(19) DANMARK

Patent- og
Varemærkestyrelsen

(10) **DK/EP 3515487 T5**

(12) Rettet oversættelse af
europæisk patentskrift

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(51) Int.Cl.: ***A 61 K 39/395 (2006.01)*** ***C 07 K 16/28 (2006.01)*** ***C 07 K 16/30 (2006.01)*** ***C 07 K 16/44 (2006.01)*** ***C 07 K 16/46 (2006.01)***

(45) Oversættelsen bekendtgjort den: **2024-09-02**

(80) Dato for Den Europæiske Patentmyndigheds
bekendtgørelse om meddelelse af patentet: **2023-07-19**

(86) Europæisk ansøgning nr.: **17784089.9**

(86) Europæisk indleveringsdag: **2017-09-22**

(87) Den europæiske ansøgnings publiceringsdag: **2019-07-31**

(86) International ansøgning nr.: **US2017053113**

(87) Internationalt publikationsnr.: **WO2018067331**

(30) Prioritet: **2016-09-23 US 201662399249 P** **2017-09-14 US 201762558711 P**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

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(54) Benævnelse: **Bispecifikke anti-MUC16-CD3-antistoffer og anti-MUC16-lægemiddelkonjugater**

(56) Fremdragne publikationer:
WO-A1-2016/149368
WO-A1-2017/053856

Fortsættes ...

DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention relates to bispecific antibodies or antigen-binding fragments thereof that bind to Mucin 16 (MUC16) and CD3, and methods of use thereof.

BACKGROUND

[0002] Mucin 16 (MUC16), also known as cancer antigen 125, carcinoma antigen 125, carbohydrate antigen 125, or CA-125, is a single transmembrane domain highly glycosylated integral membrane glycoprotein that is highly expressed in ovarian cancer. MUC16 consists of three major domains: an extracellular N-terminal domain, a large tandem repeat domain interspersed with sea urchin sperm, enterokinase, and agrin (SEA) domains, and a carboxyl terminal domain that comprises a segment of the transmembrane region and a short cytoplasmic tail. Proteolytic cleavage results in shedding of much of the extracellular portion of MUC16 into the bloodstream. MUC16 is overexpressed in cancers including ovarian cancer, breast cancer, pancreatic cancer, non-small-cell lung cancer, intrahepatic cholangiocarcinoma-mass forming type, adenocarcinoma of the uterine cervix, and adenocarcinoma of the gastric tract, and in diseases and conditions including inflammatory bowel disease, liver cirrhosis, cardiac failure, peritoneal infection, and abdominal surgery. (Haridas, D. et al., 2014, FASEB J., 28:4183-4199). Expression on cancer cells is shown to protect tumor cells from the immune system. (Felder, M. et al., 2014, Molecular Cancer, 13:129) Methods for treating ovarian cancer using antibodies to MUC16 have been investigated. Oregovomab and abgovomab are anti-MUC16 antibodies which have had limited success. (Felder, supra, Das, S. and Batra, S.K. 2015, Cancer Res. 75:4660-4674.)

[0003] CD3 is a homodimeric or heterodimeric antigen expressed on T cells in association with the T cell receptor complex (TCR) and is required for T cell activation. Functional CD3 is formed from the dimeric association of two of four different chains: epsilon, zeta, delta and gamma. The CD3 dimeric arrangements include gamma/epsilon, delta/epsilon and zeta/zeta. Antibodies against CD3 have been shown to cluster CD3 on T cells, thereby causing T cell activation in a manner similar to the engagement of the TCR by peptide-loaded MHC molecules. Thus, anti-CD3 antibodies have been proposed for therapeutic purposes involving the activation of T cells. In addition, bispecific antibodies that are capable of binding CD3 and a target antigen have been proposed for therapeutic uses involving targeting T cell immune responses to tissues and cells expressing the target antigen. The following document is relevant prior art: WO2016149368, "Anti-MUC16 antibodies and uses thereof", published on 22 September 2016.

[0004] Antigen-binding molecules that target MUC16, including antibody-drug conjugates, as well as bispecific antigen-binding molecules that bind both MUC16 and CD3 would be useful in therapeutic settings in which specific targeting and T cell-mediated killing of cells that express MUC16 is desired.

BRIEF SUMMARY OF THE INVENTION

[0005] In a first aspect, the present invention provides bispecific antibodies and antigen-binding fragments thereof that bind human MUC16 and human CD3. The bispecific antibodies 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 of cells expressing MUC16 is beneficial or desirable. For example, the bispecific antibodies can direct CD3-mediated T cell activation to specific MUC16-expressing cells, such as ovarian tumor cells.The antibodies, or antigen-binding fragments thereof, comprise an antigen-binding domain that specifically binds human MUC16 and comprises the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set of SEQ ID NOs: 20-22-24-28-30-32 (*e.g.*, H1 H8767P). The invention also provides pharmaceutical compositions that comprise the bispecific antibodies. The invention is defined by the claims.

SUMMARY OF TECHNICAL TEACHINGS

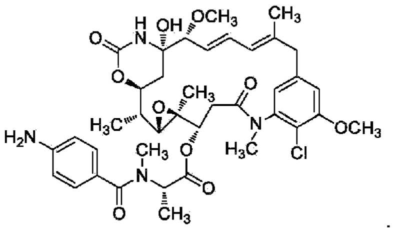
[0006] The technical information set out below may in some respects go beyond the invention, which is defined exclusively by the appended claims. The additional technical information is provided to place the actual invention in a broader technical context and to illustrate possible related technical developments. Such additional technical information which does not fall within the scope of the appended claims, is not part of the invention.

[0007] The isolated antibody or antigen-binding fragment thereof of the present disclosure binds human MUC16 within one or more of the five membrane-proximal SEA domains of human MUC 16 (SEQ ID NO: 1899). The five membrane-proximal SEA domains correspond to residues 13791-14451 of SEQ ID NO: 1899. In some cases, the antibody or antigen-binding fragment binds with a K_D of less than about 60 nM as measured in a surface plasmon resonance assay at 25°C. In some instances, the antibody or antigen-binding fragment binds within residues 14237 to 14290 of SEQ ID NO: 1899. For instance, the second antigen-binding domain that specifically binds human MUC16 may comprisea HCVR/LCVR pair comprising the amino acid sequences of SEQ ID NO: 18/26.

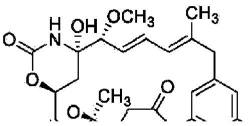
[0008] The antibodies or antigen-binding fragments thereof of the present disclosure may be used in antibody-drug conjugates comprising the antibody or antigen-binding fragment thereof and a therapeutic agent (*e.g.*, a cytotoxic agent). In some instances, the antibody or antigen-binding fragment and the cytotoxic agent are covalently attached via a linker, as discussed herein.

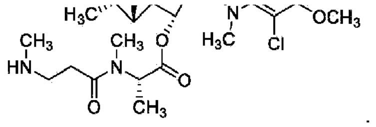
[0009] In some instances, the cytotoxic agent is selected from an auristatin, a maytansinoid, a tubulysin, a tomaymycin derivative, or a dolastatin derivative. In some cases, the cytotoxic agent is an auristatin selected from MMAE or MMAF, or a maytansinoid selected from DM1 or DM4. In some instances, the cytotoxic agent is a maytansinoid having the structure of Formula (I) or Formula (II), as discussed herein.

[0010] In some instances, the cytotoxic agent is a maytansinoid having the structure:

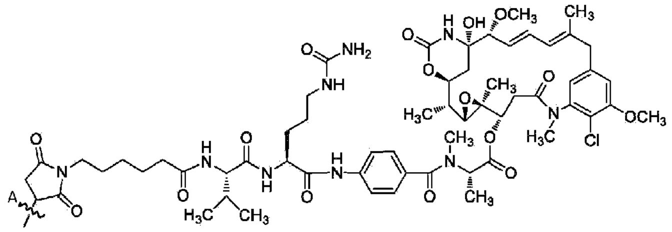


[0011] In some instances, the cytotoxic agent is a maytansinoid having the structure:

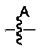




[0012] In some instances, the antibody-drug conjugate comprises an anti-MUC16 antibody or fragment thereof, and

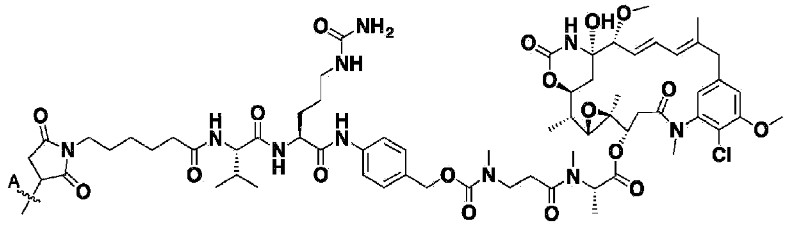


wherein



is a bond to the anti-MUC16 antibody or fragment thereof.

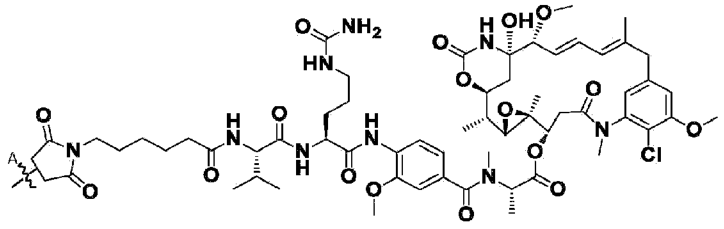
[0013] In some instances, the antibody-drug conjugate comprises an anti-MUC16 antibody or fragment thereof, and



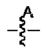
wherein

is a bond to the anti-MUC16 antibody or fragment thereof.

