

## OPTIMIZED ANTI-CD3 BISPECIFIC ANTIBODIES AND USES THEREOF

### REFERENCE TO A SEQUENCE LISTING

[0001] This application incorporates by reference the Sequence Listing submitted in Computer Readable Form as file 10151WO01\_ST25.txt, created on September 22, 2016 and containing 264,418 bytes.

### FIELD OF THE INVENTION

[0002] The invention related to bispecific antibodies, targeting an effector antigen, such as CD3 antigen, and a tumor associated antigen, and methods of tumor killing. The invention relates to methods of reducing or eliminating tumor burden and controlling the toxic side effects that may be associated with tumor immunotherapy. The present invention provides bispecific antibodies comprising an effector arm which binds to an effector antigen with weak affinity or with no detectable binding affinity, for example, an anti-CD3 antigen-binding arm which binds to CD3 with a  $K_D$  of greater than about 500 nM, in an *in vitro* affinity binding assay.

### BACKGROUND

[0003] The promise of therapeutic bispecific antibodies (bsAbs), particularly in cancer immunotherapies, aims to bridge multiple antigen targets in order to elicit a more robust innate immune response to the unwanted target-bearing cells or organism.

[0004] It is now well established that to mediate redirected lysis, a bsAb must cluster a target cell directly to a triggering molecule on an effector cell, such as a T cell. There are many factors to consider in bsAb design, for example, size and composition will affect biodistribution and stability *in vivo* (Segal, DM, Weiner, GJ, and Weiner, LM. *Current Opinion in Immunol* 1999, 11: 558-562; Chames, P. and Baty, D. *MAbs*. 2009, 1(6): 539–547). Differential outcomes are difficult to predict depending on the T cell subset being triggered to respond, as well as the state of the T cell being stimulated. It is well-known that bsAbs do not give consistent results (Manzke O, et al. *Cancer Immunol Immunother*. 1997, 45:198–202). For example, without adequate cytokine production, CD3 crosslinking can induce an apoptotic response in the T cell (Noel PJ, Boise LH, Thompson CB: Regulation of T cell activation by CD28 and CTLA4. *Adv Exp Med Biol* 1996, 406:209-217). The subset of T cells and differentiation state of such recruited T cells, e.g. naïve T cells, are important for efficacy, since naïve T cells cannot lyse target cells without preactivation (such as crosslinking with TCR in the presence of IL-2).

[0005] Certain bispecific therapies have been successful, yet, as with many cancer therapies, it comes with a price. Toxicity is the leading cause of failure among cancer

therapeutics. It is well known that toxicity of so-called chemotherapeutic drugs is the leading cause of patient side effects and secondary maladies. The act of “cell killing” itself is wrought with trouble for the patient. A plethora of cytotoxic responses can be induced by activation of effector cells *e.g.* T cells, and a cancer target cell, yet which type of response is most beneficial in tumor immunotherapy remains to be seen. A method of identifying anti-CD3 antibodies for use in a bispecific therapy having reduced side effects while maintaining efficacy and desirable pharmacokinetic (PK) properties would be advantageous.

**[0006]** Techniques such as affinity maturation have been described which, based on structure/activity relationship (SAR), utilize mutagenesis to optimize antibodies to have increased and improved binding specificity or affinity for a target antigen compared to the starting antibody (see, *e.g.* WO2011056997, published May 12, 2011). Modified OKT3 antibodies capable of binding to and interacting with CD3 with varying degrees of affinity while still exhibiting moderate to high T cell activation have been described (US7820166). However, methods of reducing binding affinity of antibody molecules to near or beyond the detectable level of binding have not been described, nor shown to have the requisite efficacy for tumor reduction or suppression.

**[0007]** Thus, there exists a need for alternative bispecific antigen-binding molecules having controlled cytotoxicity and better PK properties. Such cancer therapies would be quite useful in therapeutic settings.

#### **BRIEF SUMMARY OF THE INVENTION**

**[0008]** In a first aspect, the present invention provides antibodies and antigen-binding fragments thereof that bind human CD3 having weak or no detectable affinity for human and/or cynomolgus CD3. The antibodies according to this aspect of the invention are useful, *inter alia*, for targeting T cells expressing CD3, and for stimulating T cell activation, *e.g.*, under circumstances where T cell-mediated killing is beneficial or desirable. The anti-CD3 antibodies of the invention, or antigen-binding portions thereof, may be included as part of a bispecific antibody that directs CD3-mediated T cell activation to specific cell types such as tumor cells or infectious agents.

**[0009]** Exemplary anti-CD3 antibodies of the present invention are listed in Tables 2 and 3 herein. Table 2 sets forth the amino acid sequence identifiers of the heavy chain variable regions (HCVRs), as well as heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3). Table 3 sets forth the sequence identifiers of the nucleic acid molecules encoding the HCVRs, HCDR1, HCDR2 and HCDR3 regions of the exemplary anti-CD3 antibodies. Tables 4 and 5 set forth light chain variable regions (LCVRs), as well as complementarity determining regions (LCDR1, LCDR2 and LCDR3) of the exemplary anti-CD3 antibodies.

**[0010]** The present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCVR comprising an amino acid sequence selected from any of the HCVR amino acid sequences listed in Table 2, or a substantially similar sequence thereof having having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

**[0011]** The present invention also provides antibodies, or antigen-binding fragments thereof, comprising an HCVR and an LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 2 paired with any of the LCVR amino acid sequences listed in Table 4, or a common light chain derived from the cognate light chain of the anti-TAA heavy chain, or derived from a known or public domain light chain variable region derived from a light chain exhibiting promiscuity or ability to pair with a wide variety of non-cognate heavy chains, *i.e.* a universal or common light chain. According to certain embodiments, the present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCVR/LCVR amino acid sequence pair contained within any of the exemplary anti-CD3 antibodies listed in Table 2 paired with exemplary light chain variable regions listed in Table 4. In certain embodiments, the HCVR/LCVR amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 10/162 (*e.g.*, CD3-VH-G2); 18/162 (*e.g.*, CD3-VH-G3); 26/162 (*e.g.*, CD3-VH-G4); 34/162 (*e.g.*, CD3-VH-G5); 42/162 (*e.g.*, CD3-VH-G8); 50/162 (*e.g.*, CD3-VH-G9); 58/162 (*e.g.*, CD3-VH-G10); 66/162 (*e.g.*, CD3-VH-G11); 74/162 (*e.g.*, CD3-VH-G12); 82/162 (*e.g.*, CD3-VH-G13); 90/162 (*e.g.*, CD3-VH-G14); 98/162 (*e.g.*, CD3-VH-G15); 106/162 (*e.g.*, CD3-VH-G16); 114/162 (*e.g.*, CD3-VH-G17); 122/162 (*e.g.*, CD3-VH-G18); 130/162 (*e.g.*, CD3-VH-G19); 138/162 (*e.g.*, CD3-VH-G20); and 146/162 (*e.g.*, CD3-VH-G21).

**[0012]** The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence selected from any of the HCDR1 amino acid sequences listed in Table 2 or a substantially similar sequence thereof having at least 95%, at least 98% or at least 99% sequence identity. The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence set forth in SEQ ID NO: 178.

**[0013]** The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence selected from any of the HCDR2 amino acid sequences listed in Table 2 or a substantially similar sequence thereof having at least 95%, at least 98% or at least 99% sequence identity. The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence set forth in SEQ ID NO: 179.

**[0014]** The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence selected from any of the HCDR3 amino acid sequences listed in Table 2 or a substantially similar sequence thereof having at least 95%, at least 98% or at least 99% sequence identity. The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence set forth in SEQ ID NO: 180.

**[0015]** The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR1 (LCDR1) comprising an amino acid sequence selected from any of the LCDR1 amino acid sequences listed in Table 4 or a substantially similar sequence thereof having at least 95%, at least 98% or at least 99% sequence identity. The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR1 (LCDR1) derived from a cognate light chain of the anti-TAA heavy chain, or derived from a light chain exhibiting promiscuity or ability to pair with a wide variety of non-cognate heavy chains, *i.e.* a universal or common light chain.

**[0016]** The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR2 (LCDR2) comprising an amino acid sequence selected from any of the LCDR2 amino acid sequences listed in Table 4 or a substantially similar sequence thereof having at least 95%, at least 98% or at least 99% sequence identity. The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR2 (LCDR2) derived from a cognate light chain of the anti-TAA heavy chain, or derived from a light chain exhibiting promiscuity or ability to pair with a wide variety of non-cognate heavy chains, *i.e.* a universal or common light chain.

**[0017]** The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR3 (LCDR3) comprising an amino acid sequence selected from any of the LCDR3 amino acid sequences listed in Table 4 or a substantially similar sequence thereof having at least 95%, at least 98% or at least 99% sequence identity. The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR3 (LCDR3) derived from a cognate light chain of the anti-TAA heavy chain, or derived from a light chain exhibiting promiscuity or ability to pair with a wide variety of non-cognate heavy chains, *i.e.* a universal or common light chain.

**[0018]** The present invention also provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3 and an LCDR3 amino acid sequence pair (HCDR3/LCDR3) comprising any of the HCDR3 amino acid sequences listed in Table 2 paired with any of the LCDR3 amino acid sequences listed in Table 4. According to certain embodiments, the present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3/LCDR3 amino acid sequence pair contained within any of the exemplary anti-CD3

antibodies listed in Table 2. In certain embodiments, the HCDR3/LCDR3 amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 16/168 (*e.g.*, CD3-VH-G2); 24/168 (*e.g.*, CD3-VH-G3); 32/168 (*e.g.*, CD3-VH-G4); 40/168 (*e.g.*, CD3-VH-G5); 48/168 (*e.g.*, CD3-VH-G8); 56/168 (*e.g.*, CD3-VH-G9); 64/168 (*e.g.*, CD3-VH-G10); 72/168 (*e.g.*, CD3-VH-G11); 80/168 (*e.g.*, CD3-VH-G12); 88/168 (*e.g.*, CD3-VH-G13); 96/168 (*e.g.*, CD3-VH-G14); 104/168 (*e.g.*, CD3-VH-G15); 112/168 (*e.g.*, CD3-VH-G16); 120/168 (*e.g.*, CD3-VH-G17); 128/168 (*e.g.*, CD3-VH-G18); 136/168 (*e.g.*, CD3-VH-G19); 144/168 (*e.g.*, CD3-VH-G20); and 152/168 (*e.g.*, CD3-VH-G21).

**[0019]** The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within any of the exemplary anti-CD3 antibodies listed in Tables 2 and 4. In certain embodiments, the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set is selected from the group consisting of SEQ ID NOs: 12-14-16-164-166-168 (*e.g.*, CD3-VH-G2); 20-22-24-164-166-168 (*e.g.*, CD3-VH-G3); 28-30-32-164-166-168 (*e.g.*, CD3-VH-G4); 36-38-40-164-166-168 (*e.g.*, CD3-VH-G5); 44-46-48-164-166-168 (*e.g.*, CD3-VH-G8); 52-54-56-164-166-168 (*e.g.*, CD3-VH-G9); 60-62-64-164-166-168 (*e.g.*, CD3-VH-G10); 68-70-72-164-166-168 (*e.g.*, CD3-VH-G11); 76-78-80-164-166-168 (*e.g.*, CD3-VH-G12); 84-86-88-164-166-168 (*e.g.*, CD3-VH-G13); 92-94-96-164-166-168 (*e.g.*, CD3-VH-G14); 100-102-104-164-166-168 (*e.g.*, CD3-VH-G15); 108-110-112-164-166-168 (*e.g.*, CD3-VH-G16); 116-118-120-164-166-168 (*e.g.*, CD3-VH-G17); 124-126-128-164-166-168 (*e.g.*, CD3-VH-G18); 132-134-136-164-166-168 (*e.g.*, CD3-VH-G19); 140-142-144-164-166-168 (*e.g.*, CD3-VH-G20); and 148-150-152-164-166-168 (*e.g.*, CD3-VH-G21).

**[0020]** In a related embodiment, the present invention provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within an HCVR/LCVR amino acid sequence pair as defined by any of the exemplary anti-CD3 antibodies listed in Tables 2 and 4. For example, the present invention includes antibodies, or antigen-binding fragments thereof, comprising the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set contained within an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 10/162 (*e.g.*, CD3-VH-G2); 18/162 (*e.g.*, CD3-VH-G3); 26/162 (*e.g.*, CD3-VH-G4); 34/162 (*e.g.*, CD3-VH-G5); 42/162 (*e.g.*, CD3-VH-G8); 50/162 (*e.g.*, CD3-VH-G9); 58/162 (*e.g.*, CD3-VH-G10); 66/162 (*e.g.*, CD3-VH-G11); 74/162 (*e.g.*, CD3-VH-G12); 82/162 (*e.g.*, CD3-VH-G13); 90/162 (*e.g.*, CD3-VH-G14); 98/162 (*e.g.*, CD3-VH-G15); 106/162 (*e.g.*, CD3-VH-G16); 114/162 (*e.g.*, CD3-VH-G17); 122/162 (*e.g.*, CD3-VH-G18); 130/162 (*e.g.*, CD3-VH-G19); 138/162 (*e.g.*, CD3-VH-G20); and 146/162 (*e.g.*, CD3-VH-G21).

**[0021]** Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified

HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See, e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol.* 273:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA* 86:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

**[0022]** The present invention also provides nucleic acid molecules encoding anti-CD3 antibodies or portions thereof. For example, the present invention provides nucleic acid molecules encoding any of the HCVR amino acid sequences listed in Table 3; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

**[0023]** The present invention also provides nucleic acid molecules encoding any of the LCVR amino acid sequences listed in Table 4; or an LCVR derived from a cognate light chain of the anti-TAA heavy chain, or derived from a light chain exhibiting promiscuity or ability to pair with a wide variety of non-cognate heavy chains, *i.e.* a universal or common light chain. In certain embodiments, the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 5, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

**[0024]** The present invention also provides nucleic acid molecules encoding any of the HCDR1 amino acid sequences listed in Table 2; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR1 nucleic acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

**[0025]** The present invention also provides nucleic acid molecules encoding any of the HCDR2 amino acid sequences listed in Table 2; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR2 nucleic acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

**[0026]** The present invention also provides nucleic acid molecules encoding any of the HCDR3 amino acid sequences listed in Table 2; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR3 nucleic

acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

**[0027]** The present invention also provides nucleic acid molecules encoding any of the LCDR1 amino acid sequences listed in Table 4; or an LCDR1 derived from a cognate light chain of the anti-TAA heavy chain, or derived from a light chain exhibiting promiscuity or ability to pair with a wide variety of non-cognate heavy chains, *i.e.* a universal or common light chain. In certain embodiments, the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR1 nucleic acid sequences listed in Table 5, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

**[0028]** The present invention also provides nucleic acid molecules encoding any of the LCDR2 amino acid sequences listed in Table 4; or an LCDR2 derived from a cognate light chain of the anti-TAA heavy chain, or derived from a light chain exhibiting promiscuity or ability to pair with a wide variety of non-cognate heavy chains, *i.e.* a universal or common light chain. In certain embodiments, the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR2 nucleic acid sequences listed in Table 5, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

**[0029]** The present invention also provides nucleic acid molecules encoding any of the LCDR3 amino acid sequences listed in Table 4; or an LCDR3 derived from a cognate light chain of the anti-TAA heavy chain, or derived from a light chain exhibiting promiscuity or ability to pair with a wide variety of non-cognate heavy chains, *i.e.* a universal or common light chain. In certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR3 nucleic acid sequences listed in Table 5, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

**[0030]** The present invention also provides nucleic acid molecules encoding an HCVR, wherein the HCVR comprises a set of three CDRs (*i.e.*, HCDR1-HCDR2-HCDR3), wherein the HCDR1-HCDR2-HCDR3 amino acid sequence set is as defined by any of the exemplary anti-CD3 antibodies listed in Table 2.

**[0031]** The present invention also provides nucleic acid molecules encoding an LCVR, wherein the LCVR comprises a set of three CDRs (*i.e.*, LCDR1-LCDR2-LCDR3), wherein the LCDR1-LCDR2-LCDR3 amino acid sequence set is as defined by any of the exemplary universal light chain antibodies listed in Table 4; or the LCDR1-LCDR2-LCDR3 is derived from a cognate light chain of the anti-TAA heavy chain, or derived from a light chain exhibiting promiscuity or ability to pair with a wide variety of non-cognate heavy chains, *i.e.* a universal or common light chain.

**[0032]** The present invention also provides nucleic acid molecules encoding both an HCVR and an LCVR, wherein the HCVR comprises an amino acid sequence of any of the HCVR amino acid sequences listed in Table 2, and wherein the LCVR comprises an amino acid sequence of any of the LCVR amino acid sequences listed in Table 4; or the LCVR is derived from a cognate light chain of the anti-TAA heavy chain, or derived from a light chain exhibiting promiscuity or ability to pair with a wide variety of non-cognate heavy chains, *i.e.* a universal or common light chain. In certain embodiments, the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto, and a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 5, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto. In some embodiments, the nucleic acid molecules encoding both an HCVR and an LCVR are fully human sequences or derived from human germline immunoglobulin sequences.

**[0033]** The present invention also provides recombinant expression vectors capable of expressing a polypeptide comprising a heavy or light chain variable region of an anti-CD3 antibody. For example, the present invention includes recombinant expression vectors comprising any of the nucleic acid molecules mentioned above, *i.e.*, nucleic acid molecules encoding any of the HCVR, LCVR, and/or CDR sequences as set forth in Table 2 or 4. Also included within the scope of the present invention are host cells into which such vectors have been introduced, as well as methods of producing the antibodies or portions thereof by culturing the host cells under conditions permitting production of the antibodies or antibody fragments, and recovering the antibodies and antibody fragments so produced.

**[0034]** The present invention includes anti-CD3 antibodies and/or anti-TAA antibodies, as well as bispecific anti-CD3/anti-TAA antibodies having a modified glycosylation pattern. In some embodiments, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fucose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield *et al.* (2002) *JBC* 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

**[0035]** In one aspect, the invention provides a cytotoxic composition comprising a bispecific antigen-binding molecule that i) is not capable of specifically binding to an effector cell, and ii) specifically binds a target tumor cell, wherein specific binding is measured in an *in vitro* FACS binding assay or an *in vitro* surface plasmon resonance binding assay. In certain embodiments, the invention provides a cytotoxic composition comprising a bispecific antigen-binding molecule that exhibits no detectable binding to an effector cell, and that

specifically binds to a target tumor cell with a measurable binding affinity, wherein the binding affinity value is measured in an *in vitro* FACS binding assay or an *in vitro* surface plasmon resonance binding assay.

**[0036]** In other embodiments, the invention provides a cytotoxic composition comprising a bispecific antigen-binding molecule comprising i) a first antigen-binding fragment (Fab1) that exhibits no detectable binding to CD3, and ii) a second antigen-binding fragment (Fab2) that specifically binds to a target tumor cell with a measurable binding affinity, wherein the binding affinity value is measured in an *in vitro* FACS binding assay or an *in vitro* surface plasmon resonance binding assay. In some cases, the binding affinity is monovalent binding affinity (*e.g.*, in a bispecific antibody construct).

**[0037]** In another aspect the invention provides a cytotoxic composition comprising a bispecific antigen-binding molecule that specifically binds an effector cell with a weak binding affinity, for example exhibiting an EC<sub>50</sub> value of about or greater than about 100 nM, and that specifically binds a target tumor cell with an appreciable EC<sub>50</sub> value, or a high affinity EC<sub>50</sub> value such as less than 50 nM, wherein the EC<sub>50</sub> binding affinity value is measured in an *in vitro* FACS binding assay. In certain embodiments, the invention provides a cytotoxic composition comprising a bispecific antigen-binding molecule that specifically binds an effector cell with an EC<sub>50</sub> value of greater than about 500 nM, and that specifically binds a target tumor cell with an appreciable EC<sub>50</sub> value, or a high affinity EC<sub>50</sub> value such as less than 50 nM, wherein the EC<sub>50</sub> binding affinity value is measured in an *in vitro* FACS binding assay.

**[0038]** In some examples, the bispecific antigen-binding molecule includes a Fab1 that specifically binds human CD3 with an EC<sub>50</sub> value of greater than about 40 nM, or greater than about 100 nM, greater than about 200 nM, greater than about 300 nM, greater than about 400 nM, greater than about 500 nM, or greater than about 1  $\mu$ M (*e.g.* in a monovalent binding context). In some embodiments, the bispecific antigen-binding molecule includes a Fab2 derived from a second antibody that specifically binds the target tumor cell with high affinity, *e.g.* an EC<sub>50</sub> value of less than less than about 50 nM, less than about 40 nM, less than about 20 nM, less than about 10 nM or less than about 6 nM (*e.g.*, in a monovalent binding context). In some cases, the Fab1 specifically binds each of human CD3 and cynomolgus CD3 with an EC<sub>50</sub> value of greater than about 40 nM, or greater than about 100 nM, greater than about 200 nM, or greater than about 1  $\mu$ M. In some cases, the Fab1 specifically binds each of human CD3 and cynomolgus CD3 with weak or no measurable affinity.

**[0039]** In some embodiments, the target tumor cell is a human tumor cell. In some embodiments, the Fab1 (or the bispecific antigen-binding molecule) induces T cell-mediated

tumor cell killing with an EC<sub>50</sub> value of less than about 1.3 nM, as measured in an *in vitro* T cell-mediated tumor cell killing assay.

**[0040]** In some applications, the Fab1 or the bispecific antigen-binding molecule specifically binds human CD3 with an K<sub>D</sub> value of greater than about 11 nM, as measured in an *in vitro* surface plasmon resonance binding assay. In other instances, the Fab1 or the bispecific antigen-binding molecule specifically binds each of human CD3 and cynomolgus CD3 with an K<sub>D</sub> value of greater than about 15 nM, or greater than about 30 nM, greater than about 60 nM, greater than about 120 nM, or greater than about 300 nM, as measured in an *in vitro* surface plasmon resonance binding assay. In still some applications, the Fab1 or the bispecific antigen-binding molecule i) exhibits no detectable binding to human CD3 as measured in each of an *in vitro* surface plasmon resonance binding assay and a FACS binding assay, and ii) induces T cell-mediated tumor cell killing, as measured in an *in vitro* T cell-mediated tumor cell killing assay.

**[0041]** In some applications, the bispecific antigen-binding molecule comprises a first heavy chain comprising a HCDR1 region comprising an amino acid sequence set forth in SEQ ID NO: 12 or 20. In some embodiments, the first heavy chain comprises a HCDR2 region comprising an amino acid sequence set forth in SEQ ID NO: 14 or 54. In other embodiments, the first heavy chain comprises a HCDR3 region comprising an amino acid sequence set forth in SEQ ID NO: 16, SEQ ID NO: 24, SEQ ID NO: 32, SEQ ID NO: 40, SEQ ID NO: 48, SEQ ID NO: 56, SEQ ID NO: 64, SEQ ID NO: 72, SEQ ID NO: 80, SEQ ID NO: 88, SEQ ID NO: 96, SEQ ID NO: 104, SEQ ID NO: 112, SEQ ID NO: 120, SEQ ID NO: 128, SEQ ID NO: 136, SEQ ID NO: 144, or SEQ ID NO: 152. In other applications, the first heavy chain comprises a HCVR comprising HCDR1-HCDR2-HCDR3 having the amino acid sequences of SEQ ID NOs: 178-179-180. In other embodiments, a first heavy chain comprises a CDR1 comprising amino acid residues 1-7 of SEQ ID NO:178, a CDR2 comprising amino acid residues 1-7 of SEQ ID NO:179, a CDR3 comprising amino acid residues 4-11 of SEQ ID NO:180.

