235, G237 and N297 and a deletion of G236 (SEQ ID NO:322 of WO2011/090762, said sequence incorporated by reference herein), human IgG4 CH2 region with alanine substitutions at F234 and N297 (SEQ ID NO:343 of WO2011/090762, said sequence incorporated by reference herein), human IgG4 CH2 region with alanine substitutions at L235 and N297 (SEQ ID NO:344 of WO2011/090762, said sequence incorporated by reference herein), human IgG4 CH2 region with alanine substitutions at G236 and N297 (SEQ ID NO:345 of WO2011/090762, said sequence incorporated by reference herein), and human IgG4 CH2 region with alanine substitutions at G237 and N297 (SEQ ID NO:346 of WO2011/090762, said sequence incorporated by reference herein).

[0175] In certain embodiments, in addition to the amino acid substitutions described above, an altered CH2 region (*e.g.*, an altered human IgG1 CH2 domain) can contain one or more additional amino acid substitutions at one or more positions other than the above-noted positions. Such amino acid substitutions can be conservative or non-conservative amino acid substitutions. For example, in certain embodiments, P233 can be changed to E233 in an altered IgG2 CH2 region (*see, e.g.*, SEQ ID NO:326 of WO2011/090762, said sequence incorporated by reference herein). In addition or alternatively, in certain embodiments, the altered CH2 region can contain one or more amino acid insertions, deletions, or both. The insertion(s), deletion(s) or substitution(s) can be anywhere in an immunoglobulin CH2 region, such as at the N- or C-terminus of a wild type immunoglobulin CH2 region resulting from linking the CH2 region with another region (*e.g.*, a binding domain or an immunoglobulin heterodimerization domain) via a hinge.

[0176] In certain embodiments, an altered CH2 region in a polypeptide of the present disclosure comprises or is a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to a wild type immunoglobulin CH2 region, such as the CH2 region of wild type human IgG1, IgG2, or IgG4, or mouse IgG2a (*e.g.*, IGHG2c).

[0177] An altered immunoglobulin CH2 region in a PSMA-binding polypeptide of the present disclosure can be derived from a CH2 region of various immunoglobulin isotypes, such as IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgD, from various species (including human, mouse, rat, and other mammals). In certain embodiments, an altered immunoglobulin CH2 region in a fusion protein of the present disclosure can be derived from a CH2 region of human IgG1, IgG2 or IgG4, or mouse IgG2a (*e.g.*, IGHG2c), whose sequences are set forth in SEQ ID NOS:115, 199, 201, and 320 of WO2011/090762 (said sequences incorporated by reference herein).

[0178] In certain embodiments, an altered CH2 domain is a human IgG1 CH2 domain with alanine substitutions at positions 235, 318, 320, and 322 (*i.e.*, a human IgG1 CH2 domain with L235A, E318A, K320A and K322A substitutions) (SEQ ID NO:595 of WO2011/090762, said sequence incorporated by reference herein), and optionally an N297 mutation (*e.g.*, to alanine). In certain other embodiments, an altered CH2 domain is a human IgG1 CH2 domain with alanine substitutions at positions 234, 235, 237, 318, 320 and 322 (*i.e.*, a human IgG1 CH2 domain with L234A, L235A, G237A, E318A, K320A and K322A substitutions) (SEQ ID NO:596 of WO2011/090762, said sequence incorporated by reference herein), and optionally an N297 mutation (*e.g.*, to alanine).

[0179] In certain embodiments, an altered CH2 domain is an altered human IgG1 CH2 domain with mutations known in the art that enhance immunological activities such as ADCC, ADCP, CDC, complement fixation, Fc receptor binding, or any combination thereof.

[0180] The CH3 domain that can form an immunoglobulin constant region of a PSMA-binding polypeptide of the present disclosure can be a wild type immunoglobulin CH3 domain or an altered immunoglobulin CH3 domain thereof from certain immunoglobulin classes or subclasses (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, IgM) of various species (including human, mouse, rat, and other mammals). In certain embodiments, a CH3 domain is a wild type human immunoglobulin CH3 domain, such as wild type CH3 domains of human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM as set forth in SEQ ID NOS:116, 208-210, 204-207, and 212, respectively of WO2011/090762 (said sequences incorporated by reference herein). In certain embodiments, the CH3 domain is a wild type human IgG1 CH3 domain as set forth in SEQ ID NO:116 of WO2011/090762 (said sequence incorporated by reference herein). In certain embodiments, a CH3 domain is an altered human immunoglobulin CH3 domain, such as an altered CH3 domain based on or derived from a wild-type CH3 domain of human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM antibodies. For example, an altered CH3 domain can be a human IgG1 CH3 domain with one or two mutations at positions H433 and N434 (positions are numbered according to EU numbering). The mutations in such positions can be involved in complement fixation. In

certain other embodiments, an altered CH3 domain can be a human IgG1 CH3 domain but with one or two amino acid substitutions at position F405 or Y407. The amino acids at such positions are involved in interacting with another CH3 domain. In certain embodiments, an altered CH3 domain can be an altered human IgG1 CH3 domain with its last lysine deleted. The sequence of this altered CH3 domain is set forth in SEQ ID NO:761 of WO2011/090762 (said sequence incorporated by reference herein).

[0181] In certain embodiments, PSMA-binding polypeptides forming a polypeptide heterodimer comprise a CH3 pair that comprises so called "knobs-into-holes" mutations (*see*, Marvin and Zhu, Acta Pharmacologica Sinica 26:649-58, 2005; Ridgway *et al.*, Protein Engineering 9:617-21, 1966). More specifically, mutations can be introduced into each of the two CH3 domains of each polypeptide chain so that the steric complementarity required for CH3/CH3 association obligates these two CH3 domains to pair with each other. For example, a CH3 domain in one single chain polypeptide of a polypeptide heterodimer can contain a T366W mutation (a "knob" mutation, which substitutes a small amino acid with a larger one), and a CH3 domain in the other single chain polypeptide of the polypeptide heterodimer can contain a Y407A mutation (a "hole" mutation, which substitutes a large amino acid with a smaller one). Other exemplary knobs-into-holes mutations include (1) a T366Y mutation in one CH3 domain and a Y407T in the other CH3 domain, and (2) a T366W mutation in one CH3 domain and T366S, L368A and Y407V mutations in the other CH3 domain.

[0182] The CH4 domain that can form an immunoglobulin constant region of PSMA-binding polypeptides of the present disclosure can be a wild type immunoglobulin CH4 domain or an altered immunoglobulin CH4 domain thereof from IgE or IgM molecules. In certain embodiments, the CH4 domain is a wild type human immunoglobulin CH4 domain, such as wild type CH4 domains of human IgE and IgM molecules as set forth in SEQ ID NOS:213 and 214, respectively, of WO2011/090762 (said sequences incorporated by reference herein). In certain embodiments, a CH4 domain is an altered human immunoglobulin CH4 domain, such as an altered CH4 domain based on or derived from a CH4 domain of human IgE or IgM molecules, which have mutations that increase or decrease an immunological activity known to be associated with an IgE or IgM Fc region.

[0183] In certain embodiments, an immunoglobulin constant region of PSMA binding polypeptides of the present disclosure comprises a combination of CH2, CH3 or CH4 domains (*i.e.*, more than one constant region domain selected from CH2, CH3 and CH4). For example, the immunoglobulin constant region can comprise CH2 and CH3 domains or CH3 and CH4 domains. In certain other embodiments, the immunoglobulin constant region can comprise two CH3 domains and no CH2 or CH4 domains (*i.e.*, only two or more CH3).

The multiple constant region domains that form an immunoglobulin constant region can be based on or derived from the same immunoglobulin molecule, or the same class or subclass immunoglobulin molecules. In certain embodiments, the immunoglobulin constant region is an IgG CH2CH3 (*e.g.*, IgG1 CH2CH3, IgG2 CH2CH3, and IgG4 CH2CH3) and can be a human (*e.g.*, human IgG1, IgG2, and IgG4) CH2CH3. For example, in certain embodiments, the immunoglobulin constant region comprises (1) wild type human IgG1 CH2 and CH3 domains, (2) human IgG1 CH2 with N297A substitution (*i.e.*, CH2(N297A)) and wild type human IgG1 CH3, or (3) human IgG1 CH2(N297A) and an altered human IgG1 CH3 with the last lysine deleted.

[0184] Alternatively, the multiple constant region domains can be based on or derived from different immunoglobulin molecules, or different classes or subclasses immunoglobulin molecules. For example, in certain embodiments, an immunoglobulin constant region comprises both human IgM CH3 domain and human IgG1 CH3 domain. The multiple constant region domains that form an immunoglobulin constant region can be directly linked together or can be linked to each other via one or more (*e.g.*, about 2-10) amino acids.

[0185] Exemplary immunoglobulin constant regions are set forth in SEQ ID NOS:305-309, 321, 323, 341, 342, and 762 of WO2011/090762 (said sequences incorporated by reference herein).

[0186] In certain embodiments, the immunoglobulin constant regions of both PSMA-binding polypeptides of a polypeptide homodimer or heterodimer are identical to each other. In certain other embodiments, the immunoglobulin constant region of one polypeptide chain of a heterodimeric protein is different from the immunoglobulin constant region of the other polypeptide chain of the heterodimer. For example, one immunoglobulin constant region of a heterodimeric protein can contain a CH3 domain with a "knob" mutation, whereas the other immunoglobulin constant region of the heterodimeric protein can contain a CH3 domain with a "hole" mutation.

IV. Nucleic Acids, Host Cells, and Methods for Production

[0187] The invention also includes nucleic acids (*e.g.*, DNA or RNA) encoding a PSMA-binding polypeptide as described herein, or one or more polypeptide chains of a dimeric or heterodimeric PSMA-binding protein as described herein. Nucleic acids of the invention include nucleic acids having a region that is substantially identical to a polynucleotide as listed in Table 3, *infra*. In certain embodiments, a nucleic acid in accordance with the present invention has at least 80%, typically at least about 90%, and more typically at least about 95% or at least about 98% identity to a polypeptide-encoding polynucleotide as listed

in Table 3. Nucleic acids of the invention also include complementary nucleic acids. In some instances, the sequences will be fully complementary (no mismatches) when aligned. In other instances, there can be up to about a 20% mismatch in the sequences. In some embodiments of the invention are provided nucleic acids encoding both first and second polypeptide chains of a heterodimeric PSMA-binding protein of the invention. The nucleic acid sequences provided herein can be exploited using codon optimization, degenerate sequence, silent mutations, and other DNA techniques to optimize expression in a particular host, and the present invention encompasses such sequence modifications.

[0188] Polynucleotide molecules comprising a desired polynucleotide sequence are propagated by placing the molecule in a vector. Viral and non-viral vectors are used, including plasmids. The choice of plasmid will depend on the type of cell in which propagation is desired and the purpose of propagation. Certain vectors are useful for amplifying and making large amounts of the desired DNA sequence. Other vectors are suitable for expression in cells in culture. Still other vectors are suitable for transfer and expression in cells in a whole animal or person. The choice of appropriate vector is well within the skill of the art. Many such vectors are available commercially. The partial or full-length polynucleotide is inserted into a vector typically by means of DNA ligase attachment to a cleaved restriction enzyme site in the vector. Alternatively, the desired nucleotide sequence can be inserted by homologous recombination *in vivo*. Typically this is accomplished by attaching regions of homology to the vector on the flanks of the desired nucleotide sequence. Regions of homology are added by ligation of oligonucleotides, or by polymerase chain reaction using primers comprising both the region of homology and a portion of the desired nucleotide sequence, for example.

[0189] For expression, an expression cassette or system may be employed. To express a nucleic acid encoding a polypeptide disclosed herein, a nucleic acid molecule encoding the polypeptide, operably linked to regulatory sequences that control transcriptional expression in an expression vector, is introduced into a host cell. In addition to transcriptional regulatory sequences, such as promoters and enhancers, expression vectors can include translational regulatory sequences and a marker gene which is suitable for selection of cells that carry the expression vector. The gene product encoded by a polynucleotide of the invention is expressed in any convenient expression system, including, for example, bacterial, yeast, insect, amphibian and mammalian systems. In the expression vector, the polypeptide-encoding polynucleotide is linked to a regulatory sequence as appropriate to obtain the desired expression properties. These can include promoters, enhancers, terminators, operators, repressors, and inducers. The promoters can be regulated (*e.g.*, the promoter from the steroid inducible pIND vector (Invitrogen)) or constitutive (*e.g.*, promoters from

CMV, SV40, Elongation Factor, or LTR sequences). These are linked to the desired nucleotide sequence using the techniques described above for linkage to vectors. Any techniques known in the art can be used. Accordingly, the expression vector will generally provide a transcriptional and translational initiation region, which can be inducible or constitutive, where the coding region is operably linked under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination region.

[0190] An expression cassette ("expression unit") can be introduced into a variety of vectors, *e.g.*, plasmid, BAC, YAC, bacteriophage such as lambda, P1, M13, *etc.*, plant or animal viral vectors (*e.g.*, retroviral-based vectors, adenovirus vectors), and the like, where the vectors are normally characterized by the ability to provide selection of cells comprising the expression vectors. The vectors can provide for extrachromosomal maintenance, particularly as plasmids or viruses, or for integration into the host chromosome. Where extrachromosomal maintenance is desired, an origin sequence is provided for the replication of the plasmid, which can be low- or high copy-number. A wide variety of markers are available for selection, particularly those which protect against toxins, more particularly against antibiotics. The particular marker that is chosen is selected in accordance with the nature of the host, where in some cases, complementation can be employed with auxotrophic hosts. Introduction of the DNA construct can use any convenient method, including, *e.g.*, conjugation, bacterial transformation, calcium-precipitated DNA, electroporation, fusion, transfection, infection with viral vectors, biolistics, and the like.

[0191] Accordingly, proteins for use within the present invention can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells (including cultured cells of multicellular organisms), particularly cultured mammalian cells. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* (3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001), and Ausubel *et al.*, *Short Protocols in Molecular Biology* (4th ed., John Wiley & Sons, 1999).

[0192] For example, for recombinant expression of a homodimeric PSMA-binding protein comprising two identical PSMA-binding polypeptides as described herein, an expression vector will generally include a nucleic acid segment encoding the PSMA-binding polypeptide, operably linked to a promoter. For recombinant expression of a heterodimeric PSMA-binding protein, comprising different first and second polypeptide chains, the first and second polypeptide chains can be co-expressed from separate vectors in the host cell for expression of the entire heterodimeric protein. Alternatively, for the expression of heterodimeric PSMA-

binding proteins, the first and second polypeptide chains are co-expressed from separate expression units in the same vector in the host cell for expression of the entire heterodimeric protein. The expression vector(s) are transferred to a host cell by conventional techniques, and the transfected cells are then cultured by conventional techniques to produce the encoded polypeptide(s) to produce the corresponding PSMA-binding protein.

[0193] To direct a recombinant protein into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence) is provided in the expression vector. The secretory signal sequence can be that of the native form of the recombinant protein, or can be derived from another secreted protein or synthesized *de novo*. The secretory signal sequence is operably linked to the polypeptide-encoding DNA sequence, *i.e.*, the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain signal sequences can be positioned elsewhere in the DNA sequence of interest (*see, e.g.*, Welch *et al.*, U.S. Patent No. 5,037,743; Holland *et al.*, U.S. Patent No. 5,143,830). In certain variations, a secretory signal sequence for use in accordance with the present invention has the amino acid sequence MEAPAQLLFLLLLWLPDTTG (SEQ ID NO:85).