[0014] In some instances, the antibody-drug conjugate comprises an anti-MUC16 antibody or fragment thereof, and



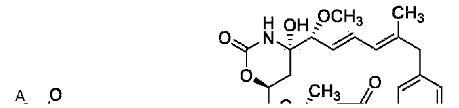
wherein

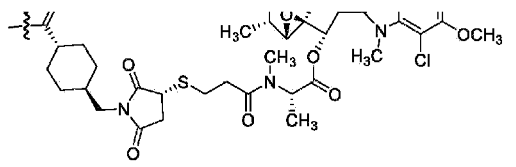


is a bond to the anti-MUC16 antibody or fragment thereof.

[0015] In some instances, the bond contacts the antibody or fragment thereof via a sulfur constituent of a cysteine residue.

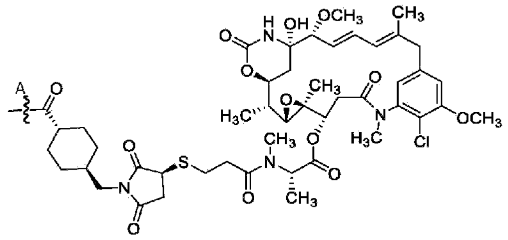
[0016] In some instances, the antibody-drug conjugate comprises an anti-MUC16 antibody or fragment thereof, and





,

or



,

or a mixture thereof,

wherein

is a bond to the anti-MUC16 antibody or fragment thereof.

[0017] In some instances, the bond contacts the antibody or fragment thereof via a nitrogen constituent of a lysine residue.

[0018] In any of the various instances of the antibody-drug conjugates discussed above or herein, the antibody-drug conjugate can comprise from 1 to 4 cytotoxic agents per antibody or fragment thereof.

[0019] The present disclosure provides bispecific antibodies, and antigen-binding fragments thereof, that bind MUC16 and CD3. Such bispecific antigen-binding molecules are also referred to herein as "anti-MUC16/anti-CD3 bispecific molecules," "anti-CD3/anti-MUC16 bispecific molecules," or "MUC16xCD3 bsAbs." The anti-MUC16 portion of the anti-MUC16/anti-CD3 bispecific molecule is useful for targeting cells (*e.g.*, tumor cells) that express MUC16 (*e.g.*, ovarian tumors), and the anti-CD3 portion of the bispecific molecule is useful for activating T-cells. The simultaneous binding of MUC16 on a tumor cell and CD3 on a T-cell facilitates directed killing (cell lysis) of the targeted tumor cell by the activated T-cell. The anti-MUC16/anti-CD3 bispecific molecules of the disclosure are therefore useful, *inter alia,* for treating diseases and disorders related to or caused by MUC16-expressing tumors (*e.g.*, ovarian cancers).

[0020] The bispecific antibodies, and antigen-binding fragments thereof, comprise a first antigen-binding domain that specifically binds human CD3, and a second antigen-binding domain that specifically binds MUC16, wherein the second antigen-binding domain comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 20-22-24-28-30-32. The present disclosure includes anti-MUC16/anti-CD3 bispecific molecules (*e.g.*, bispecific antibodies) wherein each antigen-binding domain comprises a heavy chain variable region (HCVR) paired with a light chain variable region (LCVR). In certain exemplary instances of the disclosure, the anti-CD3 antigen-binding domain and the anti-MUC16 antigen binding domain each comprise different, distinct HCVRs paired with a common LCVR. For example, as illustrated in Example 3 herein, bispecific antibodies were constructed comprising a first antigen-binding domain that specifically binds CD3, wherein the first antigen-binding domain comprises an HCVR derived from an anti-CD3 antibody paired with an LCVR derived from an anti-MUC16 antibody (*e.g.*, the same LCVR that is included in the anti-MUC16 antigen-binding domain); and a second antigen-binding domain that specifically binds MUC16, wherein the second antigen-binding domain comprises an HCVR/LCVR derived from an anti-MUC16 antibody. In other words, in the exemplary molecules disclosed herein, the pairing of an HCVR from an anti-CD3 antibody with an

LCVR from an anti-MUC16 antibody creates an antigen-binding domain that specifically binds CD3 (i.e., the MUC16-binding domain binds to MUC16, but the CD3-binding domain does not bind MUC16). In such instances, the first and second antigen-binding domains comprise distinct anti-CD3 and anti-MUC16 HCVRs but share a common anti-MUC16 LCVR. In other instances, the bispecific antibody, and antigen-binding fragment thereof, comprise distinct anti-CD3 and anti-MUC16 HCVRs, but share a common LCVR. 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. Alternatively, variable heavy chains may be paired with one common light chain and expressed recombinantly in host cells. As such, the antibodies of the disclosure can comprise immunoglobulin heavy chains associated with a single rearranged light chain. In some instances, the light chain comprises a variable domain derived from a human Vκ1-39 gene segment or a Vκ3-20 gene segment. In other instances, the light chain comprises a variable domain derived from a human Vκ1-39 gene segment rearranged with a human Jκ5 or a human Jκ1 gene segment.

[0021] In some instances, the first antigen-binding domain that specifically binds CD3 comprises any of the HCVR amino acid sequences, any of the LCVR amino acid sequences, any of the HCVR/LCVR amino acid sequence pairs, any of the heavy chain CDR1-CDR2-CDR3 amino acid sequences, or any of the light chain CDR1-CDR2-CDR3 amino acid sequences as set forth in US publication 2014/0088295 published March 27, 2014 and PCT/US2016/044732 filed July 29, 2016.

[0022] In some instances, the first antigen-binding domain that specifically binds CD3 comprises any of the HCVR amino acid sequences as set forth in Tables 16, 18, and 22 herein. The first antigen-binding domain that specifically binds CD3 may also comprise any of the LCVR amino acid sequences as set forth in Tables 1, 16, 19, and 23 herein. According to certain instance, the first antigen-binding domain that specifically binds CD3 comprises any of the HCVR/LCVR amino acid sequence pairs as set forth in Tables 16, 18, 19, 22, and 23 herein. The present disclosure also provides anti-CD3/anti-MUC16 bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises any of the heavy chain CDR1-CDR2-CDR3 amino acid sequences as set forth in Tables 16, 18, and 22 herein, and/or any of the light chain CDR1-CDR2-CDR3 amino acid sequences as set forth in Tables 1, 16, 19, and 23 herein.

[0023] In some instances, the first antigen-binding domain that specifically binds CD3 comprises a heavy chain variable region (HCVR) having an amino acid sequence as set forth in Tables16, 18, and 22 herein or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0024] In some instances, the first antigen-binding domain that specifically binds CD3 comprises a light chain variable region (LCVR) having an amino acid sequence as set forth in Tables 1, 6, 19, and 23 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0025] In some instances, the first antigen-binding domain that specifically binds CD3 comprises a HCVR and LCVR (HCVR/LCVR) amino acid sequence pair as set forth in Tables 16, 18, 19, 22, and 23 herein.

[0026] In some instances, the first antigen-binding domain that specifically binds CD3 comprises a heavy chain CDR3 (HCDR3) domain having an amino acid sequence as set forth in Tables 16, 18, and 22 herein, or a substantially similar sequence thereto having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR3 (LCDR3) domain having an amino acid sequence as set forth in Tables 1, 16, 19, and 23 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0027] In certain instances, the first antigen-binding domain that specifically binds CD3 comprises a HCDR3/LCDR3 amino acid sequence pair as set forth in Tables 16, 18, 19, 22, and 23 herein.

[0028] In some instances, the first antigen-binding domain that specifically binds CD3 comprises a heavy chain CDR1 (HCDR1) domain having an amino acid as set forth in Tables 16, 18, and 22 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR2 (HCDR2) domain having an amino acid as set forth in Tables 16, 18, and 22, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR3 (HCDR3) domain having an amino acid as set forth in Tables 16, 18, and 22, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a light chain CDR1 (LCDR1) domain having an amino acid sequence as set forth in Tables 1, 16, 19, and 23 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a light chain CDR2 (LCDR2) domain having an amino acid sequence as set forth in Tables 1, 16, 19, and 23 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity, and a light chain CDR3 (LCDR3) domain having an amino acid sequence as set forth in Tables 1, 16, 19, and 23 herein , or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0029] Certain non-limiting, exemplary anti-CD3/anti-MUC16 bispecific antigen-binding molecules of the disclosure include a first antigen-binding domain that specifically binds CD3 comprising HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, having the amino acid sequences as set forth in Tables 16, 18, 19, 22, and 23 herein.

[0030] In some instances, the first antigen-binding domain that specifically binds human CD3 comprises heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) from a heavy chain variable region (HCVR) comprising an amino acid sequence as set forth in Table 16, Table 18, or Table 22 and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) from a light chain variable region (LCVR) comprising an amino acid sequence as set forth in Table 1, Table 16, Table 19 or Table 23.

[0031] In some instances, the first antigen-binding domain that specifically binds human CD3 comprises heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) from a heavy chain variable region (HCVR) selected from the group consisting of SEQ ID NOs: 1730, 1762, 1778, 1786, and 1866, and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) from a light chain variable region (LCVR) comprising an amino acid sequence of SEQ ID NO: 26.

[0032] In some instances, the first antigen-binding domain that specifically binds human CD3 comprises three heavy chain complementarity determining regions (A1-HCDR1, A1-HCDR2 and A1-HCDR3) and three light chain complementarity determining regions (A1-LCDR1, A1-LCDR2 and A1-LCDR3), wherein A1-HCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:1732, 1764, 1780, 1788, and 1868; A1-HCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:1734, 1766, 1782, 1790, and 1870; A1-HCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:1736, 1768, 1784, 1792, and 1872; A1-LCDR1 comprises an amino acid sequence of SEQ ID NO:28; A1-LCDR2 comprises an amino acid sequence of SEQ ID NO:30; and A1-LCDR3 comprises an amino acid sequence of SEQ ID NO:32.