**[0042]** In more embodiments, the first heavy chain comprises variable domain framework regions having an amino acid sequence selected from FR1 (SEQ ID NO: 174), FR2 (SEQ ID NO: 175), FR3 (SEQ ID NO: 176), and FR4 (SEQ ID NO: 177).

**[0043]** The present invention provides bispecific antigen-binding molecules comprising a Fab1 HCVR and LCVR amino acid sequence pair (HCVR/LCVR) selected from the group consisting of: SEQ ID NOs: 10/162; 18/162; 26/162; 34/162; 42/162; 50/162; 58/162; 66/162; 74/162; 82/162; 90/162; 98/162; 106/162; 114/162; 122/162; 130/162; 138/162; 146/162.

**[0044]** The antibodies, and antigen-binding fragments and bispecific antibodies thereof were made by replacing amino acid residues of a parental in a stepwise manner based on differences between the germline sequence and the parental antibody sequence. The

present invention provides a method of making a cytotoxic composition comprising (a) identifying the amino acid sequence of the first heavy chain derived from a first antibody that specifically binds CD3 with high affinity, for example exhibits a binding affinity  $EC_{50}$  value of less than about 40 nM, (b) modifying selected amino acid residues in the heavy chain variable region of the first antibody to produce a modified antibody, (c) pairing the modified antibody with a second heavy chain derived from a second antibody that specifically binds a target tumor antigen to produce a bispecific antibody, (d) testing the bispecific antibody in a binding affinity assay, and if the binding affinity to CD3 has an  $EC_{50}$  value of greater than about 40 nM, or greater than 100 nM or greater than 300 nM or greater than 500 nM, or no detectable binding, then (e) preparing a composition comprising the bispecific antibody and a pharmaceutically acceptable carrier or diluent. In addition to modifying the heavy chain variable region of selected antibodies to engineer antigen-binding arms having weak or no affinity for, yet specifically target an effector cell, the invention provides methods herein for modifying the heavy chain constant region (e.g.  $C_H3$  domain) of each binding arm to prepare and isolate bispecific antibodies.

**[0045]** An exemplary method provides a method of producing a cytotoxic bispecific antibody, comprising: (a) identifying a first human antibody or antigen-binding fragment thereof that interacts with an effector cell antigen from multiple species; (b) identifying the germline amino acid residues of the heavy chain variable region (HCVR) of the human antibody; (c) comparing the amino acid sequence of the HCVR of the first human antibody to the amino acid sequence of the corresponding germline HCVR; (d) identifying amino acids within a modified region of the HCVR of the first antibody, whereby a modified region in the first antibody displays at least one amino acid modification by substitution, deletion or addition of a single amino acid residue compared to the same region in the germline HCVR; (e) producing a plurality of modified antibodies each comprising at least one modified region of the HCVR; (f) screening each of the plurality of modified antibodies for monovalent affinity to the effector cell antigen; (g) selecting those modified antibodies that exhibit weaker binding affinity or no detectable binding affinity to the effector cell antigen compared to the first antibody; and (h) pairing the selected first antibody with a second antibody that interacts with a tumor-associated antigen to produce a cytotoxic bispecific antibody.

**[0046]** In another aspect, the invention provides a pharmaceutical composition comprising a recombinant human bispecific antibody or fragment thereof which specifically binds CD3 and a pharmaceutically acceptable carrier. In a related aspect, the invention features a composition which is a combination of an anti-CD3 antibody and a second therapeutic agent. In one embodiment, the second therapeutic agent is any agent that is advantageously combined with an anti-CD3 antibody. Exemplary agents that may be advantageously combined with an anti-CD3 antibody include, without limitation, other agents that bind and/or

activate CD3 signaling (including other antibodies or antigen-binding fragments thereof, etc.) and/or agents which do not directly bind CD3 but nonetheless activate or stimulate immune cell activation. Additional combination therapies and co-formulations involving the anti-CD3 antibodies of the present invention are disclosed elsewhere herein.

**[0047]** In yet another aspect, the invention provides therapeutic methods for stimulating T cell activation using an anti-CD3 antibody or antigen-binding portion of an antibody of the invention, wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising a bispecific antibody of the invention, or antigen-binding fragment thereof, to a subject in need thereof. The disorder treated is any disease or condition, which is improved, ameliorated, inhibited or prevented by cytotoxic therapy targeted to a tumor-associated antigen, such as cancer.

**[0048]** According to another aspect, the present invention provides bispecific antigen-binding molecules that bind CD3 and a target antigen, especially a tumor-associated antigen (TAA).

**[0049]** The present invention also includes the use of an anti-CD3/anti-TAA bispecific antigen-binding molecule of the invention in the manufacture of a medicament for the treatment of a disease or disorder related to or caused by TAA expression. The present invention also provides use of an anti-CD3/anti-TAA bispecific antigen-binding molecule, exhibiting weak affinity to CD3-expressing effector cells and reduced clearance, in the manufacture of a medicament for the treatment of a disease or disorder related to or caused by TAA expression, compared to an anti-CD3/anti-TAA bispecific antigen-binding molecule exhibiting high affinity to CD3-expressing effector cells.

**[0050]** Other embodiments will become apparent from a review of the ensuing detailed description.

## **BRIEF DESCRIPTION OF THE FIGURES**

**[0051]** **Figure 1** shows the amino acid alignment of the following antibody heavy chain variable region (HCVR) sequences: germline hlgHV (SEQ ID NO:181); CD3-VH-P (SEQ ID NO:154); CD3-VH-G (SEQ ID NO:2); CD3-VH-G2 (SEQ ID NO:10); CD3-VH-G3 (SEQ ID NO:18); CD3-VH-G4 (SEQ ID NO:26); CD3-VH-G5 (SEQ ID NO: 34); CD3-VH-G8 (SEQ ID NO:42); CD3-VH-G9 (SEQ ID NO:50); CD3-VH-G10 (SEQ ID NO:58); CD3-VH-G11 (SEQ ID NO:66); CD3-VH-G12(SEQ ID NO:74); CD3-VH-G13 (SEQ ID NO:82); CD3-VH-G14(SEQ ID NO:90); CD3-VH-G15(SEQ ID NO:98); CD3-VH-G16 (SEQ ID NO:106); CD3-VH-G17 (SEQ ID NO:114); CD3-VH-G18 (SEQ ID NO:122); CD3-VH-G19 (SEQ ID NO:130); CD3-VH- G20 (SEQ ID NO:138); and CD3-VH-G21(SEQ ID NO:146). Each derivative HCVR is compared to the parent antibody and germline amino acid residues, with rectangular boxes denoting mutations in the CDRs.

**[0052]** Figures 2A, 2B and 2C illustrate mean concentrations of total IgG in serum following a single 0.4 mg/kg intra-peritoneal injection of BSMUC16/CD3-001, BSMUC16/CD3-005 and isotype control antibodies in wild-type mice (Fig. 2A), humanized CD3 mice (Fig. 2B) and humanized MUC16 x CD3 mice (Fig. 2C).

#### **DETAILED DESCRIPTION**

**[0053]** Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

**[0054]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

**[0055]** Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

#### Definitions

**[0056]** The expression "CD3" refers to an antigen which is expressed on T cells as part of the multimolecular T cell receptor (TCR) and which consists of a homodimer or heterodimer formed from the association of two of four receptor chains: CD3-epsilon, CD3- delta, CD3-zeta, and CD3-gamma. Human CD3-epsilon (hCD3 $\epsilon$ ) comprises the amino acid sequence as set forth in SEQ ID NO:169 (UniProtKB/Swiss-Prot: P07766.2). Human CD3-delta (hCD3 $\delta$ ) comprises the amino acid sequence as set forth in SEQ ID NO:170 (UniProtKB/Swiss-Prot: P04234.1). All references to proteins, polypeptides and protein fragments herein are intended to refer to the human version of the respective protein, polypeptide or protein fragment unless explicitly specified as being from a non-human species. Thus, the expression "CD3" means human CD3 unless specified as being from a non-human species, *e.g.*, "mouse CD3," "monkey CD3," etc.

**[0057]** The phrase "an antibody that binds CD3" or an "anti-CD3 antibody" includes antibodies and antigen-binding fragments thereof that specifically recognize and associate with a single CD3 subunit (*e.g.*, epsilon, delta, gamma or zeta), as well as antibodies and

antigen-binding fragments thereof that specifically recognize and associate with a dimeric complex of two CD3 subunits (*e.g.*, epsilon/delta, epsilon/gamma, and zeta/zeta CD3 dimers). The antibodies and antigen-binding fragments of the present invention may bind soluble CD3, bound CD3 and/or cell surface expressed CD3. Soluble CD3 includes natural CD3 proteins as well as recombinant CD3 protein variants such as, *e.g.*, monomeric and dimeric CD3 constructs, that lack a transmembrane domain or are otherwise unassociated with a cell membrane. The present invention provides antibodies that bind and activate human and cynomolgus CD3 with weak or no detectable binding affinity. "Binding to CD3 with no detectable binding affinity" means that the antibody and or antigen-binding fragment interaction with the CD3 target may not be measurable or detectable with a known assay for detection, such as a FACS (cell-based) binding assay as described herein or a surface plasmon resonance binding assay as described herein and well-known in the art. Other binding assays are well-known in the art. The antibody and or antigen-binding fragment may recognize the CD3 target by very weak protein-protein biochemical interaction, however a determination of specific  $K_D$  or  $EC_{50}$  value cannot be measured since the interaction is beyond the detection limit of the assay, *e.g.* no measurement can be determined. In another instance, "no detectable binding affinity" is determined if the affinity of an antibody corresponding to a  $K_D$  value is equal to or less than ten-fold lower than a non-specific antigen such as, BSA, casein, or the like. "Binding to CD3 with weak binding affinity" includes interactions where binding affinity measurement is at or slightly above the detection limit of the assay, or equivalent to the binding affinity to a non-specific antigen.

**[0058]** The expression "cell surface-expressed CD3" means one or more CD3 protein(s) that is/are expressed on the surface of a cell *in vitro* or *in vivo*, such that at least a portion of a CD3 protein is exposed to the extracellular side of the cell membrane and is accessible to an antigen-binding portion of an antibody. "Cell surface-expressed CD3" includes CD3 proteins contained within the context of a functional T cell receptor in the membrane of a cell. The expression "cell surface-expressed CD3" includes CD3 protein expressed as part of a homodimer or heterodimer on the surface of a cell (*e.g.*, delta/epsilon, gamma/epsilon, and zeta/zeta CD3 dimers). The expression, "cell surface-expressed CD3" also includes a CD3 chain (*e.g.*, CD3-delta, CD3-epsilon or CD3-gamma) that is expressed by itself, without other CD3 chain types, on the surface of a cell. A "cell surface-expressed CD3" can comprise or consist of a CD3 protein expressed on the surface of a cell which normally expresses CD3 protein. Alternatively, "cell surface-expressed CD3" can comprise or consist of CD3 protein expressed on the surface of a cell that normally does not express human CD3 on its surface but has been artificially engineered to express CD3 on its surface.

**[0059]** Effector cells include effector T cells (T lymphocytes), for example CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, Th1, Th2 and regulatory T cells (Tregs). Effector cells may also include natural

killer (NK) cells, macrophages, granulocytes, plasma cells or B cells (lymphocytes). It is understood that therapies may mediate a plethora of cell-mediated immune responses, or effector functions, through Ig interaction with effector cell surface receptors, such as CD3 (T cell surface receptor), CD28 (T cells), Fcγ receptors (FcγRs) (NK cells, activated macrophages and the like). Effector functions such as cell killing, complement activation, phagocytosis and opsonisation are subsequently triggered through these interactions. Binding to an effector cell and a tumor target cell allows for a valuable and effective immunotherapy design that propagates tumor cell killing and induces endogenous immune functions to fight the tumor or cancer.

**[0060]** The expression "anti-CD3 antibody" includes both monovalent antibodies with a single specificity, as well as bispecific antibodies comprising a first arm that binds CD3 and a second arm that binds a second (target) antigen, wherein the anti-CD3 arm comprises any of the HCVR/LCVR or CDR sequences as set forth in Tables 2, 3, 4 and/or 5 herein. Examples of anti-CD3 bispecific antibodies are described elsewhere herein. The term "antigen-binding molecule" includes antibodies and antigen-binding fragments of antibodies, including, *e.g.*, bispecific antibodies.

**[0061]** The term "antibody" includes any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen (*e.g.*, CD3). The term "antibody" includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or V<sub>H</sub>) and a heavy chain constant region. The heavy chain constant region comprises three domains, C<sub>H</sub>1, C<sub>H</sub>2 and C<sub>H</sub>3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V<sub>L</sub>) and a light chain constant region. The light chain constant region comprises one domain (C<sub>L</sub>1). The V<sub>H</sub> and V<sub>L</sub> regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V<sub>H</sub> and V<sub>L</sub> is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the anti-CD3 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

**[0062]** The term "antibody" also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically

obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

**[0063]** Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')<sub>2</sub> fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

**[0064]** An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V<sub>H</sub> domain associated with a V<sub>L</sub> domain, the V<sub>H</sub> and V<sub>L</sub> domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V<sub>H</sub>-V<sub>H</sub>, V<sub>H</sub>-V<sub>L</sub> or V<sub>L</sub>-V<sub>L</sub> dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V<sub>H</sub> or V<sub>L</sub> domain.

**[0065]** In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V<sub>H</sub>-C<sub>H</sub>1; (ii) V<sub>H</sub>-C<sub>H</sub>2; (iii) V<sub>H</sub>-C<sub>H</sub>3; (iv) V<sub>H</sub>-C<sub>H</sub>1-C<sub>H</sub>2; (v) V<sub>H</sub>-C<sub>H</sub>1-C<sub>H</sub>2-C<sub>H</sub>3; (vi) V<sub>H</sub>-C<sub>H</sub>2-C<sub>H</sub>3; (vii) V<sub>H</sub>-C<sub>L</sub>; (viii) V<sub>L</sub>-C<sub>H</sub>1; (ix) V<sub>L</sub>-C<sub>H</sub>2; (x) V<sub>L</sub>-C<sub>H</sub>3; (xi) V<sub>L</sub>-C<sub>H</sub>1-C<sub>H</sub>2; (xii) V<sub>L</sub>-C<sub>H</sub>1-C<sub>H</sub>2-C<sub>H</sub>3; (xiii) V<sub>L</sub>-C<sub>H</sub>2-C<sub>H</sub>3; and (xiv) V<sub>L</sub>-C<sub>L</sub>. In any configuration of variable and constant domains, including any of the

exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V<sub>H</sub> or V<sub>L</sub> domain (*e.g.*, by disulfide bond(s)).

**[0066]** As with full antibody molecules, antigen-binding fragments may be monospecific or multispecific (*e.g.*, bispecific). A multispecific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antibody format, including the exemplary bispecific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

**[0067]** The antibodies of the present invention may function through complement-dependent cytotoxicity (CDC) or antibody-dependent cell-mediated cytotoxicity (ADCC). "Complement-dependent cytotoxicity" (CDC) refers to lysis of antigen-expressing cells by an antibody of the invention in the presence of complement. "Antibody-dependent cell-mediated cytotoxicity" (ADCC) refers to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (*e.g.*, Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and thereby lead to lysis of the target cell. CDC and ADCC can be measured using assays that are well known and available in the art. (*See, e.g.*, U.S. Patent Nos 5,500,362 and 5,821,337, and Clynes *et al.* (1998) Proc. Natl. Acad. Sci. (USA) 95:652-656). The constant region of an antibody is important in the ability of an antibody to fix complement and mediate cell-dependent cytotoxicity. Thus, the isotype of an antibody may be selected on the basis of whether it is desirable for the antibody to mediate cytotoxicity.

**[0068]** In certain embodiments of the invention, the anti-CD3 antibodies of the invention (monospecific or bispecific) are human antibodies. The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR

sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

**[0069]** The term "recombinant human antibody" is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (see *e.g.*, Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V<sub>H</sub> and V<sub>L</sub> regions of the recombinant antibodies are sequences that, while derived from and related to human germline V<sub>H</sub> and V<sub>L</sub> sequences, do not necessarily naturally exist within the human antibody germline repertoire *in vivo*.

**[0070]** Human antibodies can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via inter-chain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification.

**[0071]** The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. A single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal et al. (1993) Molecular Immunology 30:105) to levels typically observed using a human IgG1 hinge. The instant invention encompasses antibodies having one or more mutations in the hinge, C<sub>H</sub>2 or C<sub>H</sub>3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form.

**[0072]** The antibodies of the invention may be isolated antibodies. An "isolated antibody," as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, or from a tissue or cell in which the antibody naturally exists or is naturally produced, is an "isolated antibody"

for purposes of the present invention. An isolated antibody also includes an antibody *in situ* within a recombinant cell. Isolated antibodies are antibodies that have been subjected to at least one purification or isolation step. According to certain embodiments, an isolated antibody may be substantially free of other cellular material and/or chemicals.

**[0073]** The present invention also includes one-arm antibodies that bind CD3. The phrase "one-arm antibody" means an antigen-binding molecule comprising a single antibody heavy chain and a single antibody light chain. The one-arm antibodies of the present invention may comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Table 2 herein.

**[0074]** The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstances, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

**[0075]** The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95%, and more preferably at least about 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

**[0076]** As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence

identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson (1994) *Methods Mol. Biol.* 24: 307-331. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) *Science* 256: 1443-1445. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

**[0077]** Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as Gap and Bestfit which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, e.g., GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, e.g., Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410 and Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389-402.

#### Germline Mutations

**[0078]** The anti-CD3 antibodies disclosed herein comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy chain variable domains as compared to the corresponding germline sequences from which the antibodies were derived.

**[0079]** The present invention also includes antibodies, and antigen-binding fragments

thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"), and having weak or no detectable binding to a CD3 antigen. Several such exemplary antibodies that recognize CD3 are described in Table 2 herein.

**[0080]** Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be tested for one or more desired properties such as, improved binding specificity, weak or reduced binding affinity, improved or enhanced pharmacokinetic properties, reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner given the guidance of the present disclosure are encompassed within the present invention.

**[0081]** The present invention also includes anti-CD3 antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes anti-CD3 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences set forth in Table 2 herein. The antibodies and bispecific antigen-binding molecules of the present invention comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which the individual antigen-binding domains were derived, while maintaining or improving the desired weak-to-no detectable binding to CD3 antigen. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein, *i.e.* the amino acid substitution maintains or improves the desired weak to no detectable binding affinity in the case of anti-CD3 binding molecules. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine

and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443-1445. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

**[0082]** The present invention also includes antigen-binding molecules comprising an antigen-binding domain with an HCVR and/or CDR amino acid sequence that is substantially identical to any of the HCVR and/or CDR amino acid sequences disclosed herein, while maintaining or improving the desired weak affinity to CD3 antigen. The term "substantial identity" or "substantially identical," when referring to an amino acid sequence means that two amino acid sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, *e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331.

**[0083]** Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as Gap and Bestfit which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, *e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (*e.g.*, FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using

default parameters. See, e.g., Altschul *et al.* (1990) J. Mol. Biol. 215:403-410 and Altschul *et al.* (1997) Nucleic Acids Res. 25:3389-402.

**[0084]** Once obtained, antigen-binding domains that contain one or more germline mutations were tested for decreased binding affinity utilizing one or more *in vitro* assays. Although antibodies that recognize a particular antigen are typically screened for their purpose by testing for high (*i.e.* strong) binding affinity to the antigen, the antibodies of the present invention exhibit weak binding or no detectable binding. Bispecific antigen-binding molecules comprising one or more antigen-binding domains obtained in this general manner are also encompassed within the present invention and were found to be advantageous as avidity-driven tumor therapies.

**[0085]** Unexpected benefits, for example, improved pharmacokinetic properties and low toxicity to the patient may be realized from the methods described herein.

#### Binding Properties of the Antibodies

**[0086]** As used herein, the term "binding" in the context of the binding of an antibody, immunoglobulin, antibody-binding fragment, or Fc-containing protein to either, e.g., a predetermined antigen, such as a cell surface protein or fragment thereof, typically refers to an interaction or association between a minimum of two entities or molecular structures, such as an antibody-antigen interaction.

**[0087]** For instance, binding affinity typically corresponds to a  $K_D$  value of about  $10^{-7}$  M or less, such as about  $10^{-8}$  M or less, such as about  $10^{-9}$  M or less when determined by, for instance, surface plasmon resonance (SPR) technology in a BIAcore 3000 instrument using the antigen as the ligand and the antibody, Ig, antibody-binding fragment, or Fc-containing protein as the analyte (or antiligand). Cell-based binding strategies, such as fluorescent-activated cell sorting (FACS) binding assays, are also routinely used, and FACS data correlates well with other methods such as radioligand competition binding and SPR (Benedict, CA, *J Immunol Methods*. 1997, 201(2):223-31; Geuijen, CA, et al. *J Immunol Methods*. 2005, 302(1-2):68-77).

**[0088]** Accordingly, the antibody or antigen-binding protein of the invention may bind to the predetermined antigen or cell surface molecule (receptor such as CD3) having an affinity corresponding to a  $K_D$  value that is at least ten-fold lower than its affinity for binding to a non-specific antigen (e.g., BSA, casein). According to the present invention, if the affinity of an antibody corresponding to a  $K_D$  value is equal to or less than ten-fold lower than a non-specific antigen, this may be considered non-detectable binding, however such an antibody may be paired with a second antigen binding arm for the production of a bispecific antibody of the invention.