[0194] Cultured mammalian cells are suitable hosts for production of recombinant proteins for use within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler *et al.*, *Cell* 14:725, 1978; Corsaro and Pearson, *Somatic Cell Genetics* 7:603, 1981: Graham and Van der Eb, *Virology* 52:456, 1973), electroporation (Neumann *et al.*, *EMBO J.* 1:841-845, 1982), DEAE-dextran mediated transfection (Ausubel *et al.*, *supra*), and liposome-mediated transfection (Hawley-Nelson *et al.*, *Focus* 15:73, 1993; Ciccarone *et al.*, *Focus* 15:80, 1993). The production of recombinant polypeptides in cultured mammalian cells is disclosed by, for example, Levinson *et al.*, U.S. Patent No. 4,713,339; Hagen *et al.*, U.S. Patent No. 4,784,950; Palmiter *et al.*, U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Examples of suitable mammalian host cells include African green monkey kidney cells (Vero; ATCC CRL 1587), human embryonic kidney cells (293-HEK; ATCC CRL 1573), baby hamster kidney cells (BHK-21, BHK-570; ATCC CRL 8544, ATCC CRL 10314), canine kidney cells (MDCK; ATCC CCL 34), Chinese hamster ovary cells (CHO-K1; ATCC CCL61; CHO DG44; CHO DXB11 (Hyclone, Logan, UT); *see also, e.g.*, Chasin *et al.*, *Som. Cell. Molec. Genet.* 12:555, 1986)), rat pituitary cells (GH1; ATCC CCL82), HeLa S3 cells (ATCC CCL2.2), rat hepatoma cells (H-4-II-E; ATCC CRL 1548) SV40-transformed monkey kidney cells (COS-1; ATCC CRL 1650) and murine embryonic cells (NIH-3T3; ATCC CRL 1658).

Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Manassas, Virginia. Strong transcription promoters can be used, such as promoters from SV-40 or cytomegalovirus. *See, e.g.*, U.S. Patent No. 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patents Nos. 4,579,821 and 4,601,978) and the adenovirus major late promoter.

[0195] Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants." Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." Exemplary selectable markers include a gene encoding resistance to the antibiotic neomycin, which allows selection to be carried out in the presence of a neomycin-type drug, such as G-418 or the like; the gpt gene for xanthine-guanine phosphoribosyl transferase, which permits host cell growth in the presence of mycophenolic acid/xanthine; and markers that provide resistance to zeocin, bleomycin, blastocidin, and hygromycin (*see, e.g.*, Gatignol *et al., Mol. Gen. Genet.* 207:342, 1987; Drocourt *et al., Nucl. Acids Res.* 18:4009, 1990). Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. An exemplary amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (*e.g.*, hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used.

[0196] Other higher eukaryotic cells can also be used as hosts, including insect cells, plant cells and avian cells. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar *et al.*, *J. Biosci. (Bangalore)* 11:47-58, 1987. Transformation of insect cells and production of foreign polypeptides therein is disclosed by Guarino *et al.*, U.S. Patent No. 5,162,222 and WIPO publication WO 94/06463.

[0197] Insect cells can be infected with recombinant baculovirus, commonly derived from *Autographa californica* nuclear polyhedrosis virus (AcNPV). *See* King and Possee, *The Baculovirus Expression System: A Laboratory Guide* (Chapman & Hall, London); O'Reilly *et al.*, *Baculovirus Expression Vectors: A Laboratory Manual* (Oxford University Press., New York 1994); and *Baculovirus Expression Protocols. Methods in Molecular Biology* (Richardson ed., Humana Press, Totowa, NJ, 1995). Recombinant baculovirus can also be produced through the use of a transposon-based system described by Luckow *et al.* (*J. Virol.* 67:4566-4579, 1993). This system, which utilizes transfer vectors, is commercially available in kit form (BAC-TO-BAC kit; Life Technologies, Gaithersburg, MD). The transfer

vector (*e.g.*, PFASTBAC1; Life Technologies) contains a Tn7 transposon to move the DNA encoding the protein of interest into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." *See* Hill-Perkins and Possee, *J. Gen. Virol.* 71:971-976, 1990; Bonning *et al.*, *J. Gen. Virol.* 75:1551-1556, 1994; and Chazenbalk and Rapoport, *J. Biol. Chem.* 270:1543-1549, 1995. In addition, transfer vectors can include an in-frame fusion with DNA encoding a polypeptide extension or affinity tag as disclosed above. Using techniques known in the art, a transfer vector containing a protein-encoding DNA sequence is transformed into *E. coli* host cells, and the cells are screened for bacmids which contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is isolated, using common techniques, and used to transfect *Spodoptera frugiperda* cells, such as Sf9 cells. Recombinant virus that expresses the protein or interest is subsequently produced. Recombinant viral stocks are made by methods commonly used in the art.

[0198] For protein production, the recombinant virus is used to infect host cells, typically a cell line derived from the fall armyworm, *Spodoptera frugiperda* (*e.g.*, Sf9 or Sf21 cells) or *Trichoplusia ni* (*e.g.*, HIGH FIVE cells; Invitrogen, Carlsbad, CA). *See generally* Glick and Pasternak, *Molecular Biotechnology, Principles & Applications of Recombinant DNA* (ASM Press, Washington, D.C., 1994). *See also* U.S. Patent No. 5,300,435. Serum-free media are used to grow and maintain the cells. Suitable media formulations are known in the art and can be obtained from commercial suppliers. The cells are grown up from an inoculation density of approximately 2-5 x 10^5 cells to a density of 1-2 x 10^6 cells, at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3. Procedures used are generally described in available laboratory manuals (*see, e.g.*, King and Possee, *supra*; O'Reilly *et al.*, *supra*; Richardson, *supra*).

[0199] Fungal cells, including yeast cells, can also be used within the present invention. Yeast species of in this regard include, e.g., *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki *et al.*, U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch *et al.*, U.S. Patent No. 5,037,743; and Murray *et al.*, U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (*e.g.*, leucine). An exemplary vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki *et al.* (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (*see,*

e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman *et al.*, U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. *See also* U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936; and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia pastoris, Pichia methanolica, Pichia guillermondii*, and *Candida maltosa* are known in the art. *See, e.g.*, Gleeson *et al.*, *J. Gen. Microbiol.* 132:3459-3465, 1986; Cregg, U.S. Patent No. 4,882,279; and Raymond *et al.*, *Yeast* 14:11-23, 1998. *Aspergillus* cells can be utilized according to the methods of McKnight *et al.*, U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino *et al.*, U.S. Patent No. 5,162,228. Methods for transforming Neurospora are disclosed by Lambowitz, U.S. Patent No. 4,486,533. Production of recombinant proteins in *Pichia methanolica* is disclosed in U.S. Patents Nos. 5,716,808; 5,736,383; 5,854,039; and 5,888,768.

[0200] Prokaryotic host cells, including strains of the bacteria *Escherichia coli*, *Bacillus*, and other genera are also useful host cells within the present invention. Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well-known in the art (*see, e.g.*, Sambrook and Russell, *supra*). When expressing a recombinant protein in bacteria such as *E. coli*, the protein can be retained in the cytoplasm, typically as insoluble granules, or can be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured protein can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the alternative, the protein can be recovered from the cytoplasm in soluble form and isolated without the use of denaturants. The protein is recovered from the cell as an aqueous extract in, for example, phosphate buffered saline. To capture the protein of interest, the extract is applied directly to a chromatographic medium, such as an immobilized antibody or heparin-Sepharose column. Secreted proteins can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding. Antibodies, including single-chain antibodies, can be produced in bacterial host cells according to known methods. *See, e.g.*, Bird *et al.*, *Science* 242:423-426, 1988; Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883, 1988; and Pantoliano *et al.*, *Biochem.* 30:10117-10125, 1991.

[0201] Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media can also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell.

[0202] PSMA-binding proteins are purified by conventional protein purification methods, typically by a combination of chromatographic techniques. *See generally Affinity Chromatography: Principles & Methods* (Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988); Scopes, *Protein Purification: Principles and Practice* (Springer-Verlag, New York 1994). Proteins comprising an immunoglobulin Fc region can be purified by affinity chromatography on immobilized protein A or protein G. Additional purification steps, such as gel filtration, can be used to obtain the desired level of purity or to provide for desalting, buffer exchange, and the like.

V. Methods of Treatment

[0203] In another embodiment, the present invention provides a method for treating a disorder characterized by overexpression of PSMA. Generally, such methods include administering to a subject in need of such treatment a therapeutically effective amount of a PSMA-binding protein as described herein. In some embodiments, the PSMA-binding protein comprises at least one effector function selected from antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), such that the PSMA-binding protein induces ADCC and/or CDC against PSMA-expressing cells in the subject. In other embodiments, where the PSMA-binding protein comprises a second binding domain that specifically binds a T cell (*e.g.*, to a TCR complex or component thereof, such as CD3ε), the PSMA-binding protein induces redirected T-cell cytotoxicity (RTCC) against PSMA-expressing cells in the subject.

[0204] In certain variations of the method, the disorder is a cancer. Exemplary cancers amenable to treatment in accordance with the present invention include, for example, prostate cancer (*e.g.*, castrate-resistant prostate cancer), colorectal cancer, gastric cancer, clear cell renal carcinoma, bladder cancer, and lung cancer. In other variations, the disorder is a prostate disorder such as, for example, prostate cancer or benign prostatic hyperplasia

(BPH). In yet other embodiments, the disorder is an neovascular disorder such as, for example, a cancer characterized by solid tumor growth. Exemplary cancers with tumor vasculatures characterized by PSMA overexpression and amenable to treatment in accordance with the present invention include, for example, clear cell renal carcinoma (CCRCC), colorectal cancer, breast cancer, bladder cancer, lung cancer, and pancreatic cancer (*see, e.g.*, Baccala *et al.*, *Urology* 70:385-390, 2007 (expression of PSMA in CCRCC); Liu *et al.*, *Cancer Res.* 57:3629-3634, 1997 (expression of PSMA in various non-prostate cancers, including renal, urothelial, lung, colon, breast, and adenocarcinaoma to the liver); and Milowsky *et al.*, *J. Clin. Oncol.* 25:540-547, 2007 (expression in, *e.g.*, kidney, colon, bladder, and pancreatic cancers, and demonstration of specific targeting of tumor vasculature in humans using an anti-PSMA mAb).

[0205] In each of the embodiments of the treatment methods described herein, the PSMA-binding protein is delivered in a manner consistent with conventional methodologies associated with management of the disease or disorder for which treatment is sought. In accordance with the disclosure herein, an effective amount of the PSMA-binding protein is administered to a subject in need of such treatment for a time and under conditions sufficient to prevent or treat the disease or disorder.

[0206] Subjects for administration of PSMA-binding proteins as described herein include patients at high risk for developing a particular disorder characterized by PSMA overexpression as well as patients presenting with an existing such disorder. Typically, the subject has been diagnosed as having the disorder for which treatment is sought. Further, subjects can be monitored during the course of treatment for any change in the disorder (*e.g.*, for an increase or decrease in clinical symptoms of the disorder). Also, in some variations, the subject does not suffer from another disorder requiring treatment that involves targeting PSMA-expressing cells.

[0207] In prophylactic applications, pharmaceutical compositions or medicants are administered to a patient susceptible to, or otherwise at risk of, a particular disorder in an amount sufficient to eliminate or reduce the risk or delay the onset of the disorder. In therapeutic applications, compositions or medicants are administered to a patient suspected of, or already suffering from such a disorder in an amount sufficient to cure, or at least partially arrest, the symptoms of the disorder and its complications. An amount adequate to accomplish this is referred to as a therapeutically effective dose or amount. In both prophylactic and therapeutic regimes, agents are usually administered in several dosages until a sufficient response (*e.g.*, inhibition of inappropriate angiogenesis activity) has been achieved. Typically, the response is monitored and repeated dosages are given if the desired response starts to fade.

[0208] To identify subject patients for treatment according to the methods of the invention, accepted screening methods can be employed to determine risk factors associated with specific disorders or to determine the status of an existing disorder identified in a subject. Such methods can include, for example, determining whether an individual has relatives who have been diagnosed with a particular disorder. Screening methods can also include, for example, conventional work-ups to determine familial status for a particular disorder known to have a heritable component. For example, various cancers are also known to have certain inheritable components. Inheritable components of cancers include, for example, mutations in multiple genes that are transforming (*e.g.*, Ras, Raf, EGFR, cMet, and others), the presence or absence of certain HLA and killer inhibitory receptor (KIR) molecules, or mechanisms by which cancer cells are able to modulate immune suppression of cells like NK cells and T cells, either directly or indirectly (*see, e.g.*, Ljunggren and Malmberg, *Nature Rev. Immunol.* 7:329-339, 2007; Boyton and Altmann, *Clin. Exp. Immunol.* 149:1-8, 2007). Toward this end, nucleotide probes can be routinely employed to identify individuals carrying genetic markers associated with a particular disorder of interest. In addition, a wide variety of immunological methods are known in the art that are useful to identify markers for specific disorder. For example, various ELISA immunoassay methods are available and well-known in the art that employ monoclonal antibody probes to detect antigens associated with specific tumors. Screening can be implemented as indicated by known patient symptomology, age factors, related risk factors, etc. These methods allow the clinician to routinely select patients in need of the methods described herein for treatment. In accordance with these methods, targeting pathological, PSMA-expressing cells can be implemented as an independent treatment program or as a follow-up, adjunct, or coordinate treatment regimen to other treatments.

[0209] For administration, the PSMA-binding protein is formulated as a pharmaceutical composition. A pharmaceutical composition comprising a PSMA-binding protein can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic molecule is combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known to those in the art. (*See, e.g.*, Gennaro (ed.), *Remington's Pharmaceutical Sciences* (Mack Publishing Company, 19th ed. 1995).) Formulations can further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, *etc.*

[0210] A pharmaceutical composition comprising a PSMA-binding protein is administered to a subject in a therapeutically effective amount. According to the methods of the present invention, a PSMA-binding protein can be administered to subjects by a variety of administration modes, including, for example, by intramuscular, subcutaneous, intravenous, intra-atrial, intra-articular, parenteral, intranasal, intrapulmonary, transdermal, intrapleural, intrathecal, and oral routes of administration. For prevention and treatment purposes, an antagonist can be administered to a subject in a single bolus delivery, via continuous delivery (*e.g.*, continuous transdermal delivery) over an extended time period, or in a repeated administration protocol (*e.g.*, on an hourly, daily, or weekly basis).

[0211] A "therapeutically effective amount" of a composition is that amount that produces a statistically significant effect in amelioration of one or more symptoms of the disorder, such as a statistically significant reduction in disease progression or a statistically significant improvement in organ function. The exact dose will be determined by the clinician according to accepted standards, taking into account the nature and severity of the condition to be treated, patient traits, *etc.* Determination of dose is within the level of ordinary skill in the art.