[0033] In some instances, the first antigen-binding domain that specifically binds human CD3 comprises the heavy and light chain CDRs of a HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 1730/26, 1762/26, 1778/26, 1786/26, and 1866/26

[0034] In some instances, the first antigen-binding domain that specifically binds human CD3 comprises three heavy chain complementarity determining regions (A1-HCDR1, A1-HCDR2 and A1-HCDR3) and three light chain complementarity determining regions (A1-LCDR1, A1-LCDR2 and A1-LCDR3), and wherein the second antigen-binding domain that specifically binds human MUC16 comprises three heavy chain complementarity determining regions (A2-HCDR1, A2-HCDR2 and A2-HCDR3) and three light chain complementarity determining regions (A2-LCDR1, A2-LCDR2 and A2-LCDR3); wherein A1-HCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1732, 1764, 1780, 1788, and 1868; A1-HCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1734, 1766, 1782, 1790, and 1870; A1-HCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1736, 1768, 1784, 1792, and 1872; A1-LCDR1 comprises an amino acid sequence of SEQ ID NO:28; A1-LCDR2 comprises an amino acid sequence of SEQ ID NO:30; and A1-LCDR3 comprises an amino acid sequence of SEQ ID NO:32; and wherein A2-HCDR1 comprises an amino acid sequence of SEQ ID NO:20; A2-HCDR2 comprises an amino acid sequence of SEQ ID NO:22; A2-HCDR3 comprises an amino acid sequence of SEQ ID NO:24; A2-LCDR1 comprises an amino acid sequence of SEQ ID NO:28; A2-LCDR2 comprises an amino acid sequence of SEQ ID NO:30; and A2-LCDR3 comprises an amino acid sequence of SEQ ID NO:32.

[0035] Certain non-limiting, exemplary anti-CD3/anti-MUC16 bispecific antigen-binding molecules of the disclosure include a first antigen-binding domain that specifically binds CD3 comprising a heavy chain comprising variable domain framework regions having an amino acid sequence selected from FR1 (SEQ ID NO: 1903), FR2 (SEQ ID NO: 1904), FR3 (SEQ ID NO: 1905), and FR4 (SEQ ID NO: 1906).

[0036] In more instances, exemplary anti-CD3/anti-MUC16 bispecific antigen-binding molecules of the disclosure include a bispecific antigen-binding molecule wherein the first antigen-binding domain that specifically binds human CD3 comprises a HCVR comprising HCDR1-HCDR2-HCDR3 having the amino acid sequences of SEQ ID NOs: 1907-1908-1909.

[0037] In some instances, the second antigen-binding domain that specifically binds MUC16 comprises a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO: 18, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0038] In some instances, the second antigen-binding domain that specifically binds MUC16 comprises a light chain variable region (LCVR) having the amino acid sequence of SEQ ID No:26, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0039] In some instances, the second antigen-binding domain that specifically binds MUC16 comprises a HCVR and LCVR (HCVR/LCVR) amino acid sequence pair of SEQ ID NOs:18/26.

[0040] The bispecific antibody or antigen-binding fragment thereof of the disclosure may have the following properties: binds human cells expressing human CD3 and cynomolgus monkey cells expressing cynomolgus CD3; binds human cells expressing human MUC16; inhibits tumor growth in immunocompromised mice bearing human ovarian cancer xenografts; suppresses tumor growth of established tumors in immunocompromised mice bearing human ovarian cancer xenografts; the first antigen-binding domain specifically binds an effector cell with an EC_{50} value of greater than about 4 nM and, and the second antigen-binding domain specifically binds a target human ovarian tumor cell with an

EC_{50} value of less than 3 nM, wherein such EC_{50} binding value is measured in an *in vitro* FACS binding assay; the second antigen-binding domain specifically binds the target ovarian tumor cell with an EC_{50} value of less than about 2 nM; the first antigen-binding domain specifically binds each of human CD3 and cynomolgus CD3 with an EC_{50} value of greater than about 40 nM, 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 μM; the first antigen-binding domain specifically binds each of human CD3 and cynomolgus CD3 with weak or no measurable binding or binding affinity; induces T cell-mediated tumor cell killing with an EC_{50} value of less than about 31 pM, as measured in an *in vitro* T cell-mediated tumor cell killing assay, for example, where the tumor cells are OVCAR3 cells; the first antigen-binding domain binds human CD3 with an K_D value of greater than about 11 nM, as measured in an *in vitro* surface plasmon resonance binding assay; the first antigen-binding domain binds each of human CD3 and cynomolgus CD3 with an K_D value of greater than about 15 nM, greater than about 30 nM, greater than about 60 nM, greater than about 120 nM, greater than about 300 nM, or greater than about 500 nM as measured in an *in vitro* surface plasmon resonance binding assay.

[0041] In certain instances, antibodies of the disclosure, and antigen-binding fragments 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.

[0042] In some instances, the first antigen-binding domain competes for binding to human CD3 with a reference antigen-binding protein comprising three heavy chain complementarity determining regions (A1-HCDR1, A1-HCDR2 and A1-HCDR3) and three light chain complementarity determining regions (A1-LCDR1, A1-LCDR2 and A1-LCDR3), A1-HCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1732, 1764, 1780, 1788, and 1868; A1-HCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1734, 1766, 1782, 1790, and 1870; A1-HCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1736, 1768, 1784, 1792, and 1872; A1-LCDR1 comprises an amino acid sequence SEQ ID NO: 28; A1-LCDR2 comprises an amino acid sequence of SEQ ID NO: 30; and A1-LCDR3 comprises an amino acid sequence of SEQ ID NO: 32. In some instances, the first antigen-binding domain competes for binding to human CD3 with a reference antigen-binding protein comprising a heavy chain variable region (HCVR) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1730, 1762, 1778, 1786, and 1866, and a light chain variable region (LCVR) comprising an amino acid sequence of SEQ ID NO:26.

[0043] In one aspect, the disclosure provides a pharmaceutical composition comprising an anti-MUC16/anti-CD3 bispecific antigen-binding molecule of the disclosure and a pharmaceutically acceptable carrier or diluent. The disclosure further provides the anti-MUC16/anti-CD3 bispecific antigen-binding molecule, or antigen-binding fragment thereof, of the disclosure for use in a method for treating a cancer in a subject, the method comprising administering to the subject the pharmaceutical composition comprising an anti-MUC16 antigen-binding molecule or anti-MUC16/anti-CD3 bispecific antigen-binding molecule and a pharmaceutically acceptable carrier or diluent. In some instances, the cancer is selected from the group consisting of cancers including ovarian cancer, breast cancer, pancreatic cancer, non-small-cell lung cancer, intrahepatic cholangiocarcinoma-mass forming type, adenocarcinoma of the uterine cervix, and adenocarcinoma of the gastric tract. In some cases, the cancer is ovarian cancer. However, methods of treatment are not part of the claimed invention.

[0044] The present disclosure provides nucleic acid molecules encoding any of the HCVR, LCVR or CDR sequences of the anti-CD3/anti-MUC16 bispecific antigen-binding molecules disclosed herein. Disclosed are nucleic acid molecules comprising the polynucleotide sequences as set forth in Tables 2, 17, 20, 21, 23, and 25 herein, as well as nucleic acid molecules comprising two or more of the polynucleotide sequences as set forth in Tables 2, 17, 20, 21, 23, and 25 in any functional combination or arrangement thereof. Recombinant expression vectors carrying the nucleic acids of the disclosure, and host cells into which such vectors have been introduced, are also described, as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced. However, nucleic acids, vectors, host cells and methods of producing antibodies are not part of the claimed invention.

[0045] The present disclosure includes anti-CD3/anti-MUC16 bispecific antigen-binding molecules having a modified glycosylation pattern. In some applications, 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).

[0046] In another aspect, the disclosure provides a pharmaceutical composition comprising an anti-CD3/anti-MUC16 bispecific antigen-binding molecule as disclosed herein and a pharmaceutically acceptable carrier. Also described herein is a composition which is a combination of an anti-CD3/anti-MUC16 bispecific antigen-binding molecule and a second therapeutic agent. For instance, the second therapeutic agent may be any agent that is advantageously combined with an anti-CD3/anti-MUC16 bispecific antigen-binding molecule. Exemplary agents that may be advantageously combined with an anti-CD3/anti-MUC16 bispecific antigen-binding molecule are discussed in detail elsewhere herein.

[0047] In yet another aspect, the disclosure provides the bispecific antibody or antigen-binding fragment thereof of the disclosure for use in therapeutic methods for targeting/killing tumor cells expressing MUC16, wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an anti-CD3/anti-MUC16 bispecific antigen-binding molecule of the disclosure to a subject in need thereof. Therapeutic methods are not part of the claimed invention.

[0048] Methods of using the antibodies of the disclosure are described herein, including a method of detecting MUC16 in a biological sample, comprising: obtaining a biological sample from a subject, and detecting whether MUC16 is present in the biological sample by contacting the biological sample with an anti-MUC16 antibody or antigen-binding fragment thereof and detecting binding between MUC16 and the anti-MUC16 antibody or antigen-binding fragment. In some cases, the biological sample is a tissue or fluid sample selected from plasma, serum, ascites, ovary, uterus, cervix, liver, bladder, pancreas, stomach, small or large intestine, gall bladder, breast, lung, kidney, salivary, and lacrimal glands, or any epithelioid malignancy thereof.

[0049] Also described is a method of detecting MUC16 in a patient, comprising: obtaining a tissue sample from the patient; and detecting whether MUC16 is present in the tissue sample by contacting the tissue sample with an anti-MUC16 antibody and detecting binding between MUC16 and the anti-MUC16 antibody. In some cases, the method further comprises diagnosing the patient with a cancer when the presence of MUC16 in the tissue sample is detected. In one instance, the tissue sample is ovarian tissue. However, only the antibodies defined in the claims fall within the scope of the invention.