**[0089]** The term " $K_D$ " (M) refers to the dissociation equilibrium constant of a particular antibody-antigen interaction, or the dissociation equilibrium constant of an antibody or antibody-binding fragment binding to an antigen. There is an inverse relationship between  $K_D$  and binding affinity, therefore the smaller the  $K_D$  value, the higher, *i.e.* stronger, the affinity. Thus, the terms "higher affinity" or "stronger affinity" relate to a higher ability to form an interaction and therefore a smaller  $K_D$  value, and conversely the terms "lower affinity" or "weaker affinity" relate to a lower ability to form an interaction and therefore a larger  $K_D$  value. In some circumstances, a higher binding affinity (or  $K_D$ ) of a particular molecule (*e.g.* antibody) to its interactive partner molecule (*e.g.* antigen X) compared to the binding affinity of the molecule (*e.g.* antibody) to another interactive partner molecule (*e.g.* antigen Y) may be expressed as a binding ratio determined by dividing the larger  $K_D$  value (lower, or weaker, affinity) by the smaller  $K_D$  (higher, or stronger, affinity), for example expressed as 5-fold or 10-fold greater binding affinity, as the case may be. For example, "low affinity" refers to less strong binding interaction. In some embodiments, the low binding affinity corresponds to greater than about 1 nM  $K_D$ , greater than about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or greater than about 40 nM  $K_D$ , wherein such  $K_D$  binding affinity value is measured in an *in vitro* surface plasmon resonance binding assay, or equivalent biomolecular interaction sensing assay. In some embodiments, the low binding affinity corresponds to greater than about 10 nM  $EC_{50}$ , greater than about 15 nM  $EC_{50}$ , 20 nM  $EC_{50}$ , greater than about 25 nM  $EC_{50}$ , 30 nM  $EC_{50}$ , greater than about 35 nM  $EC_{50}$ , or greater than about 40 nM  $EC_{50}$ , wherein such  $EC_{50}$  binding affinity value is measured in an *in vitro* FACS binding assay, or equivalent cell-based binding assay. "Weak affinity" refers to weak binding interaction. In some embodiments, the weak binding affinity corresponds to greater than about 100 nM  $K_D$  or  $EC_{50}$ , greater than about 200, 300, or greater than about 500 nM  $K_D$  or  $EC_{50}$ , wherein such  $K_D$  binding affinity value is measured in an *in vitro* surface plasmon resonance binding assay, or equivalent biomolecular interaction sensing assay, and such  $EC_{50}$  binding affinity value is measured in an *in vitro* FACS binding assay, or equivalent cell-based interaction detecting assay to detect monovalent binding. No detectable binding means that the affinity between the two biomolecules, for example, especially between the monovalent antibody binding arm and its target antigen, is beyond the detection limit of the assay being used.

**[0090]** The term " $k_d$ " (sec<sup>-1</sup> or 1/s) refers to the dissociation rate constant of a particular antibody-antigen interaction, or the dissociation rate constant of an antibody or antibody-binding fragment. Said value is also referred to as the  $k_{off}$  value.

**[0091]** The term " $k_a$ " (M<sup>-1</sup> x sec<sup>-1</sup> or 1/M) refers to the association rate constant of a particular antibody-antigen interaction, or the association rate constant of an antibody or antibody-binding fragment.

**[0092]** The term " $K_A$ " ( $M^{-1}$  or  $1/M$ ) refers to the association equilibrium constant of a particular antibody-antigen interaction, or the association equilibrium constant of an antibody or antibody-binding fragment. The association equilibrium constant is obtained by dividing the  $k_a$  by the  $k_d$ .

**[0093]** The term "EC<sub>50</sub>" or "EC<sub>50</sub>" refers to the half maximal effective concentration, which includes the concentration of an antibody which induces a response halfway between the baseline and maximum after a specified exposure time. The EC<sub>50</sub> essentially represents the concentration of an antibody where 50% of its maximal effect is observed. In certain embodiments, the EC<sub>50</sub> value equals the concentration of an antibody of the invention that gives half-maximal binding to cells expressing CD3 or tumor-associated antigen, as determined by *e.g.* a FACS binding assay. Thus, reduced or weaker binding is observed with an increased EC<sub>50</sub> value, or half maximal effective concentration value such that 500 nM EC<sub>50</sub> is indicative of a weaker binding affinity than 50 nM EC<sub>50</sub>.

**[0094]** In one embodiment, decreased binding can be defined as an increased EC<sub>50</sub> antibody concentration which enables binding to the half-maximal amount of target cells.

**[0095]** In other experimental measurements, the EC<sub>50</sub> value represents the concentration of an antibody of the invention that elicits half-maximal depletion of target cells by T cell cytotoxic activity. Thus, increased cytotoxic activity (*e.g.* T cell-mediated tumor cell killing) is observed with a decreased EC<sub>50</sub>, or half maximal effective concentration value.

#### Bispecific Antigen-Binding Molecules

**[0096]** The antibodies of the present invention may be bi-specific, or multispecific. Multispecific antibodies may be specific for one effector molecule, such as CD3, in combination with different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, *e.g.*, Tutt et al., 1991, J. Immunol. 147:60-69; Kufer *et al.*, 2004, Trends Biotechnol. 22:238-244. The anti-CD3 antibodies of the present invention can be linked to or co-expressed with another functional molecule, *e.g.*, another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment to produce a bi-specific or a multispecific antibody with a second binding specificity.

**[0097]** Use of the expression "anti-CD3 antibody" herein is intended to include both monospecific anti-CD3 antibodies as well as bispecific antibodies comprising a CD3-binding arm and a second arm that binds a target antigen. Thus, the present invention includes bispecific antibodies wherein one arm of an immunoglobulin binds human CD3, and the other arm of the immunoglobulin is specific for a target antigen. The target antigen that the

other arm of the CD3 bispecific antibody binds can be any antigen expressed on or in the vicinity of a cell, tissue, organ, microorganism or virus, against which a targeted immune response is desired. The CD3-binding arm can comprise any of the HCVR or CDR amino acid sequences as set forth in Table 2 herein. In certain embodiments, the CD3-binding arm binds weakly to human CD3 and induces human T cell activation. In other embodiments, the CD3-binding arm binds weakly to human CD3 and induces tumor-associated antigen-expressing cell killing in the context of a bispecific or multispecific antibody. In other embodiments, the CD3-binding arm binds or associated weakly with human and cynomolgus (monkey) CD3, yet the binding interaction is not detectable by *in vitro* assays known in the art. In some embodiments of the invention, the CD3-binding arm does not bind or associate with human and cynomolgus (monkey) CD3, yet the bispecific molecule still elicits tumor-associated cell killing.

**[0098]** In the context of bispecific antibodies of the present invention wherein one arm of the antibody binds CD3 and the other arm binds a target antigen, the target antigen can be a tumor-associated antigen (TAA). Non-limiting examples of specific tumor-associated antigens include, *e.g.*, AFP, ALK, BAGE proteins, BIRC5 (survivin), BIRC7,  $\beta$ -catenin, bcr-abl, BRCA1, BORIS, CA9, carbonic anhydrase IX, caspase-8, CALR, CCR5, CD19, CD20 (MS4A1), CD22, CD30, CD40, CDK4, CEA, CTLA4, cyclin-B1, CYP1B1, EGFR, EGFRvIII, ErbB2/Her2, ErbB3, ErbB4, ETV6-AML, EpCAM, EphA2, Fra-1, FOLR1, GAGE proteins (*e.g.*, GAGE-1, -2), GD2, GD3, GloboH, glypican-3, GM3, gp100, Her2, HLA/B-raf, HLA/k-ras, HLA/MAGE-A3, hTERT, LMP2, MAGE proteins (*e.g.*, MAGE-1, -2, -3, -4, -6, and -12), MART-1, mesothelin, ML-IAP, Muc1, Muc2, Muc3, Muc4, Muc5, Muc16 (CA-125), MUM1, NA17, NY-BR1, NY-BR62, NY-BR85, NY-ESO1, OX40, p15, p53, PAP, PAX3, PAX5, PCTA-1, PLAC1, PRLR, PRAME, PSMA (FOLH1), RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, STEAP1, STEAP2, TAG-72, TGF- $\beta$ , TMPRSS2, Thompson-nouvelle antigen (Tn), TRP-1, TRP-2, tyrosinase, and uroplakin-3.

**[0099]** The inventors envision that the present invention includes numerous examples of bispecific antibodies having a weak anti-CD3 binding arm made in accordance with the invention.

**[0100]** According to certain exemplary embodiments, the present invention includes bispecific antigen-binding molecules that specifically bind CD3 and PSMA. Such molecules may be referred to herein as, *e.g.*, "anti-CD3/anti-PSMA," or "anti-CD3xPSMA" or "CD3xPSMA" bispecific molecules, and so forth. The term "PSMA," as used herein, refers to the human PSMA protein unless specified as being from a non-human species (*e.g.*, "mouse PSMA," "monkey PSMA," etc.).

**[0101]** The term "PSMA" refers to prostate-specific membrane antigen, also known as folate hydrolase 1 (FOLH1) (UniProtKB/Swiss-Prot. No. Q04609; SEQ ID NO: 171). PSMA is

an integral, non-shed membrane glycoprotein that is highly expressed in prostate epithelial cells and is a cell-surface marker for prostate cancer.

**[0102]** According to other exemplary embodiments, the present invention includes bispecific antigen-binding molecules that specifically bind CD3 and EGFRvIII. Such molecules may be referred to herein as, *e.g.*, "anti-CD3/anti-EGFRvIII" or "anti-CD3xEGFRvIII" or "CD3xEGFRvIII" bispecific molecules, and so forth. The term "EGFRvIII" refers to the human EGFRvIII protein unless specified as being from a non-human species (*e.g.*, "mouse EGFRvIII," "monkey EGFRvIII," etc.).

**[0103]** The term "EGFRvIII" refers to the class III variant of the epidermal growth factor receptor (EGFRvIII; SEQ ID NO: 172) which is the most frequently found EGFR variant in glioblastoma (Bigner *et al.*, 1990, *Cancer Res* 50:8017-8022; Humphrey *et al.*, 1990, *Proc Natl Acad Sci USA* 87:4207-4211; Yamazaki *et al.*, 1990, *Jap J Cancer Res* 81:773-779; Ekstrand *et al.*, 1992, *Proc Natl Acad Sci USA* 89:4309-4313; Wikstrand *et al.*, 1995, *Cancer Res* 55:3140-3148; and Frederick *et al.*, 2000, *Cancer Res* 60:1383-1387). EGFRvIII is characterized by a deletion of exons 2-7 of the EGFR gene, resulting in an in-frame deletion of 801 base pairs of the coding region, *i.e.*, deletion of 6-273 amino acid residues (based on the residue numbers of mature EGFR; see UniProtKB/Swiss-Prot. No. P00533), as well as the generation of a new glycine at the fusion junction (Humphrey *et al.*, 1988, *Cancer Res* 48:2231-2238; Yamazaki *et al.*, 1990, *supra*). EGFRvIII has been shown to have a ligand-independent, weak but constitutively active kinase activity as well as enhanced tumorigenicity (Nishikawa *et al.*, 1994, *Proc Natl Acad Sci USA* 91:7727-7731; and Batra *et al.*, 1995, *Cell Growth and Differentiation* 6:1251-1259). In addition to gliomas, EGFRvIII has been detected in ductal and intraductal breast carcinoma (Wikstrand *et al.*, 1995, *Cancer Res* 55:3140-3148), non-small cell lung carcinomas (Garcia de Palazzo *et al.*, 1993, *Cancer Res* 53:3217-3220), ovarian carcinomas (Moscatello *et al.*, 1995, *Cancer Res* 55:5536-5539), prostate cancer (Olapade-Olaopa *et al.*, 2000, *British J Cancer* 82:186-194), and squamous cell carcinoma of the head and neck (Tinhofer *et al.*, 2011, *Clin Cancer Res* 17(15):5197-5204).

**[0104]** In still other exemplary embodiments, the present invention includes bispecific antigen-binding molecules that specifically bind CD3 and MUC16. Such molecules may be referred to herein as, *e.g.*, "anti-CD3/anti-MUC16" or "anti-CD3xMUC16" or "CD3xMUC16" bispecific molecules, and so forth. The term "MUC16" refers to the human MUC16 protein unless specified as being from a non-human species (*e.g.*, "mouse MUC16," "monkey MUC16," etc.).

**[0105]** Mucin 16 (MUC16; NCBI Reference Sequence: NP\_078966.2, SEQ ID NO: 173), otherwise known as cancer antigen 125 (CA-125), is a mucin encoded by *MUC16* gene in humans. The family of mucin proteins are known to protect the body from infection by

pathogen binding to oligosaccharides in the extracellular domain, preventing the pathogen from reaching the cell surface. For many years, overexpression of MUC16/CA125 has been used as a prognostic and diagnostic marker for ovarian cancer (Yin and Lloyd, 2001, *J. Biol. Chem.* 276 (29), 27371-27375; O'Brien, TJ, *et al.*, 2001, *Tumour Biol.* 22 (6), 348-366; Leggieri, C. *et al.*, 2014, *Eur. J. Gynaecol. Oncol.* 35 (4), 438-441). MUC16 has been shown to protect tumor cells from the immune system with its heavily glycosylated tandem repeat domain which can bind to galectin-1 (an immunosuppressive protein) (Seelenmeyer, C., *et al.*, 2003, *J. Cell. Sci.* 116 (Pt 7): 1305-18; O'Brien, TJ, *et al.*, 2002, *Tumour Biol.* 23 (3), 154-169). Natural killer cells and monocytes are unable to attack tumor cells expressing high levels of MUC16. In its normal physiologic role, MUC16-galectin interaction serves as a barrier for bacterial and viral infection, however MUC16 is believed to be immunoprotective in the context of tumor cells, thereby preventing cancer cell cytolysis (Felder, M. *et al.*, 2014, *Molecular Cancer*, 13:129). MUC16 is therefore a desirable target for immunotherapeutic bispecific antibody molecules administered to treat ovarian cancer by activating immune effector cells.

**[0106]** In still other exemplary embodiments, the present invention includes bispecific antigen-binding molecules that specifically bind CD3 and STEAP2. Such molecules may be referred to herein as, *e.g.*, "anti-CD3/anti-STEAP2" or "anti-CD3xSTEAP2" or "CD3xSTEAP2" bispecific molecules, and so forth. The term "STEAP2" refers to the human STEAP2 protein unless specified as being from a non-human species (*e.g.*, "mouse STEAP2," "monkey STEAP2," etc.). Six transmembrane epithelial antigen of the prostate 2 (STEAP2; UniProtKB/Swiss-Prot: Q8NFT2.3) is a 490-amino acid protein encoded by *STEAP2* gene located at the chromosomal region 7q21 in humans.

**[0107]** The aforementioned bispecific antigen-binding molecules that specifically bind tumor-associated antigen comprise an anti-CD3 antigen-binding molecule which binds to CD3 with a weak or no detectable binding affinity such as exhibiting a  $K_D$  of greater than about 100 nM, 300 nM or 500 nM, as measured by an *in vitro* affinity binding assay.

**[0108]** As used herein, the expression "antigen-binding molecule" means a protein, polypeptide or molecular complex comprising or consisting of at least one complementarity determining region (CDR) that alone, or in combination with one or more additional CDRs and/or framework regions (FRs), specifically binds to a particular antigen. In certain embodiments, an antigen-binding molecule is an antibody or a fragment of an antibody, as those terms are defined elsewhere herein.

**[0109]** As used herein, the expression "bispecific antigen-binding molecule" means a protein, polypeptide or molecular complex comprising at least a first antigen-binding domain and a second antigen-binding domain. Each antigen-binding domain within the bispecific antigen-binding molecule comprises at least one CDR that alone, or in combination with one

or more additional CDRs and/or FRs, specifically binds to a particular antigen. In the context of the present invention, the first antigen-binding domain specifically binds a first antigen (*e.g.*, CD3), and the second antigen-binding domain specifically binds a second, distinct antigen (*e.g.*, PSMA, MUC16, EGFRvIII or STEAP2).

**[0110]** In certain exemplary embodiments of the present invention, the bispecific antigen-binding molecule is a bispecific antibody. Each antigen-binding domain of a bispecific antibody comprises a heavy chain variable domain (HCVR) and a light chain variable domain (LCVR). In the context of a bispecific antigen-binding molecule comprising a first and a second antigen-binding domain (*e.g.*, a bispecific antibody), the CDRs of the first antigen-binding domain may be designated with the prefix "A1" and the CDRs of the second antigen-binding domain may be designated with the prefix "A2". Thus, the CDRs of the first antigen-binding domain may be referred to herein as A1-HCDR1, A1-HCDR2, and A1-HCDR3; and the CDRs of the second antigen-binding domain may be referred to herein as A2-HCDR1, A2-HCDR2, and A2-HCDR3.

**[0111]** The first antigen-binding domain and the second antigen-binding domain may be directly or indirectly connected to one another to form a bispecific antigen-binding molecule of the present invention. Alternatively, the first antigen-binding domain and the second antigen-binding domain may each be connected to a separate multimerizing domain. The association of one multimerizing domain with another multimerizing domain facilitates the association between the two antigen-binding domains, thereby forming a bispecific antigen-binding molecule. As used herein, a "multimerizing domain" is any macromolecule, protein, polypeptide, peptide, or amino acid that has the ability to associate with a second multimerizing domain of the same or similar structure or constitution. For example, a multimerizing domain may be a polypeptide comprising an immunoglobulin C<sub>H</sub>3 domain. A non-limiting example of a multimerizing component is an Fc portion of an immunoglobulin (comprising a C<sub>H</sub>2-C<sub>H</sub>3 domain), *e.g.*, an Fc domain of an IgG selected from the isotypes IgG1, IgG2, IgG3, and IgG4, as well as any allotype within each isotype group.

**[0112]** Bispecific antigen-binding molecules of the present invention will typically comprise two multimerizing domains, *e.g.*, two Fc domains that are each individually part of a separate antibody heavy chain. The first and second multimerizing domains may be of the same IgG isotype such as, *e.g.*, IgG1/IgG1, IgG2/IgG2, IgG4/IgG4. Alternatively, the first and second multimerizing domains may be of different IgG isotypes such as, *e.g.*, IgG1/IgG2, IgG1/IgG4, IgG2/IgG4, etc.

**[0113]** In certain embodiments, the multimerizing domain is an Fc fragment or an amino acid sequence of 1 to about 200 amino acids in length containing at least one cysteine residues. In other embodiments, the multimerizing domain is a cysteine residue, or a short

cysteine-containing peptide. Other multimerizing domains include peptides or polypeptides comprising or consisting of a leucine zipper, a helix-loop motif, or a coiled-coil motif.

**[0114]** Any bispecific antibody format or technology may be used to make the bispecific antigen-binding molecules of the present invention. For example, an antibody or fragment thereof having a first antigen binding specificity can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment having a second antigen-binding specificity to produce a bispecific antigen-binding molecule. Specific exemplary bispecific formats that can be used in the context of the present invention include, without limitation, *e.g.*, scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.*, common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED)body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab<sup>2</sup> bispecific formats (*see, e.g.*, Klein *et al.* 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats).

**[0115]** In the context of bispecific antigen-binding molecules of the present invention, the multimerizing domains, *e.g.*, Fc domains, may comprise one or more amino acid changes (*e.g.*, insertions, deletions or substitutions) as compared to the wild-type, naturally occurring version of the Fc domain. For example, the invention includes bispecific antigen-binding molecules comprising one or more modifications in the Fc domain that results in a modified Fc domain having a modified binding interaction (*e.g.*, enhanced or diminished) between Fc and FcRn. In one embodiment, the bispecific antigen-binding molecule comprises a modification in a C<sub>H</sub>2 or a C<sub>H</sub>3 region, wherein the modification increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.*, in an endosome where pH ranges from about 5.5 to about 6.0). Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (*e.g.*, E or Q); 250 and 428 (*e.g.*, L or F); 252 (*e.g.*, L/Y/F/W or T), 254 (*e.g.*, S or T), and 256 (*e.g.*, S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.*, L/R/S/P/Q or K) and/or 434 (*e.g.*, H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.*, 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.*, M428L) and 434S (*e.g.*, N434S) modification; a 428L, 259I (*e.g.*, V259I), and 308F (*e.g.*, V308F) modification; a 433K (*e.g.*, H433K) and a 434 (*e.g.*, 434Y) modification; a 252, 254, and 256 (*e.g.*, 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.*, T250Q and M428L); and a 307 and/or 308 modification (*e.g.*, 308F or 308P).

**[0116]** The present invention also includes bispecific antigen-binding molecules comprising a first C<sub>H</sub>3 domain and a second Ig C<sub>H</sub>3 domain, wherein the first and second Ig C<sub>H</sub>3 domains differ from one another by at least one amino acid, and wherein at least one

amino acid difference reduces binding of the bispecific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C<sub>H</sub>3 domain binds Protein A and the second Ig C<sub>H</sub>3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C<sub>H</sub>3 may further comprise a Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C<sub>H</sub>3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies.

**[0117]** In certain embodiments, the Fc domain may be chimeric, combining Fc sequences derived from more than one immunoglobulin isotype. For example, a chimeric Fc domain can comprise part or all of a C<sub>H</sub>2 sequence derived from a human IgG1, human IgG2 or human IgG4 C<sub>H</sub>2 region, and part or all of a C<sub>H</sub>3 sequence derived from a human IgG1, human IgG2 or human IgG4. A chimeric Fc domain can also contain a chimeric hinge region. For example, a chimeric hinge may comprise an "upper hinge" sequence, derived from a human IgG1, a human IgG2 or a human IgG4 hinge region, combined with a "lower hinge" sequence, derived from a human IgG1, a human IgG2 or a human IgG4 hinge region. A particular example of a chimeric Fc domain that can be included in any of the antigen-binding molecules set forth herein comprises, from N- to C-terminus: [IgG4 C<sub>H</sub>1] - [IgG4 upper hinge] - [IgG2 lower hinge] - [IgG4 CH2] - [IgG4 CH3]. Another example of a chimeric Fc domain that can be included in any of the antigen-binding molecules set forth herein comprises, from N- to C-terminus: [IgG1 C<sub>H</sub>1] - [IgG1 upper hinge] - [IgG2 lower hinge] - [IgG4 CH2] - [IgG1 CH3]. These and other examples of chimeric Fc domains that can be included in any of the antigen-binding molecules of the present invention are described in PCT International Publication No. WO2014/121087 A1, published August 7, 2014, which is herein incorporated by reference in its entirety. Chimeric Fc domains having these general structural arrangements, and variants thereof, can have altered Fc receptor binding, which in turn affects Fc effector function.

**[0118]** In certain embodiments, the invention provides an antibody heavy chain wherein the heavy chain constant region (CH) region comprises an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to any one of SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 189, SEQ ID NO: 190 or SEQ ID NO: 191. In some embodiments, the heavy chain constant region (CH) region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID

NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 189, SEQ ID NO: 190 and SEQ ID NO: 191.

**[0119]** In other embodiments, the invention provides an antibody heavy chain wherein the Fc domain comprises an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to any one of SEQ ID NO: 192, SEQ ID NO: 193, SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197, SEQ ID NO: 198, SEQ ID NO: 199, SEQ ID NO: 200 or SEQ ID NO: 201. In some embodiments, the Fc domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 192, SEQ ID NO: 193, SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197, SEQ ID NO: 198, SEQ ID NO: 199, SEQ ID NO: 200 and SEQ ID NO: 201.

#### Other Fc Variants

**[0120]** According to certain embodiments of the present invention, anti-CD3 antibodies, and anti-CD3/anti-TAA bispecific antigen-binding molecules, are provided comprising an Fc domain comprising one or more mutations which enhance or diminish antibody binding to the FcRn receptor, *e.g.*, at acidic pH as compared to neutral pH. For example, the present invention includes antibodies comprising a mutation in the C<sub>H</sub>2 or a C<sub>H</sub>3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.*, in an endosome where pH ranges from about 5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the antibody when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (*e.g.*, E or Q); 250 and 428 (*e.g.*, L or F); 252 (*e.g.*, L/Y/F/W or T), 254 (*e.g.*, S or T), and 256 (*e.g.*, S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.*, H/L/R/S/P/Q or K) and/or 434 (*e.g.*, H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.*, 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.*, M428L) and 434S (*e.g.*, N434S) modification; a 428L, 259I (*e.g.*, V259I), and 308F (*e.g.*, V308F) modification; a 433K (*e.g.*, H433K) and a 434 (*e.g.*, 434Y) modification; a 252, 254, and 256 (*e.g.*, 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.*, T250Q and M428L); and a 307 and/or 308 modification (*e.g.*, 308F or 308P).