[0212] Determination of effective dosages in this context is typically based on animal model studies followed up by human clinical trials and is guided by determining effective dosages and administration protocols that significantly reduce the occurrence or severity of the subject disorder in model subjects. Effective doses of the compositions of the present invention vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, whether treatment is prophylactic or therapeutic, as well as the specific activity of the composition itself and its ability to elicit the desired response in the individual. Usually, the patient is a human, but in some diseases, the patient can be a nonhuman mammal. Typically, dosage regimens are adjusted to provide an optimum therapeutic response, *i.e.*, to optimize safety and efficacy. Accordingly, a therapeutically effective amount is also one in which any undesired collateral effects are outweighed by the beneficial effects of administering a PSMA-binding protein as described herein. For administration of the PSMA-binding protein, a dosage typically ranges from about 0.1 µg to 100 mg/kg or 1 µg/kg to about 50 mg/kg, and more usually 10 µg to 5 mg/kg of the subject's body weight. In more specific embodiments, an effective amount of the agent is between about 1 µg/kg and about 20 mg/kg, between about 10 µg/kg and about 10 mg/kg, or between about 0.1 mg/kg and about 5 mg/kg. Dosages within this range can be achieved by single or multiple administrations, including, *e.g.*, multiple administrations per day or daily, weekly, bi-weekly, or monthly administrations. For example, in certain variations, a regimen consists of an initial administration followed by multiple, subsequent administrations at

weekly or bi-weekly intervals. Another regimen consists of an initial administration followed by multiple, subsequent administrations at monthly or bi-monthly intervals. Alternatively, administrations can be on an irregular basis as indicated by monitoring clinical symptoms of the disorder.

[0213] Dosage of the pharmaceutical composition can be varied by the attending clinician to maintain a desired concentration at a target site. For example, if an intravenous mode of delivery is selected, local concentration of the agent in the bloodstream at the target tissue can be between about 1-50 nanomoles of the composition per liter, sometimes between about 1.0 nanomole per liter and 10, 15, or 25 nanomoles per liter depending on the subject's status and projected measured response. Higher or lower concentrations can be selected based on the mode of delivery, *e.g.*, trans-epidermal delivery versus delivery to a mucosal surface. Dosage should also be adjusted based on the release rate of the administered formulation, *e.g.*, nasal spray versus powder, sustained release oral or injected particles, transdermal formulations, *etc.* To achieve the same serum concentration level, for example, slow-release particles with a release rate of 5 nanomolar (under standard conditions) would be administered at about twice the dosage of particles with a release rate of 10 nanomolar.

[0214] Pharmaceutical compositions as described herein can also be used in the context of combination therapy. The term "combination therapy" is used herein to denote that a subject is administered at least one therapeutically effective dose of a PSMA-binding protein and another therapeutic agent.

[0215] For example, in the context of cancer immunotherapy, a PSMA-binding protein of the present invention can be used in combination with chemotherapy or radiation. A PSMA-binding protein as described herein can work in synergy with conventional types of chemotherapy or radiation. The PSMA-binding protein can further reduce tumor burden and allow more efficient killing by a chemotherapeutic.

[0216] Compositions of the present invention can also be used in combination with immunomodulatory compounds including various cytokines and co-stimulatory/inhibitory molecules. These can include, but are not limited to, the use of cytokines that stimulate anti-cancer immune responses (*e.g.*, IL-2, IL-12, or IL-21). In addition, PSMA-binding proteins can be combined with reagents that co-stimulate various cell surface molecules found on immune-based effector cells, such as the activation of CD137 (*see* Wilcox *et al.*, *J. Clin. Invest.* 109:651-9, 2002) or inhibition of CTLA4 (*see* Chambers et al., *Ann. Rev. Immunol.* 19:565-94, 2001). Alternatively, PSMA-binding proteins could be used with reagents that induce tumor cell apoptosis by interacting with TNF superfamily receptors (*e.g.*, TRAIL-

related receptors, DR4, DR5, Fas, or CD37). (*See, e.g.*, Takeda *et al.*, *J. Exp. Med.* 195:161-9, 2002; Srivastava, *Neoplasia* 3:535-46, 2001.) Such reagents include ligands of TNF superfamily receptors, including ligand-Ig fusions, and antibodies specific for TNF superfamily receptors (*e.g.*, TRAIL ligand, TRAIL ligand-Ig fusions, anti-TRAIL antibodies, and the like).

[0217] With particular regard to treatment of solid tumors, protocols for assessing endpoints and anti-tumor activity are well-known in the art. While each protocol may define tumor response assessments differently, the RECIST (Response evaluation Criteria in solid tumors) criteria is currently considered to be the recommended guidelines for assessment of tumor response by the National Cancer Institute (*see* Therasse *et al.*, *J. Natl. Cancer Inst.* 92:205-216, 2000). According to the RECIST criteria tumor response means a reduction or elimination of all measurable lesions or metastases. Disease is generally considered measurable if it comprises lesions that can be accurately measured in at least one dimension as $\geq$ 20mm with conventional techniques or $\geq$ 10mm with spiral CT scan with clearly defined margins by medical photograph or X-ray, computerized axial tomography (CT), magnetic resonance imaging (MRI), or clinical examination (if lesions are superficial). Non-measurable disease means the disease comprises of lesions < 20mm with conventional techniques or < 10mm with spiral CT scan, and truly non-measurable lesions (too small to accurately measure). Non-measureable disease includes pleural effusions, ascites, and disease documented by indirect evidence.

[0218] The criteria for objective status are required for protocols to assess solid tumor response. Representative criteria include the following: (1) Complete Response (CR), defined as complete disappearance of all measurable disease; no new lesions; no disease related symptoms; no evidence of non-measurable disease; (2) Partial Response (PR) defined as 30% decrease in the sum of the longest diameter of target lesions (3) Progressive Disease (PD), defined as 20% increase in the sum of the longest diameter of target lesions or appearance of any new lesion; (4) Stable or No Response, defined as not qualifying for CR, PR, or Progressive Disease. (*See* Therasse *et al.*, *supra.*)

[0219] Additional endpoints that are accepted within the oncology art include overall survival (OS), disease-free survival (DFS), objective response rate (ORR), time to progression (TTP), and progression-free survival (PFS) (*see Guidance for Industry: Clinical Trial Endpoints for the Approval of Cancer Drugs and Biologics*, April 2005, Center for Drug Evaluation and Research, FDA, Rockville, MD.)

[0220] Pharmaceutical compositions can be supplied as a kit comprising a container that comprises the pharmaceutical composition as described herein. A pharmaceutical

composition can be provided, for example, in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively, such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of a pharmaceutical composition. Such a kit can further comprise written information on indications and usage of the pharmaceutical composition.

EXAMPLES

EXAMPLE 1: Isolation of murine variable domains from 107-1A4 and preparation of humanized variants

[0221] Murine variable domains were cloned from hybridoma cells expressing the 107-1A4 monoclonal antibody specific for human PSMA (see Brown et al, 1998, Prostate Cancer and Prostatic Diseases. 1: 208-215). Total RNA was isolated from the hybridoma using RNeasy® Protect Mini kit (QIAGEN Inc., 74124) according to the manufacturer's instructions. SMART™ RACE cDNA amplification kit (Clontech Laboratories, Inc., 634914) was used to generate 5'RACE-ready cDNA with oligo(dT) primer according to the manufacturer's instructions. V_H and V_L regions of antibody were PCR-amplified from cDNA by SMART™ RACE protocol using pools of proprietary degenerate gene specific primers for different murine VK or VH gene families. PCR amplification products were confirmed by gel electrophoresis, and correct sized bands were isolated and cloned into pCR®2.1-TOPO® plasmid vector using the TOPO® TA Cloning kit according to manufacturer's instructions (Invitrogen Corporation). The resulting recombinant vector was transformed into TOP10 *E. coli*. Sequencing DNA from clones revealed multiple isolates of a heavy chain region with a murine VH1 framework with high homology (92.7%) to the murine germline framework L17134 (Genbank), and a kappa chain region with a murine Vk16 framework with very high homology (98.6%) to the murine germline framework AJ235936 (EMBL). Two restriction sites – one HindIII and one EcoRI site – were removed by neutral mutations from the DNA coding for the parent murine kappa (light) variable domain to simplify cloning into destination mammalian expression vectors, and the native murine secretion/leader sequences were also not used in favor of the human Vk3 leader sequence. The polynucleotide sequence of PSMA-specific murine VH region (107-1A4) is given in SEQ ID NO:1, and the amino acid sequence is given in SEQ ID NO:2. The polynucleotide sequence of PSMA-specific murine VL region (107-1A4) with the restriction sites is given in SEQ ID NO:3. The polynucleotide sequence of PSMA-specific murine VL region (107-1A4) modified to remove the restriction sites is given in SEQ ID NO:4, and the amino acid sequence is given in SEQ ID NO:5.

[0222] DNA sequences coding for these murine scFv sequences and cassetted for insertion into appropriate scaffolds (*e.g.*, SMIP, SCORPION, and mono-specific or multispecific heterodimer polypeptides) were designed. The constructs were then synthesized by Blue Heron (Bothell, WA) and standard, restriction-digest-based cloning techniques were used to produce the gene sequences corresponding to TSC084 (SEQ ID NO:44; amino acid sequence SEQ ID NO:46), TSC085 (SEQ ID NO:36; amino acid sequence SEQ ID NO:38), and TSC092 (SEQ ID NO:37; amino acid sequence SEQ ID NO:39).

[0223] Humanized sequences designed through CDR grafting to human frameworks were similarly synthesized by Blue Heron and cloned into similar vectors using restriction digests to produce the the following gene sequences using two approaches: (A) three piece ligation using a *HindIII/BamHI* fragment, a *BamHI/XhoI* fragment, and a destination vector cut with *HindIII/XhoI* to produce the gene sequences corresponding to TSC188 (SEQ ID NO:40; amino acid sequence SEQ ID NO:42) and TSC189 (SEQ ID NO:41; amino acid sequence SEQ ID NO:43); and (B) two piece ligation using a *HindIII/XhoI* fragment and a destination vector cut with *HindIII/XhoI* to produce the gene sequences corresponding to TSC192 (SEQ ID NO:53; amino acid sequence SEQ ID NO:58), TSC193 (SEQ ID NO:54; amino acid sequence SEQ ID NO:59), TSC194 (SEQ ID NO:48; amino acid sequence SEQ ID NO:49), TSC195 (SEQ ID NO:55; amino acid sequence SEQ ID NO:60), TSC196 (SEQ ID NO:56; amino acid sequence SEQ ID NO:61), TSC199 (SEQ ID NO:50; amino acid sequence SEQ ID NO:51), TSC210 (SEQ ID NO:69; amino acid sequence SEQ ID NO:70), TSC211 (SEQ ID NO:71; amino acid sequence SEQ ID NO:72), TSC212 (SEQ ID NO:73; amino acid sequence SEQ ID NO:74), TSC213 (SEQ ID NO:75; amino acid sequence SEQ ID NO:76); TSC249 (SEQ ID NO:77; amino acid sequence SEQ ID NO:78), TSC250 (SEQ ID NO:79; amino acid sequence SEQ ID NO:80), TSC251 (SEQ ID NO:81; amino acid sequence SEQ ID NO:82), and TSC252 (SEQ ID NO:83; amino acid sequence SEQ ID NO:84); and (C) two piece ligation using a *BsrGI/EcoRI* fragment and one of two destination vectors cut with *BsrGI/EcoRI* to produce the gene sequences corresponding to TSC295 (SEQ ID NO:157; amino acid sequence SEQ ID NO:158), TSC296 (SEQ ID NO:159; amino acid sequence SEQ ID NO:160), TSC301 (SEQ ID NO:161; amino acid sequence SEQ ID NO:162), and TSC302 (SEQ ID NO:163; amino acid sequence SEQ ID NO:164). The humanized PSMA-specific (107-1A4) VL region polynucleotide sequence is given in SEQ ID NO:22, and the amino acid sequence is given in SEQ ID NO:23. A humanized PSMA-specific (107-1A4) VH region #1 polynucleotide sequence is given in SEQ ID NO:24, and the amino acid sequence is given in SEQ ID NO:25. A humanized PSMA-specific (107-1A4) VH region #2

polynucleotide sequence is given in SEQ ID NO:26, and the amino acid sequence is given in SEQ ID NO:27.

[0224] Sequences for the various cloned sequences and components are also presented in Table 3. Amino acid sequences given for polypeptide constructs (*e.g.*, SMIP, SCORPION, mono- or multi-specific heterodimeric proteins) do not include the human Vk3 leader sequence.

Table 3: Binding Polypeptide Sequences and Components

Name	Nucleotide Sequence	Amino Acid Sequence	SEQ ID NOs: (amino acid)
Murine 107-1A4 VH region	gagatccagctgcaacagtctggacctgagctggtgaagcctggggcttca gtgaagatgtcctgcaaggcttctggatacacattcactgactactacatgcac tgggtgaagcagaacaatggagagagccttgagtggattggatatttttaatcc ttataatgattatactagatacaaccagaatttcaatggcaaggccacattgact gtagacaagtcctccagcacagcctacatgcagctcaacagcctgacatctg aggactctgcattctattactgtgcaagatcggatggttactacgatgctatgg actactggggtcaaggaacctcagtcaccgtctcctcg	eiqlqqsgpelvkpgasvk msckasgytftdyymhw vkqnngeslewigyfnpy ndytrynqnfngkatltvdk ssstaymqlnsltsedsafy ycarsdgyydamdywgq	SEQ ID NO:1 (SEQ ID NO:2)
Murine 107-1A4 VL region w/ additional restriction sites	Gatgtccagataacccagtctccatcttatcttgctgcatctcctggagaaacc attactattaattgcagggcaagtaagagcattagcaaatatttagcctggtatc aagagaaacctgggaaagctaataagcttcttatccattctggatccactttgc aatctggaattccatcaaggttcagtggcagtggatctggtacagatttcactct caccatcagtagcctggagcctgaagattttgcaatgtattactgtcaacagca tattgaatacccgtggacgttcggtggtggcaccaaactggaaattaaacgg gct		SEQ ID NO:3
Murine 107-1A4 VL region modified	gatgtccagataacccagtctccatcttatcttgctgcatctcctggagaaacc attactattaattgcagggcaagtaagagcattagcaaatatttagcctggtatc aagagaaacctgggaaagctaataagctacttatccattctggatccactttgc aatctggaataccatcaaggttcagtggcagtggatctggtacagatttcactc tcaccatcagtagcctggagcctgaagattttgcaatgtattactgtcaacagc atattgaatacccgtggacgttcggtggtggcaccaaactggaaattaaacg ggcc	dvqitqspsylaaspgetiti ncrasksiskylawyqekp gkankllihsgstlqsgipsr fsgsgsgtdftltisslepedf amyycqqhieypwtfggg tkleikra	SEQ ID NO:4 (SEQ ID NO:5)
107-1A4 VH CDR1	tctggatacacattcactgactactacatgcac	sgytftdyymh	SEQ ID NO:6 (SEQ ID NO:9)
107-1A4 VH CDR2	tattttaatccttataatgattatactaga	Yfnpyndytr	SEQ ID NO:7 (SEQ ID NO:10)
107-1A4 VH CDR3	tgtgcaagatcggatggttactacgatgctatggactactgg	carsdgyydamdyw	SEQ ID NO:8 (SEQ ID NO:11)
107-1A4 VL CDR1	Aagagcattagcaaatat	Ksisky	SEQ ID NO:12 (SEQ ID NO:15)
107-1A4 VL CDR2	Tctggatcc	Sgs	SEQ ID NO:13 (SEQ ID NO:16)
107-1A4 VL CDR3	Caacagcatattgaatacccgtggacg	Qqhieypwt	SEQ ID NO:14 (SEQ ID NO:17)
107-1A4	gagatccagctgcaacagtctggacctgagctggtgaagcctggggcttca gtgaagatgtcctgcaaggcttctggatacacattcactgactactacatgcac	eiqlqqsgpelvkpgasvk msckasgytftdyymhw	SEQ ID NO:18