[0050] Also described is a method of detecting MUC16 in a patient, comprising: obtaining a plasma sample from the patient; and detecting whether MUC16 is present in the plasma sample by contacting the plasma sample with an anti-MUC16 antibody, and detecting binding between MUC16 and the anti-MUC16 antibody. In some cases, the method further comprises diagnosing the patient with a cancer when the presence of MUC16 in the plasma sample is detected. In some instances, the method further comprises administering an effective amount of an anti-CD3xMUC16 bispecific antibody to the diagnosed patient. In some instances, the method further comprises administering an effective amount of an ADC comprising an anti-MUC16 antibody or antigen-binding fragment thereof and a cytotoxic agent to the diagnosed patient. However, only the antibodies defined in the claims fall within the scope of the invention.

[0051] Other instances will become apparent from a review of the ensuing detailed description. The invention is defined in the independent claims. Further aspects and preferred embodiments are defined in the dependent claims. Any aspects, embodiments and examples of the present disclosure which do not fall under the scope of the appended claims do not form part of the invention and are merely provided for illustrative purposes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0052]

Figures 1, 2, and 3 illustrate pharmacokinetic profiles of anti-MUC16 x CD3 bispecific antibodies in wild-type mice (Fig. 1), humanized CD3 mice (Fig. 2) or humanized MUC16 x CD3 mice (Fig. 3).

Figure 4 shows the results of the OVCAR-3 model study 1 (Avg Radiance [p/s/cm^2/sr] at Day 6). All groups had similar tumor burden as assessed by BLI before dosing started. Data shown is tumor burden as assessed by BLI on Day 6 post tumor implantation. Statistical significance was determined using unpaired nonparametric Mann-Whitney t-tests. There was no significant difference in tumor burden between groups.

Figure 5 shows the results of the OVCAR-3 model study 1 (Avg Radiance [p/s/cm2^2/sr] at Day 20). BSMUC16/CD3-001 significantly reduces tumor burden at 0.1 and 0.5 mg/kg. NSG mice engrafted with human T cells were implanted with human OVCAR-3/Luc cells. Treatment began 6 days post tumor implantation. Mice were treated on Days 6, 10, 13, 16 and 21 with 0.01, 0.1, or 0.5 mg/kg BSMUC16/CD3-001 administered IP or treated with a CD3-binding control or non-binding control (0.5 mg/kg IP). Data shown is tumor burden as assessed by BLI on Day 20 post tumor implantation. Statistical significance was determined using unpaired nonparametric Mann-Whitney t-tests. Treatment with BSMUC16/CD3-001 was compared to the non-binding control (** $p < 0.01$ for 0.5 mg/kg, # $p < 0.05$ for 0.1 mg/kg BSMUC16/CD3-001).

Figure 6 shows the results of the OVCAR-3 model study 1 (Fold change in BLI-evident tumors between D6 and D20). BSMUC16/CD3-001 significantly reduces fold change in tumor burden at 0.01, 0.1, and 0.5 mg/kg. NSG mice engrafted with human T cells were implanted with human OVCAR-3/Luc cells. Mice were treated on Days 6, 10, 13, 16 and 21 with 0.01, 0.1 or 0.5 mg/kg BSMUC16/CD3-001 administered IP or treated with a CD3-binding control or non-binding control (0.5 mg/kg IP). Data shown is fold change in tumor burden from first measurement (taken before treatment began) and on Day 20 at end of study. Statistical significance was determined using unpaired nonparametric Mann-Whitney t-tests. Treatment with BSMUC16/CD3-001 was compared to the non-binding control (** $p < 0.01$ for 0.5 mg/kg, # $p < 0.05$ for 0.1 mg/kg, $ $p < 0.05$ for 0.01 mg/kg BSMUC16/CD3-001).

Figure 7 shows the results of the OVCAR-3 model study 2 (Avg Radiance [p/s/cm^2/sr] at Day 4). All groups had similar tumor burden as assessed by BLI before dosing started. Data shown is tumor burden as assessed by BLI on Day 4 post tumor implantation. Statistical significance was determined using unpaired nonparametric Mann-Whitney t-tests. There was no significant difference in tumor burden at Day 4 between groups.

Figure 8 shows the results of the OVCAR-3 model study 2 (Avg Radiance [p/s/cm2^2/sr] at Day 25). BSMUC16/CD3-005 significantly reduces tumor burden at 0.5, 1 and 5 mg/kg. NSG mice engrafted with human T cells were implanted with human OVCAR-3/Luc cells. Treatment began 5 days post tumor implantation. Mice were treated on Days 5, 8, 12, 15, 19, and 22 with 0.1, 0.5, 1, or 5 mg/kg REGN4019 administered IV or administered a CD3-binding control or non-binding control (5 mg/kg IV). Data shown is tumor burden as assessed by BLI on Day 25 post tumor implantation. Statistical significance was determined using unpaired nonparametric Mann-Whitney t-tests. Treatment with BSMUC16/CD3-005 was compared to the non-binding control (** $p < 0.01$ for 5 mg/kg, ## $p < 0.01$ for 1 mg/kg, $$ $p < 0.01$ for 0.5 mg/kg BSMUC16/CD3-005).

Figure 9 shows the results of the OVCAR-3 model study 2 (Fold change in BLI-evident tumors between D4 and D25). BSMUC16/CD3-005 significantly reduces tumor growth at 0.5, 1 and 5 mg/kg. NSG mice engrafted with human T cells were implanted with human OVCAR-3/Luc cells. Mice were treated on Days 5, 8, 12, 15, 19, and 22 with 0.1, 0.5, 1, or 5 mg/kg REGN4019 administered IV or treated with a CD3-binding control or non-binding control (5 mg/kg IV). Data shown is fold change in tumor burden from first measurement (taken the day before treatment began) and on Day 25, at end of study. Statistical significance was determined using unpaired nonparametric Mann-Whitney t-tests. Treatment with BSMUC16/CD3-005 was compared to the non-binding control (** $p < 0.01$ for 5 mg/kg, ## $p < 0.01$ for 1 mg/kg, $$ $p < 0.01$ for 0.5 mg/kg REGN4019).

Figure 10 shows the results of the ID8-VEGF/huMUC16 model. Tumor size at Day 47 post implantation BSMUC16/CD3-001 significantly reduces tumor growth in a syngeneic model when treatment begins either on day of implantation or 10 days post tumor implantation. Mice expressing human CD3 in place of mouse CD3 and a chimeric MUC16 molecule were implanted with the murine ovarian tumor line expressing a portion of human MUC16. Mice were administered BSMUC16/CD3-001 (100ug IP) on day of implantation or 10 days post implantation or administered CD3-binding control (100ug IP) on day of implantation. Mice were treated on Days 0, 4, 7, 10, 13, 17, 20 or 24 for immediate-treatment groups and on days 10, 13, 17, 20 and 24 for the group where dosing started on D10. Data shown is tumor volume on Day 47 post implantation. Statistical significance was determined using unpaired nonparametric Mann-Whitney t-tests. Treatment with BSMUC16/CD3-001 was compared to the CD3-binding control (** $p < 0.01$ starting at D0, * $p < 0.05$ starting at D10).

Figures 11A-11C illustrate the results of flow cytometric analysis (or FACS) of the binding of select bispecific antibodies to PEO-1, OVCAR3-Luc, Jurkat cells, and cynomolgus T cells. The titration analysis was done by testing a range of serial dilutions of each antibody: either MUC16xCD3 bispecific antibodies BSMUC16/CD3-001, BSMUC16/CD3-002, or BSMUC16/CD3-003, or a first or second isotype control antibody (having no cross-reactivity to CD3 or MUC16).

Figures 12A and 12B depict examples of PEO-1 (Fig. 12A) or OVCAR3-Luc (Fig. 12B) cell killing in a 48hour cytotoxicity assay following anti-MUC16xanti-CD3 treatment in the presence of human PBMCs.

DETAILED DESCRIPTION

[0053] The technical information set out below may in some respects go beyond the disclosure of the disclosure, which is defined exclusively by the appended claims. The additional technical information is provided to place the actual disclosure in a broader technical context and to illustrate possible related technical developments. Such additional technical information which does not fall within the scope of the appended claims, is not part of the disclosure.

[0054] Before the present disclosure is described, it is to be understood that this disclosure 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 aspects only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0055] 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.).

[0056] 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

[0057] The expression "CD3," as used herein, 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 comprises the amino acid sequence as set forth in SEQ ID NO:1897; human CD3-delta comprises the amino acid sequence as set forth in SEQ ID NO:1898. 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.

[0058] As used herein, "an antibody that binds CD3" or an "anti-CD3 antibody" includes antibodies and antigen-binding fragments thereof that specifically recognize a single CD3 subunit (e.g., epsilon, delta, gamma or zeta), as well as antibodies and antigen-binding fragments thereof that specifically recognize a dimeric complex of two CD3 subunits (e.g., gamma/epsilon, delta/epsilon, and zeta/zeta CD3 dimers). The antibodies and antigen-binding fragments may bind soluble 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.

[0059] As used herein, 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.*, gamma/epsilon, delta/epsilon, and zeta/zeta CD3 dimers). The expression, "cell surface-expressed CD3" also includes a CD3 chain (*e.g.*, CD3-epsilon, CD3-delta 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.

[0060] The expression "MUC16," as used herein, refers to mucin 16. MUC16 is a single transmembrane domain highly glycosylated integral membrane glycoprotein that is highly expressed in ovarian cancer. The amino acid sequence of human MUC16 is set forth in SEQ ID NO:1899.

[0061] As used herein, "an antibody that binds MUC16" or an "anti-MUC16 antibody" includes antibodies and antigen-binding fragments thereof that specifically recognize MUC16.

[0062] The term "antigen-binding molecule" includes antibodies and antigen-binding fragments of antibodies, including, *e.g.*, bispecific antibodies.

[0063] The term "antibody", as used herein, means 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.*, MUC16 or 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_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (C_L1). The V_H and V_L 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_H and V_L 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 instances , the FRs of the anti-MUC16 antibody or 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.

[0064] The term "antibody", as used herein, 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.

[0065] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 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.