**[0121]** For example, the present invention includes anti-CD3 antibodies, and anti-CD3/anti-TAA bispecific antigen-binding molecules, comprising an Fc domain comprising one or more pairs or groups of mutations selected from the group consisting of: 250Q and 248L (*e.g.*, T250Q and M248L); 252Y, 254T and 256E (*e.g.*, M252Y, S254T and T256E); 428L and 434S (*e.g.*, M428L and N434S); and 433K and 434F (*e.g.*, H433K and N434F). All possible combinations of the foregoing Fc domain mutations, and other mutations within the

antibody variable domains disclosed herein, are contemplated within the scope of the present invention.

#### Biological Characteristics of the Antibodies and Bispecific Antigen-Binding Molecules

**[0122]** The present invention includes bispecific antigen-binding molecules (*e.g.*, bispecific antibodies) which are capable of simultaneously binding to human CD3 and a human TAA. According to certain embodiments, the bispecific antigen-binding molecules of the invention specifically interact with cells that express CD3 and/or TAA, such as PSMA, EGFRvIII or MUC16. The binding arm that interacts with cells that express CD3 may have weak to no detectable binding as measured in a suitable *in vitro* binding assay. The extent to which a bispecific antigen-binding molecule binds cells that express CD3 and/or TAA can be assessed by fluorescence activated cell sorting (FACS), as illustrated in Example 4 herein.

**[0123]** For example, the present invention includes antibodies, antigen-binding fragments, and bispecific antibodies thereof which specifically bind human T-cell lines which express CD3 but not the TAA (*e.g.*, Jurkat), primate T-cells (*e.g.*, cynomolgus peripheral blood mononuclear cells [PBMCs]), and/or TAA-expressing cells. The present invention includes bispecific antigen-binding molecules which bind any of the aforementioned T cells and T cell lines with an  $EC_{50}$  value of from about  $1.8 \times 10^{-8}$  (18 nM) to about  $2.1 \times 10^{-7}$  (210 nM), or more (*i.e.* weaker affinity), and includes bispecific antibodies for which  $EC_{50}$  is undetectable, as determined using a FACS binding assay as set forth in Example 4 or a substantially similar assay. In certain embodiments, the antibodies, antigen-binding fragments, and bispecific antibodies of the present invention bind CD3 with an  $EC_{50}$  of greater than about 30 nM, greater than about 40 nM, greater than about 45 nM, greater than about 50 nM, greater than about 55 nM, greater than about 60 nM, greater than about 65 nM, greater than about 70 nM, greater than about 75 nM, at least 80 nM, greater than about 90 nM, greater than about 100 nM, greater than about 110 nM, at least 120 nM, greater than about 130 nM, greater than about 140 nM, greater than about 150 nM, at least 160 nM, greater than about 170 nM, greater than about 180 nM, greater than about 190 nM, greater than about 200 nM, greater than about 250 nM, greater than about 300 nM, greater than about 500 nM, greater than about 1  $\mu$ M, greater than about 2  $\mu$ M, or greater than about 3  $\mu$ M, or no detectable binding affinity, as measured by FACS binding, *e.g.*, using an assay format as defined in Example 4 herein, or a substantially similar assay.

**[0124]** The present invention also includes antibodies, antigen-binding fragments, and bispecific antibodies thereof which bind to TAA-expressing cells and cell lines, such as PSMA-, EGFRvIII-, STEAP2- and MUC16-expressing cell lines, with an  $EC_{50}$  value of less than about 100 nM, or even less concentration necessary for binding (*i.e.* stronger affinity) such as less than 5.6 nM ( $5.6 \times 10^{-9}$ ), as determined using a FACS binding assay as set forth

in Example 4 or a substantially similar cell-based assay. The present invention includes bispecific antigen-binding molecules which bind any of the aforementioned tumor cell lines with an EC<sub>50</sub> value of less than about 50 nM, less than about 45 nM, less than about 40 nM, less than about 35 nM, less than about 30 nM, less than about 25 nM, less than about 20 nM, less than about 15 nM, less than about 10 nM, less than about 6 nM, less than about 5 nM, or less than about 1 nM, *e.g.* using the aforementioned assay.

**[0125]** The present invention includes antibodies, antigen-binding fragments, and bispecific antibodies thereof that bind human CD3 with low, weak or even no detectable affinity. According to certain embodiments, the present invention includes antibodies and antigen-binding fragments of antibodies that bind human CD3 (*e.g.*, at 37°C) with a K<sub>D</sub> of greater than about 11 nM, includes antibodies that bind CD3 with a K<sub>D</sub> of greater than about 100 nM or 500 nM, and also includes antibodies having no detectable binding affinity, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 5 herein. In certain embodiments, the antibodies or antigen-binding fragments of the present invention bind CD3 with a K<sub>D</sub> of greater than about 15 nM, greater than about 20 nM, greater than about 25 nM, greater than about 30 nM, greater than about 35 nM, greater than about 40 nM, greater than about 45 nM, greater than about 50 nM, greater than about 55 nM, greater than about 60 nM, greater than about 65 nM, greater than about 70 nM, greater than about 75 nM, at least 80 nM, greater than about 90 nM, greater than about 100 nM, greater than about 110 nM, at least 120 nM, greater than about 130 nM, greater than about 140 nM, greater than about 150 nM, at least 160 nM, greater than about 170 nM, greater than about 180 nM, greater than about 190 nM, greater than about 200 nM, greater than about 250 nM, greater than about 300 nM, greater than about 1 μM, greater than about 2 μM, or greater than about 3 μM, or no detectable affinity, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 5 herein (*e.g.*, mAb-capture or antigen-capture format), or a substantially similar assay.

**[0126]** The present invention includes antibodies, antigen-binding fragments, and bispecific antibodies thereof that bind monkey (*i.e.* cynomolgus) CD3 with low, weak, or even no detectable affinity. According to certain embodiments, the present invention includes antibodies, antigen-binding fragments, and bispecific antibodies thereof that bind human CD3 (*e.g.*, at 37°C) with a K<sub>D</sub> of greater than about 10 nM, includes antibodies that bind CD3 with a K<sub>D</sub> of greater than about 100 nM or 500 nM, and also includes antibodies having no detectable binding affinity, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 5 herein. In certain embodiments, the antibodies or antigen-binding fragments of the present invention bind CD3 with a K<sub>D</sub> of greater than about 15 nM, greater than about 20 nM, greater than about 25 nM, greater than about 30 nM, greater than about 35 nM, greater than about 40 nM, greater than about 45

nM, greater than about 50 nM, greater than about 55 nM, greater than about 60 nM, greater than about 65 nM, greater than about 70 nM, greater than about 75 nM, at least 80 nM, greater than about 90 nM, greater than about 100 nM, greater than about 110 nM, at least 120 nM, greater than about 130 nM, greater than about 140 nM, greater than about 150 nM, at least 160 nM, greater than about 170 nM, greater than about 180 nM, greater than about 190 nM, greater than about 200 nM, greater than about 250 nM, greater than about 300 nM, greater than about 1  $\mu$ M, greater than about 2  $\mu$ M, or greater than about 3  $\mu$ M, or no detectable affinity, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 5 herein (*e.g.*, mAb-capture or antigen-capture format), or a substantially similar assay.

**[0127]** The present invention includes antibodies, antigen-binding fragments, and bispecific antibodies thereof that bind human CD3 and induce T cell activation. For example, the present invention includes anti-CD3 antibodies that induce human T cell activation with an EC<sub>50</sub> value of less than about 113 pM, as measured by an *in vitro* T cell activation assay, *e.g.*, using the assay format as defined in Example 6 herein [*e.g.*, assessing the percent activated (CD69+) cells out of total T cells (CD2+) in the presence of anti-CD3 antibodies], or a substantially similar assay that assesses T cell in their activated state. In certain embodiments, the antibodies or antigen-binding fragments of the present invention induce human T cell activation [*e.g.*, percent activated (CD69+) T cells] with an EC<sub>50</sub> value of less than about 100 pM, less than about 50 pM, less than about 20 pM, less than about 19 pM, less than about 18 pM, less than about 17 pM, less than about 16 pM, less than about 15 pM, less than about 14 pM, less than about 13 pM, less than about 12 pM, less than about 11 pM, less than about 10 pM, less than about 9 pM, less than about 8 pM, less than about 7 pM, less than about 6 pM, less than about 5 pM, less than about 4 pM, less than about 3 pM, less than about 2 pM, or less than about 1 pM, as measured by an *in vitro* T cell activation assay, *e.g.*, using the assay format as defined in Example 6 herein, or a substantially similar assay. Anti-CD3 antibodies that have weak or no detectable binding to CD3 have the ability to induce T cell activation with high potency (*i.e.* pM range), despite having weak or no detectable binding affinity to CD3, as exemplified in Example 6 herein.

**[0128]** The present invention also includes antibodies, antigen-binding fragments, and bispecific antibodies that bind human CD3 and induce T cell-mediated killing of tumor antigen-expressing cells. For example, the present invention includes anti-CD3 antibodies that induce T cell-mediated killing of tumor cells with an EC<sub>50</sub> of less than about 1.3 nM, as measured in an *in vitro* T cell-mediated tumor cell killing assay, *e.g.*, using the assay format as defined in Example 6 herein (*e.g.*, assessing the extent of tumor antigen-expressing cells, such as PSMA-expressing, EGFRvIII-expressing or MUC16-expressing cell killing by human PBMCs in the presence of anti-CD3 antibodies), or a substantially similar assay. In certain

embodiments, the antibodies or antigen-binding fragments of the present invention induce T cell-mediated tumor cell killing (*e.g.*, PBMC-mediated killing of OVCAR3 cells) with an EC<sub>50</sub> value of less than about 1 nM, less than about 400 pM, less than about 250 pM, less than about 100 pM, less than about 50 pM, less than about 40 pM, less than about 30 pM, less than about 20 pM, less than about 10 pM, less than about 9 pM, less than about 8 pM, less than about 7 pM, less than about 6 pM, less than about 5 pM, less than about 4 pM, less than about 3 pM, less than about 2 pM, or less than about 1 pM, as measured by an *in vitro* T cell-mediated tumor cell killing assay, *e.g.*, using the assay format as defined in Example 6 herein, or a substantially similar assay.

**[0129]** The present invention also includes antibodies, antigen-binding fragments, and bispecific antibodies that bind CD3 with a dissociative half-life ( $t_{1/2}$ ) of less than about 10 minutes as measured by surface plasmon resonance at 25°C or 37°C, *e.g.*, using an assay format as defined in Example 5 herein, or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments of the present invention bind CD3 with a  $t_{1/2}$  of less than about 9 minutes, of less than about 8 minutes, of less than about 7 minutes, of less than about 6 minutes, of less than about 5 minutes, of less than about 4 minutes, of less than about 3 minutes, of less than about 2 minutes, of less than about 1.9 minutes, or less than about 1.8 minutes, or exhibit very weak or no detectable binding as measured by surface plasmon resonance at 25°C or 37°C, *e.g.*, using an assay format as defined in Example 5 herein (*e.g.*, mAb-capture or antigen-capture format), or a substantially similar assay.

**[0130]** The anti-CD3/anti-TAA bispecific antigen-binding molecules of the present invention may additionally exhibit one or more characteristics selected from the group consisting of: (a) inducing PBMC proliferation *in vitro*; (b) activating T-cells via inducing IFN- $\gamma$  release and CD25 up-regulation in human whole blood; and (c) inducing T-cell mediated cytotoxicity on anti-TAA-resistant cell lines.

**[0131]** The present invention includes anti-CD3/anti-TAA bispecific antigen-binding molecules which are capable of depleting tumor antigen-expressing cells in a subject (*see, e.g.*, Example 7). For example, according to certain embodiments, anti-CD3/anti-PSMA, anti-CD3/anti-MUC16, or anti-CD3/anti-STEAP2 bispecific antigen-binding molecules are provided, wherein a single administration of 1  $\mu$ g, or 10  $\mu$ g, or 100  $\mu$ g of the bispecific antigen-binding molecule to a subject (*e.g.*, at a dose of about 0.1 mg/kg, about 0.08 mg/kg, about 0.06 mg/kg, about 0.04 mg/kg, about 0.04 mg/kg, about 0.02 mg/kg, about 0.01 mg/kg, or less) causes a reduction in the number of tumor antigen-expressing cells in the subject (*e.g.*, tumor growth in the subject is suppressed or inhibited) below detectable levels. In certain embodiments, a single administration of the anti-CD3/anti-PSMA bispecific antigen-binding molecule at a dose of about 0.4 mg/kg causes a reduction in tumor growth in the

subject below detectable levels by about day 7, about day 6, about day 5, about day 4, about day 3, about day 2, or about day 1 after administration of the bispecific antigen-binding molecule to the subject. According to certain embodiments, a single administration of an anti-CD3/anti-PSMA bispecific antigen-binding molecule of the invention, at a dose of at least about 0.01 mg/kg, causes the number of PSMA-expressing tumor cells to remain below detectable levels until at least about 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days or more, following the administration. As used herein, the expression "below detectable levels" means that no tumor cells can be directly or indirectly detected growing subcutaneously in a subject using standard caliper measurement methods, *e.g.*, as set forth in Example 7, herein. In certain embodiments, a single administration of the anti-CD3/anti-MUC16 bispecific antigen-binding molecule at a dose of about 10 µg causes a suppression of tumor growth in the subject at about day 6, and maintains tumor suppression until at least day 26 after administration of the bispecific antigen-binding molecule to the subject. In subjects receiving a single administration of the anti-CD3/anti-MUC16 bispecific antigen-binding molecule at a dose of about 10 µg at least 7 days after tumor implantation, the bispecific antigen-binding molecule exhibits efficacy in suppression of established tumors from further growth in the subject at about day 26 after tumor implantation in the subject. According to certain embodiments, a single administration of an anti-CD3/anti-MUC16 bispecific antigen-binding molecule of the invention, at a dose of at least about 0.1 mg/kg, inhibits growth of MUC16-expressing tumor cells for at least about 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or more, following administration of the bispecific molecule. See, *e.g.* Example 8.

**[0132]** In certain embodiments, a single administration of the anti-CD3/anti-STEAP2 bispecific antigen-binding molecule at a dose of about 0.1 mg/kg or 0.01 mg/kg maintains a suppression of tumor growth until at least day 46 after administration of the bispecific antigen-binding molecule and tumor to the subject. According to certain embodiments, a single administration of an anti-CD3/anti-STEAP2 bispecific antigen-binding molecule of the invention, at a dose of at least about 0.1 mg/kg, about 0.08 mg/kg, about 0.06 mg/kg, about 0.05 mg/kg, about 0.04 mg/kg, about 0.03 mg/kg, about 0.02 mg/kg, about 0.01 mg/kg, or less inhibits growth of STEAP2-expressing tumor cells for at least about 20 days, 30 days, 35 days, 40 days, 45 days or more, following administration of the bispecific molecule. See, *e.g.* Example 10.

**[0133]** In other embodiments, anti-CD3/anti-TAA bispecific antigen-binding molecules having a CD3 targeted binding arm having weak binding affinity to effector cells exhibit reduced drug elimination rates compared to the bispecific antibodies comprising the same anti-TAA binding arm and a strong CD3 binding arm administered in an *in vivo*

pharmacokinetic study. The results suggest that the bispecific molecules comprising weaker binding of the CD3 targeting arm may exhibit beneficial drug exposure levels ( $AUC_{last}$ ) and drug elimination profiles (antibody clearance). See, *e.g.*, Example 9.

**[0134]** The present invention provides anti-CD3/anti-PSMA, anti-CD3/anti-MUC16 and anti-CD3/anti-STEAP2 bispecific antigen-binding molecules (*i.e.* anti-CD3/anti-TAA bispecific antigen-binding molecules) which exhibit one or more characteristics selected from the group consisting of: (a) inhibiting tumor growth in immunocompromised mice bearing human prostate cancer xenografts; (b) inhibiting tumor growth in immunocompetent mice bearing human prostate cancer xenografts; (c) suppressing tumor growth of established tumors in immunocompromised mice bearing human prostate cancer xenografts; and (d) reducing tumor growth of established tumors in immunocompetent mice bearing human prostate cancer xenografts (*see, e.g.*, Examples 7, 8 and 10). The present invention also provides anti-CD3/anti-PSMA, anti-CD3/anti-MUC16 and anti-CD3/anti-STEAP2 bispecific antibodies (*i.e.* anti-CD3/anti-TAA bispecific antibodies) comprising a first heavy chain directed to an effector T cell (*i.e.* CD3), and ii) a second heavy chain directed to a target tumor cell, wherein the bispecific antibodies exhibit weak binding or no detectable binding to the effector cells, and exhibit tumor growth suppression and reduced antibody clearance (*i.e.* elimination) from the body compared to bispecific antibodies that exhibit strong binding to effector cells.

#### Epitope Mapping and Related Technologies

**[0135]** The epitope on CD3 to which the antigen-binding molecules of the present invention bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids of a CD3 protein. Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) of CD3. The antibodies of the invention may interact with amino acids contained within a single CD3 chain (*e.g.*, CD3-epsilon, CD3-delta or CD3-gamma), or may interact with amino acids on two or more different CD3 chains. The term "epitope," as used herein, refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstances, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

**[0136]** Various techniques known to persons of ordinary skill in the art can be used to determine whether an antigen-binding domain of an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, *e.g.*, routine cross-blocking assay such as that described Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY), alanine scanning mutational analysis, peptide blots analysis (Reineke, 2004, *Methods Mol Biol* 248:443-463), and peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer, 2000, *Protein Science* 9:487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antigen-binding domain of an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water to allow hydrogen-deuterium exchange to occur at all residues except for the residues protected by the antibody (which remain deuterium-labeled). After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. *See, e.g.*, Ehrling (1999) *Analytical Biochemistry* 267(2):252-259; Engen and Smith (2001) *Anal. Chem.* 73:256A-265A. X-ray crystallography of the antigen/antibody complex may also be used for epitope mapping purposes.

**[0137]** The present invention further includes anti-PSMA antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein (*e.g.* antibodies comprising any of the amino acid sequences as set forth in Table 6 herein). Likewise, the present invention also includes anti-PSMA antibodies that compete for binding to PSMA with any of the specific exemplary antibodies described herein (*e.g.* antibodies comprising any of the amino acid sequences as set forth in Table 6 herein). Anti-PSMA antibodies disclosed in U.S. Application No. 15/223,434 are herein incorporated by reference into this application.

**[0138]** The present invention also includes bispecific antigen-binding molecules comprising a first antigen-binding domain that specifically binds human CD3 and/or cynomolgus CD3 with low or detectable binding affinity, and a second antigen binding domain that specifically binds human tumor-associated antigen (TAA), wherein the first antigen-binding domain binds to the same epitope on CD3 as any of the specific exemplary CD3-specific antigen-binding domains described herein.

**[0139]** Likewise, the present invention also includes bispecific antigen-binding molecules comprising a first antigen-binding domain that specifically binds human CD3 and/or cynomolgus CD3 with low or detectable binding affinity, and a second antigen binding domain that specifically binds human tumor-associated antigen (TAA), wherein the first

antigen-binding domain competes for binding to CD3 with any of the specific exemplary CD3-specific antigen-binding domains described herein.

**[0140]** One can easily determine whether a particular antigen-binding molecule (*e.g.*, antibody) or antigen-binding domain thereof binds to the same epitope as, or competes for binding with, a reference antigen-binding molecule of the present invention by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope on CD3 (or TAA) as a reference bispecific antigen-binding molecule of the present invention, the reference bispecific molecule is first allowed to bind to a CD3 protein (or TAA protein). Next, the ability of a test antibody to bind to the CD3 molecule is assessed. If the test antibody is able to bind to CD3 (or TAA) following saturation binding with the reference bispecific antigen-binding molecule, it can be concluded that the test antibody binds to a different epitope of CD3 (or TAA) than the reference bispecific antigen-binding molecule. On the other hand, if the test antibody is not able to bind to the CD3 (or TAA) molecule following saturation binding with the reference bispecific antigen-binding molecule, then the test antibody may bind to the same epitope of CD3 (or TAA) as the epitope bound by the reference bispecific antigen-binding molecule of the invention. Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference bispecific antigen-binding molecule or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. If the reference antibody is one that has no measurable binding as exemplified herein, then the reference antibody may be mutated back to germline sequence in order to determine binding to the CD3 for purpose of comparing epitope interaction, or comparing its binding properties to the test antibody as described herein. Experiments of this sort can be performed using ELISA, RIA, Biacore, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art. In accordance with certain embodiments of the present invention, two antigen-binding proteins bind to the same (or overlapping) epitope if, *e.g.*, a 1-, 5-, 10-, 20- or 100-fold excess of one antigen-binding protein inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.*, Junghans et al., Cancer Res. 1990:50:1495-1502). Alternatively, two antigen-binding proteins are deemed to bind to the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antigen-binding protein reduce or eliminate binding of the other. Two antigen-binding proteins are deemed to have "overlapping epitopes" if only a subset of the amino acid mutations that reduce or eliminate binding of one antigen-binding protein reduce or eliminate binding of the other.

**[0141]** To determine if an antibody or antigen-binding domain thereof competes for binding with a reference antigen-binding molecule, the above-described binding methodology is

performed in two orientations: In a first orientation, the reference antigen-binding molecule is allowed to bind to a CD3 protein (or TAA protein) under saturating conditions followed by assessment of binding of the test antibody to the CD3 (or TAA) molecule. In a second orientation, the test antibody is allowed to bind to a CD3 (or TAA) molecule under saturating conditions followed by assessment of binding of the reference antigen-binding molecule to the CD3 (or TAA) molecule. If, in both orientations, only the first (saturating) antigen-binding molecule is capable of binding to the CD3 (or TAA) molecule, then it is concluded that the test antibody and the reference antigen-binding molecule compete for binding to CD3 (or TAA). As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antigen-binding molecule may not necessarily bind to the same epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope. If the reference antibody is one that has no measurable binding as exemplified herein, then the reference antibody may be mutated back to germline sequence in order to determine binding to the CD3 for purpose of comparing epitope interaction, or comparing its binding properties or blocking interaction with the test antibody as described herein.

#### Preparation of Antigen-Binding Domains and Construction of Bispecific Molecules

**[0142]** Antigen-binding domains specific for particular antigens can be prepared by any antibody generating technology known in the art. Once obtained, two different antigen-binding domains, specific for two different antigens (*e.g.*, CD3 and TAA), can be appropriately arranged relative to one another to produce a bispecific antigen-binding molecule of the present invention using routine methods. (A discussion of exemplary bispecific antibody formats that can be used to construct the bispecific antigen-binding molecules of the present invention is provided elsewhere herein). In certain embodiments, one or more of the individual components (*e.g.*, heavy and light chains) of the multispecific antigen-binding molecules of the invention are derived from chimeric, humanized or fully human antibodies. Methods for making such antibodies are well known in the art. For example, one or more of the heavy and/or light chains of the bispecific antigen-binding molecules of the present invention can be prepared using VELOCIMMUNE™ technology. Using VELOCIMMUNE™ technology (or any other human antibody generating technology), high affinity chimeric antibodies to a particular antigen (*e.g.*, CD3 or TAA) are initially isolated having a human variable region and a mouse constant region. The antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate fully human heavy and/or light chains that can be incorporated into the bispecific antigen-binding molecules of the present invention.