Name	Nucleotide Sequence	Amino Acid Sequence	SEQ ID NOs: (amino acid)
VH-VL scFv	tgggtgaagcagaacaatggagagagccttgagtggattggatattttaatcc ttataatgattatactagatacaaccagaatttcaatggcaaggccacattgact gtagacaagtcctccagcacagcctacatgcagctcaacagcctgacatctg aggactctgcattctattactgtgcaagatcggatggttactacgatgctatgg actactggggtcaaggaacctcagtcaccgtctcctcaggcggcggcggaa gcggcggtggcggcagcagcggcggcggcggcagcgatgtccagataa cccagtctccatcttatcttgctgcatctcctggagaaaccattactattaattgc agggcaagtaagagcattagcaaatatttagcctggtatcaagagaaacctg ggaaagctaataagctacttatccattctggatccacttttgcaatctggaataacc atcaaggttcagtggcagtggatctggtacagatttcactctcaccatcagtag cctggagcctgaagattttgcaatgtattactgtcaacagcatattgaataccc gtggacgttcggtggtggcaccaaactggaaattaaacgggcctcg	vkqnngeslewigyfnpy ndytrynqnfngkatltvdk ssstaymqlnsltsedsafy ycarsdgyydamdywgq gtsvtvssggggsggggss ggggsdvqitqspsylaasp getitincrasksiskylawy qekpgkankllihsgstlqs gipsrfsgsgsgtdftltissle pedfamyycqqhieypwt fgggtkleikras	(SEQ ID NO:19)
107-1A4 VL-VH scFv	gatgtccagataacccagtctccatcttatcttgctgcatctcctggagaaaacc attactattaattgcagggcaagtaagagcattagcaaatatttagcctggtatc aagagaaacctgggaaagctaataagctacttatccattctggatccactttgc aatctggaataccatcaaggttcagtggcagtggatctggtacagatttcactc tcaccatcagtagcctggagcctgaagattttgcaatgtattactgtcaacagc atattgaatacccgtggacgttcggtggtggcaccaaactggaaattaaacg ggccggcggcggcggaagcggcggtggcggcagcagcggcggcggcg gcagcgagatccagctgcaacagtctggacctgagctggtgaagcctggg gcttcagtgaagatgtcctgcaaggcttctggatacacattcactgactactac atgcactgggtgaagcagaacaatggagagagccttgagtggattggatatt ttaatccttataatgattatactagatacaaccagaatttcaatggcaaggccac attgactgtagacaagtcctccagcacagcctacatgcagctcaacagcctg acatctgaggactctgcattctattactgtgcaagatcggatggttactacgatg ctatggactactggggtcaaggaacctcagtcaccgtctcctcg	diqmtqspsylaaspgetiti ncrasksiskylawyqekp gkankllihsgstlqsgipsr fsgsgsgtdftltissiepedf amyycqqhieypwtfggg tkleikraggggsggggssg gggseiqlqqsgpelvkpg asvkmsckasgytftdyy mhwvkqnngeslewigy fnpyndytrynqnfngkatl tvdkssstaymqlnsltsed safyycarsdgyydamdy wgqgtsvtvss	SEQ ID NO:20 (SEQ ID NO:21)
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Humanized 107-1A4 VH#1	gaggtccagctggtacagtctggggctgaggtgaagaagcctggggctac agtgaagatctcctgcaaggcttctggatacacattcactgactactacatgca ctgggtgcaacaggcccctggaaaagggcttgagtggatgggatattttaat ccttataatgattatactagatacgcagagaagttccagggcagagtcaccat aaccgcggacacgtctacagacacagcctacatggagctgagcagcctga gatctgaggacacggccgtgtattactgtgcaagatcggatggttactacgat gctatggactactggggtcaaggaaccacagtcaccgtctcctcg	evqlvqsgaevkkpgatvk isckasgytftdyymhwv qqapgkglewmgyfnpy ndytryaekfqgrvtitadts tdtaymelsslrsedtavyy carsdgyydamdywgqg ttvtvss	SEQ ID NO:24 (SEQ ID NO:25)
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Humanized 107-1A4 VL-VH#1	gatatccagatgacccagtctccatccgccatgtctgcatctgtaggagacag agtcaccatcacttgccgggcgagtaagagcattagcaaatatttagcctggt ttcagcagaaaccagggaaagttcctaagctccgcatccattctggatctactt	diqmtqspsamsasvgdr vtitcrasksiskylawfqqk pgkvpklrihsgstlqsgvp	SEQ ID NO:28 (SEQ ID NO:30)

Name	Nucleotide Sequence	Amino Acid Sequence	SEQ ID NOs: (amino acid)
scFv	tgcaatcaggggtcccatctcggttcagtggcagtggatctgggacagaatttactctcaccatcagcagcctgcagcctgaagattttgcaacttattactgtcaacagcatattgaatacccgtggacgttcggccaagggaccaaggtggaaatcaaacgaggtggcggagggtctgggggtggcggatccggaggtggtggctctgaggtccagctggtacagtctggggctgaggtgaagaagcctggggctacagtgaagatctcctgcaaggcttctggatacacattcactgactactacatgcactgggtgcaacaggcccctggaaaagggcttgagtggatgggatattttaatccttataatgattatactagatacgcagagaagttccagggcagagtcaccataaccgcggacacgtctacagacacagcctacatggagctgagcagcctgagatctgaggacacggccgtgtattactgtgcaagatcggatggttactacgatgctatggactactggggtcaaggaaccacagtcaccgtctcctcg	srfsgsgsgteftltisslqpedfatyycqqhieypwtfgqgtkveikrggggsggggsggggsevqlvqsgaevkkpgatvkisckasgytftdyymhwvqqapgkglewmgyfnpyndytryaekfqgrvtitadtstdtaymelsslrsedtavyycarsdgyydamdywgqgttvtvss	
Humanized 107-1A4 VL-VH#2 scFv	gatatccagatgacccagtctccatccgccatgtctgcatctgtaggagacagagtcaccatcacttgccgggcgagtaagagcattagcaaatatttagcctggttcagcagaaaccagggaaagttcctaagctccgcatccattctggatctacttgcaatcaggggtcccatctcggttcagtggcagtggatctgggacagaatttactctcaccatcagcagcctgcagcctgaagattttgcaacttattactgtcaacagcatattgaatacccgtggacgttcggccaagggaccaaggtggaaatcaaacgaggtggcggagggtctgggggtggcggatccggaggtggtggctctcaggtccagctggtacagtctggggctgaggtgaagaagcctggggcttcagtgaaggtctcctgcaaggcttctggatacacattcactgactactacatgcactgggtgcgacaggccccttgacaagggcttgagtggatgggatattttaatcccttataatgattatactagatacgcacagaagttccagggcagagtcaccatgaccagggacacgtctatcagcacagcctacatggagctgagcagcctgagatctgacgacacggccgtgtattactgtgcaagatcggatggttactacgatgctatggactactggggtcaaggaaccacagtcaccgtctcctcg	diqmtqspsamsasvgdrvtitcrasksiskylawfqqkpgkvpklrihsgstlqsgvpsrfsgsgsgteftltisslqpedfatyycqqhieypwtfgqgtkveikrggggsggggsgggsqvqlvqsgaevkkpgasvkvsckasgytftdyymhwvrqapgqglewmgyfnpyndytryaqkfqgrvtmtrdtsistaymelsslrsddtavyycarsdgyydamdywgqgttvtvss	SEQ ID NO:29 (SEQ ID NO:31)
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Name	Nucleotide Sequence	Amino Acid Sequence	SEQ ID NOs: (amino acid)
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Name	Nucleotide Sequence	Amino Acid Sequence	SEQ ID NOs: (amino acid)
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TSC188 humanized SMIP (107-1A4 VL-VH#1 scFv-Fc)	atggaagcaccagcgcagcttctcttcctcctgctactctggctcccagatac caccggtgatatccagatgacccagtctccatccgccatgtctgcatctgtag gagacagagtcaccatcacttgccgggcgagtaagagcattagcaaatatttt agcctggtttcagcagaaaccagggaaagttcctaagctccgcatccattctg gatctactttgcaatcaggggtcccatctcggttcagtggcagtggatctggg acagaatttactctcaccatcagcagcctgcagcctgaagattttgcaacttatt actgtcaacagcatattgaatacccgtggacgttcggccaagggaccaaggt ggaaatcaaacgaggtggcggagggtctgggggtggcggatccggaggt ggtggctctgaggtccagctggtacagtctggggctgaggtgaagaagcct ggggctcacagtgaagatctcctgcaaggcttctggatacacattcactgacta ctacatgcactgggtgcaacaggccccctggaaaagggcttgagtggatggg atattttaatccttataatgattatactagatacgcagagaagttccagggcaga gtcaccataaccgcggacacgtctacagacacagcctacatggagctgagc agcctgagatctgaggacacggccgtgtattactgtgcaagatcggatggtt actacgatgctatggactactggggtcaaggaaccacagtcaccgtctcctc gagtgagcccaaatcttctgacaaaactcacacatgcccaccgtgcccagca cctgaagccgcgggtgcaccgtcagtcttcctcttccccccaaaacccaagg acaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgt gagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtgga ggtgcataatgccaagacaaagccgcgcgggaggagcagtacaacagcacgt accgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaa ggcgtacgcgtgcgcggtctccaacaaagccctcccagccccatcgaga aaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacc ctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgc ctggtcaaaggcttctatccaagcgacatcgccgtggagtgggagagcaat gggcagccggagaacaactacaagaccacgcctcccgtgctggactccga cggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcag caggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccacta cacgcagaagagcctctccctgtctccgggtaaatga	diqmtqspsamsasvgdr vtitcrasksiskylawfqqk pgkvpklrihsgstlqsgvp srfsgsgsgteftltisslqpe dfatyycqqhieypwtfgq gtkveikrggggsggggsg gggsevqlvqsgaevkkp gatvkiscksasgytftdyy mhwvqqapgkglewmg yfnpyndytryaekfqgrvt itadtstdtaymelsslrsedt avyycarsdgyydamdy wgqgttvtvsssepkssdkt htcppcpapeaagapsvflf ppkpkdtlmisrtpevtcvv vdvshedpevkfnwyvd gvevhnaktkpreeqynst yrvvsvltvlhqdwlngka yacavsnkalpapiektisk akgqprepqvytlppsrdel tknqvsltclvkgfypsdia vewesngqpennykttpp vldsdgsfflyskltvdksr wqqgnvfscsvmhealhn hytqkslslspgk	SEQ ID NO:40 (SEQ ID NO:42)
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TSC084 chimeric Interceptor (murine VL-VH 107-1A4 scFv-Fc-CH1)	atggaagcaccagcgcagcttctcttcctcctgctactctggctcccagatac caccggtgatgtccagataacccagtctccatcttatcttgctgcatctcctgga gaaaccattactattaattgcagggcaagtaagagcattagcaaatatttagcc tggtatcaagagaaacctgggaaagctaataagctacttatccattctggatcc actttgcaatctggaataccatcaaggttcagtggcagtggatctggtacagat ttcactctcaccatcagtagcctggagcctgaagattttgcaatgtattactgtc aacagcatattgaatacccgtggacgttcggtggtggcaccaaactggaaatt aaacgggccggcggcggcggcggaagcggcggtggcggcagcagcggcgg cggcggcagcgagatccagctgcaacagtctggacctgagctggtgaagc ctggggcttcagtgaagatgtcctgcaaggcttctggatacacattcactgact actacatgcactgggtgaagcagaacaatggagagagccttgagtggattg gatattttaatccttataatgattatactagatacaaccagaatttcaatggcaag gccacattgactgtagacaagtcctccagcacagcctacatgcagctcaaca gcctgacatctgaggactctgcattctattactgtgcaagatcggatggttacta cgatgctatggactactggggtcaaggaacctcagtcaccgtctcctcgagc gagcccaaatcttctgacaaaactcacacatgcccaccgtgcccagcacctg aagccgcgggtgcaccgtcagtcttcctcttccccccaaaacccaaggacac cctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagc cacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtg cataatgccaagacaaagccgcgcgggaggagcagtacaacagcacgtaccg tgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggc gtacgcgtgcgcggtctccaacaaagccctcccagcccccatcgagaaaac catctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgc ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctgg tcaaaggcttctatccaagcgacatcgccgtggagtgggagagcaatgggc agccggagaacaactacaagaccacgcctcccgtgctggactccgacggc tccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcagg ggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacg cagaagagcctctccctgtctccgggtaaatctagagctccaccaagggcc catcggtcttcccctggcaccctcctccaagagcacctctgggggcacagc ggccctgggctgcctggtcaaggactacttccccgagccggtgacggtgtc gtggaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcct acagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagc agcttgggcacccagacctacatctgcaacgtgaatcacaagcccagcaac accaaggtggacaagaaagtttga	dvqltqspsylaaspgetiti ncrasksiskylawyqekp gkankllihsgstlqsgipsr fsgsgsgtdftltisslepedf amyycqqhieypwtfggg tkleikraggggsggggssg gggseiqlqqsgpelvkpg asvkmsckasgytftdyy mhwvkqnngeslewigy fnpyndytrynqnfngkatl tvdkssstaymqlnsltsed safyycarsdgyydamdy wgqgtsvtvsssepkssdk thtcppcpapeaagapsvfl fppkpkdtlmisrtpevtcv vvdvshedpevkfnwyv dgvevhnaktkpreeqyns tyrvvsvltvlhqdwlngka yacavsnkalpapiektisk akgqprepqvytlppsrdel tknqvsltclvkgfypsdia vewesngqpennykttpp vldsdgsfflyskltvdksr wqqgnvfscsvmhealhn hytqkslslspgksrastkg psvfplapsskstsggtaalg clvkdyfpepvtvswnsga ltsgvhtfpavlqssglysls vvtvpssslgtqtyicnvnh kpsntkvdkkv	
TSC093 Interceptor (Cris7 scFv-Fc-$C\kappa_{YAE}$)	atggaagcaccagcgcagcttctcttcctcctgctactctggctcccagatac caccggtcaggtccagctggtgcagtctgggggcggagtggtgcagcctg ggcggtcactgaggctgtcctgcaaggcttctggctacaccttactagatcta cgatgcactgggtaaggcaggcccctggaaagggtctggaatggattggat acattaatcctagcagtgcttatactaattacaatcagaaattcaaggacaggtt cacaatcagcgcagacaaatccaagagcacagccttcctgcagatggacag cctgaggcccgaggacaccggcgtctatttctgtgcacggccccaagtcca ctatgattacaacgggtttccttactggggccaagggactccgtcactgtctc tagcggtggcggagggtctgggggtggcggatccggaggtggtggctctg cacaagacatccagatgacccagtctccaagcagcctgtctgcaagcgtgg gggacagggtcaccatgacctgcagtgccagctcaagtgtaagttacatgaa ctggtaccagcagaagcccggcaaggccccaaaagatggatttatgactc atccaaactggcttctggagtccctgctcgcttcagtggcagtgggtctggga ccgactataccctcacaatcagcagcctgcagcccgaagatttcgccacttat tactgccagcagtggagtcgtaacccacccacgttcggaggggggaccaa gctacaaattacacgctcgagtgagcccaaatcttctgacaaaactcacacat	qvqlvqsgggvvqpgrslrl sckasgytftrstmhwvrq apgkglewigyinpssayt nynqkfkdrftisadkskst aflqmdslrpedtgvyfcar pqvhydyngfpywgqgt pvtvssggggsggggsgg ggsaqdiqmtqspsslsas vgdrvtmtcsasssvsymn wyqqkpgkapkrwiydss klasgvparfsgsgsgtdytl tisslqpedfatyycqqwsr npptfgggtklqitrssepks sdkthtcppcpapeaagap svflfppkpkdtlmisrtpe vtcvvvdvshedpevkfn	