[0066] 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_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H-V_H, V_H-V_L or V_L-V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0067] In certain instances, 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 include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. 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 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_H or V_L domain (*e.g.*, by disulfide bond(s)).

[0068] 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 using routine techniques available in the art.

[0069] The antibodies 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 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.

[0070] In certain instances, the anti-MUC16 monospecific antibodies or anti-MUC16/anti-CD3 bispecific antibodies 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 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.

[0071] The antibodies may, in some instances, be recombinant human antibodies. The term "recombinant human antibody", as used herein, 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 instances, 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_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo.*

[0072] 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.

[0073] 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 disclosure encompasses antibodies having one or more mutations in the hinge, C_H2 or C_H3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form.

[0074] The antibodies 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 disclosure. 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 instances, an isolated antibody may be substantially free of other cellular material and/or chemicals. Only an isolated antibody or antigen-binding fragment thereof, as defined in the claims, is within the scope of an antibody of the claimed invention.

[0075] Also described are one-arm antibodies that bind MUC16. As used herein, a "one-arm antibody" means an antigen-binding molecule comprising a single antibody heavy chain and a single antibody light chain. The one-arm antibodies may comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Table 1. However, one-arm antibodies that fall outside of the scope of the claims are not part of the invention.

[0076] The anti-MUC16/anti-CD3 antibodies disclosed herein may 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 antibodies were derived. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present disclosure 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"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain instances, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other instances, only certain residues are mutated back to the original germline sequence, e.g., only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other instances, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (i.e., a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies 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 easily tested for one or more desired property such as, improved binding specificity, increased binding (*e.g.*, as measured by cell binding titration or FACS binding) or binding affinity (*e.g.*, K_D), improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present disclosure. However, only an antibody or antigen-binding fragment thereof that comprises a first antigen-binding domain that specifically binds human CD3, and a second antigen-binding domain that specifically binds human MUC16, wherein the second antigen-binding domain comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 20-22-24-28-30-32, as defined in the claims, is within the scope of the antibodies of the claimed invention.

[0077] Also described are anti-MUC16 or anti-MUC16/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, anti-MUC16 or anti-MUC16/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 1 herein or as described in Tables 16, 18, 19, 22, and 23 herein.

[0078] 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 circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

[0079] 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.

[0080] 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.

[0081] 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 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

[0082] The antibodies of the invention are defined by the claims. Other antibodies are also described herein. The anti-CD3 antibodies disclosed herein may 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.

[0083] Described herein are 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 Tables 16, 18, 19, 22, and 23 herein. However, only an antibody or antigen-binding fragment thereof that comprises a first antigen-binding domain that specifically binds human CD3, and a second antigen-binding domain that specifically binds human MUC16, wherein the second antigen-binding domain comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 20-22-24-28-30-32, as defined in the claims, is within the scope of the antibodies of the claimed invention.

[0084] Furthermore, the antibodies 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 or binding affinity, improved or enhanced pharmacokinetic properties, reduced immunogenicity, etc.

[0085] Also described herein are 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, 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 Tables 16, 18, 19, 22, and 23 herein. The antibodies and bispecific antigen-binding molecules may 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 or 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.

[0086] Also disclosed herein are 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.

[0087] 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 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.

[0088] Once obtained, antigen-binding domains that contain one or more germline mutations were tested for decreased binding or 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 or binding affinity to the antigen, the antibodies exhibit weak binding or no detectable binding. Bispecific antigen-binding molecules comprising one or more antigen-binding domains obtained in this general manner were found to be advantageous as avidity-driven tumor therapies.

[0089] 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

[0090] 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.

[0091] 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).

[0092] Accordingly, the antibody or antigen-binding protein binds to the predetermined antigen or cell surface molecule (receptor) 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). The affinity of an antibody corresponding to a K_D value that is equal to or less than ten-fold lower than a non-specific antigen 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.

[0093] 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.

[0094] The term "k_d" (sec -1 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.

[0095] The term "k_a" (M-1 x sec-1 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.

[0096] 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.

[0097] The term "EC50" or "EC_{50}" 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_{50} essentially represents the concentration of an antibody where 50% of its maximal effect is observed. In certain instances, the EC_{50} value equals the concentration of an antibody 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_{50}, or half maximal effective concentration value.

[0098] For instance, decreased binding can be defined as an increased EC_{50} antibody concentration which enables binding to the half-maximal amount of target cells.

[0099] In another instance, the EC_{50} value represents the concentration of an antibody 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_{50}, or half maximal effective concentration value.

Bispecific Antigen-Binding Molecules

[0100] The antibodies described herein may be monospecific, bi-specific, or multispecific. The antibodies of the invention are bispecific, as defined in the claims. Multispecific antibodies may be specific for 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-MUC16 monospecific antibodies or anti-MUC16/anti-CD3 bispecific antibodies 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 or additional binding specificity.

[0101] Use of the expression "anti-CD3 antibody" or "anti-MUC16 antibody" herein is intended to include both monospecific anti-CD3 or anti-MUC16 antibodies as well as bispecific antibodies comprising a CD3-binding arm and a MUC16-binding arm. Thus, the present disclosure relates to bispecific antibodies wherein one arm of an immunoglobulin binds human CD3, and the other arm of the immunoglobulin is specific for human MUC16. The CD3-binding arm can comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Tables 1, 16, 18, 19, 22, and 23 herein.

[0102] In certain instances, the CD3-binding arm binds to human CD3 and induces human T cell activation. In certain instances, the CD3-binding arm binds weakly to human CD3 and induces human T cell activation. In other instances, 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 instances, 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. The MUC16-binding arm can comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Table 1 herein.

[0103] The present invention relates to bispecific antibodies and antigen-binding fragments thereof that specifically bind CD3 and MUC16, as defined in the claims. Such molecules may be referred to herein as, *e.g.*, "anti-CD3/anti-MUC16," or "anti-CD3xMUC16" or "CD3xMUC16" bispecific molecules, or other similar terminology (*e.g.*, anti-MUC16/anti-CD3). Described herein are antigen-binding molecules constructed with a first antigen-binding arm that binds MUC16 and a second antigen-binding arm that binds CD3. In some instances, the anti-CD3 arm comprises a heavy chain derived from IGHV3-9*01, IGHJ6*02, IGHD5-12*01.In other instances, the bispecific antigen-binding molecule activates human PBMC cells and/or induces cytotoxic activity on tumor antigen-expressing cell lines.

[0104] The term "MUC16," as used herein, refers to the human MUC16 protein unless specified as being from a non-human species (*e.g.*, "mouse MUC16," "monkey MUC16," etc.). The human MUC16 protein has the amino acid sequence shown in SEQ ID NO:1899.

[0105] The aforementioned bispecific antigen-binding molecules that specifically bind CD3 and MUC16 may comprise an anti-CD3 antigen-binding molecule which binds to CD3 with a weak binding affinity such as exhibiting a K_D of greater than about 40 nM, as measured by an *in vitro* affinity binding assay. The aforementioned bispecific antigen-binding molecules may comprise an anti-CD3 antigen-binding molecule which binds to CD3 and exhibits an EC50 of greater than about 100 nM, as measured by a FACS titration assay. The aforementioned bispecific antigen-binding molecules may comprise an anti-CD3 antigen-binding molecule which exhibits no measurable or observable binding to CD3, as measured by an *in vitro* affinity binding assay or a FACS titration assay, yet retains ability to activate human PBMC cells and/or induce cytotoxic activity on tumor antigen-expressing cell lines.

[0106] 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 instances, an antigen-binding molecule is an antibody or a fragment of an antibody, as those terms are defined elsewhere herein.

[0107] 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 (CD3), and the second antigen-binding domain specifically binds a second, distinct antigen (MUC16).

[0108] In the present invention, the bispecific antigen-binding molecule is a bispecific antibody or antigen binding fragment thereof, as defined in the claims. 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.

[0109] 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. 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_H3 domain. A non-limiting example of a multimerizing component is an Fc portion of an immunoglobulin (comprising a C_H2-C_H3 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.

[0110] Bispecific antigen-binding molecules 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.

[0111] In certain instances, the multimerizing domain is an Fc fragment or an amino acid sequence of from 1 to about 200 amino acids in length containing at least one cysteine residue. In other instances, 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.

[0112] Any bispecific antibody format or technology may be used to make the bispecific antigen-binding molecules described herein. 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 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^2 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).

[0113] 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, 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. For instance, the bispecific antigen-binding molecule comprises a modification in a C_H2 or a C_H3 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. For instance, 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).

[0114] Also described are bispecific antigen-binding molecules comprising a first C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 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. For instance, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 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_H3 may further comprise a Y96F modification (by IMGT; Y436F by EU). See, for example, US Patent No. 8,586,713. Further modifications that may be found within the second C_H3 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.

[0115] In certain instances, 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_H2 sequence derived from a human IgG1, human IgG2 or human IgG4 C_H2 region, and part or all of a C_H3 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_H1] - [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_H1] - [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 are described in US Publication 2014/0243504, published August 28, 2014. Chimeric Fc domains having these general structural arrangements, and variants thereof, can have altered Fc receptor binding, which in turn affects Fc effector function.

[0116] Described herein is 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: 1911, SEQ ID NO: 1912, SEQ ID NO: 1913, SEQ ID NO: 1914, SEQ ID NO: 1915, SEQ ID NO: 1916, SEQ ID NO: 1917, SEQ ID NO: 1918, SEQ ID NO: 1919 or SEQ ID NO: 1920. In some instances, the heavy chain constant region (CH) region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1911, SEQ ID NO: 1912, SEQ ID NO: 1913, SEQ ID NO: 1914, SEQ ID NO: 1915, SEQ ID NO: 1916, SEQ ID NO: 1917, SEQ ID NO: 1918, SEQ ID NO: 1919 and SEQ ID NO: 1920.