**[0143]** Genetically engineered animals may be used to make human bispecific antigen-binding molecules. For example, a genetically modified mouse can be used which is incapable of rearranging and expressing an endogenous mouse immunoglobulin light chain variable sequence, wherein the mouse expresses only one or two human light chain variable domains encoded by human immunoglobulin sequences operably linked to the mouse kappa ( $\kappa$ ) constant gene at the endogenous mouse kappa ( $\kappa$ ) locus. Such genetically modified mice can be used to produce fully human bispecific antigen-binding molecules comprising two different heavy chains that associate with an identical light chain that comprises a variable domain derived from one of two different human light chain variable region gene segments. (See, e.g., US 2011/0195454 for a detailed discussion of such engineered mice and the use thereof to produce bispecific antigen-binding molecules). Antibodies of the invention may comprise immunoglobulin heavy chains associated with a common light chain. The common light chain may be derived from a cognate light chain of the anti-TAA heavy chain, or derived from a known or public domain light chain variable region derived from a light chain exhibiting promiscuity or ability to pair with a wide variety of non-cognate heavy chains, *i.e.* a universal or common light chain. Antibodies of the invention may comprise immunoglobulin heavy chains associated with a single rearranged light chain. In some embodiments, the light chain a variable domain derived from a human Vk1-39 gene segment or a Vk3-20 gene segment. In other embodiments, the light chain comprises a variable domain derived from a human Vk1-39 gene segment rearranged with a human Jk5 or a human Jk1 gene segment, or a Vk3-20 gene segment rearranged with a human Jk1 gene segment, or a Vk1-39 gene segment rearranged with a human Jk1 gene segment.

#### Bioequivalents

**[0144]** The present invention encompasses antigen-binding molecules having amino acid sequences that vary from those of the exemplary molecules disclosed herein but that retain the ability to bind or interact with CD3 and/or TAA. Such variant molecules may comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but exhibit biological activity that is essentially equivalent to that of the described bispecific antigen-binding molecules.

**[0145]** The present invention includes antigen-binding molecules that are bioequivalent to any of the exemplary antigen-binding molecules set forth herein. Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single dose or multiple dose. Some antigen-binding proteins will be considered equivalents or pharmaceutical alternatives if they are equivalent in the

extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, e.g., chronic use, and are considered medically insignificant for the particular drug product studied.

**[0146]** In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

**[0147]** In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

**[0148]** In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

**[0149]** Bioequivalence may be demonstrated by *in vivo* and *in vitro* methods. Bioequivalence measures include, e.g., (a) an *in vivo* test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an *in vitro* test that has been correlated with and is reasonably predictive of human *in vivo* bioavailability data; (c) an *in vivo* test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antigen-binding protein.

**[0150]** Bioequivalent variants of the exemplary bispecific antigen-binding molecules set forth herein may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antigen-binding proteins may include variants of the exemplary bispecific antigen-binding molecules set forth herein comprising amino acid changes which modify the glycosylation characteristics of the molecules, e.g., mutations which eliminate or remove glycosylation.

#### Species Selectivity and Species Cross-Reactivity

**[0151]** According to certain embodiments of the invention, antigen-binding molecules are provided which display weak or no interaction with human CD3 and weak or no interaction

with CD3 from other species, such as cynomolgous monkey CD3. Also provided are antigen-binding molecules which bind to human TAA but not to TAA from other species. The present invention also includes antigen-binding molecules that bind to human CD3 and to CD3 from one or more non-human species; and/or antigen-binding molecules that bind to human TAA and to TAA from one or more non-human species.

**[0152]** According to certain exemplary embodiments of the invention, antigen-binding molecules are provided which bind weakly to human CD3 and/or human TAA and may bind or not bind, as the case may be, to one or more of mouse, rat, guinea pig, hamster, gerbil, pig, cat, dog, rabbit, goat, sheep, cow, horse, camel, cynomolgus, marmoset, rhesus or chimpanzee CD3 and/or TAA. For example, in certain exemplary embodiments of the present invention bispecific antigen-binding molecules are provided comprising a first antigen-binding domain that weakly binds human CD3 and cynomolgus CD3, and a second antigen-binding domain that specifically binds human PSMA, MUC16, EGFRvIII or STEAP2.

#### Immunoconjugates

**[0153]** The present invention encompasses antigen-binding molecules conjugated to a therapeutic moiety ("immunoconjugate"), such as a cytotoxin, a chemotherapeutic drug, an immunosuppressant or a radioisotope. Cytotoxic agents include any agent that is detrimental to cells. Examples of suitable cytotoxic agents and chemotherapeutic agents for forming immunoconjugates are known in the art, (see for example, WO 05/103081).

#### Therapeutic Formulation and Administration

**[0154]** The present invention provides pharmaceutical compositions comprising the antigen-binding molecules of the present invention. The pharmaceutical compositions of the invention are formulated with suitable carriers, excipients, and other agents that provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™, Life Technologies, Carlsbad, CA), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

**[0155]** The dose of antigen-binding molecule administered to a patient may vary depending upon the age and the size of the patient, target disease, conditions, route of administration, and the like. The preferred dose is typically calculated according to body weight or body surface area. When a bispecific antigen-binding molecule of the present

invention is used for therapeutic purposes in an adult patient, it may be advantageous to intravenously administer the bispecific antigen-binding molecule of the present invention normally at a single dose of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. Effective dosages and schedules for administering a bispecific antigen-binding molecule may be determined empirically; for example, patient progress can be monitored by periodic assessment, and the dose adjusted accordingly. Moreover, interspecies scaling of dosages can be performed using well-known methods in the art (*e.g.*, Mordenti *et al.*, 1991, *Pharmaceut. Res.* 8:1351).

**[0156]** Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu *et al.*, 1987, *J. Biol. Chem.* 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

**[0157]** A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

**[0158]** Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX

75/25<sup>TM</sup> pen, HUMALOG<sup>TM</sup> pen, HUMALIN 70/30<sup>TM</sup> pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN<sup>TM</sup> I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR<sup>TM</sup> (Novo Nordisk, Copenhagen, Denmark), BD<sup>TM</sup> pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN<sup>TM</sup>, OPTIPEN PRO<sup>TM</sup>, OPTIPEN STARLET<sup>TM</sup>, and OPTICLIK<sup>TM</sup> (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to the SOLOSTAR<sup>TM</sup> pen (sanofi-aventis), the FLEXPEN<sup>TM</sup> (Novo Nordisk), and the KWIKPEN<sup>TM</sup> (Eli Lilly), the SURECLICK<sup>TM</sup> Autoinjector (Amgen, Thousand Oaks, CA), the PENLET<sup>TM</sup> (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA<sup>TM</sup> Pen (Abbott Labs, Abbott Park IL), to name only a few.

**[0159]** In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201). In another embodiment, polymeric materials can be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Florida. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527-1533.

**[0160]** The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (*e.g.*, ethanol), a polyalcohol (*e.g.*, propylene glycol, polyethylene glycol), a nonionic surfactant [*e.g.*, polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, *e.g.*, sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

**[0161]** Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills,

capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

#### Therapeutic Uses of the Antigen-Binding Molecules

**[0162]** The present invention includes methods comprising administering to a subject in need thereof a therapeutic composition comprising an anti-tumor antibody or antigen-binding fragment thereof, or a bispecific antigen-binding molecule that specifically binds weakly or has no detectable binding to CD3 and binds a tumor-associated antigen. The therapeutic composition can comprise any of the antibodies or bispecific antigen-binding molecules as disclosed herein and a pharmaceutically acceptable carrier or diluent. As used herein, the expression "a subject in need thereof" means a human or non-human animal that exhibits one or more symptoms or indicia of cancer (*e.g.*, a subject expressing a tumor or suffering from any of the cancers mentioned herein below), or who otherwise would benefit from an inhibition or reduction in tumor activity or a depletion of tumor cells (*e.g.*, PSMA++ prostate cancer cells).

**[0163]** The antibodies and bispecific antigen-binding molecules of the invention (and therapeutic compositions comprising the same) are useful, *inter alia*, for treating any disease or disorder in which stimulation, activation and/or targeting of an immune response would be beneficial. In particular, the bispecific antigen-binding molecules of the present invention may be used for the treatment, prevention and/or amelioration of any disease or disorder associated with or mediated by a cell expressing a TAA, *e.g.* PSMA expression or activity or the proliferation of PSMA+ cells. The mechanism of action by which the therapeutic methods of the invention are achieved include killing of the cells expressing tumor-associated antigens, in the presence of effector cells, for example, by CDC, apoptosis, ADCC, phagocytosis, or by a combination of two or more of these mechanisms. Cells expressing tumor-associated antigens, such as PSMA, MUC16, STEAP2 or EGFRvIII, which can be inhibited or killed using the bispecific antigen-binding molecules of the invention include, for example, prostate tumor cells.

**[0164]** The antigen-binding molecules of the present invention may be used to treat, *e.g.*, primary and/or metastatic tumors arising in the brain and meninges, head and neck, oropharynx, lung and bronchial tree, gastrointestinal tract, male and female reproductive tract, muscle, bone, skin and appendages, connective tissue, spleen, immune system, blood forming cells and bone marrow, liver and urinary tract, kidney, bladder and/or special sensory organs such as the eye. In certain embodiments, the bispecific antigen-binding molecules of the invention are used to treat one or more of, but not limited to, the following cancers: pancreatic carcinoma, head and neck cancer, prostate cancer, malignant gliomas,

osteosarcoma, colorectal cancer, gastric cancer (*e.g.*, gastric cancer with MET amplification), malignant mesothelioma, multiple myeloma, ovarian cancer, small cell lung cancer, non-small cell lung cancer, synovial sarcoma, thyroid cancer, breast cancer, melanomaglioma, breast cancer (*e.g.* ductal or intraductal breast carcinoma, squamous cell carcinoma, esophageal cancer, clear cell renal cell carcinoma, chromophobe renal cell carcinoma, (renal) oncocytoma, (renal) transitional cell carcinoma, urothelial carcinoma, (bladder) adenocarcinoma, or (bladder) small cell carcinoma. According to certain embodiments of the present invention, the bispecific antibodies are useful for treating a patient afflicted with a refractory or treatment-resistant cancer, *e.g.* castrate-resistant prostate cancer. According to exemplary embodiments of the invention, methods are provided comprising administering an anti-CD3/anti-PSMA bispecific antigen-binding molecule as disclosed herein to a patient who is afflicted with a castrate-resistant prostate cancer. Analytic/diagnostic methods known in the art, such as tumor scanning, etc., may be used to ascertain whether a patient harbors a tumor that is castrate-resistant.

**[0165]** The present invention also includes methods for treating residual cancer in a subject. As used herein, the term "residual cancer" means the existence or persistence of one or more cancerous cells in a subject following treatment with an anti-cancer therapy, such as a first-line or standard therapy.

**[0166]** According to certain aspects, the present invention provides methods for treating a cancer associated with TAA expression (*e.g.*, prostate cancer associated with PSMA expression or STEAP2 expression, glioblastoma associated with EGFRvIII expression, or ovarian cancer associated with MUC16 expression) comprising administering one or more of the bispecific antigen-binding molecules described elsewhere herein to a subject after the subject has been determined to have the cancer. For example, the present invention includes methods for treating prostate cancer comprising administering an anti-CD3/anti-TAA bispecific antigen-binding molecule to a patient 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks or 4 weeks, 2 months, 4 months, 6 months, 8 months, 1 year, or more after the subject has received a previous therapy.

#### Combination Therapies and Formulations

**[0167]** The present invention provides methods which comprise administering a pharmaceutical composition comprising any of the exemplary antibodies and bispecific antigen-binding molecules described herein in combination with one or more additional therapeutic agents. Exemplary additional therapeutic agents that may be combined with or administered in combination with an antigen-binding molecule of the present invention include, *e.g.*, an anti-Programmed Cell Death 1 antibody (*e.g.* an anti-PD1 antibody as described in U.S. Pat. Appln. Pub. No. US2015/0203579A1), an anti-Programmed Cell

Death Ligand-1 (e.g. an anti-PD-L1 antibody as described in U.S. Pat. Appln. Pub. No. US2015/0203580A1), an EGFR antagonist (e.g., an anti-EGFR antibody [e.g., cetuximab or panitumumab] or small molecule inhibitor of EGFR [e.g., gefitinib or erlotinib]), an antagonist of another EGFR family member such as Her2/ErbB2, ErbB3 or ErbB4 (e.g., anti-ErbB2, anti-ErbB3 or anti-ErbB4 antibody or small molecule inhibitor of ErbB2, ErbB3 or ErbB4 activity), an antagonist of EGFRvIII (e.g., an antibody that specifically binds EGFRvIII), a cMET antagonist (e.g., an anti-cMET antibody), an IGF1R antagonist (e.g., an anti-IGF1R antibody), a B-raf inhibitor (e.g., vemurafenib, sorafenib, GDC-0879, PLX-4720), a PDGFR- $\alpha$  inhibitor (e.g., an anti-PDGFR- $\alpha$  antibody), a PDGFR- $\beta$  inhibitor (e.g., an anti-PDGFR- $\beta$  antibody), a VEGF antagonist (e.g., a VEGF-Trap, *see, e.g.*, US 7,087,411 (also referred to herein as a "VEGF-inhibiting fusion protein"), anti-VEGF antibody (e.g., bevacizumab), a small molecule kinase inhibitor of VEGF receptor (e.g., sunitinib, sorafenib or pazopanib), a DLL4 antagonist (e.g., an anti-DLL4 antibody disclosed in US 2009/0142354 such as REGN421), an Ang2 antagonist (e.g., an anti-Ang2 antibody disclosed in US 2011/0027286 such as H1H685P), a FOLH1 (PSMA) antagonist, a PRLR antagonist (e.g., an anti-PRLR antibody), a STEAP1 or STEAP2 antagonist (e.g., an anti-STEAP1 antibody or an anti-STEAP2 antibody), a TMPRSS2 antagonist (e.g., an anti-TMPRSS2 antibody), a MSLN antagonist (e.g., an anti-MSLN antibody), a CA9 antagonist (e.g., an anti-CA9 antibody), a uroplakin antagonist (e.g., an anti-uroplakin antibody), etc. Other agents that may be beneficially administered in combination with the antigen-binding molecules of the invention include cytokine inhibitors, including small-molecule cytokine inhibitors and antibodies that bind to cytokines such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-11, IL-12, IL-13, IL-17, IL-18, or to their respective receptors. The pharmaceutical compositions of the present invention (e.g., pharmaceutical compositions comprising an anti-CD3/anti-PSMA bispecific antigen-binding molecule as disclosed herein) may also be administered as part of a therapeutic regimen comprising one or more therapeutic combinations selected from "ICE": ifosfamide (e.g., Ifex®), carboplatin (e.g., Paraplatin®), etoposide (e.g., Etopophos®, Toposar®, VePesid®, VP-16); "DHAP": dexamethasone (e.g., Decadron®), cytarabine (e.g., Cytosar-U®, cytosine arabinoside, ara-C), cisplatin (e.g., Platinol®-AQ); and "ESHAP": etoposide (e.g., Etopophos®, Toposar®, VePesid®, VP-16), methylprednisolone (e.g., Medrol®), high-dose cytarabine, cisplatin (e.g., Platinol®-AQ).

**[0168]** The present invention also includes therapeutic combinations comprising any of the antigen-binding molecules mentioned herein and an inhibitor of one or more of VEGF, Ang2, DLL4, EGFR, ErbB2, ErbB3, ErbB4, EGFRvIII, cMet, IGF1R, B-raf, PDGFR- $\alpha$ , PDGFR- $\beta$ , FOLH1 (PSMA), PRLR, STEAP1, STEAP2, TMPRSS2, MSLN, CA9, uroplakin, or any of the aforementioned cytokines, wherein the inhibitor is an aptamer, an antisense molecule, a ribozyme, an siRNA, a peptibody, a nanobody or an antibody fragment (e.g., Fab fragment;

F(ab')<sub>2</sub> fragment; Fd fragment; Fv fragment; scFv; dAb fragment; or other engineered molecules, such as diabodies, triabodies, tetrabodies, minibodies and minimal recognition units). The antigen-binding molecules of the invention may also be administered and/or co-formulated in combination with antivirals, antibiotics, analgesics, corticosteroids and/or NSAIDs. The antigen-binding molecules of the invention may also be administered as part of a treatment regimen that also includes radiation treatment and/or conventional chemotherapy.

**[0169]** The additional therapeutically active component(s) may be administered just prior to, concurrent with, or shortly after the administration of an antigen-binding molecule of the present invention; (for purposes of the present disclosure, such administration regimens are considered the administration of an antigen-binding molecule "in combination with" an additional therapeutically active component).

**[0170]** The present invention includes pharmaceutical compositions in which an antigen-binding molecule of the present invention is co-formulated with one or more of the additional therapeutically active component(s) as described elsewhere herein.

#### Administration Regimens

**[0171]** According to certain embodiments of the present invention, multiple doses of the bispecific antigen-binding molecule (*e.g.*, an anti-TAA bispecific antigen-binding molecule) may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject multiple doses of an antigen-binding molecule of the invention. As used herein, "sequentially administering" means that each dose of an antigen-binding molecule is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of an antigen-binding molecule, followed by one or more secondary doses of the antigen-binding molecule, and optionally followed by one or more tertiary doses of the antigen-binding molecule.

**[0172]** The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the antigen-binding molecule of the invention. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of the antigen-binding molecule, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of an antigen-binding molecule contained in the initial, secondary and/or tertiary doses varies from one another (*e.g.*, adjusted up or down as appropriate) during the course

of treatment. In certain embodiments, two or more (e.g., 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (e.g., "maintenance doses").

**[0173]** In one exemplary embodiment of the present invention, each secondary and/or tertiary dose is administered 1 to 26 (e.g., 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21, 21½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of antigen-binding molecule which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

**[0174]** The methods according to this aspect of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of an antigen-binding molecule (e.g., an anti-TAA bispecific antigen-binding molecule). For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (e.g., 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (e.g., 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

**[0175]** In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 2 weeks after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 2 to 4 weeks after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

## **EXAMPLES**

**[0176]** The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and

deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

#### **Example 1: Generation of Anti-CD3 Antibodies**

**[0177]** The following procedures were aimed at identifying antibodies that specifically recognized CD3 (T cell co-receptor) as an antigen.

**[0178]** A pool of anti-CD3 antibodies were derived by immunizing genetically modified mice. Briefly, mice genetically engineered to express reverse chimeric (human variable, mouse constant) and immunoglobulin heavy chains associated with a single rearranged light chain (e.g., a V $\kappa$ 1-39/J or a V $\kappa$ 3-20/J), were immunized with a CD3 antigen and generated B cells that comprised a diversity of human VH rearrangements in order to express a diverse repertoire of high-affinity antigen-specific antibodies. Certain exemplified antibodies described in the subject application have been made recombinantly and express the same light chain sequence of V $\kappa$ 1-39JK5 (LCVR set forth in SEQ ID NO: 162), while other antibodies made recombinantly express a cognate light chain of one of the heavy chain arms (e.g. the tumor target arm).

**[0179]** Generated antibodies were tested for affinity to human and cynomolgus monkey CD3 antigen in an *in vitro* binding assay, and e.g. one CD3 antibody: designated CD3-VH-P (HCVR set forth in SEQ ID NO: 154) was identified, amongst a few others, that were found to bind to both human and cyno CD3 having an EC<sub>50</sub> between 1 and 40 nM affinity (+++), as determined in a FACS titration of Jurkat cells and cynomolgus T cells, respectively. See, e.g. FACS binding experiments outlined in Example 4 herein below.

**[0180]** The germline amino acid residues of CD3-VH-P were subsequently identified and an antibody designated "CD3-VH-G" was engineered to contain only germline frameworks. Other antibody derivatives were engineered by well-known molecular cloning techniques to replace amino acid residues in a stepwise manner based on differences between the germline sequence and the CD3-VH-P sequence. Each antibody derivative is given a "CD3-VH-G" number designation. See Table 1 and Figure 1.

**[0181]** Bispecific antibodies, comprising a first binding arm derived from the engineered anti-CD3 antibodies with the designations and descriptions shown in Table 1, and a second binding arm derived from anti-TAA antibodies, were prepared and tested for monovalent affinity to CD3-bearing cells in a FACS assay (as described in Example 4). The monovalent binding affinity results of these bispecific antibodies are shown in the two right columns of Table 1. In specific examples, bispecific antibodies having a TAA-binding arm and a CD3-binding arm with designations "CD3-VH-G," "CD3-VH-G5," and "CD3-VH-G20," respectively, bound Jurkat cells with an EC<sub>50</sub> of 2.7E-08, no detectable binding, and 5.5E-07, respectively.

**Table 1: Mutations to CDRs Based on the Germline Sequence and Corresponding FACS Binding Affinity for Each Engineered Antibody**

| <b>Antibody CD3-VH Designation</b> | <b>Description of Mutations compared to antibody CD3-VH-G*</b> | <b>JURKAT</b> | <b>cyno T cells</b> |
|------------------------------------|----------------------------------------------------------------|---------------|---------------------|
| CD3-VH-G                           | Germline (GL) only framework regions (FRs); CD3-VHP CDRs       | (+ + +)       | (+ + +)             |
| CD3-VH-G2                          | All GL (FRs and CDRs).                                         | (-)           | (-)                 |
| CD3-VH-G3                          | All GL (FRs and CDRs).<br>Add back A33S.                       | (-)           | (-)                 |
| CD3-VH-G4                          | All GL (FRs and CDRs).<br>Add back Y105K                       | (-)           | (-)                 |
| CD3-VH-G5                          | All GL (FRs and CDRs).<br>Add back A33S and Y105K.             | (-)           | (-)                 |
| CD3-VH-G8                          | Germline frameworks.<br>Add back K58I                          | (+ + +)       | (+)                 |
| CD3-VH-G9                          | Germline frameworks.<br>Add back Y99D                          | (+)           | (-)                 |
| CD3-VH-G10                         | Germline frameworks.<br>Add back H108Y                         | (+)           | (-)                 |
| CD3-VH-G11                         | Germline frameworks.<br>Add back L111M                         | (+ + +)       | (+)                 |
| CD3-VH-G12                         | Germline frameworks.<br>Add back K58I, Y99D                    | (+ +)         | (+/-)               |
| CD3-VH-G13                         | Germline frameworks.<br>Add back K58I, H108Y                   | (+ +)         | (+)                 |
| CD3-VH-G14                         | Germline frameworks.<br>Add back K58I, L111M                   | (+ + +)       | (+ +)               |
| CD3-VH-G15                         | Germline frameworks.<br>Add back Y99D, H108Y                   | (+)           | (-)                 |
| CD3-VH-G16                         | Germline frameworks.<br>Add back Y99D, L111M                   | (+ +)         | (+/-)               |
| CD3-VH-G17                         | Germline frameworks.<br>Add back H108Y, L111M                  | (+/-)         | (+/-)               |
| CD3-VH-G18                         | Germline frameworks.<br>Add back K58I, Y99D, H108Y             | (+/-)         | (-)                 |
| CD3-VH-G19                         | Germline frameworks.<br>Add back K58I, Y99D, L111M             | (+/-)         | (-)                 |
| CD3-VH-G20                         | Germline frameworks.<br>Add back K58I, H108Y, L111M            | (+/-)         | (+/-)               |
| CD3-VH-G21                         | Germline frameworks.<br>Add back Y99D, H108Y, L111M            | (+/-)         | (-)                 |

\* Sequential numbering based on 7221G (CD3-VH-G) mature protein

**[0182]** While CD3-VH-G and some other engineered antibodies retained their binding affinity as seen in the FACS assays, several anti-CD3 antibodies bound to human or cyno CD3 *in vitro* with weak (+/-) to no (-) measurable affinity. Binding affinities, binding kinetics, and other biological properties to elucidate toxicity and pharmacokinetic (pK) profiles were subsequently investigated for bispecific antibodies comprising the exemplary anti-CD3 antibodies generated in accordance with the methods of this Example, and are described in detail in the Examples set forth below.