Name	Nucleotide Sequence	Amino Acid Sequence	SEQ ID NOs: (amino acid)
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TSC194 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-Cris7 scFv)	atggaagcaccagcgcagcttctcttcctcctgctactctggctcccagatac caccggtgatatccagatgacccagtctccatccgccatgtctgcatctgtag gagacagagtcaccatcacttgccgggcgagtaagagcattagcaaatatttt agcctggtttcagcagaaaccagggaaagttcctaagctccgcatccattctg gatctactttgcaatcaggggtcccatctcggttcagtggcagtggatctggg acagaatttactctcaccatcagcagcctgcagcctgaagattttgcaacttatt actgtcaacagcatattgaatacccgtggacgttcggccaagggaccaaggt ggaaatcaaacgaggtggcggagggtctgggggtggcggatccggaggt ggtggctctcaggtccagctggtacagtctggggctgaggtgaagaagcct ggggcttcagtgaaggtctcctgcaaggcttctggatacacattcactgacta ctacatgcactgggtgcgacaggcccctggacaagggcttgagtggatggg atattttaatccttataatgattatactagatacgcacagaagttccagggcaga gtcaccatgaccagggacacgtctatcagcacagcctacatggagctgagc agcctgagatctgacgacacggccgtgtattactgtgcaagatcggatggtta ctacgatgctatggactactggggtcaaggaaccacagtcaccgtctcctcg agtgagcccaaatcttctgacaaaactcacacatgcccaccgtgcccagcac ctgaagccgcggggtgcaccgtcagtcttcctcttccccccaaaacccaagga caccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtg agccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggag gtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgta ccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaag gcgtacgcgtgcgcggtctccaacaaagccctcccagccccatcgagaaa accatctccaaagccaaagggcagccccgagaaccacaggtgtacaccct gcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcct ggtcaaaggcttctatccaagcgacatcgccgtggagtgggagagcaatgg gcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg gctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagca ggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactaca cgcagaagagcctctccctgtctccgggtcagaggcacaacaattcttcact gaatacaggaactcagatggcaggtcattctccgaattctcaggtccagctgg tgcagtctgggggcggagtggtgcagcctgggcggtcactgaggctgtcct gcaaggcttctggctacaccttactagatctacgatgcactgggtaaggcag gcccctggaaagggtctggaatggattggatacattaatcctagcagtgcttat actaattacaatcagaaattcaaggacaggttcacaatcagcgcagacaaatc caagagcacagccttcctgcagatggacagcctgaggccgaggacaccg gcgtctatttctgtgcacggccccaagtccactatgattacaacgggtttcctta ctggggccaagggactcccgtcactgtctctagcggtggcggagggtctgg	diqmtqspsamsasvgdr vtitcrasksiskylawfqqk pgkvpklrihsgstlqsgvp srfsgsgsgteftltisslqpe dfatyycqqhieypwtfgq gtkveikrggggsggggsg gggsqvqlvqsgaevkkp gasvkvsckasgytftdyy mhwvrqapgqglewmg yfnpyndytryaqkfqgrv tmtrdtsistaymelsslrsd dtavyycarsdgyydamd ywgqgttvtvsssepkssd kthtcppcpapeaagapsv flfppkpkdtlmisrtpevtc vvvdvshedpevkfnwy vdgvevhnaktkpreeqy nstyrvvsvltvlhqdwlng kayacavsnkalpapiekti skakgqprepqvytlppsr deltknqvsltclvkgfyps diavewesngqpennyktt ppvldsdgsfflyskltvdk srwqqgnvfscsvmheal hnhytqkslslspgqrhnns slntgtqmaghspnsqvql vqsgggvvqpgrslrlscka sgytftrstmhwvrqapgk glewigyinpssaytnynq kfkdrftisadkskstaflqm dslrpedtgvyfcarpqvhy dyngfpywgqgtpvtvss ggggsggggsggggsaqd iqmtqspsslsasvgdrvt mtcsasssvsymnwyqq kpgkapkrwiydssklasg vparfsgsgsgtdytltisslq	SEQ ID NO:48 (SEQ ID NO:49)

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Name	Nucleotide Sequence	Amino Acid Sequence	SEQ ID NOs: (amino acid)
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humanized TSC250 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, with H81 linker)	atggaagcaccagcgcagcttctcttcctcctgctactctggctcccagatac caccggtgatatccagatgacccagtctccatccgccatgtctgcatctgtag gagacagagtcaccatcacttgccgggcgagtaagagcattagcaaatattt agcctggtttcagcagaaaccagggaaagttcctaagctccgcatccattctg gatctactttgcaatcaggggtcccatctcggttcagtggcagtggatctggg acagaatttactctcaccatcagcagcctgcagcctgaagattttgcaacttatt actgtcaacagcatattgaatacccgtggacgttcggccaagggaccaaggt ggaaatcaaacgaggtggcggagggtctgggggtggcggatccggaggt ggtggctctcaggtccagctggtacagtctggggctgaggtgaagaagcct ggggcttcagtgaaggtctcctgcaaggcttctggatacacattcactgacta ctacatgcactgggtgcgacaggcccctggacaagggcttgagtggatggg atattttaatccttataatgattatactagatacgcacagaagttccagggcaga gtcaccatgaccagggacacgtctatcagcacagcctacatggagctgagc agcctgagatctgacgacacggccgtgtattactgtgcaagatcggatggtta ctacgatgctatggactactggggtcaaggaaccacagtcaccgtctcctcg agtgagcccaaatcttctgacaaaactcacacatgcccaccgtgcccagcac ctgaagccgcgggtgcaccgtcagtcttcctcttccccccaaaacccaagga caccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtg agccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggag gtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgta ccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaag gcgtacgcgtgcgcggtctccaacaaagccctcccagcccccatcgagaaa accatctccaaagccaaagggcagccccgagaaccacaggtgtacaccct gcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcct ggtcaaaggcttctatccaagcgacatcgccgtggagtgggagagcaatgg gcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg gctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagca ggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactaca cgcagaagagcctctccctgtctccgggtgaagttcaaattccctgaccgaa agttacagcccgaattctcaggtccagctggtggagtctgggggcggagtg gtgcagcctgggcggtcactgaggctgtcctgcaaggcttctggctacacct ttactagatctacgatgcactgggtaaggcaggccctggacaaggtctgga	Diqmtqspsamsasvgdr vtitcrasksiskylawfqqk pgkvpklrihsgstlqsgvp srfsgsgsgteftltisslqpe dfatyycqqhieypwtfgq gtkveikrggggsggggsg gggsqvqlvqsgaevkkp gasvkvsckasgytftdyy mhwvrqapgqglewmg yfnpyndytryaqkfqgrv tmtrdtsistaymelsslrsd dtavyycarsdgyydamd ywgqgttvtvsssepkssd kthtcppcpapeaagapsv flfppkpkdtlmisrtpevtc vvvdvshedpevkfnwy vdgvevhnaktkpreeqy nstyrvvsvltvlhqdwlng kayacavsnkalpapiekti skakgqprepqvytlppsr deltknqvsltclvkgfyps diavewesngqpennyktt ppvldsdgsfflyskltvdk srwqqgnvfscsvmheal hnhytqkslslspgevqiplt esyspnsqvqlvesgggvv qpgrslrlsckasgytftrst mhwvrqapgqglewigyi npssaytnynqkfkdrftis adkskstaflqmdslrpedt gvyfcarpqvhydyngfp ywgqgtpvtvssggggsg	SEQ ID NO:79 (SEQ ID NO:80)

Name	Nucleotide Sequence	Amino Acid Sequence	SEQ ID NOs: (amino acid)
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humanized TSC251 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, with H83 linker)	atggaagcaccagcgcagcttctcttcctcctgctactctggctcccagatac caccggtgatatccagatgacccagtctccatccgccatgtctgcatctgtag gagacagagtcaccatcacttgccgggcgagtaagagcattagcaaatattt agcctggtttcagcagaaaccagggaaagttcctaagctccgcatccattctg gatctactttgcaatcaggggtcccatctcggttcagtggcagtggatctggg acagaatttactctcaccatcagcagcctgcagcctgaagattttgcaacttatt actgtcaacagcatattgaatacccgtggacgttcggccaagggaccaaggt ggaaatcaaacgaggtggcggagggtctgggggtggcggatccggaggt ggtggctctcaggtccagctggtacagtctggggctgaggtgaagaagcct ggggcttcagtgaaggtctcctgcaaggcttctggatacacattcactgacta ctacatgcactgggtgcgacaggccccctggacaagggcttgagtggatggg atattttaatccttataatgattatactagatacgcacagaagttccagggcaga gtcaccatgaccagggacacgtctatcagcacagcctacatggagctgagc agcctgagatctgacgacacggccgtgtattactgtgcaagatcggatggtta ctacgatgctatggactactggggtcaaggaaccacagtcaccgtctcctcg agtgagcccaaatcttctgacaaaactcacacatgcccaccgtgcccagcac ctgaagccgcgggtgcaccgtcagtcttcctcttccccccaaaacccaagga caccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtg agccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggag gtgcataatgccaagacaaagccgcgcgggaggagcagtacaacagcacgta ccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaag gcgtacgcgtgcgcggtctccaacaaagccctcccagccccatcgagaaa accatctccaaagccaaagggcagccccgagaaccacaggtgtacaccct gcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcct ggtcaaaggcttctatccaagcgacatcgccgtggagtgggagagcaatgg gcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg gctccttctttcctctacagcaagctcaccgtggacaagagcaggtggcagca ggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactaca cgcagaagagcctctccctgtctccgggttcttccctgaatacaggaactcag atggcaggtcattctccgaattctcaggtccagctggtggagtctgggggcg gagtggtgcagcctgggcggtcactgaggctgtcctgcaaggcttctggcta caccttactagatctacgatgcactgggtaaggcaggccctggacaaggt ctggaatggattggatacattaatcctagcagtgcttatactaattacaatcaga aattcaaggacaggttcacaatcagcgcagacaaatccaagagcacagcctt cctgcagatggacagcctgaggcccgaggacaccggcgtctatttctgtgca cggccccaagtccactatgattacaacgggtttccttactggggccaaggga ctcccgtcactgtctctagcggtggcggagggtctgggggtggcggatccg gaggtggtggctctgcacaagacatccagatgacccagtctccaagcagcc tgtctgcaagcgtgggggacagggtcaccatgacctgcagtgccagctcaa gtgtaagttacatgaactggtaccagcagaagccgggcaaggccccaaaa gatggatttatgactcatccaaactggcttctggagtccctgctcgcttcagtg gcagtgggtctgggaccgactataccctcacaatcagcagcctgcagcccg aagatttcgccacttattactgccagcagtggagtcgtaacccacccacgttc ggaggggggaccaagctacaaattacatcctccagctaa	Diqmtqspsamsasvgdr vtitcrasksiskylawfqqk pgkvpklrihsgstlqsgvp srfsgsgsgtefltlissiqpe dfatyycqqhieypwtfgq gtkveikrggggsggggsg gggsqvqlvqsgaevkkp gasvkvsckasgytftdyy mhwvrqapgqglewmg yfnpyndytryaqkfqgrv tmtrdtsistaymelsslrsd dtavyycarsdgyydamd ywgqgttvtvsssepkssd kthtcppcpapeaagapsv flfppkpkdtlmisrtpevtc vvvdvshedpevkfnwy vdgvevhnaktkpreeqy nstyrvvsvltvlhqdwlng kayacavsnkalpapiekti skakgqprepqvytlppsr deltknqvsltclvkgfyps diavewesngqpennyktt ppvldsdgsfflyskltvdk srwqqgnvfscsvmheal hnhytqkslslspgsslntgt qmaghspnsqvqlvesgg gvvqpgrslrlsckasgytft rstmhwvrqapgqglewi gyinpssaytnynqkfkdrf tisadkskstaflqmdslrpe dtgvyfcarpqvhydyngf pywgqgtpvtvssggggs ggggsggggsaqdiqmtq spssIsasvgdrvtmtcsas ssvsymnwyqqkpgkap krwiydsskIasgvparfsg sgsgtdytltisslqpedfaty ycqqwsrnpptfgggtklq itsss	SEQ ID NO:81 (SEQ ID NO:82)

Name	Nucleotide Sequence	Amino Acid Sequence	SEQ ID NOs: (amino acid)
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Humanized TSC295 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, with H9 linker)	atggaagcaccagcgcagcttctcttcctcctgctactctggctcccagatac caccggtgatatccagatgacccagtctccatccgccatgtctgcatctgtag gagacagagtcaccatcacttgccgggcgagtaagagcattagcaaatatttt agcctggtttcagcagaaaccagggaaagttcctaagctccgcatccattctg gatctactttgcaatcaggggtcccatctcggttcagtggcagtggatctggg acagaatttactctcaccatcagcagcctgcagcctgaagatttgcaacttatt actgtcaacagcatattgaatacccgtggacgttcggccaagggaccaaggt ggaaatcaaacgaggtggcggagggtctgggggtggcggatccggaggt ggtggctctcaggtccagctggtacagtctggggctgaggtgaagaagcct ggggcttcagtgaaggtctcctgcaaggcttctggatacacattcactgacta ctacatgcactgggtgcgacaggccccctggacaagggcttgagtggatggg atattttaatccttataatgattatactagatacgcacagaagttccagggcaga gtcaccatgaccagggacacgtctatcagcacagcctacatggagctgagc	diqmtqspsamsasvgdr vtitcrasksiskylawfqqk pgkvpklrihsgstlqsgvp srfsgsgsgteftltisslqpe dfatyycqqhieypwtfgq gtkveikrggggsggggsg gggsqvqlvqsgaevkkp gasvkvsckasgytftdyy mhwvrqapgqglewmg yfnpyndytryaqkfqgrv tmtrdtsistaymelsslrsd dtavyycarsdgyydamd ywgqgttvtvsssepkssd	SEQ ID NO:157 (SEQ ID NO:158)