[0117] Also described herein is 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: 1921, SEQ ID NO: 1922, SEQ ID NO: 1923 SEQ ID NO: 1924 SEQ ID NO: 1925, SEQ ID NO: 1926, SEQ ID NO: 1927, SEQ ID NO: 1928, SEQ ID NO: 1929 or SEQ ID NO: 1930. In some instances, the Fc domain comprises an amino acid sequence selected form the group consisting of SEQ ID NO: 1921, SEQ ID NO: 1922, SEQ ID NO: 1923 SEQ ID NO: 1924 SEQ ID NO: 1925, SEQ ID NO: 1926, SEQ ID NO: 1927, SEQ ID NO: 1928, SEQ ID NO: 1929 and SEQ ID NO: 1930. However, only an antibody or antigen-binding fragment thereof that comprises a first antigen-binding domain that specifically binds human CD3, and a second antigen-binding domain that specifically binds human MUC16, wherein the second antigen-binding domain comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 20-22-24-28-30-32, as defined in the claims, is within the scope of the antibodies of the claimed invention.**Sequence Variants**

[0118] The antibodies of the invention are defined by the claims. Other antibodies are also described herein. The antibodies and bispecific antigen-binding molecules described herein may 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. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The antigen-binding molecules may comprise antigen-binding domains which are derived from any of the exemplary 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"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain instances, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antigen-binding domain was originally derived. In other instances, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other instances, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antigen-binding domain was originally derived). Furthermore, the antigen-binding domains 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, antigen-binding domains that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding or binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc.

[0119] Also described herein are antigen-binding molecules wherein one or both antigen-binding domains comprise variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, antigen-binding molecules comprising an antigen-binding domain 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 disclosed herein. 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. 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.

[0120] Also described herein are antigen-binding molecules comprising an antigen-binding domain with an HCVR, LCVR, and/or CDR amino acid sequence that is substantially identical to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein. 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.

[0121] 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 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.

pH-Dependent Binding

[0122] Described herein are anti-MUC16 antibodies, and anti-CD3/anti-MUC16 bispecific antigen-binding molecules, with pH-dependent binding characteristics. For example, an anti-MUC16 antibody may exhibit reduced binding to MUC16 at acidic pH as compared to neutral pH. Alternatively, anti-MUC16 antibodies may exhibit enhanced binding to MUC16 at acidic pH as compared to neutral pH. The expression "acidic pH" includes pH values less than about 6.2, e.g., about 6.0, 5.95, 5,9, 5.85, 5.8, 5.75, 5.7, 5.65, 5.6, 5.55, 5.5, 5.45, 5.4, 5.35, 5.3, 5.25, 5.2, 5.15, 5.1, 5.05, 5.0, or less. As used herein, the expression "neutral pH" means a pH of about 7.0 to about 7.4. The expression "neutral pH" includes pH values of about 7.0, 7.05, 7.1, 7.15, 7.2, 7.25, 7.3, 7.35, and 7.4.

[0123] In certain instances, "reduced binding ... at acidic pH as compared to neutral pH" is expressed in terms of a ratio of the K_D value of the antibody binding to its antigen at acidic pH to the K_D value of the antibody binding to its antigen at neutral pH (or vice versa). For example, an antibody or antigen-binding fragment thereof may be regarded as exhibiting "reduced binding to MUC16 at acidic pH as compared to neutral pH" if the antibody or antigen-binding fragment thereof exhibits an acidic/neutral K_D ratio of about 3.0 or greater. In certain exemplary instances, the acidic/neutral K_D ratio for an antibody or antigen-binding fragment can be about 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 20.0. 25.0, 30.0, 40.0, 50.0, 60.0, 70.0, 100.0 or greater.

[0124] Antibodies with pH-dependent binding characteristics may be obtained, *e.g.*, by screening a population of antibodies for reduced (or enhanced) binding to a particular antigen at acidic pH as compared to neutral pH. Additionally, modifications of the antigen-binding domain at the amino acid level may yield antibodies with pH-dependent characteristics. For example, by substituting one or more amino acids of an antigen-binding domain (*e.g.*, within a CDR) with a histidine residue, an antibody with reduced antigen-binding at acidic pH relative to neutral pH may be obtained.

Antibodies Comprising Fc Variants

[0125] Anti-MUC16 antibodies, and anti-CD3/anti-MUC16 bispecific antigen-binding molecules, are described herein 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, antibodies comprising a mutation in the C_H2 or a C_H3 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. For instance, 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).

[0126] For example, anti-MUC16 antibodies, and anti-CD3/anti-MUC16 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.

Biological Characteristics of the Antibodies and Bispecific Antigen-Binding Molecules

[0127] Described herein are antibodies and antigen-binding fragments thereof that bind human MUC16 with high affinity (*e.g.*, sub-nanomolar K_D values).

[0128] According to certain instances, the present disclosure includes antibodies and antigen-binding fragments of antibodies that bind human MUC16 (*e.g.*, at 25°C) with a K_D of less than about 60 nM as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 4 herein. In certain instances, the antibodies or antigen-binding fragments of the present disclosure bind MUC16 with a K_D of less than about 60 nM, less than about 40 nM, less than about 20 nM, less than about 10 nM, less than about 8 nM, less than about 7 nM, less than about 6 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM, less than about 800 pM, less than about 700 pM, less than about 500 pM, less than about 400 pM, or less than about 300 pM, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 4 herein (*e.g.*, mAb-capture or antigen-capture format), or a substantially similar assay. The present disclosure includes bispecific antigen-binding molecules (e.g., bispecific antibodies which bind MUC16 with a K_D of less than about 7 nM, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 4 herein (*e.g.*, mAb-capture or antigen-capture format), or a substantially similar assay..

[0129] The present disclosure also includes antibodies and antigen-binding fragments thereof that bind MUC16 with a dissociative half-life (t½) of greater than about 10 minutes or greater than about 125 minutes as measured by surface plasmon resonance at 25°C, *e.g.*, using an assay format as defined in Example 4 herein, or a substantially similar assay. In certain instances, the antibodies or antigen-binding fragments bind MUC16 with a t½ of greater than about 10 minutes, greater than about 20 minutes, greater than about 30 minutes, greater than about 40 minutes, greater than about 50 minutes, greater than about 60 minutes, greater than about 70 minutes, greater than about 80 minutes, greater than about 90 minutes, greater than about 100 minutes, greater than about 110 minutes, or greater than about 120 minutes, as measured by surface plasmon resonance at 25°C, *e.g.*, using an assay format as defined in Example 4 herein (*e.g.*, mAb-capture or antigen-capture format), or a substantially similar assay. The present disclosure includes bispecific antigen-binding molecules (e.g., bispecific antibodies which bind MUC16 with a of greater than about 10 minutes or greater than about 20 minutes as measured by surface plasmon resonance at 25°C, *e.g.*, using an assay format as defined in Example 4 herein, or a substantially similar assay.

[0130] The present disclosure also includes antibodies and antigen-binding fragments thereof which bind specifically to human cell lines which express endogenous MUC16 (e.g., OVCAR-3), as determined by an electrochemoluminescence-based detection assay as set forth in Example 2 or a substantially similar assay.

[0131] The present disclosure also includes anti-CD3/anti-MUC16 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 ovarian cancer xenografts; and (b);suppressing tumor growth of established tumors in immunocompromised mice bearing human ovarian cancer xenografts (*see, e.g.*, Example 8).

[0132] The present disclosure includes antibodies and antigen-binding fragments thereof that bind human CD3 with high affinity. The present disclosure also includes antibodies and antigen-binding fragments thereof that bind human CD3 with medium or low affinity, depending on the therapeutic context and particular targeting properties that are desired. In some cases, the low affinity includes antibodies that bind CD3 with a K_D or EC_{50} (*e.g.*, as measured in a surface plasmon resonance assay) of greater than 300 nM, greater than 500 nM or greater than 1 µM. The present disclosure also includes antibodies and antigen-binding fragments thereof that bind human CD3 with no measureable affinity. For example, in the context of a bispecific antigen-binding molecule, wherein one arm binds CD3 and another arm binds a target antigen (*e.g.*,MUC16), it may be desirable for the target antigen-binding arm to bind the target antigen with high affinity while the anti-CD3 arm binds CD3 with only moderate or low affinity or no affinity. In this manner, preferential targeting of the antigen-binding molecule to cells expressing the target antigen may be achieved while avoiding general/untargeted CD3 binding and the consequent adverse side effects associated therewith.

[0133] The present disclosure includes bispecific antigen-binding molecules (*e.g.*, bispecific antibodies) which are capable of simultaneously binding to human CD3 and a human 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 MUC16 can be assessed by fluorescence activated cell sorting (FACS), as illustrated in Example 5 herein.

[0134] For example, the present disclosure includes antibodies, antigen-binding fragments, and bispecific antibodies thereof which specifically bind human T-cell lines which express CD3 but do not express MUC16 (*e.g.*, Jurkat), primate T-cells (*e.g.*, cynomolgus peripheral blood mononuclear cells [PBMCs]), and/or MUC16-expressing cells.

[0135] The present disclosure includes antibodies, antigen-binding fragments, and bispecific antibodies thereof that bind human CD3 with weak (*i.e.* low) or even no detectable binding or binding affinity.

[0136] The present disclosure includes antibodies, antigen-binding fragments, and bispecific antibodies thereof that bind monkey (*i.e.* cynomolgus) CD3 with weak (*i.e.* low) or even no detectable binding or binding affinity.

[0137] The present disclosure includes antibodies, antigen-binding fragments, and bispecific antibodies thereof that bind human CD3 and induce T cell activation.

[0138] The present disclosure includes anti-CD3/anti- MUC16 bispecific antigen-binding molecules which are capable of depleting tumor antigen-expressing cells in a subject (*see, e.g.*, Example 8, in a bioluminescent imaging assay, or a substantially similar assay). For example, according to certain instances, anti-CD3/anti-MUC16 bispecific antigen-binding molecules are provided, wherein a single administration of 10 µg of the bispecific antigen-binding molecule to a subject causes a reduction in the number of MUC16-expressing cells in the subject (*e.g.*, tumor growth in the subject is suppressed or inhibited). Unless otherwise indicated, bioluminescent radiance refers to [p/s/cm2^2/sr].