**Example 2: Heavy and Light Chain Variable Regions (Amino Acid and Nucleic Acid Sequences of the CDRs)**

**[0183]** Amino acid and nucleic acid sequences were determined for each antibody heavy chain sequence. Each antibody heavy chain, as a derivative of the germline sequence IGHV3-9\*01/D5-12\*01/J6\*02 (SEQ ID NO: 181) was assigned a “G” number designation for consistent nomenclature. Table 2 sets forth the amino acid sequence identifiers of the heavy chain variable regions and CDRs of the engineered anti-CD3 antibodies of the invention. The corresponding nucleic acid sequence identifiers are set forth in Table 3. The amino acid and nucleic acid sequence identifiers of the light chain variable region and CDRs to construct each recombinant antibody are also identified below in Tables 4 and 5, respectively.

**Table 2: Heavy Chain Amino Acid Sequence Identifiers**

| Antibody<br>CD3-VH<br>Designation | SEQ ID NOs: |      |      |      |
|-----------------------------------|-------------|------|------|------|
|                                   | HCVR        | CDR1 | CDR2 | CDR3 |
| CD3-VH-G                          | 2           | 4    | 6    | 8    |
| CD3-VH-G2                         | 10          | 12   | 14   | 16   |
| CD3-VH-G3                         | 18          | 20   | 22   | 24   |
| CD3-VH-G4                         | 26          | 28   | 30   | 32   |
| CD3-VH-G5                         | 34          | 36   | 38   | 40   |
| CD3-VH-G8                         | 42          | 44   | 46   | 48   |
| CD3-VH-G9                         | 50          | 52   | 54   | 56   |
| CD3-VH-G10                        | 58          | 60   | 62   | 64   |
| CD3-VH-G11                        | 66          | 68   | 70   | 72   |
| CD3-VH-G12                        | 74          | 76   | 78   | 80   |
| CD3-VH-G13                        | 82          | 84   | 86   | 88   |
| CD3-VH-G14                        | 90          | 92   | 94   | 96   |
| CD3-VH-G15                        | 98          | 100  | 102  | 104  |
| CD3-VH-G16                        | 106         | 108  | 110  | 112  |

|            |     |     |     |     |
|------------|-----|-----|-----|-----|
| CD3-VH-G17 | 114 | 116 | 118 | 120 |
| CD3-VH-G18 | 122 | 124 | 126 | 128 |
| CD3-VH-G19 | 130 | 132 | 134 | 136 |
| CD3-VH-G20 | 138 | 140 | 142 | 144 |
| CD3-VH-G21 | 146 | 148 | 150 | 152 |
| CD3-VH-P   | 154 | 156 | 158 | 160 |

**Table 3: Heavy Chain Nucleic Acid Sequence Identifiers**

| Antibody<br>CD3-VH<br>Designation | SEQ ID NOs: |      |      |      |
|-----------------------------------|-------------|------|------|------|
|                                   | HCVR        | CDR1 | CDR2 | CDR3 |
| CD3-VH-G                          | 1           | 3    | 5    | 7    |
| CD3-VH-G2                         | 9           | 11   | 13   | 15   |
| CD3-VH-G3                         | 17          | 19   | 21   | 23   |
| CD3-VH-G4                         | 25          | 27   | 29   | 31   |
| CD3-VH-G5                         | 33          | 35   | 37   | 39   |
| CD3-VH-G8                         | 41          | 43   | 45   | 47   |
| CD3-VH-G9                         | 49          | 51   | 53   | 55   |
| CD3-VH-G10                        | 57          | 59   | 61   | 63   |
| CD3-VH-G11                        | 65          | 67   | 69   | 71   |
| CD3-VH-G12                        | 73          | 75   | 77   | 79   |
| CD3-VH-G13                        | 81          | 83   | 85   | 87   |
| CD3-VH-G14                        | 89          | 91   | 93   | 95   |
| CD3-VH-G15                        | 97          | 99   | 101  | 103  |
| CD3-VH-G16                        | 105         | 107  | 109  | 111  |
| CD3-VH-G17                        | 113         | 115  | 117  | 119  |
| CD3-VH-G18                        | 121         | 123  | 125  | 127  |
| CD3-VH-G19                        | 129         | 131  | 133  | 135  |
| CD3-VH-G20                        | 137         | 139  | 141  | 143  |
| CD3-VH-G21                        | 145         | 147  | 149  | 151  |
| CD3-VH-P                          | 153         | 155  | 157  | 159  |

**Table 4: Light Chain Amino Acid Sequence Identifiers**

| Antibody<br>ULC<br>Designation | SEQ ID NOs: |      |      |      |
|--------------------------------|-------------|------|------|------|
|                                | LCVR        | CDR1 | CDR2 | CDR3 |
| V <sub>k</sub> 1-39JK5         | 162         | 164  | 166  | 168  |

**Table 5: Light Chain Nucleic Acid Sequence Identifiers**

| Antibody<br>ULC<br>Designation | SEQ ID NOs: |      |      |      |
|--------------------------------|-------------|------|------|------|
|                                | LCVR        | CDR1 | CDR2 | CDR3 |
| Vk1-39JK5                      | 161         | 163  | 165  | 167  |

**[0184]** Control 1 antibody designated "CD3-L2K" was constructed based on a known anti-CD3 antibody (*i.e.*, the anti-CD3 antibody "L2K" as set forth in WO2004/106380).

**[0185]** Isotype Control Antibody, referred to in the Examples hereinbelow, is an isotype matched (modified IgG4) antibody that interacts with an irrelevant antigen, *i.e.* FeID1 antigen.

### **Example 3: Generation of ULC Bispecific Antibodies that Bind CD3 and Tumor-Associated Antigens (TAA)**

**[0186]** Bispecific antibodies comprising an anti-CD3-specific binding domain and an anti-TAA-specific binding domain, such as PSMA, EGFRvIII, MUC16, or STEAP2, were constructed using standard molecular biology methodologies utilizing a heavy chain from an anti-CD3 antibody described herein, a heavy chain from an anti-TAA antibody and a common light chain or a universal light chain (ULC). The anti-TAA antibodies used to construct the bispecific antibodies of this invention were obtained by immunizing genetically modified mice.

**[0187]** A summary of the component parts of the antigen-binding domains of the various bispecific antibodies made in accordance with this Example is set forth below in Tables 6, 7 and 8. All bispecific antibodies were manufactured having a modified (chimeric) IgG4 Fc domain as set forth in US Patent Application Publication No. US20140243504A1, published on August 28, 2014. Exemplary EGFRvIIIxCD3 bispecific antibodies can be prepared using any of the heavy chain and light chain variable regions (or CDRs) of any of the EGFRvIII antibodies discussed in US Patent Application Publication NO. US20150259423, which is hereby incorporated by reference in its entirety, in combination with the variable regions or CDRs of any of the anti-CD3 antibodies discussed herein.

**Table 6: Construction of PSMAxCD3 Bispecific Antibodies**

| Bispecific Antibody Identifier | Anti-PSMA Antigen-Binding Domain   | Anti-CD3 Antigen-Binding Domain    | Common Light Chain Variable Region |
|--------------------------------|------------------------------------|------------------------------------|------------------------------------|
|                                | <i>Heavy Chain Variable Region</i> | <i>Heavy Chain Variable Region</i> |                                    |

|                 |           |            |           |
|-----------------|-----------|------------|-----------|
| BSPSMA/CD3-003  | PSMA-VH-B | CD3-VH-G   | Vk1-39JK5 |
| BSPSMA/CD3-200  |           | CD3-VH-G2  |           |
| BSPSMA/CD3-300  |           | CD3-VH-G3  |           |
| BSPSMA/CD3-400  |           | CD3-VH-G4  |           |
| BSPSMA/CD3-004  |           | CD3-VH-G5  |           |
| BSPSMA/CD3-800  |           | CD3-VH-G8  |           |
| BSPSMA/CD3-900  |           | CD3-VH-G9  |           |
| BSPSMA/CD3-1000 |           | CD3-VH-G10 |           |
| BSPSMA/CD3-1100 |           | CD3-VH-G11 |           |
| BSPSMA/CD3-1200 |           | CD3-VH-G12 |           |
| BSPSMA/CD3-1300 |           | CD3-VH-G13 |           |
| BSPSMA/CD3-1400 |           | CD3-VH-G14 |           |
| BSPSMA/CD3-1500 |           | CD3-VH-G15 |           |
| BSPSMA/CD3-1600 |           | CD3-VH-G16 |           |
| BSPSMA/CD3-1700 |           | CD3-VH-G17 |           |
| BSPSMA/CD3-1800 |           | CD3-VH-G18 |           |
| BSPSMA/CD3-1900 |           | CD3-VH-G19 |           |
| BSPSMA/CD3-005  |           | CD3-VH-G20 |           |
| BSPSMA/CD3-2100 |           | CD3-VH-G21 |           |

**Table 7: Construction of EGFRvIII xCD3 Bispecific Antibodies**

| Bispecific Antibody Identifier | Anti-EGFRvIII Antigen-Binding Domain | Anti-CD3 Antigen-Binding Domain    | Common Light Chain Variable Region |
|--------------------------------|--------------------------------------|------------------------------------|------------------------------------|
|                                | <i>Heavy Chain Variable Region</i>   | <i>Heavy Chain Variable Region</i> |                                    |
| BSV3/CD3-001                   | EGFRvIII-VH-A                        | CD3-VH-G                           | EGFRvIII-VL-A                      |
| BSV3/CD3-002                   |                                      | CD3-VH-G5                          |                                    |
| BSV3/CD3-003                   |                                      | CD3-VH-G9                          |                                    |
| BSV3/CD3-004                   |                                      | CD3-VH-G10                         |                                    |

**Table 8: Construction of MUC16xCD3 Bispecific Antibodies**

| Bispecific Antibody Identifier | Anti-MUC16 Antigen-Binding Domain | Anti-CD3 Antigen-Binding Domain | Common Light Chain Variable Region |
|--------------------------------|-----------------------------------|---------------------------------|------------------------------------|
|--------------------------------|-----------------------------------|---------------------------------|------------------------------------|

|                 | <b>Heavy Chain<br/>Variable Region</b> | <b>Heavy Chain<br/>Variable Region</b> |            |
|-----------------|----------------------------------------|----------------------------------------|------------|
| BSMUC16/CD3-001 | MUC16-VH-A                             | CD3-VH-G                               | MUC16-VL-A |
| BSMUC16/CD3-002 |                                        | CD3-VH-G5                              |            |
| BSMUC16/CD3-003 |                                        | CD3-VH-G9                              |            |
| BSMUC16/CD3-004 |                                        | CD3-VH-G10                             |            |
| BSMUC16/CD3-005 |                                        | CD3-VH-G20                             |            |

**Table 9: Construction of STEAP2xCD3 Bispecific Antibodies**

| <b>Bispecific<br/>Antibody Identifier</b> | <b>Anti-STEAP2<br/>Antigen-Binding<br/>Domain</b> | <b>Anti-CD3<br/>Antigen-Binding<br/>Domain</b> | <b>Common Light<br/>Chain Variable<br/>Region</b> |
|-------------------------------------------|---------------------------------------------------|------------------------------------------------|---------------------------------------------------|
|                                           | <b>Heavy Chain<br/>Variable Region</b>            | <b>Heavy Chain<br/>Variable Region</b>         |                                                   |
| BSSTEAP2/CD3-001                          | STEAP2-VH-A                                       | CD3-VH-G                                       | STEAP2-VL-A                                       |
| BSSTEAP2/CD3-002                          |                                                   | CD3-VH-G5                                      |                                                   |
| BSSTEAP2/CD3-003                          |                                                   | CD3-VH-G20                                     |                                                   |

[0188] Each of the exemplary bispecific antibodies were tested in various bioassays as described herein below.

#### **Example 4: Binding Affinities of Exemplified Bispecific Antibodies as Measured by FACS Analysis**

[0189] In this example, the ability of CD3xTAA bispecific antibodies to bind to human and cynomolgus CD3-expressing cell lines via FACS was determined. Additionally, the ability of these bispecific antibodies to bind to target-specific (TAA-specific) cell lines was also confirmed. As described above, the various bispecific antibodies of this invention utilized a single TAA-specific binding arm (PSMA, EGFRvIII, MUC16, or STEAP2; see Example 3, Tables 6, 7 and 8) paired with one of a panel of anti-CD3 binding arms (see Examples 1 and 2 hereinabove) and a common light chain. As is also shown in Example 5, the CD3xTAA bispecific antibodies displayed a range of affinities to human soluble heterodimeric hCD3 $\epsilon$ / $\delta$ .mFc protein via surface plasmon resonance.

[0190] Briefly,  $2 \times 10^5$  cells/well of human CD3-expressing Jurkat, cynomolgus T or TAA-specific expressing cells were incubated with a serial dilution of bispecific antibodies for 30 min at 4°C. After incubation, cells were washed and a goat F(ab')<sub>2</sub> anti-human Fc $\gamma$ PE

labeled secondary (Jackson Immunolabs) was added to the cells for an additional 30 min. Next, cells were washed, re-suspended in cold PBS + 1% BSA and analyzed via flow cytometry on a BD FACS Canto II.

**[0191]** For FACS analysis, cells were gated by forward scatter height vs. forward scatter area for single events selection, followed by side and forward scatters. The EC<sub>50</sub> for cell binding titration was determined using PRISM™ software (GraphPad Software, Inc., La Jolla, CA). Values were calculated using 4-parameter non-linear regression analysis.

**Table 10A: FACS Binding on CD3 and PSMA-Specific Cell lines**

| Bispecific Antibody Identifier | Anti-CD3-Binding Arm | Jurkat               | Cyno T-cells         | B16F10.9/PSMA        |
|--------------------------------|----------------------|----------------------|----------------------|----------------------|
|                                |                      | EC <sub>50</sub> [M] | EC <sub>50</sub> [M] | EC <sub>50</sub> [M] |
| BSPSMA/CD3-003                 | CD3-VH-G             | 1.65E-08             | 1.42E-08             | 2.26E-09             |
| BSPSMA/CD3-200                 | CD3-VH-G2            | NB                   | NB                   | 1.88E-09             |
| BSPSMA/CD3-300                 | CD3-VH-G3            | NB                   | NB                   | 1.90E-09             |
| BSPSMA/CD3-400                 | CD3-VH-G4            | NB                   | NB                   | 1.72E-09             |
| BSPSMA/CD3-004                 | CD3-VH-G5            | Very weak            | NB                   | 1.31E-09             |
| BSPSMA/CD3-800                 | CD3-VH-G8            | 1.93E-08             | 1.96E-08             | 1.31E-09             |
| BSPSMA/CD3-900                 | CD3-VH-G9            | 2.74E-07             | NB                   | 1.43E-09             |
| BSPSMA/CD3-1000                | CD3-VH-G10           | 2.77E-07             | NB                   | 1.19E-09             |
| BSPSMA/CD3-1100                | CD3-VH-G11           | 1.83E-08             | 8.90E-07             | 1.03E-09             |
| BSPSMA/CD3-1200                | CD3-VH-G12           | 4.72E-08             | NB                   | 1.16E-09             |
| BSPSMA/CD3-1300                | CD3-VH-G13           | 1.02E-07             | 2.17E-06             | 1.25E-09             |
| BSPSMA/CD3-1400                | CD3-VH-G14           | 3.19E-08             | 1.70E-07             | 1.30E-09             |
| BSPSMA/CD3-1500                | CD3-VH-G15           | 9.30E-08             | NB                   | 1.21E-09             |
| BSPSMA/CD3-1600                | CD3-VH-G16           | 5.68E-08             | NB                   | 1.03E-09             |
| BSPSMA/CD3-1700                | CD3-VH-G17           | 2.00E-07             | 3.35E-06             | 1.34E-09             |
| BSPSMA/CD3-1800                | CD3-VH-G18           | 1.26E-07             | NB                   | 2.16E-09             |
| BSPSMA/CD3-1900                | CD3-VH-G19           | 6.07E-08             | NB                   | 1.35E-09             |
| BSPSMA/CD3-005                 | CD3-VH-G20           | 2.10E-07             | 6.14E-06             | 2.09E-09             |
| BSPSMA/CD3-2100                | CD3-VH-G21           | 1.06E-07             | NB                   | 1.14E-09             |

**Table 10B: FACS Binding on CD3 and EGFRvIII-Specific Cell lines**

| Bispecific Antibody Identifier | Anti-CD3 Binding Arm | Jurkat               | Cyno T-Cells         | U87/EGFRvIII         |
|--------------------------------|----------------------|----------------------|----------------------|----------------------|
|                                |                      | EC <sub>50</sub> [M] | EC <sub>50</sub> [M] | EC <sub>50</sub> [M] |
| BSV3/CD3-001                   | CD3-VH-G             | 1.46E-09             | NT                   | 2.40E-09             |
| BSV3/CD3-002                   | CD3-VH-G5            | Very weak            | NT                   | 5.60E-09             |

**Table 10C: FACS Binding on CD3 and MUC16-Specific Cell lines**

| Bispecific Antibody Identifier | Anti-CD3 Binding Arm | Jurkat               | Cyno T-cells         | OVCAR3 (MUC16+)      |
|--------------------------------|----------------------|----------------------|----------------------|----------------------|
|                                |                      | EC <sub>50</sub> [M] | EC <sub>50</sub> [M] | EC <sub>50</sub> [M] |
| BSMUC16/CD3-001                | CD3-VH-G             | 3.21E-09             | NT                   | 1.20E-09             |
| BSMUC16/CD3-002                | CD3-VH-G5            | Very weak            | NT                   | 2.69E-09             |

[0192] As shown in Table 10A, the CD3 binding arms of each CD3xPSMA bispecific antibody displayed a range of cell binding affinity to human CD3 expressing Jurkat cells (15 to 300 nM EC<sub>50</sub> range). Importantly, the CD3 arms that showed weak-to-no binding to human CD3 heterodimeric protein via surface plasmon resonance (see Table 11 hereinbelow) also correlated with weak to no observable binding on Jurkat cells (*i.e.* CD3-VH-G2, CD3-VH-G3, CD3-VH-G5). Non-detectable binding, or no detectable binding, in the FACS assay or equivalent assay means that the affinity between the antibody and its target antigen is beyond the detection limit of the assay (*e.g.* > 1  $\mu$ M). Several CD3-binding arms also displayed cross reactivity to cynomolgus T-cells. All tested bispecific antibodies displayed similar cell binding on respective PSMA, EGFRvIII and MUC16-expressing cell lines, confirming that bispecific pairing with individual CD3 arms did not affect or diminish TAA-specific binding (TAA-specific binding was less than or equal to 5.6 nM (high affinity) in all examples tested).

[0193] Antibodies exhibiting weak-to-no detectable binding to human CD3, and also exhibiting weak-to-no binding to cynomolgus CD3, are considered advantageous for avidity-driven bispecific pairing in accordance with the present invention, and were further tested for cytotoxicity in *in vitro* and *in vivo* assays.

#### **Example 5: Binding Affinities of Exemplified Antibodies as Measured by a Surface Plasmon Resonance Binding Assay**

[0194] Binding affinities and kinetic constants of anti-TAA x anti-CD3 bispecific antibodies to soluble heterodimeric hCD3 $\epsilon$ / $\delta$ .mFc protein (hCD3 $\epsilon$  =UniProtKB/Swiss-Prot: P07766.2;

SEQ\_ID NO: 169; hCD3δ = UniProtKB/Swiss-Prot: P04234.1, SEQ ID NO: 170) were determined by surface plasmon resonance at 37°C using either an antigen-capture format (Table 11) or an antibody-capture format (data not shown). In this example, BSPSMA/CD3 bispecific antibodies were utilized as these pairings represented the use of a wider panel of antibodies for the CD3 binding arm. Measurements were conducted on a Sierra Sensors MASS-1 instrument.

**[0195]** In the antigen-capture format, the MASS-1 high-density amine sensor surface was derivatized with a goat anti-mouse IgG2a polyclonal antibody (Southern Biotech). Soluble heterodimeric CD3 protein was captured and the respective antibodies were injected over the captured antigen.

**[0196]** Kinetic association ( $k_a$ ) and dissociation ( $k_d$ ) rate constants were determined by processing and fitting the data to a 1:1 binding model using MASS-1 AnalyserR2 curve fitting software. Binding dissociation equilibrium constants ( $K_D$ ) and dissociative half-lives ( $t_{1/2}$ ) were calculated from the kinetic rate constants as:  $K_D$  (M) =  $k_d / k_a$ ; and  $t_{1/2}$  (min) =  $(\ln 2 / (60 * k_d))$ .

