(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 ~~OF THE INVENTION~~

[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

[0024] ~~In another embodiment, the present disclosure provides~~ In one aspect, there is provided a dimeric PSMA-binding protein comprising first and second polypeptide chains, wherein each of said polypeptide chains is ~~a~~ the PSMA-binding polypeptide as ~~in any of the embodiments disclosed herein~~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

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 ~~another embodiment~~one aspect, ~~the present disclosure provides~~there is provided an isolated nucleic acid encoding ~~a~~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 ~~invention~~disclosure will become evident upon reference to the following detailed description of the ~~invention~~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