[0139] The present disclosure also includes anti-MUC16 antibody drug conjugates which inhibit tumor growth in in vivo MUC16 positive ovarian cancer xenograft models (*see, e.g.*, Reference Example 2, in a bioluminescent imaging assay, or a substantially similar assay). In certain instances, anti-MUC16 antibody drug conjugates with Compound 7 are provided wherein four once weekly doses administered at a dose of 85 µg/kg inhibit intraperitoneal OVCAR3/luc tumor growth in in vivo. In certain instances, anti-MUC16 antibody drug conjugates with Compound 7 are provided wherein four once weekly doses administered at a dose of 85 µg/kg inhibit subcutaneous OVCAR3/luc tumor growth in in vivo. In certain instances, anti-MUC16 antibody drug conjugates with Compound 10 are provided wherein a single dose at a dose of 85 µg/kg, 170 µg/kg, or 340 µg/kg inhibit intraperitoneal OVCAR3/luc tumor growth in in vivo. Unless otherwise indicated, bioluminescent radiance refers to [p/s/cm2^2/sr].

[0140] The present disclosure also includes anti-CD3/anti- MUC16 bispecific antigen-binding molecules which exhibit pharmacokinetic profiles in humanized MUC16 x CD3 mice (mice homozygous for human MUC16 and CD3 expression, MUC16 $^{hu/hu}$ x CD3 $^{hu/hu}$), CD3 humanized mice (mice homozygous for human CD3 expression, CD3 $^{hu/hu}$) and strain-matched (75% C57BL, 25%129Sv) wild-type (WT) mice, as described in Example 7 and shown in Figures 1, 2, and 3.

Epitope Mapping and Related Technologies

[0141] The epitope on CD3 and/or MUC16 to which the antigen-binding molecules 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 or MUC16 protein. Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) of CD3 or MUC16. The antibodies 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.

[0142] 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.*, Ehring (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.

[0143] The antibodies of the invention are defined by the claims. Other antibodies are also described herein. The present disclosure includes anti-MUC16 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 1 herein). Likewise, the present disclosure also includes anti-MUC16 antibodies that compete for binding to MUC16 with any of the specific exemplary antibodies described herein (*e.g.* antibodies comprising any of the amino acid sequences as set forth in Table 1 herein). However, only an antibody or antigen-binding fragment thereof that comprises a first antigen-binding domain that specifically binds human CD3, and a second antigen-binding domain that specifically binds human MUC16, wherein the second antigen-binding domain comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 20-22-24-28-30-32, as defined in the claims, is within the scope of the antibodies of the claimed invention.

[0144] The present disclosure also includes bispecific antigen-binding molecules comprising a first antigen-binding domain that specifically binds human CD3 and/or cynomolgus CD3 with low or no detectable binding or binding affinity, and a second antigen binding domain that specifically binds human MUC16, 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, and/or wherein the second antigen-binding domain binds to the same epitope on MUC16 as any of the specific exemplary MUC16-specific antigen-binding domains described herein.

[0145] Likewise, the present disclosure also includes bispecific antigen-binding molecules comprising a first antigen-binding domain that specifically binds human CD3, and a second antigen binding domain that specifically binds human MUC16, wherein the first antigen-binding domain competes for binding to CD3 with any of the specific exemplary CD3-specific antigen-binding domains described herein, and/or wherein the second antigen-binding domain competes for binding to MUC16 with any of the specific exemplary MUC16-specific antigen-binding domains described herein.

[0146] 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 by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope on MUC16 (or CD3) as a reference bispecific antigen-binding molecule, the reference bispecific molecule is first allowed to bind to a MUC16 protein (or CD3 protein). Next, the ability of a test antibody to bind to the MUC16 (or CD3) molecule is assessed. If the test antibody is able to bind to MUC16 (or CD3) following saturation binding with the reference bispecific antigen-binding molecule, it can be concluded that the test antibody binds to a different epitope of MUC16 (or CD3) than the reference bispecific antigen-binding molecule. On the other hand, if the test antibody is not able to bind to the MUC16 (or CD3) molecule following saturation binding with the reference bispecific antigen-binding molecule, then the test antibody may bind to the same epitope of MUC16 (or CD3) as the epitope bound by the reference bispecific antigen-binding molecule. 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. 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 instances, 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.

[0147] 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 MUC16 protein (or CD3 protein) under saturating conditions followed by assessment of binding of the test antibody to the MUC16 (or CD3) molecule. In a second orientation, the test antibody is allowed to bind to a MUC16 (or CD3) molecule under saturating conditions followed by assessment of binding of the reference antigen-binding molecule to the MUC16 (or CD3) molecule. If, in both orientations, only the first (saturating) antigen-binding molecule is capable of binding to the MUC16 (or CD3) molecule, then it is concluded that the test antibody and the reference antigen-binding molecule compete for binding to MUC16 (or CD3). 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.

Preparation of Antigen-Binding Domains and Construction of Bispecific Molecules

[0148] 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 MUC16), can be appropriately arranged relative to one another to produce a bispecific antigen-binding molecule using routine methods. (A discussion of exemplary bispecific antibody formats that can be used to construct the bispecific antigen-binding molecules is provided elsewhere herein). In certain instances, one or more of the individual components (e.g., heavy and light chains) of the multispecific antigen-binding molecules 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 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 MUC16) 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.

[0149] 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 constant gene at the endogenous mouse 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). Fully human refers to an antibody, or antigen-binding fragment or immunoglobulin domain thereof, comprising an amino acid sequence encoded by a DNA derived from a human sequence over the entire length of each polypeptide of the antibody or antigen-binding fragment or immunoglobulin domain thereof. In some instances, the fully human sequence is derived from a protein endogenous to a human. In other instances, the fully human protein or protein sequence comprises a chimeric sequence wherein each component sequence is derived from human sequence. While not being bound by any one theory, chimeric proteins or chimeric sequences are generally designed to minimize the creation of immunogenic epitopes in the junctions of component sequences, e.g. compared to any wild-type human immunoglobulin regions or domains.

Bioequivalents

[0150] The antibodies of the invention are defined by the claims. Other antibodies are also described herein. The present disclosure 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 CD3 and/or MUC16. 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.

[0151] The present disclosure 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 does 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. However, only an antibody or antigen-binding fragment as defined in the claims is within the scope of the antibodies of the claimed invention.

[0152] For instance, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[0153] For instance, 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.

[0154] For instance, 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.

[0155] 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.

[0156] 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

[0157] According to certain instances, antigen-binding molecules are described which bind to human CD3 but not to CD3 from other species. Also provided are antigen-binding molecules which bind to human MUC16. The present described 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 MUC16.

[0158] According to certain exemplary instances, antigen-binding molecules are provided which bind to human CD3 and/or human MUC16 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 MUC16. For example, in a particular exemplary instance bispecific antigen-binding molecules are provided comprising a first antigen-binding domain that binds human CD3 and cynomolgus CD3, and a second antigen-binding domain that specifically binds human MUC16.

Antibody-Drug Conjugates (ADCs)

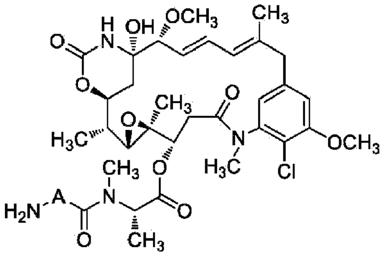
[0159] The antibodies of the invention are defined in the claims. Also described herein are antibody-drug conjugates (ADCs) comprising an anti-MUC16 antibody or antigen-binding fragment thereof conjugated to a therapeutic moiety such as a cytotoxic agent, a chemotherapeutic drug, immunosuppressant or a radioisotope. In general terms, the ADCs comprise: $A - [L - P]_y$, in which A is an antigen-binding molecule, *e.g.* an anti-MUC16 antibody, or a fragment thereof (*e.g.*, a fragment comprising at least a HCDR3 selected from any of the HCDR3 amino acid sequences listed in Table 1), L is a linker, P is the payload or therapeutic moiety (*e.g.*, cytotoxic agent), and y is an integer from 1 to 30. In various instances, the ADC comprises an anti-MUC16 antibody or antigen-binding fragment thereof that comprises the CDRs of a HCVR and a LCVR having the amino acid sequences of the SEQ ID NOs (*e.g.*, SEQ ID NOs: 2 and 10) set forth in Table 1, or specific HCVR/LCVR pairs (*e.g.*, SEQ ID NOs: 2/10). In some cases, the anti-MUC16 antibody or fragment comprises CDRs with the amino acid sequences of the SEQ ID NOs (*e.g.*, SEQ ID NOs: 4-6-8-12-14-16) set forth in Table 1. In some cases, the anti-MUC16 antibody or fragment comprises a HCVR and a LCVR having the amino acid sequences of the SEQ ID NOs (*e.g.*, SEQ ID NOs: 2 and 10) set forth in Table 1, or specific amino acid sequence pairs (*e.g.*, SEQ ID NOs: 2/10). In some cases, the anti-MUC16 antibody is an antibody or antigen-binding fragment that binds human MUC16 within one or more of five membrane-proximal SEA domains of human MUC16 corresponding to residues 13791-14451 of SEQ ID NO: 1899. In some cases, the anti-MUC16 antibody is an antibody or antigen-binding fragment that binds human MUC16 within residues 13810-14451 of SEQ ID NO: 1899. In some cases, the anti-MUC16 antibody is an antibody or antigen-binding fragment that binds to any one of more of SEA1, SEA2, SEA3, SEA4, SEA5, SEA6, SEA7, SEA8, SEA9, SEA10, SEA11, SEA12, SEA13, SEA14, SEA15 or SEA16 of human MUC16. In one instance, the anti-MUC16 antibody or fragment binds within SEA1 (residues 12074 to 12229 of SEQ ID NO: 1899). In one instance, the anti-MUC16 antibody or fragment binds within SEA2 (residues 12230 to 12387 of SEQ ID NO: 1899). In one instance, the anti-MUC16 antibody or fragment binds within SEA3 (residues 12388 to 12543 of SEQ ID NO: 1899). In one instance, the anti-MUC16 antibody or fragment binds within SEA4 (residues 12544 to 12698 of SEQ ID NO: 1899). In one instance, the anti-MUC16 antibody or fragment binds within SEA5 (residues 12699 to 12854 of SEQ ID NO: 1899). In one instance, the anti-MUC16 antibody or fragment binds within SEA6 (residues 12855 to 13010 of SEQ ID NO: 1899). In one instance, the anti-MUC16 antibody or fragment binds within SEA7 (residues 13011 to 13166 of SEQ ID NO: 1899). In one instance, the anti-MUC16 antibody or fragment binds within SEA8 (residues 13167 to 13323 of SEQ ID NO: 1899). In one instance, the anti-MUC16 antibody or fragment binds within SEA9 (residues 13324 to 13478 of SEQ ID NO: 1899). In one instance, the anti-MUC16 antibody or fragment binds within SEA10 (residues 13479 to 13634 of SEQ ID NO: 1899). In one instance, the anti-MUC16 antibody or fragment binds within SEA11 (residues 13635 to 13790 of SEQ ID NO: 1899). In one instance, the anti-MUC16 antibody or fragment binds within SEA12 (residues 13791 to 13923 of SEQ ID NO: 1899). In one instance, the anti-MUC16 antibody or fragment binds within SEA13 (residues 13924 to 14074 of SEQ ID NO: 1899). In one instance, the anti-MUC16 antibody or fragment binds within SEA14 (residues 14075 to 14227 of SEQ ID NO: 1899). In one instance, the anti-