**Table 11: Affinities of anti-CD3 Bispecific Antibodies to Soluble Human CD3**

| Binding at 37°C / Antigen-Capture Format |                                                        |                                       |                          |           |                 |
|------------------------------------------|--------------------------------------------------------|---------------------------------------|--------------------------|-----------|-----------------|
| Bispecific Antibody Identifier           | Corresponding anti-CD3 Antigen-Binding HCVR Identifier | $k_a$ (M <sup>s</sup> <sup>-1</sup> ) | $k_d$ (s <sup>-1</sup> ) | $K_D$ (M) | $T_{1/2}$ (min) |
| BSPSMA/CD3-003                           | CD3-VH-G                                               | 1.32E+05                              | 7.62E-04                 | 5.78E-09  | 15.2            |
| BSPSMA/CD3-200                           | CD3-VH-G2                                              | NB                                    | NB                       | NB        | NB              |
| BSPSMA/CD3-300                           | CD3-VH-G3                                              | NB                                    | NB                       | NB        | NB              |
| BSPSMA/CD3-400                           | CD3-VH-G4                                              | NB                                    | NB                       | NB        | NB              |
| BSPSMA/CD3-004                           | CD3-VH-G5                                              | NB                                    | NB                       | NB        | NB              |
| BSPSMA/CD3-800                           | CD3-VH-G8                                              | 5.95E+04                              | 1.15E-03                 | 1.94E-08  | 10.0            |
| BSPSMA/CD3-900                           | CD3-VH-G9                                              | 4.38E+04                              | 4.95E-03                 | 1.13E-07  | 2.3             |
| BSPSMA/CD3-1000                          | CD3-VH-G10                                             | 3.44E+04                              | 6.37E-03                 | 1.85E-07  | 1.8             |
| BSPSMA/CD3-1100                          | CD3-VH-G11                                             | 9.21E+04                              | 1.02E-03                 | 1.11E-08  | 11.3            |
| BSPSMA/CD3-1200                          | CD3-VH-G12                                             | 3.85E+04                              | 2.47E-03                 | 6.42E-08  | 4.7             |
| BSPSMA/CD3-1300                          | CD3-VH-G13                                             | 2.03E+04                              | 2.48E-03                 | 1.22E-07  | 4.7             |
| BSPSMA/CD3-1400                          | CD3-VH-G14                                             | 6.21E+04                              | 3.31E-03                 | 5.33E-08  | 3.5             |
| BSPSMA/CD3-1500                          | CD3-VH-G15                                             | 7.36E+04                              | 6.11E-03                 | 8.29E-08  | 1.9             |

|                 |            |          |          |          |     |
|-----------------|------------|----------|----------|----------|-----|
| BSPSMA/CD3-1600 | CD3-VH-G16 | 6.43E+04 | 2.43E-03 | 3.78E-08 | 4.7 |
| BSPSMA/CD3-1700 | CD3-VH-G17 | 4.70E+04 | 3.07E-03 | 6.52E-08 | 3.8 |
| BSPSMA/CD3-1800 | CD3-VH-G18 | NB       | NB       | NB       | NB  |
| BSPSMA/CD3-1900 | CD3-VH-G19 | 4.43E+04 | 5.09E-03 | 1.15E-07 | 2.3 |
| BSPSMA/CD3-005  | CD3-VH-G20 | 1.73E+04 | 5.77E-03 | 3.34E-07 | 2.0 |
| BSPSMA/CD3-2100 | CD3-VH-G21 | 3.02E+04 | 2.34E-03 | 7.75E-08 | 4.9 |
| Control 1       | CD3-L2K    | 3.68E+05 | 2.66E-03 | 7.22E-09 | 4.3 |

NB: No binding detected

**[0197]** As shown in Table 11, all of the derived anti-CD3xanti-PSMA bispecific antibodies maintained very weak binding to soluble CD3 in the surface plasmon resonance binding assay, e.g. having a  $K_D$  value greater than 11 nM up to 334 nM which is weaker than that of the bispecific anti-CD3 arm derived from germline frameworks, CD3-VH-G.

**[0198]** Several bispecific antibodies exhibited greater than 50 nM  $K_D$  values, and some were greater than 100 nM ( $>1 \times 10^{-7}$ )  $K_D$  values (*i.e.* BSPSMA/CD3-900, BSPSMA/CD3-1000, BSPSMA/CD3-1900), greater than 300 nM ( $>3 \times 10^{-7}$ )  $K_D$  values (*i.e.* BSPSMA/CD3-005) and even beyond the detection limit of the assay ( $> 500$  nM;  $>5 \times 10^{-7}$ ), *i.e.* showed no detectable binding to soluble human CD3 (*i.e.* BSPSMA/CD3-200, BSPSMA/CD3-300, BSPSMA/CD3-400, BSPSMA/CD3-004 and BSPSMA/CD3-1800).

#### **Example 6: T Cell Activation and Tumor-specific Cytotoxicity Exhibited by Bispecific Antibodies of the Invention as Measured *In Vitro***

**[0199]** In this example, the specific killing of PSMA, EGFRvIII or MUC16-expressing TAA target cells in the presence of CD3-based bispecific antibodies was monitored via flow cytometry. As reported previously, the bispecific antibodies displayed a range of affinity to CD3 protein and CD3-expressing cell lines (*i.e.* weak, moderate and strong binding). This same panel of bispecific antibodies was tested for the ability to induce naïve human T-cells to re-direct killing toward target-expressing cells.

**[0200]** Briefly, PSMA-expressing (C4-2, 22Rv1 and TRAMPC2\_PSMA), EGFRvIII-expressing (U87/EGFRvIII) or MUC16-expressing (OVCAR3) cell lines were labeled with 1 $\mu$ M of the fluorescent tracking dye Violet Cell Tracker. After labeling, cells were plated overnight at 37°C. Separately, human PBMCs were plated in supplemented RPMI media at  $1 \times 10^6$  cells/mL and incubated overnight at 37°C in order to enrich for lymphocytes by depleting adherent macrophages, dendritic cells, and some monocytes. The next day, target cells were co-incubated with adherent cell-depleted naïve PBMC (Effector/Target cell 4:1 ratio) and a serial dilution of relevant bispecific antibodies or Isotype control (concentration

range: 66.7nM to 0.25pM) for 48 hours at 37°C. Cells were removed from cell culture plates using an enzyme-free cell dissociation buffer, and analyzed by FACS.

**[0201]** For FACS analysis, cells were stained with a dead/live far red cell tracker (Invitrogen).  $5 \times 10^5$  counting beads were added to each well immediately before FACS analysis.  $1 \times 10^4$  beads were collected for each sample. For the assessment of specificity of killing, cells were gated on live Violet labeled populations. Percent of live population was recorded and used for the calculation of normalized survival.

**[0202]** T cell activation was assessed by incubating cells with directly conjugated antibodies to CD2 and CD69, and by reporting the percent of activated (CD69+) T cells out of total T cells (CD2+).

**[0203]** As the results in Tables 12A-12C show, depletion of TAA-expressing cells was observed with anti-PSMA, EGFRvIII or MUC16xCD3 bispecifics. Most of the tested bispecific antibodies activated and directed human T cells to deplete the target cells with  $EC_{50}$ s in picomolar range. Additionally, the observed target-cell lysis (depletion) was associated with an up-regulation of CD69 cells on CD2+ T cells, with picomolar (pM)  $EC_{50}$ s.

**[0204]** Importantly, the results of this example demonstrate that several bispecifics which utilized a CD3 binding arm that displayed weak-to-non-observable binding to CD3 protein or CD3-expressing cells (*i.e.* CD3-VH-G5) still retained the ability to activate T-cells and exhibited potent cytotoxicity of tumor antigen-expressing cells.

**Table 12A: Cytotoxicity and T-cell activation properties of selected PSMAxCD3 Bispecific Antibodies**

| <b>Bispecific Antibody Identifier</b> | <b>Anti-CD3 Binding Arm</b> | <b>C4-2 Cell depletion <math>EC_{50}</math> [M]</b> | <b>22RV1 Cell depletion <math>EC_{50}</math> [M]</b> | <b>TrampC2.PSMA Cell depletion <math>EC_{50}</math> [M]</b> | <b>T cell activation <math>EC_{50}</math> [M]</b> |
|---------------------------------------|-----------------------------|-----------------------------------------------------|------------------------------------------------------|-------------------------------------------------------------|---------------------------------------------------|
| BSPSMA/CD3-003                        | CD3-VH-G                    | 1.03E-11                                            | NT                                                   | 6.43E-12                                                    | 1.23E-12                                          |
| BSPSMA/CD3-200                        | CD3-VH-G2                   | NT                                                  | No activity                                          | NT                                                          | No activity                                       |
| BSPSMA/CD3-300                        | CD3-VH-G3                   | NT                                                  | Very weak                                            | NT                                                          | 1.85E-11                                          |
| BSPSMA/CD3-400                        | CD3-VH-G4                   | NT                                                  | Very weak                                            | NT                                                          | Very weak                                         |
| BSPSMA/CD3-004                        | CD3-VH-G5                   | 2.15E-11                                            | 6.31E-12                                             | 1.15E-11                                                    | 1.34E-11                                          |
| BSPSMA/CD3-800                        | CD3-VH-G8                   | NT                                                  | NT                                                   | 9.27E-12                                                    | 1.76E-12                                          |

|                     |                |          |          |          |          |
|---------------------|----------------|----------|----------|----------|----------|
| BSPSMA/<br>CD3-900  | CD3-VH-G9      | NT       | NT       | 3.50E-12 | 1.12E-12 |
| BSPSMA/<br>CD3-1000 | CD3-VH-<br>G10 | NT       | NT       | 5.97E-12 | 1.28E-12 |
| BSPSMA/<br>CD3-1100 | CD3-VH-<br>G11 | NT       | NT       | 3.86E-12 | 1.11E-12 |
| BSPSMA/<br>CD3-1300 | CD3-VH-<br>G13 | 8.74E-12 | NT       | NT       | 2.31E-12 |
| BSPSMA/<br>CD3-1700 | CD3-VH-<br>G17 | 7.37E-12 | 2.07E-12 | NT       | 3.89E-12 |
| BSPSMA/<br>CD3-005  | CD3-VH-<br>G20 | 1.39E-11 | 8.32E-12 | NT       | 6.11E-12 |

NT= not tested

**Table 12B: Cytotoxicity and T-cell activation properties of selected EGFRvIIIxCD3 Bispecific Antibodies**

| <b>Bispecific Antibody Identifier</b> | <b>Anti-CD3 Binding Arm</b> | <b>U87_EGFRvIII cell depletion EC<sub>50</sub> [M]</b> | <b>T cell activation EC<sub>50</sub> [M]</b> |
|---------------------------------------|-----------------------------|--------------------------------------------------------|----------------------------------------------|
| BSV3/CD3-001                          | CD3-VH-G                    | 3.64E-10                                               | 3.33E-11                                     |
| BSV3/CD3-002                          | CD3-VH-G5                   | 1.30E-09                                               | 1.13E-10                                     |

**Table 12C: Cytotoxicity and T-cell activation properties of selected MUC16xCD3 Bispecific Antibodies**

| <b>Bispecific Antibody Identifier</b> | <b>Anti-CD3 Binding Arm</b> | <b>OVCAR3 cell depletion EC<sub>50</sub> [M]</b> | <b>T cell activation EC<sub>50</sub> [M]</b> |
|---------------------------------------|-----------------------------|--------------------------------------------------|----------------------------------------------|
| BSV3/CD3-001                          | CD3-VH-G                    | 2.24E-11                                         | 5.88E-12                                     |
| BSV3/CD3-002                          | CD3-VH-G5                   | 3.06E-11                                         | 1.01E-11                                     |

**Example 7: Anti-PSMA/anti-CD3 bispecific antibodies display potent anti-tumor efficacy *in vivo***

[0205] To determine the *in vivo* efficacy of exemplary anti-PSMA/anti-CD3 bispecific antibodies identified as having weak or no detectable binding affinity to human and cynomolgus CD3, studies were performed in immunocompromised mice bearing human prostate cancer xenografts. Additional studies were also carried out in immunocompetent mice bearing mouse prostate cancer xenografts engineered to express human PSMA.

*Efficacy of anti-PSMA/anti-CD3 bispecific antibodies in human tumor xenograft Models*

**[0206]** To assess the in vivo efficacy of the anti-PSMA/anti-CD3 bispecifics in human tumor xenograft studies, NOD *scid* gamma (NSG) mice (Jackson Laboratories, Bar Harbor, Maine) were co-implanted with human peripheral blood mononuclear cells (PBMCs) along with 22Rv1 or C4-2 human prostate tumor cells which endogenously express PSMA.

**[0207]** Briefly,  $4 \times 10^6$  22Rv1 or  $5 \times 10^6$  C4-2 cells (MD Anderson, TX) cells were co-implanted s.c. with  $1 \times 10^6$  human PBMCs (ReachBio, LLC., Seattle, WA) in a 50:50 mix of matrigel matrix (BD Biosciences) into the right flank of male NSG mice. In the C4-2 study, mice were treated i.p. on days 0, 4, and 7 post tumor implantation with 0.1 mg/kg BSPSMA/CD3-003 or BSPSMA/CD3-005.

**[0208]** In an additional xenogenic model, anti-PSMA/anti-CD3 bispecifics were tested in mice engrafted with human hematopoietic CD34+ stem cells. Briefly, newborn SIRP $\alpha$  BALB/c-Rag2- IL2 $\gamma$ - (BRG) pups were engrafted with hCD34+ fetal liver cells. 3-6 months later hCD34-engrafted SIRP $\alpha$  BRG mice were then implanted with C4-2 cells ( $5 \times 10^6$  s.c. in matrigel). 8 days later, mice were treated with 10  $\mu$ g of BSPSMA/CD3-004 or an isotype control antibody, followed by 2x/week doses throughout the study.

**[0209]** In all studies, tumor size was measured 2x/week using calipers and tumor volume calculated as Volume = (length x width<sup>2</sup>)/2.

**[0210]** As the results in Table 13 show, the bispecific antibodies tested in the xenogenic models described above were all effective at suppressing tumor growth compared to treatment with the isotype control.

**Table 13: Suppression of Tumor Growth by Administering Anti-PSMA/Anti-CD3 Bispecific Antibodies to Xenogenic Mouse Models**

| Xenogenic model: suppression of tumor growth                                         |                                 |                                   |                              |                                                           |
|--------------------------------------------------------------------------------------|---------------------------------|-----------------------------------|------------------------------|-----------------------------------------------------------|
| Tumor Model/<br>Mouse Strain                                                         | N<br># mice/<br>treatment group | Bispecific Antibody<br>Identifier | Dose                         | Final Tumor Volume<br>(mm <sup>3</sup> )<br>Mean $\pm$ SD |
| C4-2/<br>NSG                                                                         | 5                               | BSPSMA/CD3-003                    | 0.1 mg/kg<br>on day 0,4 & 7  | 0 $\pm$ 0                                                 |
|                                                                                      | 5                               | BSPSMA/CD3-005                    |                              | 0 $\pm$ 0                                                 |
|                                                                                      | 5                               | Isotype Control                   |                              | 960 $\pm$ 660                                             |
| C4-2/<br>SIRP $\alpha$ Balb/c-Rag2-<br>IL2 $\gamma$ BRG engrafted<br>with hCD34+ HSC | 5                               | BSPSMA/CD3-004                    | 1.0 $\mu$ g/mouse<br>2x/week | 70 $\pm$ 60                                               |
|                                                                                      | 5                               | Isotype Control                   |                              | 260 $\pm$ 180                                             |

*Efficacy of anti-PSMA/anti-CD3 bispecific antibodies in immune-competent tumor model*

**[0211]** Additionally, anti-PSMA/anti-CD3 bispecifics were assessed for anti-tumor activity in an immune-competent model (U.S. Provisional Application No. 62/083,653, filed November 24, 2014). Mice humanized for the three chains ( $\delta\gamma\epsilon$ ) of CD3 were also humanized for PSMA and implanted with a variant murine prostate cancer cell line TRAMP-C2 transfected with human PSMA.

**[0212]** Prior to study initiation, the tumorigenic cell line variant TRAMP-C2\_hPSMAv#1 was generated. Briefly,  $7.5 \times 10^6$  TRAMP-C2\_hPSMA cells were implanted s.c. into the right flank of male mice humanized for CD3 and PSMA. A tumor was excised and cut into 3 mm fragments and subsequently implanted into the right flank of new male humanized mice. A tumor arising from the implanted tumor fragments was then harvested and disaggregated into a single cell suspension. These cells (TRAMP-C2\_hPSMAv#1) were then cultured in vitro under G418 selection.  $4 \times 10^6$  cells of this variant cell line were then implanted into the right flank of male PSMA/CD3 humanized mice for the bispecific antibody efficacy studies.

**[0213]** Humanized PSMA/CD3 mice implanted with TRAMP-C2\_hPSMAv#1 were treated with 100  $\mu$ g or 10  $\mu$ g of anti-PSMA/anti-CD3 bispecific antibody BSPSMA/CD3-004 or an isotype control 2x/week starting from the day of tumor implantation. Serum cytokine levels 4h post-injection were also examined, as well as spleen T-cell levels. Study was terminated at Day 27.

**[0214]** As the results in Table 14 show, the anti-PSMA/anti-CD3 bispecific molecule tested, BSPSMA/CD3-004, showed efficacy in significantly delaying tumor growth across treatment groups. Minimal cytokine release was observed after administration of BSPSMA/CD3-004, possibly due to the weak binding of the anti-CD3. Both antibodies tested showed anti-tumor efficacy without depleting T cells in the spleen.

**Table 14: Efficacy of anti-PSMA/anti-CD3 Bispecific antibodies in immune-competent syngeneic model**

| Tumor Model/<br>Mouse Strain                               | Bispecific<br>Antibody Identifier | Dose<br>( $\mu\text{g}/\text{mouse}$ )<br>2x/ week* | N<br># mice/<br>treatment<br>group | Tumor Volume<br>( $\text{mm}^3$ )<br>at Day 27<br>(Mean $\pm$ SD) | Mean Serum Cytokine Concentrations,<br>(pg/mL) |      |      |          |      | Spleen T-cell<br>level %, (mean $\pm$ SD )# |                   |
|------------------------------------------------------------|-----------------------------------|-----------------------------------------------------|------------------------------------|-------------------------------------------------------------------|------------------------------------------------|------|------|----------|------|---------------------------------------------|-------------------|
|                                                            |                                   |                                                     |                                    |                                                                   | IFNg                                           | TNFA | IL-2 | IL-12p70 | IL-6 | CD4+                                        | CD8+              |
| TRAMP-C2/<br>PSMA <sup>Hum/hum</sup><br>CD3 <sup>Hum</sup> |                                   |                                                     |                                    |                                                                   |                                                |      |      |          |      |                                             |                   |
|                                                            | BSPSMA/CD3-<br>004                | 100                                                 | 4                                  | 50 $\pm$ 60                                                       | 30                                             | 60   | 60   | 40       | 370  | 8.0 $\pm$<br>1.0                            | 12.0<br>$\pm$ 2.0 |
|                                                            |                                   | 10                                                  | 5                                  | 380 $\pm$ 650                                                     | 10                                             | 50   | 50   | 10       | 330  | 8.0 $\pm$<br>3.0                            | 14.0 $\pm$<br>4.0 |
|                                                            |                                   |                                                     |                                    |                                                                   |                                                |      |      |          |      |                                             |                   |
|                                                            | Isotype Control                   | 100                                                 | 5                                  | 1740 $\pm$ 560                                                    | 4                                              | 30   | 30   | 10       | 230  | 5.0 $\pm$<br>1.0                            | 8.0<br>$\pm$ 2.0  |
|                                                            |                                   |                                                     |                                    |                                                                   |                                                |      |      |          |      |                                             |                   |

\* Mice were dosed with antibody or isotype control 2x/week starting on the day of tumor implantation

# Measured as the percentage of CD4+ or CD8+ cells in spleen out of live mCD45+ cells

[0215] In summary, the anti-PSMA/anti-CD3 bispecific antibodies of this invention display potent anti-tumor efficacy in both immune-compromised and immune-competent tumor models, despite having low to no detectable binding to CD3 antigen.

**Example 8: Anti-MUC16/anti-CD3 bispecific antibodies display potent anti-tumor efficacy *in vivo***

[0216] To determine the *in vivo* efficacy of exemplary anti-MUC16/anti-CD3 bispecific antibodies identified as having weak or no detectable binding affinity to human and cynomolgus CD3, studies were performed in immunocompromised mice bearing human prostate cancer xenografts. The efficacy of selected bispecific antibodies was tested in both immediate treatment and therapeutic treatment dosing models.

*Efficacy of anti-MUC16/anti-CD3 bispecific antibodies in human tumor xenograft models*

[0217] To assess the *in vivo* efficacy of the anti-MUC16/anti-CD3 bispecifics in human tumor xenograft studies, NOD *scid* gamma (NSG) mice (Jackson Laboratories, Bar Harbor, Maine) were pre-implanted with human peripheral blood mononuclear cells (PBMCs; ReachBio LLC., Seattle, WA) and then given ascites cells from the human ovarian cancer cell line OVCAR-3 (American Type Tissue Culture, Manassas, VA) transduced with luciferase (OVCAR-3/Luc). OVCAR-3 cells endogenously express MUC-16.

[0218] Briefly, NSG mice were injected intraperitoneally (i.p.) with  $5.0 \times 10^6$  human PBMCs. 8d later,  $1.5 \times 10^6$  ascites cells from the OVCAR-3/Luc cell line, previously passaged *in vivo*, were administered i.p. to the NSG mice engrafted with PBMCs. In the immediate treatment group, mice were treated i.p. on the day of OVCAR-3/Luc cell implantation with MUC16/CD3 Bispecific antibodies BSMUC16/CD3-001 or BSMUC16/CD3-005, or an isotype control, at a dose of 10ug/mouse (N=5 mice/treatment group). In the therapeutic dose model, mice were treated i.p. 7d post tumor implantation with the MUC16/CD3 Bispecific or control antibodies described above, at a dose of 10ug/mouse (N=5/treatment group).

[0219] In all studies, tumor growth was monitored via bioluminescent imaging (BLI). Mice were injected i.p. with the luciferase substrate D-luciferin suspended in PBS (150 mg/kg) and imaged under isoflurane anesthesia after 10 min. BLI was performed using the Xenogen IVIS system (Perkin Elmer, Hopkinton, MA) and BLI signals were extracted using Living Image software (Xenogen/Perkin Elmer). Regions of interest were drawn around each cell mass and photon intensities were recorded as photons(p)/sec(s)/cm<sup>2</sup>/steradian(sr). For the immediate-

treatment group, data is shown as BLI levels 26d post tumor implantation (Table 15). For the therapeutic-treatment group, data is shown as fold-change in BLI between day 6 (1 d before treatment) and at study endpoint (26d post tumor implantation; Table 16).

**[0220]** As the results show, both BSMUC16/CD3-001 and BSMUC16/CD3-005 showed similar efficacy in suppressing tumor growth compared to the isotype control when BLI was measured at Day 26 in the immediate dosing model. Both anti-MUC16/anti-CD3 bispecific antibodies also suppressed the growth of established tumors when administered 7d post tumor implantation, compared to the control. In summary, the bispecific anti-MUC16/anti-CD3 antibodies of this invention display potent anti-tumor efficacy in several models.