Name	Nucleotide Sequence	Amino Acid Sequence	SEQ ID NOs: (amino acid)
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Humanized TSC296 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, with H94 linker)	atggaagcaccagcgcagcttctcttcctcctgctactctggctcccagatac caccggtgatatccagatgacccagtctccatccgccatgtctgcatctgtag gagacagagtcaccatcacttgccgggcgagtaagagcattagcaaatatttt agcctggtttcagcagaaaccagggaaagttcctaagctccgcatccattctg gatctactttgcaatcagggtcccatctcggttcagtggcagtggatctggg acagaatttactctcaccatcagcagcctgcagcctgaagattttgcaacttatt actgtcaacagcatattgaatacccgtggacgttcggccaagggaccaaggt ggaaatcaaacgaggtggcggagggtctgggggtggcggatccggaggt ggtggctctcaggtccagctggtacagtctggggctgaggtgaagaagcct ggggcttcagtgaaggtctcctgcaaggcttctggatacacattcactgacta ctacatgcactgggtgcgacaggcccctggacaagggcttgagtggatggg atattttaatccttataatgattatactagatacgcacagaagttccagggcaga gtcaccatgaccgaggacacgtctatcagcacagcctacatggagctgagc agcctgagatctgacgacacggccgtgtattactgtgcaagatcggatggtta ctacgatgctatggactactggggtcaaggaaccacagtcaccgtctcctcg agtgagcccaaatcttctgacaaaactcacacatgcccaccgtgcccagcac ctgaagccgcgggtgcaccgtcagtcttcctcttcccccaaaacccaagga caccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtg agccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggag gtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgta ccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaag gcgtacgcgtgcgcggtctccaacaaagccctcccagcccccatcgagaaa accatctccaaagccaaagggcagccccgagaaccacaggtgtacaccct gccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcct ggtcaaaggcttctatccaagcgacatcgccgtggagtgggagagcaatgg gcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg	diqmtqspsamsasvgdr vtitcrasksiskylawfqqk pgkvpklrihsgstlqsgvp srfsgsgsgtefiltisslqpe dfatyycqqhieypwtfgq gtkveikrggggsggggsg gggsqvqlvqsgaevkkp gasvkvsckasgytftdyy mhwvrqapgqglewmg yfnpyndytryaqkfqgrv tmtrdtsistaymelsslrsd dtavyycarsdgyydamd ywgqgttvtvsssepkssd kthtcppcpapeaagapsv flfppkpkdtlmisrtpevtc vvvdvshedpevkfnwy vdgvevhnaktkpreeqy nstyrvvsvltvlhqdwlng kayacavsnkalpapiekti skakgqprepqvytlppsr deltknqvsltclvkgfyps diavewesngqpennyktt ppvldsdgsfflyskltvdk srwqqgnvfscsvmheal hnhytqkslslspgsggggs ggggsggggspnsqvqlv	SEQ ID NO:159 (SEQ ID NO:160)

Name	Nucleotide Sequence	Amino Acid Sequence	SEQ ID NOs: (amino acid)
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Humanized TSC301 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, with H105 linker)	atggaagccaccagcgcagcttctcttcctcctgctactctggctcccagatac caccggtgatatccagatgacccagtctccatccgccatgtctgcatctgtag gagacagagtcaccatcacttgccgggcgagtaagagcattagcaaatatttt agcctggtttcagcagaaaccagggaaagttcctaagctccgcatccattctg gatctactttgcaatcaggggtcccatctcggttcagtggcagtggatctggg acagaatttactctcaccatcagcagcctgcagcctgaagattttgcaacttatt actgtcaacagcatattgaatacccgtggacgttcggccaagggaccaaggt ggaaatcaaacgaggtggcggagggtctggggtggcggatccggaggt ggtggctctcaggtccagctggtacagtctggggctgaggtgaagaagcct ggggcttcagtgaaggtctcctgcaaggcttctggatacacattcactgacta ctacatgcactgggtgcgacaggcccctggacaagggcttgagtggatggg atattttaatccttataatgattatactagatacgcacagaagttccagggcaga gtcaccatgaccagggacacgtctatcagcacagcctacatggagctgagc agcctgagatctgacgcacggccgtgtattactgtgcaagatcggatggtta ctacgatgctatggactactggggtcaaggaaccacagtcaccgtctcctcg agtgagcccaaatcttctgacaaaactcacacatgcccaccgtgcccagcac ctgaagccgcgggtgcaccgtcagtcttcctcttccccccaaaacccaagga caccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtg agccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggag gtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgta ccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaag gcgtacgcgtgcgcggtctccaacaaagccctcccagccccatcgagaaa accatctccaaagccaaagggcagccccgagaaccacaggtgtacaccct gccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcct ggtcaaaggcttctatccaagcgacatcgccgtggagtgggagagcaatgg gcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg gctccttctttcctctacagcaagctcaccgtggacaagagcaggtggcagca ggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactaca cgcagaagagcctctccctgtctccgggttctggtggaggcggttcaggcg gaggtggctccggcggtggcggatcgcaggtccagctggtggagtctggg ggcggagtggtgcagcctgggcggtcactgaggctgtcctgcaaggcttct ggctacaccttactagatctacgatgcactgggtaaggcaggccccctggac aaggtctggaatggattggatacattaatcctagcagtgcttatactaattacaa tcagaaattcaaggacaggttcacaatcagcgcagacaaatccaagagcac agccttcctgcagatggacagcctgaggccccgaggacaccggcgtctatttc tgtgcacggccccaagtccactatgattacaacgggtttccttactggggcca agggactccgtcactgtctctagcggtggcggagggtctgggggtggcgg atccggaggtggtggctctgcacaagacatccagatgacccagtctccaa	Diqmtqspsamsasvgdr vtitcrasksiskylawfqqk pgkvpklrihsgstlqsgvp srfsgsgsgteftltisslqpe dfatyycqqhieypwtfgq gtkveikrggggsggggsg gggsqvqlvqsgaevkkp gasvkvsckasgytftdyy mhwvrqapgqglewmg yfnpyndytryaqkfqgrv tmtrdtsistaymelsslrsd dtavyycarsdgyydamd ywgqgttvtvsssepkssd kthtcppcpapeaagapsv flfppkpkdtlmisrtpevtc vvvdvshedpevkfnwy vdgvevhnaktkpreeqy nstyrvvsvltvlhqdwlng kayacavsnkalpapiekti skakgqprepqvytlppsr deltknqvsltclvkgfyps diavewesngqpennyktt ppvldsdgsfflyskltvdk srwqqgnvfscsvmheal hnhytqkslslspgsggggs ggggsggggsqvqlvesg ggvvqpgrslrlsckasgyt ftrstmhwvrqapgqgle wigyinpssaytnynqkfk drftisadkskstaflqmdsl rpedtgvyfcarpqvhydy ngfpywgqgtpvtvssgg ggsggggsggggsaqdiq mtqspsslsasvgdrvtmt csasssvsymnwyqqkp gkapkrwiydssklasgvp arfsgsgsgtdytltisslqpe dfatyycqqwsrnpptfgg	SEQ ID NO:161 (SEQ ID NO:162)

Name	Nucleotide Sequence	Amino Acid Sequence	SEQ ID NOs: (amino acid)
	gcagcctgtctgcaagcgtgggggacagggtcaccatgacctgcagtgcca gctcaagtgtaagttacatgaactggtaccagcagaagccgggcaaggccc ccaaaagatggatttatgactcatccaaactggcttctggagtccctgctcgct tcagtggcagtgggtctgggaccgactatacccctcacaatcagcagcctgca gcccgaagatttcgccacttattactgccagcagtggagtcgtaacccaccca cgttcggaggggggaccaagctacaaattacatcctccagctaa	gtklqitsss	
Humanized TSC302 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, with H106 linker)	atggaagcaccagcgcagcttctcttcctcctgctactctggctcccagatac cacggtgatatccagatgacccagtctccatccgccatgtctgcatctgtag gagacagagtcaccatcacttgccgggcgagtaagagcattagcaaatattt agcctggtttcagcagaaaccagggaaagttcctaagctccgcatccattctg gatctactttgcaatcaggggtcccatctcggttcagtggcagtggatctggg acagaatttactctcaccatcagcagcctgcagcctgaagattttgcaacttatt actgtcaacagcatattgaatacccgtggacgttcggccaagggaccaaggt ggaaatcaaacgaggtggcggagggtctgggggtggcggatccggaggt ggtggctctcaggtccagctggtacagtctggggctgaggtgaagaagcct ggggcttcagtgaaggtctcctgcaaggcttctggatacacattcactgacta ctacatgcactgggtgcgacaggcccctggacaagggcttgagtggatggg atattttaatccttataatgattatactagatacgcacagaagttccagggcaga gtcaccatgaccagggacacgtctatcagcacagcctacatggagctgagc agcctgagatctgacgcacggccgtgtattactgtgcaagatcggatggtta ctacgatgctatggactactggggtcaaggaaccacagtcaccgtctcctcg agtgagcccaaatcttctgacaaaactcacacatgcccaccgtgcccagcac ctgaagccgcgggtgcaccgtcagtcttcctcttccccccaaaacccaagga caccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtg agccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggag gtgcataatgccaagacaaagccgcgcgggaggagcagtacaacagcacgta ccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaag gcgtacgcgtgcgcggtctccaacaaagccctcccagccccatcgagaaa accatctccaaagccaaagggcagccccgagaaccacaggtgtacaccct gcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcct ggtcaaaggcttctatccaagcgacatcgccgtggagtgggagagcaatgg gcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg gctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagca ggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactaca cgcagaagagcctctccctgtctccgggtcagaggcacaacaattcttcact gaatacaggaactcagatggcaggtcattctcaggtccagctggtggagtct gggggcggagtggtgcagcctgggcggtcactgaggctgtcctgcaaggc ttctggctacaccittactagatctacgatgcactgggtaaggcaggccctg gacaaggtctggaatggattggatacattaatcctagcagtgcttatactaatta caatcagaaattcaaggacaggtcacaatcagcgcagacaaatccaagag cacagccttcctgcagatggacagcctgaggcccgaggacaccggcgtcta tttctgtgcacggccccaagtccactatgattacaacgggtttccttactgggg ccaagggactcccgtcactgtctctagcggtggcggagggtctgggggtgg cggatccggaggtggtggctctgcacaagacatccagatgacccagtctcc aagcagcctgtctgcaagcgtgggggacagggtcaccatgacctgcagtgc cagctcaagtgtaagttacatgaactggtaccagcagaagccgggcaaggc ccccaaaagatggatttatgactcatccaaactggcttctggagtccctgctcg cttcagtggcagtgggtctgggaccgactataccctcacaatcagcagcctg cagcccgaagatttcgccacttattactgccagcagtggagtcgtaacccacc cacgttcggaggggggaccaagctacaaattacatcctccagctaa	diqmtqspsamsasvgdr vtitcrasksiskylawfqqk pgkvpklrihsgstlqsgvp srfsgsgsgteftltisslqpe dfatyycqqhieypwtfgq gtkveikrggggsggggsg gggsqvqlvqsgaevkkp gasvkvsckasgytftdyy mhwvrqapgqglewmg yfnpyndytryaqkfqgrv tmtrdtsistaymelssirsd dtavyycarsdgyydamd ywgqgttvtvsssepkssd kthtcppcpapeaagapsv flfppkpkdtlmisrtpevtc vvvdvshedpevkfnwy vdgvevhnaktkpreeqy nstyrvvsvltvlhqdwlng kayacavsnkalpapiekti skakgqprepqvytlppsr deltknqvsltclvkgfyps diavewesngqpennyktt ppvldsdgsfflyskltvdk srwqqgnvfscsvmheal hnhytqkslslspgqrhnns slntgtqmaghsqvqlves gggvvqpgrslrlsckasgy tftrstmhwvrqapgqgle wigyinpssaytnynqkfk drftisadkskstaflqmdsl rpedtgvyfcarpqvhydy ngfpywgqgtpvtvssgg ggsggggsggggsaqdiq mtqspsslsasvgdrvtmt csasssvsymnwyqqkp gkapkrwiydssklasgvp arfsgsgsgtdytltisslqpe dfatyycqqwsrnpptfgg gtklqitsss	SEQ ID NO:163 (SEQ ID NO:164)

EXAMPLE 2: Heterodimeric Molecules

[0225] PSMA-specific Interceptor molecules were made using Interceptor scaffolding as generally disclosed in International Appl. Nos. PCT/US2010/62436 and PCT/US2010/62404. Briefly, PSMA-specific polypeptide heterodimers were made by co-expressing two different polypeptides chains, one polypeptide chain comprising an immunoglobulin CH1 heterodimerization domain and the other polypeptide chain comprising an immunoglobulin CL heterodimerization domain. The day before transfection HEK293 cells were suspended at a cell concentration of 0.5 x 10^6 cells/ml in GIBCO® FreeStyle™ 293 expression medium (Invitrogen). 250mls of cells were used for a large transfection, and 60mls of cells were used for a small transfection. On transfection day, 320ul of 293fectin™ transfectin reagent (Invitrogen) was mixed with 8 mls of media. At the same time, 250 ug of DNA of each of the single chain polypeptide was mixed with the 8 mls of media and incubated for 5 minutes. After 15 minutes of incubation, the DNA-293fectin mixture was added to the 250 mls of 293 cells and returned to the shaker at 37°C and shaken at a speed of 120 RPM. For the smaller transfection using 60 mls of cells, a fourth of the DNA, 293fectin, and media were used.

[0226] Protein A affinity chromatography was used to purify the proteins. 2ml of packed protein A agarose (Repligen) was added to a Econo-Column® chromatography column, size 2.5 x 10cm (Bio-Rad Laboratories), washed extensively with PBS (10X column volume), and the supernatants were loaded, washed with PBS again, and eluted with 3 column volumes of Pierce IgG elution buffer. Proteins were then dialyzed extensively against PBS. Proteins were then concentrated using Amicon® Centricon® centrifugal filter devices (Millipore Corp.) to a final volume around 0.5ml.

[0227] Purified proteins were analyzed on a 10% SDS-PAGE gel using XCell SureLock™ Mini-Cell electrophoresis system (Invitrogen).

[0228] Bivalent polypeptide heterodimer TSC122 was made by co-expressing single chain polypeptides TSC084 and TSC093. Single chain polypeptide TSC084 comprises from its amino- to carboxyl-terminus: murine 107-1A4 (anti-PSMA) VL-VH scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human CH1. The nucleotide and amino acid sequences for TSC084 are set forth in SEQ ID NOs:44 and 46, respectively. Single chain polypeptide TSC093 comprises from its amino- to carboxyl-terminus: Cris7 (anti-CD3) scFv, human IgG1 SCC-P hinge, human IgG1CH2, human IgG1 CH3, and human Cκ(YAE)(*i.e.*, human Cκ without the first Arg or last Cys, but with N30Y, V55A, and T70E substitutions). The nucleotide and amino acid sequences for TSC093 are set forth in SEQ ID NOs:45 and 47, respectively.

[0229] Bivalent polypeptide heterodimer TSC200 was made by co-expressing polypeptide chains TSC192 and TSC125. TSC192 comprises from its amino- to carboxyl-terminus: humanized 107-1A4 (anti-PSMA) VL-VH#2 scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human Cκ(YAE). The nucleotide and amino acid sequences for TSC192 are set forth in SEQ ID NOs:53 and 58, respectively. TSC125 comprises from its amino- to carboxyl-terminus: Cris7 (anti-CD3) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human CH1. The nucleotide and amino acid sequences for TSC125 are set forth in SEQ ID NOs:52 and 57, respectively.