**Table 15: Efficacy of anti-MUC16/anti-CD3 Bispecific Antibodies in Immune-Compromised Xenograft Model: Immediate Dosing**

| <b>Tumor Model/<br/>Mouse Strain/<br/>Dose</b> | <b>Bispecific<br/>Antibody Identifier</b> | <b>N</b> | <b>Avg Bioluminescent Radiance<br/>(photons/sec/cm<sup>2</sup>/ steradian)<br/>Day 26<br/><br/>(mean ± SD)</b> |
|------------------------------------------------|-------------------------------------------|----------|----------------------------------------------------------------------------------------------------------------|
| OVCAR-3/Luc /<br>NSG /<br>10ug / mouse         | BSMUC16/CD3-001                           | 5        | $1.4 \times 10^3 \pm 3.5 \times 10^2$                                                                          |
|                                                | BSMUC16/CD3-005                           | 5        | $1.5 \times 10^3 \pm 9.7 \times 10^2$                                                                          |
|                                                | Isotype Control                           | 5        | $2.0 \times 10^7 \pm 1.0 \times 10^6$                                                                          |

**Table 16: Efficacy of anti-Muc16/anti-CD3 Bispecific Antibodies in Immune-Compromised Xenograft Model: Therapeutic Treatment**

| <b>Tumor Model/<br/>Mouse Strain/<br/>Dose</b> | <b>Bispecific Antibody<br/>Identifier</b> | <b>N</b> | <b>Fold change in<br/>Avg Bioluminescent<br/>Radiance [p/s/cm<sup>2</sup>/sr]<br/>at Day 26 relative to Day 6</b> |
|------------------------------------------------|-------------------------------------------|----------|-------------------------------------------------------------------------------------------------------------------|
|------------------------------------------------|-------------------------------------------|----------|-------------------------------------------------------------------------------------------------------------------|

|                                        |                 |   | (mean ± SD) |
|----------------------------------------|-----------------|---|-------------|
| OVCAR-3/Luc /<br>NSG /<br>10ug / mouse | BSMUC16/CD3-001 | 5 | 2.0 ± 5.0   |
|                                        | BSMUC16/CD3-005 | 5 | 0.01 ± 0.02 |
|                                        | Isotype Control | 5 | 21.0 ± 8.0  |

### Example 9: Pharmacokinetic Assessment of anti-MUC16 x CD3 Bispecific Antibodies

**[0221]** Assessment of the pharmacokinetics of anti-MUC16 x CD3 bispecific antibodies BSMUC16/CD3-001 and BSMUC16/CD3-005 and an isotype control were conducted in humanized MUC16 x CD3 mice (mice homozygous for human MUC16 and CD3 expression, MUC16<sup>hu/hu</sup> x CD3<sup>hu/hu</sup>), CD3 humanized mice (mice homozygous for human CD3 expression, CD3<sup>hu/hu</sup>) and strain-matched (75% C57BL, 25%129Sv) wild-type (WT) mice. Cohorts contained 4-5 mice per tested antibody and per mouse strain. All mice received a single intra-peritoneal (i.p.) 0.4 mg/kg dose. Blood samples were collected at 3 and 6 hours, 1, 3, 7, 14 and 28 days post dosing. Blood was processed into serum and frozen at -80 °C until analyzed.

**[0222]** Circulating antibody concentrations were determined by total human IgG antibody analysis using the GyroLab xPlore™ (Gyros, Uppsala, Sweden). Briefly, a biotinylated goat anti-human IgG polyclonal antibody (Jackson ImmunoResearch, West Grove, PA) was captured onto streptavidin coated beads on a Gyrolab Bioaffy 200 CD (Gyros) in order to capture the human IgG present in the sera. After affinity column capture, bound human IgG antibody in samples was detected with Alexa-647 labeled goat anti-human IgG (Jackson ImmunoResearch). Fluorescent signal on the column allowed for the detection of bound IgG and response units (RU) were read by the instrument. Sample concentrations were determined by interpolation from a standard curve that was fit using a 5-parameter logistic curve fit using the Gyrolab Evaluator Software.

**[0223]** PK parameters were determined by non-compartmental analysis (NCA) using Phoenix®WinNonlin® software Version 6.3 (Certara, L.P., Princeton, NJ) and an extravascular dosing model. Using the respective mean concentration values for each antibody, all PK parameters including observed maximum concentration in serum ( $C_{max}$ ), estimated half-life observed ( $t_{1/2}$ ), and area under the concentration curve versus time up to the last measureable

concentration ( $AUC_{last}$ ) were determined using a linear trapezoidal rule with linear interpolation and uniform weighting

**[0224]** Following i.p. administration of antibodies in WT mice, the total IgG concentration-time profiles of BSMUC16/CD3-001, BSMUC16/CD3-005 and the isotype control were all similar, characterized first by a brief drug distribution followed by a single drug elimination phase throughout the remainder of the study. Maximum serum concentrations ( $C_{max}$ ) and calculated drug exposure ( $AUC_{last}$ ) of the three antibodies were comparable (within 1.3-fold of each other). Following i.p. administration of antibodies in CD3<sup>hu/hu</sup> mice, BSMUC16/CD3-001, BSMUC16/CD3-005 and isotype control had comparable  $C_{max}$  concentrations (4.6, 3.6 and 4.1  $\mu\text{g/mL}$ , respectively). BSMUC16/CD3-005 and the isotype control exhibited similar drug elimination curves, while BSMUC16/CD3-001 exhibited steeper drug elimination than both, suggesting that human CD3 target binding drives clearance. Terminal antibody concentration for BSMUC16/CD3-001 was 0.03  $\mu\text{g/mL}$ , which is about 28-fold less than terminal antibody concentrations determined for the isotype control (0.85  $\mu\text{g/mL}$ ) and 22-fold less than BSMUC16/CD3-005 (0.66  $\mu\text{g/mL}$ ) serum concentrations.

**[0225]** In MUC16<sup>hu/hu</sup> x CD3<sup>hu/hu</sup> double-humanized mice, the Muc16xCD3 bispecific and isotype control antibodies had comparable  $C_{max}$  concentrations ( $C_{max}$  range: 4.5-6.9  $\mu\text{g/mL}$ ). Both bispecific antibodies exhibited steeper drug elimination than the isotype control suggesting a target-mediated effect. Terminal antibody concentrations for BSMUC16/CD3-001 and BSMUC16/CD3-005 were about 29-fold and 2.9-fold less, respectively, than terminal antibody concentrations determined for the isotype control (0.86  $\mu\text{g/mL}$ ).

**[0226]** A summary of the data for total anti-MUC16 x CD3 bispecific antibodies and isotype control antibody concentrations are summarized in Table 17. Mean PK parameters are described in Tables 18A and 18B. Mean total antibody concentrations versus time are shown in Figures 2A, 2B and 2C. In conclusion, MUC16xCD3 bispecific antibodies exhibited similar  $C_{max}$  and drug elimination curves in WT mice, but BSMUC16/CD3-001 displayed steeper elimination rates than BSMUC16/CD3-005 and the isotype control in CD3 single-humanized mice and MUC16/CD3 double humanized mice. Since the bispecific antibodies administered in this PK study are comprised of the same anti-MUC16 binding arm, the results suggest that the strength of binding of the CD3 targeting arm may play a role in drug exposure levels ( $AUC_{last}$ ) and drug elimination rates. Neither BSMUC16/CD3-001 or BSMUC16/CD3-005 bind mouse MUC16 or mouse CD3.

**Table 17: Mean Concentrations of Total IgG in Serum Following a Single 0.4 mg/kg Intra-peritoneal Injection of BSMUC16/CD3-001, BSMUC16/CD3-005 and Isotype Control Antibodies in WT Mice, Humanized CD3 Mice and Humanized MUC16 x CD3 mice**

| Antibody        | Time (d) | Total mAb Concentration In Mouse Serum |        |                      |        |                                               |        |
|-----------------|----------|----------------------------------------|--------|----------------------|--------|-----------------------------------------------|--------|
|                 |          | WT                                     |        | CD3 <sup>hu/hu</sup> |        | MUC16 <sup>hu/hu</sup> x CD3 <sup>hu/hu</sup> |        |
|                 |          | Mean (µg/mL)                           | +/- SD | Mean (µg/mL)         | +/- SD | Mean (µg/mL)                                  | +/- SD |
| BSMUC16/CD3-001 | 0.13     | 5.39                                   | 0.34   | 4.30                 | 0.29   | 6.77                                          | 1.52   |
|                 | 0.25     | 5.80                                   | 0.36   | 4.26                 | 1.07   | 6.63                                          | 1.06   |
|                 | 1.00     | 4.13                                   | 0.43   | 2.87                 | 0.71   | 4.89                                          | 0.53   |
|                 | 3.00     | 3.19                                   | 0.53   | 1.44                 | 0.27   | 2.50                                          | 0.22   |
|                 | 7.00     | 2.61                                   | 0.73   | 0.72                 | 0.13   | 1.20                                          | 0.22   |
|                 | 14.00    | 1.44                                   | 0.69   | 0.18                 | 0.05   | 0.28                                          | 0.08   |
|                 | 21.00    | 0.93                                   | ND     | 0.07                 | 0.02   | 0.06                                          | 0.05   |
|                 | 28.00    | 0.60                                   | ND     | 0.04                 | 0.01   | 0.03                                          | 0.02   |
|                 |          |                                        |        |                      |        |                                               |        |
| BSMUC16/CD3-005 | 0.13     | 4.23                                   | 0.62   | 3.35                 | 1.15   | 4.35                                          | 0.24   |
|                 | 0.25     | 4.53                                   | 0.55   | 3.40                 | 0.96   | 4.45                                          | 0.49   |
|                 | 1.00     | 3.47                                   | 0.32   | 2.72                 | 0.42   | 3.00                                          | 0.61   |
|                 | 3.00     | 2.51                                   | 0.13   | 1.95                 | 0.37   | 1.98                                          | 0.41   |
|                 | 7.00     | 2.02                                   | 0.24   | 2.31                 | 0.67   | 1.58                                          | 0.36   |
|                 | 14.00    | 1.19                                   | 0.17   | 1.01                 | 0.23   | 0.78                                          | 0.26   |
|                 | 21.00    | 1.19                                   | 0.29   | 1.19                 | 0.11   | 0.66                                          | 0.29   |
|                 | 28.00    | 0.71                                   | 0.20   | 0.66                 | 0.28   | 0.30                                          | 0.22   |
|                 |          |                                        |        |                      |        |                                               |        |
| Isotype Control | 0.13     | 5.07                                   | 1.16   | 5.43                 | 1.30   | 6.56                                          | 0.70   |
|                 | 0.25     | 5.91                                   | 1.10   | 5.67                 | 1.91   | 6.48                                          | 0.90   |
|                 | 1.00     | 2.64                                   | 0.24   | 2.98                 | 1.14   | 2.82                                          | 0.30   |
|                 | 3.00     | 2.05                                   | 0.06   | 2.29                 | 0.83   | 1.57                                          | 0.37   |
|                 | 7.00     | 1.80                                   | 0.25   | 2.14                 | 0.85   | 1.96                                          | 0.37   |

|  |       |      |      |      |      |      |      |
|--|-------|------|------|------|------|------|------|
|  | 14.00 | 1.22 | 0.28 | 1.48 | 0.66 | 1.34 | 0.37 |
|  | 21.00 | 1.20 | 0.58 | 1.43 | 0.72 | 1.24 | 0.44 |
|  | 28.00 | 0.73 | 0.24 | 0.85 | 0.29 | 0.86 | 0.41 |

Time : (h, when noted)= time in hours post single-dose injection; D= Day of study; SD = Standard deviation; ND = Not determined due to exclusion of mice with drug clearing anti-drug titers

**Table 18A: Summary of Pharmacokinetic Parameters: CD3<sup>hu/hu</sup> humanized mice**

| Parameter           | Units   | WT mice         |                 |                 | CD3 <sup>hu/hu</sup> mice |                 |                 |
|---------------------|---------|-----------------|-----------------|-----------------|---------------------------|-----------------|-----------------|
|                     |         | Isotype Control | BSMUC16/CD3-001 | BSMUC16/CD3-005 | Isotype Control           | BSMUC16/CD3-001 | BSMUC16/CD3-005 |
| C <sub>max</sub>    | µg/mL   | 5 ± 3           | 6 ± 0.4         | 5 ± 0.5         | 4.1 ± 3                   | 4.6 ± 0.8       | 3.5 ± 1         |
| T <sub>1/2</sub>    | d       | 11 ± 4          | 7 ± 3           | 12 ± 2          | 14 ± 0.5                  | 3.9 ± 0.6       | 11 ± 5          |
| AUC <sub>last</sub> | d•µg/mL | 35 ± 18         | 40 ± 11         | 45 ± 5          | 49 ± 20                   | 16 ± 3          | 36 ± 13         |

C<sub>max</sub>= Peak concentration; AUC = Area under the concentration-time curve; AUC<sub>last</sub> = AUC computed from time zero to the time of the last positive concentration; T<sub>1/2</sub> = Estimated half-life observed; d=day

**Table 18B: Summary of Pharmacokinetic Parameters: MUC16<sup>hu/hu</sup> x CD3<sup>hu/hu</sup> double-humanized mice**

| Parameter           | Units   | WT mice         |                 |                 | MUC16 <sup>hu/hu</sup> x CD3 <sup>hu/hu</sup> mice |                 |                 |
|---------------------|---------|-----------------|-----------------|-----------------|----------------------------------------------------|-----------------|-----------------|
|                     |         | Isotype Control | BSMUC16/CD3-001 | BSMUC16/CD3-005 | Isotype Control                                    | BSMUC16/CD3-001 | BSMUC16/CD3-005 |
| C <sub>max</sub>    | µg/mL   | 5 ± 3           | 6 ± 0.4         | 5 ± 0.5         | 6.7 ± 0.7                                          | 6.9 ± 1         | 4.5 ± 4         |
| T <sub>1/2</sub>    | d       | 11 ± 4          | 7 ± 3           | 12 ± 2          | 12.9 ± 4                                           | 3.3 ± 0.8       | 8.2 ± 4         |
| AUC <sub>last</sub> | d•µg/mL | 35 ± 18         | 40 ± 11         | 45 ± 5          | 46 ± 10                                            | 27 ± 3          | 34 ± 11         |

C<sub>max</sub>= Peak concentration; AUC = Area under the concentration-time curve; AUC<sub>last</sub> = AUC computed from time zero to the time of the last positive concentration; T<sub>1/2</sub> = Estimated half-life observed; d=day

#### **Example 10: Anti-STEAP2/anti-CD3 bispecific antibodies display potent anti-tumor efficacy *in vivo***

[0227] To determine the *in vivo* efficacy of exemplary anti-STEAP2/anti-CD3 bispecific antibodies identified as having weak or no detectable binding affinity to human and cynomolgus

CD3, studies were performed in immunocompromised mice bearing human prostate cancer xenografts.

**[0228]** To assess the in vivo efficacy of the anti-STEAP2/anti-CD3 bispecifics in human tumor xenograft studies, NOD *scid* gamma (NSG) mice (Jackson Laboratories, Bar Harbor, Maine) were co-implanted with human peripheral blood mononuclear cells (PBMCs; ReachBio LLC., Seattle, WA) along with human prostate cancer C4-2 cells (MD Anderson Cancer Center, Houston TX) which endogenously express STEAP2.

**[0229]** Briefly,  $5.0 \times 10^6$  C4-2 cells were co-implanted subcutaneously (s.c.) with  $1.25 \times 10^6$  human PBMCs in a 50:50 mix of matrigel matrix (BD Biosciences, San Jose, CA) into the right flank of male NSG mice. Mice were treated intraperitoneally (i.p.) on the day of implantation (immediate treatment model) with anti-STEAP2/anti-CD3 bispecifics BSSTEAP2/CD3-001, BSSTEAP2/CD3-002 or BSSTEAP2/CD3-003, or an isotype control (that does not bind C4-2 tumor cells), at a dose of 0.1 or 0.01 mg/kg (N=5 mice/group).

**[0230]** Tumor size was measured 2x/week using calipers and tumor volume calculated as  $\text{Volume} = (\text{length} \times \text{width}^2) / 2$ . Data is shown as tumor size ( $\text{mm}^3$ ) at study endpoint, 46d post-tumor implantation (Table 19).

**[0231]** As the results in Table 19 show, BSSTEAP2/CD3-001, BSSTEAP2/CD3-002 and BSSTEAP2/CD3-003 significantly suppressed tumor growth compared to an isotype control when tumor sizes were measured at study endpoint. Importantly, the anti-STEAP2/anti-CD3 bispecific antibodies were efficacious in inhibiting C4-2 tumor growth even at the lowest dose of 0.1 mg/kg.

**Table 19: Efficacy of anti-STEAP2/anti-CD3 Bispecific Antibodies in Immune-Compromised Xenograft Model: Immediate Dosing**

| Tumor Model /<br>Mouse Strain | Bispecific Antibody<br>Identifier | Dose (mg/kg) | N | Tumor Size ( $\text{mm}^3$ )<br>46 d post-tumor<br>implantation<br>(mean $\pm$ SD) |
|-------------------------------|-----------------------------------|--------------|---|------------------------------------------------------------------------------------|
| C4-2 /<br>NSG                 | BSSTEAP2/CD3-001                  | 0.1          | 5 | 18.0 $\pm$ 14.0                                                                    |
|                               |                                   | 0.01         | 5 | 23.0 $\pm$ 220                                                                     |
|                               | BSSTEAP2/CD3-002                  | 0.1          | 5 | 15.0 $\pm$ 12.0                                                                    |

|  |                    |      |   |                    |
|--|--------------------|------|---|--------------------|
|  |                    | 0.01 | 5 | $17.0 \pm 8.0$     |
|  | BSSTEAP2/CD3-003   | 0.1  | 5 | $19.0 \pm 12.0$    |
|  |                    | 0.01 | 5 | $25.0 \pm 21.0$    |
|  | Control Bispecific | 0.1  | 5 | $1020.0 \pm 922.0$ |

**[0232]** The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

What is claimed:

1. Use of a cytotoxic bispecific antibody in the manufacture of a medicament for treating a cancer in a subject, wherein the bispecific antibody comprises a first antigen binding arm that exhibits weak to no detectable binding to human CD3 and cynomolgus CD3, and a second antigen binding arm that binds a tumor-associated antigen, wherein the first arm comprises a heavy chain variable region (HCVR) comprising complementarity determining regions HCDR1-HCDR2-HCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 36-38-40 or SEQ ID NOs: 140-142-144, and a common light chain.
2. The use of claim 1, wherein the bispecific antibody exhibits T cell activation *in vitro*.
3. The use of claim 1 or 2, wherein the tumor-associated antigen is expressed on a human tumor cell.
4. The use of any one of claims 1-3, wherein the bispecific antibody induces T cell-mediated tumor cell killing with an EC<sub>50</sub> value of less than about 1.3 nM, as measured in an *in vitro* T cell-mediated tumor cell killing assay.
5. The use of any one of claims 1-4, wherein the target tumor cell expresses a tumor-associated antigen selected from the group consisting of AFP, ALK, BAGE proteins, BIRC5 (survivin), BIRC7,  $\beta$ -catenin, bcr-abl, BRCA1, BORIS, CA9, carbonic anhydrase IX, caspase-8, CALR, CCR5, CD19, CD20 (MS4A1), CD22, CD30, CD40, CDK4, CEA, CTLA4, cyclin-B1, CYP1B1, EGFR, EGFRvIII, ErbB2/Her2, ErbB3, ErbB4, ETV6-AML, EpCAM, EphA2, Fra-1, FOLR1, GAGE proteins (*e.g.*, GAGE-1, -2), GD2, GD3, GloboH, glypican-3, GM3, gp100, Her2, HLA/B-raf, HLA/k-ras, HLA/MAGE-A3, hTERT, LMP2, MAGE proteins (*e.g.*, MAGE-1, -2, -3, -4, -6, and -12), MART-1, mesothelin, ML-IAP, Muc1, Muc2, Muc3, Muc4, Muc5, Muc16 (CA-125), MUM1, NA17, NY-BR1, NY-BR62, NY-BR85, NY-ESO1, OX40, p15, p53, PAP, PAX3, PAX5, PCTA-1, PLAC1, PRLR, PRAME, PSMA (FOLH1), RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, STEAP1, STEAP2, TAG-72, TGF- $\beta$ , TMPRSS2, Thompson-nouvelle antigen (Tn), TRP-1, TRP-2, tyrosinase, and uroplakin-3.

6. The use of claim 5, wherein the tumor-associated antigen is CD20, EGFRvIII, PSMA (FOLH1), STEAP2, or MUC16.

7. The use of any one of claims 1-6, wherein the common light chain comprises a light chain variable region (LCVR) comprising complementarity determining regions LCDR1-LCDR2-LCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 164-166-168.

8. The use of claim 7, wherein the LCVR comprises the amino acid sequence of SEQ ID NO: 162.

9. The use of any one of claims 1-6, wherein the common light chain is a cognate light chain of the second antigen-binding arm that binds a tumor-associated antigen.

10. The use of claim 7, wherein the first antigen binding arm comprises complementarity determining regions HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 36-38-40-164-166-168.

11. The use of claim 7, wherein the first antigen binding arm comprises complementarity determining regions HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 140-142-144-164-166-168.

12. The use of claim 8, wherein the bispecific antibody comprises a HCVR and LCVR amino acid sequence pair (HCVR/LCVR) comprising the amino acid sequences of SEQ ID NOs: 34/162.

13. The use of claim 8, wherein the bispecific antibody comprises a HCVR and LCVR amino acid sequence pair (HCVR/LCVR) comprising the amino acid sequences of SEQ ID NOs: 138/162.

14. A cytotoxic bispecific antibody comprising a first antigen binding arm that exhibits weak to no detectable binding to human CD3 and cynomolgus CD3, and a second antigen binding arm that binds a tumor-associated antigen, wherein the first arm comprises a heavy chain variable region (HCVR) comprising complementarity determining regions HCDR1-

HCDR2-HCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 36-38-40 or SEQ ID NOs: 140-142-144, and a common light chain.

15. The bispecific antibody of claim 14, wherein the common light chain comprises a light chain variable region (LCVR) comprising complementarity determining regions LCDR1-LCDR2-LCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 164-166-168.

16. The bispecific antibody of claim 15, wherein the LCVR comprises the amino acid sequence of SEQ ID NO: 162.

17. The bispecific antibody of claim 14, wherein the common light chain is a cognate light chain of the second antigen-binding arm that binds a tumor-associated antigen.

18. The bispecific antibody of claim 15, wherein the first antigen binding arm comprises complementarity determining regions HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 36-38-40-164-166-168.

19. The bispecific antibody of claim 15, wherein the first antigen binding arm comprises complementarity determining regions HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 140-142-144-164-166-168.

20. The bispecific antibody of claim 16, wherein the bispecific antibody comprises a HCVR and LCVR amino acid sequence pair (HCVR/LCVR) comprising the amino acid sequences of SEQ ID NOs: 34/162.

21. The bispecific antibody of claim 16, wherein the bispecific antibody comprises a HCVR and LCVR amino acid sequence pair (HCVR/LCVR) comprising the amino acid sequences of SEQ ID NOs: 138/162.

22. A pharmaceutical composition comprising the cytotoxic bispecific antibody of any one of claims 14-21, and a pharmaceutically acceptable carrier or diluent.

23. Use of the pharmaceutical composition of claim 22 in the manufacture of a medicament for treating cancer in a subject.

24. The use of claim 23, wherein the cancer is selected from the group consisting of: pancreatic cancer, melanoma, glioblastoma, head and neck cancer, prostate cancer, malignant gliomas, osteosarcoma, colorectal cancer, gastric cancer, malignant mesothelioma, multiple myeloma, ovarian cancer, small cell lung cancer, non-small cell lung cancer, synovial sarcoma, thyroid cancer, breast cancer, melanomaglioma, breast cancer, squamous cell carcinoma, esophageal cancer, clear cell renal cell carcinoma, chromophobe renal cell carcinoma, renal oncocytoma, renal transitional cell carcinoma, urothelial carcinoma, adenocarcinoma, or small cell carcinoma.

25. The use of claim 23 or 24, wherein the subject is afflicted with a tumor that is resistant to, or incompletely responsive to monospecific antibody therapy alone.