[0230] Bivalent polypeptide heterodimer TSC202 was made by co-expressing polypeptide chains TSC193 and TSC125. TSC193 comprises from its amino- to carboxyl-terminus: humanized 107-1A4 (anti-PSMA) VL-VH#1 scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human Cκ(YAE). The nucleotide and amino acid sequences for TSC193 are set forth in SEQ ID NOs: 54 and 59, respectively. TSC125 comprises from its amino- to carboxyl-terminus: Cris7 (anti-CD3) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human CH1. The nucleotide and amino acid sequences for TSC125 are set forth in SEQ ID NOs:52 and 57, respectively.

[0231] Bivalent polypeptide heterodimer TSC204 was made by co-expressing polypeptide chains TSC195 and TSC093. TSC195 comprises from its amino- to carboxyl-terminus: humanized 107-1A4 (anti-PSMA) VL-VH#2 scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human CH1. The nucleotide and amino acid sequences for TSC195 are set forth in SEQ ID NOs:55 and 60, respectively. TSC093 comprises from its amino- to carboxyl-terminus: Cris7 (anti-CD3) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human Cκ(YAE). The nucleotide and amino acid sequences for TSC093 are set forth in SEQ ID NOs: 45 and 47, respectively.

[0232] Bivalent polypeptide heterodimer TSC205 was made by co-expressing polypeptide chains TSC196 and TSC093. TSC196 comprises from its amino- to carboxyl-terminus: humanized 107-1A4 (anti-PSMA) VL-VH#1 scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human CH1. The nucleotide and amino acid sequences for TSC196 are set forth in SEQ ID NOs:56 and 61, respectively. TSC093 comprises from its amino- to carboxyl-terminus: Cris7 (anti-CD3) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human Cκ(YAE). The nucleotide and amino acid sequences for TSC093 are set forth in SEQ ID NOs: 45 and 47, respectively.

EXAMPLE 3: SCORPION Molecule Construction

[0233] PSMA-specific SCORPION molecules (TSC194 (SEQ ID NO:48 (nucleic acid), SEQ ID NO:49 (amino acid); TSC199 (SEQ ID NO:50 (nucleic acid), SEQ ID NO:51 (amino acid)); TSC 212 (SEQ ID NO:73 (nucleic acid), SEQ ID NO:74 (amino acid)); TSC213 (SEQ ID NO:75 (nucleic acid), SEQ ID NO:76 (amino acid)); TSC249 (SEQ ID NO:77 (nucleic acid), SEQ ID NO:78 (amino acid)); TSC250 (SEQ ID NO:79 (nucleic acid), SEQ ID NO:80 (amino acid)); TSC251 (SEQ ID NO:81 (nucleic acid), SEQ ID NO:82 (amino acid)); and TSC252 (SEQ ID NO:83 (nucleic acid), SEQ ID NO:84 (amino acid))) were made using standard molecular biology techniques, starting with existing SCORPION scaffolding as templates and using the methods generally disclosed in, *e.g.*, PCT Application Publication No. WO 2007/146968, U.S. Patent Application Publication No. 2006/0051844, PCT Application Publication No. WO 2010/040105, PCT Application Publication No. WO 2010/003108, and U.S. Patent No. 7,166,707 (*see also* Table 3). Insertion of the N-terminal scFv binding domain was accomplished through digestion of the parental template and scFv insert with either the restriction enzymes HindIII and XhoI or AgeI and XhoI, desired fragments were identified and isolated by agarose gel purification, and ligation. Insertion of the C-terminal scFv binding domain was accomplished through digestion of the parental template and scFv insert with the restriction enzymes EcoRI and NotI, desired fragments were identified and isolated by agarose gel purification, and ligation.

EXAMPLE 4: Binding of chimeric and humanized molecules to PSMA(+) and PSMA(-) cell lines

[0234] Monoclonal antibodies were purified from hybridoma cell culture media by standard procedures. SMIP, Interceptor, and SCORPION molecules disclosed herein were produced by transient transfection of human HEK293 cells, and purified from cell culture supernatants by Protein A affinity chromatography. If aggregates were detected after affinity chromatography, secondary size exclusion chromatography was also performed to ensure homogeneity of the protein.

[0235] Binding studies on PSMA+ (C4-2, Wu et al., 1994 Int. J. Cancer 57:406-12) and PSMA- (DU-145, Stone et al., 1978, Intl. J. Cancer 21:274-81) prostate cancer cell lines were performed by standard FACS-based staining procedures. A typical experiment would label 300,000 cells per well with a range of 200 nM to 0.1 nM binding molecule in 100 ul of FACS buffer (PBS + 2% normal goat serum + 2% fetal bovine serum + 0.1% sodium azide) on ice, followed by washes and incubation with fluorescently-labeled secondary antibody, goat anti-human IgG (1:400 dilution of Invitrogen #11013 = 5 ug/ml). After washing

secondary antibody off cells, cells were incubated with 7-Aminoactinomycin D (7-AAD) staining solution (BD Pharmingen™ cat# 559925)(6 ul of 7AAD to 100 ul of FACS Buffer) for 20 minutes. Signal from bound molecules was detected using a FACSCalibur™ flow cytometer (BD Biosciences) and analyzed by FlowJo flow cytometry analysis software. 7-AAD+ cells were excluded from analysis. Nonlinear regression analysis to determine EC50s was performed in GraphPad Prism® graphing and statistics software.

[0236] Binding studies (**Figure 1**) were used to compare the parent 107-1A4 murine antibody (*i.e.*, TSC045) with the chimeric SMIP molecules (TSC085, TSC092) and the bispecific, chimeric Interceptor molecule TSC122. Both chimeric SMIP molecules showed comparable affinity to PSMA+ cells as the parent murine antibody, although one (TSC085, with a VL-VH scFv orientation) showed a lower level of saturation on the surface of the cell. The bispecific, chimeric Interceptor molecule (TSC122), which had only a single 107-1A4 binding domain, showed a lower binding affinity to the PSMA+ cells. All showed little to no binding to the PSMA- cell line DU-145. Binding studies of humanized SMIP molecules TSC188 and TSC189 (**Figure 2A**) showed comparable affinities to those previously determined (data not shown) for the parent monoclonal antibody, with similarly high levels of saturation and selectivity for PSMA+ C4-2 cells over PSMA- DU-145 cells. Binding studies of humanized SCORPION molecules TSC194 and TSC199 showed comparable affinities to the parental humanized SMIP molecules TSC188 and TSC189 (**Figure 2B**), with no binding to the PSMA- DU-145 cell line.

EXAMPLE 5: Differential cellular internalization seen between 107-1A4 antibody, SMIP and Interceptor scaffolds

[0237] The binding proteins for internalization studies were directly labeled with CypHer™5E Mono NHS Ester (GE Healthcare, #PA15401) according to manufacturer's instructions. CypHer5E is a pH-sensitive red excited dye that fluoresces at low pH, which is typically encountered inside of endosomes and lysosomes; CypHer5E fluorescence can be used as a proxy for cellular internalization as a result. Dye dissolved in fresh DMSO was added to purified protein in PBS/sodium carbonate buffer, pH 8.3 (9:1), at a dye:protein molar ratio of 20:1. After at least 1 hour incubation in the dark at room temperature, labeled protein was separated from unreacted dye by dialysis at 4°C. Absorbance at 280 nm and 500 nm was used to calculate protein and dye concentration for the labeled material. The resulting dye:protein ratio ranged from 6 to 14, and this value was used to normalize the imaging data. To ensure that the presence of protein aggregates did not bias the internalization data, when individual molecules had detectable levels of aggregates (>5%),

secondary size exclusion chromatography was used to purify molecules to very high levels of homogeneity (>95%).

[0238] Cells were plated 2 days before experiment at 4000 cells per well in poly-D-lysine-coated 96-well plates, black with clear bottoms (BD Biocoat, 356640) in usual culture media. Media changes during experiment were conducted carefully to maintain cell adhesion to surface. Nuclei were stained with Hoechst 33342 (Invitrogen, H3570) in serum-free phenol red-free RPMI media (Invitrogen, 11835) plus 20mM HEPES (Invitrogen, 15630) (called PRF-RPMI) for an hour. Wells were washed with PRF-RPMI plus 10% FBS, and 100 ul warm PRF-RPMI + 10% FBS was added. Plates were moved to ice for 5 minutes then labeled binding proteins at various dilutions were added from 5x stock solutions for one hour binding on ice. Plates were moved to a 37°C CO_2 incubator for 60 minutes to allow internalization to proceed. Before imaging, media was replaced with PRF-RPMI + 1 % FBS.

[0239] Wells were scanned on IN Cell Analyzer 1000 automated cellular and subcellular imaging system (GE Healthcare) to quantitate internalized protein, data was collected from 8 fields in each well. The acquisition protocol was set to collect data with suitable filter sets for Hoechst and CypHer5E, and bright field images. Data was analyzed by IN Cell Investigator software, using a protocol developed to detect fluorescent granules within a zone of cytoplasm encircling each nucleus and measuring their area. Total granule area detected was normalized to compensate for the relative level of dye substitution per labeled protein.

[0240] Internalization experiments using the parental 107-1A4 murine antibody, or the chimeric SMIP and Interceptor molecules, showed no internalization in the PSMA- DU-145 cell line (data not shown), but some internalization could be detected on the PSMA+ LNCaP (CRL-1740™, American Type Culture Collection) or C4-2 cell lines (**Figure 3**). Internalization of the parental antibody was greater than the SMIP or Interceptor molecules at all concentrations tested. Apparent internalization from the (monovalent) Interceptor molecule was higher than from the (bivalent) SMIP molecule, which could be due to the higher potential binding stoichiometry – each Interceptor molecule can only engage one molecule of PSMA, whereas each SMIP molecule can engage two molecules of PSMA, potentially leading to twice as much Interceptor molecule accumulating on the cell surface. If both the Interceptor and SMIP molecules have similar levels of internalization, a higher apparent signal would always be seen from the Interceptor molecule.

EXAMPLE 6: Redirected T-cell cytotoxicity against PSMA(+) cell lines

[0241] Peripheral blood mononuclear cells (PBMC) were isolated from human blood from two different donors (labeled as AG or VV) using standard ficoll gradients. The isolated cells

were washed in saline buffer. T cells were additionally isolated using a Pan T-cell Isolation Kit II from Miltenyi Biotec (Bergisch Gladbach, Germany) using the manufacturer's protocol. T cells were used with or without stimulation, as noted in the figures (see Figures 4-6), and added at a 10:1 ratio (T cell : target cell) unless indicated otherwise.

[0242] C4-2 castration-resistant prostate cancer (CRPC) cells were labeled with CellTracker™ Green cytoplasmic dye (Invitrogen, C7025) following manufacturer's protocol in order to distinguish them from T cells. Labeled C4-2 cells were seeded into poly-D-lysine-coated 96-well plates, as used in Example 3, at 8000 cells per well in standard growth media, one day before addition of T cells and Interceptor molecule. Ten ul of concentrated bispecific Interceptor molecule (TSC122, TSC200, TSC202, or TSC204) was added to 100 ul of media per well, plus 50 ul of T cells (80,000 cells) in standard growth media. Cell cultures were kept in CO_2 incubator at 37°C overnight. After 24 hr exposure to Interceptor molecule, cells were stained with 7-AAD and Hoechst dyes to enable quantitation of dead cells. Media was changed to 100 ul RPMI + 1% FBS + 10 ug/ml 7-AAD + Hoechst at 1:1000 dilution of stock, and incubated for an additional 30 minutes.

[0243] Imaging and quantitation was performed by use of an InCell Analyzer microscope (GE), collecting data from 10 fields per well. The acquisition protocol was set to collect data with suitable filter sets for: a) nuclei detection via Hoechst stain, b) cell type discrimination via CellTracker™ Green detection, c) live/dead cell status determination via 7-AAD staining, and bright field images. Quantitation was performed by InCell Workstation software, using a decision tree application. Individual cells were detected by presence of nuclear stain by Hoechst. Threshold values of signal in the green channel (CellTracker™ Green) were used to split cells into C4-2 (positive) and T cell (negative) populations. Threshold values of signal in red channel (7-AAD) were used to split cells into dead (positive) and live (negative) populations.

[0244] Bispecific Interceptor molecules featuring either the 107-1A4 murine scFv or humanized 107-1A4 scFv as well as an anti-CD3 scFv (Cris7) were tested for the ability to cross-link T-cells to target PSMA+ tumor cells and enable target-dependent cytotoxic T cell responses (so-called 'redirected T cell cytotoxicity', or RTCC). Potent target-dependent cytotoxic activity over 24 hours was observed with the chimeric TSC122 Interceptor molecule (Figure 4) with T-cells from two different donors; roughly 60% of target cells were lysed by treatment with as little as 100 pM of TSC122 Interceptor molecule. No direct cytotoxicity on PSMA+ cells was observed in the absence of effector T-cells (Figure 4); no cytotoxicity was similarly observed on PSMA- cells in the presence of effector T-cells (data not shown). The cytotoxic activity of humanized Interceptor molecules (TSC200, TSC202, and TSC204) was also tested alongside the parent chimeric Interceptor molecule (TSC122)

(Figure 5). Two of the humanized Interceptor molecules (TSC200, TSC204) showed lower RTCC activity than the parent; one humanized Interceptor molecule (TSC202) showed equal RTCC activity to the parent chimeric Interceptor molecule (TSC122) with similar, low pM potency. These results demonstrated that certain humanized Interceptor molecules had similar T cell cytotoxicity as the parental chimeric Interceptor molecule.

[0245] The cytotoxic activity of humanized SCORPION molecules (TSC194, TSC199, TSC212, TSC213), compared to that of the chimeric Interceptor molecule TSC122, was also examined (Figure 6). The humanized SCORPION molecules with anti-PSMA scFvs in the VL-VH orientation (TSC194, TSC199) had comparable cytotoxic activity to the bispecific chimeric Interceptor molecule (also with an scFv in the VL-VH orientation, and also including an anti-CD3 scFv (Cris7)). The humanized SCORPION molecules with anti-PSMA scFvs in the VH-VL orientation, on the other hand, had lower overall cytotoxicity.

EXAMPLE 7: Target-dependent T-cell activation and proliferation induced against PSMA+ cell lines directed by bispecific 107-1A4-derived molecules

[0246] To compare the effectiveness of different bispecific polypeptide molecules at inducing target-dependent T-cell activation and proliferation, four different anti-PSMA and anti-CD3 bispecific molecules including TSC122 (a chimeric Interceptor molecule), TSC202 (humanized Interceptor molecule), TSC194 (a humanized SCORPION molecule), and TSC199 (a humanized SCORPION molecule) were compared.

[0247] C4-2 prostate cancer cells (PSMA+) were obtained from MD Anderson Cancer Center (Houston, TX) and cultured according to the provided protocol. Peripheral blood mononuclear cells (PBMC) were isolated from human blood using standard ficoll gradients. The isolated cells were washed in saline buffer. T cells were further isolated using a Pan T-cell Isolation Kit II from Miltenyi Biotec (Bergisch Gladbach, Germany) using the manufacturer's protocol.

[0248] Proliferation was assessed by labeling isolated T cell populations with carboxyfluorescein diacetate succinimidyl ester (CFSE). CFSE-labeled T cells were plated in U-bottom 96-well plates at 100,000 cells/well, respectively, with 30,000 C4-2 tumor cells/well, to achieve T cell to tumor cell ratios of roughly 3:1. Concentrations of test molecules ranging from 10 nM to 0.1 pM were added to the cell mixtures in a total of 200 ul/well in RPMI 1640 media supplemented with 10% human or bovine serum, sodium pyruvate and non-essential amino acids. Plates were incubated at 37°C, 5% CO_2 in humidified incubators. After 3 days, cells were labeled with antibodies for flow cytometric analysis. Cells were labeled and washed in their original plates to minimize cell losses

during transfers, and all labeling was done in saline buffer with 0.2% bovine serum albumin. First, cells were pre-incubated with 100 ug/ml human IgG at room temperature for 15 min. Subsequently, cells were incubated with a mixture (total volume 50 ul) of the following dye-labeled antibodies: CD5-PE, CD4-APC, CD8-Pacific Blue, CD25-PE-Cy7, as well as 7-Amino Actinomycin D (7AAD hereafter) for 40 min. Cells were washed twice, resuspended in 80 to 120 ul volumes, and measured immediately in a BD LSRII flow cytometer to acquire 80% of the contents of each well. The sample files were analyzed using FlowJo software to calculate the percentages and numbers of cells that had undergone at least one cell division, according to their CFSE profile, by gating sequentially on activated, live CD4+ or CD8+ T cells (7AAD-, CD5+ CD25+ CD4+ or 7AAD- CD5+ CD25+ CD8+, respectively). Mean values and standard deviations were calculated using Microsoft Excel software. Graphs were plotted using Microsoft Excel or GraphPad Prism.

[0249] Analysis of live CD4+ and CD8+ populations from wells with C4-2 cells treated with T-cells (**Figure 7A** and **Figure 7B**) revealed a significant increase in both the total number of cells and percent proliferating cells in the presence of C4-2 cells displaying the target PSMA antigen. Proliferation was slightly higher for CD4+ T-cells than CD8+ T-cells, and the proliferation induced by the Interceptor molecules TSC122 and TSC202 saturated at a higher level than the responses induced by the SCORPION molecules TSC194 and TSC199. All molecules showed induction of T-cell proliferation at low concentrations (100 pM). No significant differences in relative induction of CD4+ versus CD8+ cell proliferation were apparent between molecules.

EXAMPLE 8: Competitive binding studies of anti-PSMA molecules confirms 107-1A4 binds a unique epitope on PSMA

[0250] To show that anti-PSMA murine monoclonal antibody 107-1A4, chimeric 107-1A4 SMIP molecule (TSC085) and humanized 107-1A4 SMIP molecule (TSC189) binds a unique epitope on PSMA, which is not recognized by common literature antibodies (J415, J591), and that the conversion of murine monoclonal antibody 107-1A4 to SMIP format did not result in a shift in that binding epitope, competition binding experiments were carried out. Hybridomas producing the J591, Hu591 (a humanized version of J591) and J415 antibodies were obtained from ATCC. Monoclonal antibodies were purified from hybridoma cell culture media by standard procedures. SMIP molecules were produced by transient transfection of human 293 cells, and purified from cell culture supernatants by Protein A affinity chromatography. If aggregates were detected after affinity chromatography, secondary size exclusion chromatography was also performed to ensure homogeneity of the protein.

[0251] Competitive binding studies on the PSMA+ C4-2 prostate cancer cell line were performed by standard FACS-based staining procedures. To simplify binding measurements, molecules with human Fc domains were used to compete against molecules with murine Fc domains, and either an anti-human or anti-mouse antibody was used to detect binding to the target cell line.

[0252] In a typical experiment, molecule X (binder) would be mixed with molecule Y (competitor), placed on ice, and then used to label 300,000 cells per well with 4 nM of molecule X and a range of 250 nM to 0.4 nM molecule Y in 100 ul of FACS buffer (PBS + 2% normal goat serum + 2% fetal bovine serum + 0.1% sodium azide) on ice, followed by washes and incubation with fluorescently-labeled secondary antibody specific for molecule X, either goat anti-human IgG (1:400 dilution of Invitrogen 11013 = 5 ug/ml) or goat anti-mouse IgG (1:400 dilution of Invitrogen 11017). After washing secondary antibody off cells, cells were incubated with 7AAD (6 ul of BD Pharmingen 7AAD, cat# 559925) to 100 ul of FACS Buffer) for 20 minutes. Signal from bound molecules was detected using a FACSCalibur flow cytometer and analyzed by FlowJo. 7AAD+ cells were excluded from analysis. Nonlinear regression analysis to determine EC50s was performed in GraphPad Prism.

[0253] Competitive binding studies (**Figure 8A**) were used to see if the humanized J591 antibody, Hu591, could compete with the binding of 107-1A4, J591 or J415 murine antibodies to cells. No competition was observed for the binding of 107-1A4, suggesting it binds a non-competitive epitope; competition was observed for the binding of both J591 and J415 antibodies, however. Next, additional binding studies were carried out to see if the three murine antibodies could compete with the binding of the chimeric 107-1A4 SMIP molecule TSC085 to cells (**Figure 8B**). Strong competition was seen from binding of the parental 107-1A4 antibody, confirming that the SMIP molecule bound to the same epitope. No effective competition was seen from the J591 or J415 murine antibodies. Last, binding studies were carried out to see if the three murine antibodies could compete with the binding of the humanized 107-1A4 SMIP molecule TSC189 to cells (**Figure 8C**). Again, similarly strong competition was seen from binding of the parental 107-1A4 antibody, but no effective competition was seen from the J591 or J415 murine antibodies. This confirms that 107-1A4 binds a unique epitope on PSMA. It also shows that any shift in behavior of 107-1A4-based SMIP and Interceptor molecules from that of the parental 107-1A4 antibody is not due to a shift in binding epitopes.

EXAMPLE 9: Inhibition of tumor growth *in vivo* using an anti-PSMA bispecific molecule

[0254] To confirm the effectiveness of an anti-PSMA bispecific molecule of the present disclosure (*e.g.*, anti-PSMA and anti-CD3 bispecific molecules) at inhibiting tumor growth *in vivo*, the anti-PSMA bispecific molecule is evaluated as follows.

[0255] **Prophylactic treatment, or prevention of tumor engraftment of subcutaneous tumors:** Cultured, PSMA-expressing tumor cell lines (such as LNCaP, LNCaP C4-2, LNCaP C4-2B, VCaP, CWR22Rv1, LAPC4, MDA-PCa-2b, LuCaP 23.1, LuCaP 58, LuCaP 70, LuCaP 77) are mixed with human lymphocytes (either human peripheral blood mononuclear cells or purified T-cells) and injected subcutaneously into immunodeficient mice (such as SCID, NOD/SCID, etc). An anti-PSMA bispecific molecule is injected intravenously on the day of injection and on several subsequent days. Dose-dependent inhibition of tumor outgrowth, as assessed by tumor volume, indicates that the respective molecule has efficacy against PSMA-expressing tumors *in vivo*.

[0256] **Therapeutic treatment, or regression of previously established subcutaneous tumors:** Cultured, PSMA-expressing tumor cell lines (such as LNCaP, LNCaP C4-2, LNCaP C4-2B, VCaP, CWR22Rv1, LAPC4, MDA-PCa-2b, LuCaP 23.1AI, LuCaP 58, LuCaP 70, LuCaP 77) are injected subcutaneously into immunodeficient mice (such as SCID, NOD/SCID, etc). Tumor growth is monitored, and the study is initiated when tumors show signs of established growth (typically a volume of ~200 mm3). Human lymphocytes (either human peripheral blood mononuclear cells or purified T-cells) are injected intravenously along with an anti-PSMA bispecific molecule on the day of injection. The anti-PSMA bispecific molecule is injected several subsequent days. Dose-dependent inhibition of tumor growth, as assessed by tumor volume, indicates that the respective molecule has efficacy against PSMA-expressing tumors *in vivo*.

[0257] **Prophylactic treatment, or prevention of tumor engraftment of intra-tibial tumors:** Cultured, PSMA-expressing tumor cell lines (such as LNCaP C4-2, LNCaP C4-2B, VCaP, CWR22Rv1, LAPC4, MDA-PCa-2b, LuCaP 23.1, LuCaP 58, LuCaP 70, LuCaP 77) are mixed with human lymphocytes (either human peripheral blood mononuclear cells or purified T-cells) and injected intra-tibially into immunodeficient mice (such as SCID, NOD/SCID, etc). An anti-PSMA bispecific molecule is injected intravenously on the day of injection and on several subsequent days. Dose-dependent inhibition of tumor growth, as assessed by serum biomarkers, radiography, fluorescent imaging, weight loss, and other proxy measurements of tumor volume, indicates that the respective molecule has efficacy against PSMA-expressing tumors *in vivo*.

[0258] **Therapeutic treatment, or regression of previously established intra-tibial tumors:** Cultured, PSMA-expressing tumor cell lines (such as LNCaP C4-2, LNCaP C4-2B, VCaP, CWR22Rv1, LAPC4, MDA-PCa-2b, LuCaP 23.1AI, LuCaP 58, LuCaP 70, LuCaP 77) are injected intra-tibially into immunodeficient mice (such as SCID, NOD/SCID, etc). Tumor growth is monitored, and the study is initiated when tumors show signs of established growth (typically a volume of ~200 mm3). Human lymphocytes (either human peripheral blood mononuclear cells or purified T-cells) are injected intravenously along with an anti-PSMA bispecific molecule on the day of injection. The anti-PSMA bispecific molecule is injected several subsequent days. Dose-dependent inhibition of tumor growth, as assessed by serum biomarkers, radiography, fluorescent imaging, weight loss, and other proxy measurements of tumor volume, indicates that the respective molecule has efficacy against PSMA expressing tumors *in vivo*.

CLAIMS:

1. A prostate-specific membrane antigen (PSMA)-binding polypeptide comprising a humanized PSMA-binding domain wherein the PSMA binding domain comprises (i) an immunoglobulin light chain variable region comprising LCDR1, LCDR2, and LCDR3, and (ii) an immunoglobulin heavy chain variable region comprising HCDR1, HCDR2, and HCDR3, wherein the LCDR1, LCDR2 and LCDR3 has the amino acid sequences set forth in SEQ ID NO:15, 16 and 17, respectively, and the HCDR1, HCDR2, and HCDR3 has the amino acid sequences set forth in SEQ ID NO:9, 10 and 11, respectively.

2. The PSMA-binding polypeptide of claim 1, wherein said PSMA-binding polypeptide comprises from amino-terminus to carboxyl-terminus

(a) the PSMA binding domain,

(b) a first hinge region, and

(c) an immunoglobulin constant region.

3. The PSMA-binding polypeptide of claim 1, wherein said PSMA-binding polypeptide further comprises a second binding domain, wherein the second binding domain specifically binds a T cell, CD3, CD3ε or a T cell receptor (TCR) complex or a component thereof.

4. The PSMA-binding polypeptide of claim 3, wherein the PSMA-binding polypeptide comprises, in order from amino-terminus to carboxyl-terminus or in order from carboxyl-terminus to amino-terminus

(a) the PSMA binding domain,

(b) a first hinge region,

(c) an immunoglobulin constant region,

(d) a second hinge region, and

(e) a second binding domain.

5. The PSMA-binding protein of claim 3 or 4, wherein the second binding domain is amino-terminal to the second hinge region.

6. The PSMA-binding polypeptide of any one of the previous claims, wherein the immunoglobulin light chain variable region comprises an amino acid sequence that is at least 95% identical to the amino acid sequence set forth in SEQ ID NO:5 or SEQ ID NO:23 and the heavy chain variable region comprises an amino acid sequence that is at least 95% or 100% identical to the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:25, or SEQ ID NO:27.

7. The PSMA-binding polypeptide of any one of the previous claims, wherein the PSMA-binding domain competes for binding to human PSMA with a single chain Fv (scFv) having the amino acid sequence set forth in SEQ ID NO:21.

8. The PSMA-binding polypeptide of any one of claims 2-6, wherein the first hinge region is derived from (i) a stalk region of a type II C lectin or (ii) an immunoglobulin hinge region.

9. The PSMA-binding polypeptide of any one of claims 4-7, wherein the second hinge region is derived from (i) a stalk region of a type II C lectin or (ii) an immunoglobulin hinge region.

10. The PSMA-binding polypeptide of any one of the previous claims, wherein the PSMA-binding domain is a single chain Fv (scFv).

11. The PSMA-binding polypeptide of claim 10, wherein said scFv comprises an amino acid sequence that is at least 95% identical to the amino acid sequence set forth in SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:35.

12. The PSMA-binding polypeptide of any one of the preceding claims, wherein said PSMA-binding polypeptide comprises an amino acid sequence that is at least 95% identical to the amino acid sequence set forth in SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:70, or SEQ ID NO:72.

13. The PSMA-binding polypeptide of any one of claims 3-12, wherein the second binding domain competes for binding to CD3ε with a monoclonal antibody selected from the group consisting of CRIS-7and HuM291.

14. The PSMA-binding polypeptide of any one of claims 3-13, wherein the second binding domain comprises an immunoglobulin light chain variable region and an immunoglobulin heavy chain

variable region derived from a monoclonal antibody selected from the group consisting of CRIS-7 and HuM291.

15. The PSMA-binding polypeptide of claim 14, wherein the light and heavy chain variable regions of the second binding domain are selected from the group consisting of

(a) a light chain variable region comprising an amino acid sequence that is at least 95% or 100% identical to the amino acid sequence set forth in residues 139-245 of SEQ ID NO:47 and a heavy chain variable region comprising an amino acid sequence that is at least 95% or 100% identical to the amino acid sequence set forth in residues 1-121 of SEQ ID NO:47; and

(b) a light chain variable region comprising an amino acid sequence that is at least 95% or 100% identical to the amino acid sequence set forth in residues 634-740 of SEQ ID NO:78 and a heavy chain variable region comprising an amino acid sequence that is at least 95% or 100% identical to the amino acid sequence set forth in residues 496-616 of SEQ ID NO:78.

16. The PSMA-binding polypeptide of claim 1, wherein said PSMA-binding polypeptide comprises an amino acid sequence that is at least 95% or 100% identical to the amino acid sequence set forth in SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:162, or SEQ ID NO:164.

17. A dimeric PSMA-binding protein comprising first and second polypeptide chains, wherein each of said polypeptide chains is the PSMA-binding polypeptide of any one of the previous claims.

18. An isolated nucleic acid encoding the PSMA-binding polypeptide of any one of the previous claims.

19. A recombinant host cell comprising the nucleic acid of claim 18.

20. A composition comprising the PSMA-binding protein of claim 17; and a pharmaceutically acceptable carrier, diluent, or excipient.

21. An *ex vivo* method for inducing redirected T-cell cytotoxicity (RTCC) against a cell expressing prostate-specific membrane antigen (PSMA), the method comprising contacting said PSMA-expressing cell with the PSMA-binding protein of any one of claims 3-12, wherein said contacting is under

conditions whereby RTCC against the PSMA-expressing cell is induced.

22. Use of the dimeric PSMA-binding protein of claim 8 in the manufacture of a medicament for treating a disorder in a subject characterized by overexpression of prostate-specific membrane antigen (PSMA), wherein preferably, the disorder is a cancer, a prostate disorder, a neovascular disorder, a prostate cancer, colorectal cancer, gastric cancer, castrate-resistant prostate cancer, benign prostatic hyperplasia, solid tumor growth, clear cell renal carcinoma, colorectal cancer, bladder cancer, or lung cancer.