MUC16 antibody or fragment binds within SEA15 (residues 14228 to 14320 of SEQ ID NO: 1899). In one instance, the anti-MUC16 antibody or fragment binds within SEA16 (residues 14321 to 14464 of SEQ ID NO: 1899). For instance, the isolated antibody or antigen-binding fragment thereof binds human MUC16 within an epitope ranging from residue 428 to residue 481 of SEQ ID NO: 1902. In some cases, the isolated antibody or antigen-binding fragment interacts with amino acid residues 428-434, 429-434, 453-467, 459-467, 460-467 and/or 474-481 of SEQ ID NO: 1902. In some instances, the antibody or antigen-binding fragment interacts with amino acid residues 428-434, 429-434, 453-467, 459-467, 460-467 and 474-481 of SEQ ID NO: 1902. In some instances the isolated antibody or antigen-binding fragment thereof binds human MUC16 within an epitope ranging from residue 126 to residue 138 of SEQ ID NO: 1902. In some cases, the isolated antibody or antigen-binding fragment interacts with amino acid residues 126-131, 127-131 and/or 132-138 of SEQ ID NO: 1902. In some instance, the antibody or antigen-binding fragment interacts with amino acid residues 126-131, 127-131 and 132-138 of SEQ ID NO: 1902. The disclosure further provides an isolated antibody or antigen-binding fragment thereof that binds human MUC16 within an epitope ranging from residue 357 to residue 369 of SEQ ID NO: 1902. In some cases, the isolated antibody or antigen-binding fragment interacts with amino acid residues 357-369, 358-366, 358-369 and/or 361-369 of SEQ ID NO: 1902. In some instance, the antibody or antigen-binding fragment interacts with amino acid residues 357-369, 358-366, 358-369 and 361-369 of SEQ ID NO: 1902. In one instance, the disclosure provides an anti-CD3/anti-MUC16 bispecific antibody, comprising an anti-MUC16 binding arm that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 1959 and a light chain comprising the amino acid sequence of SEQ ID NO: 1960, and an anti-CD3 binding arm that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 1961 and a light chain comprising the amino acid sequence of SEQ ID NO: 1960. In another instance, the disclosure provides an anti-CD3/anti-MUC16 bispecific antibody, comprising an anti-MUC16 binding arm that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 1959 and a light chain comprising the amino acid sequence of SEQ ID NO: 1960, and an anti-CD3 binding arm that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 1962 and a light chain comprising the amino acid sequence of SEQ ID NO: 1960. However, only an antibody or antigen-binding fragment thereof as defined in the claims is within the scope of the antibodies of the claimed invention.

[0160] Cytotoxic agents include any agent that is detrimental to the growth, viability or propagation of cells. The antigen-binding molecules or antibodies deliver these cytotoxic agents, referred to herein as "payloads", to the target cells. Examples of suitable cytotoxic agents and chemotherapeutic agents for forming ADCs are known in the art.

[0161] Examples of suitable cytotoxic agents and chemotherapeutic agents that can be conjugated to anti-MUC16 antibodies in accordance with this aspect include, e.g., 1-(2chloroethyl)-1,2-dimethanesulfonyl hydrazide, 1,8-dihydroxy-bicyclo[7.3.1]trideca-4,9-diene-2,6-diyne-13-one, 1-dehydrotestosterone, 5-fluorouracil, 6-mercaptopurine, 6-thioguanine, 9-amino camptothecin, actinomycin D, amanitins, aminopterin, anguidine, anthracycline, anthramycin (AMC), auristatins (monomethyl auristatin E or monomethyl auristatin F), bleomycin, busulfan, butyric acid, calicheamicins, camptothecin, carminomycins, carmustine, cemadotins, cisplatin, colchicin, combretastatins, cyclophosphamide, cytarabine, cytochalasin B, dactinomycin, daunorubicin, decarbazine, diacetoxypentyldoxorubicin, dibromomannitol, dihydroxy anthracin dione, disorazoles, dolastatin, doxorubicin, duocarmycin, echinomycins, eleutherobins, emetine, epothilones, esperamicin, estramustines, ethidium bromide, etoposide, fluorouracils, geldanamycins, gramicidin D, glucocorticoids, irinotecans, leptomycins, leurosines, lidocaine, lomustine (CCNU), maytansinoids, mechlorethamine, melphalan, mercatopurines, methopterins, methotrexate, mithramycin, mitomycin, mitoxantrone, N8-acetyl spermidine, podophyllotoxins, procaine, propranolol, pteridines, puromycin, rhizoxins, streptozotocin, tallysomycins, taxol, tenoposide, tetracaine, thioepa chlorambucil, tomaymycins, topotecans, tubulysin, vinblastine, vincristine, vindesine, vinorelbines, and derivatives of any of the foregoing.

[0162] According to certain instances, the cytotoxic agent that is conjugated to an anti-MUC16 antibody is an auristatin such as monomethyl auristatin E (MMAE) or monomethyl auristatin F (MMAF), a tubulysin such as TUB-OH or TUB-OMOM, a tomaymycin derivative, a dolastatin derivative, or a maytansinoid such as DM1 or DM4. In some instances, the cytotoxic agent is a maytansinoid having the structure of Formula (I), including stereoisomers of the compounds of Formula (I):



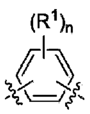
(Formula I)

wherein A is arylene or heteroarylene.

[0163] In some instances, A is a divalent radical of benzene, of pyridine, of naphthalene, or of quinolone, which are optionally substituted.

[0164] In some instances, A is arylene.

[0165] In some instances, A is: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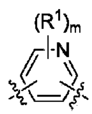
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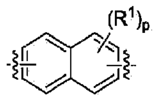
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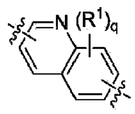
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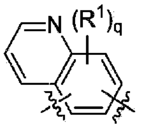
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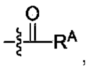
wherein:

R^1 is, independently at each occurrence, alkyl, alkenyl, alkynyl, aryl, alkaryl, aralkyl, halo,

heteroaryl, heterocycloalkyl, hydroxyl, cyano, nitro,

$-\S-OR^A$, $-\S-SO_2R^A$,  $-\S-\overset{O}{\|}-R^A$,

or azido, wherein R^A is alkyl or heteroalkyl;

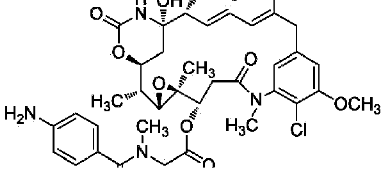
n is an integer from 0 to 4;

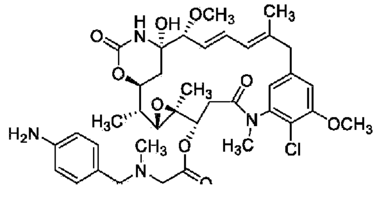
m is and integer from 0 to 3;

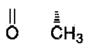
p is an integer from 0 to 6; and

q is an integer from 0 to 5.

[0166] In some instances, the compound of Formula I is selected from the group consisting of:







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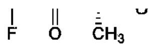

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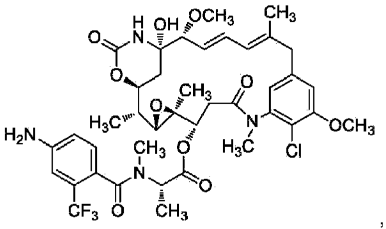

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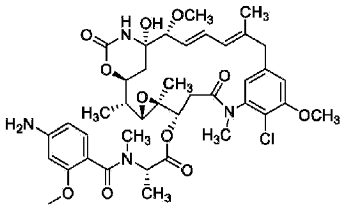

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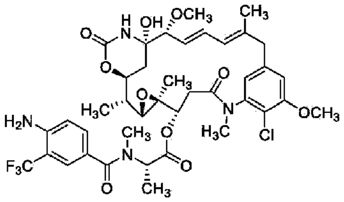

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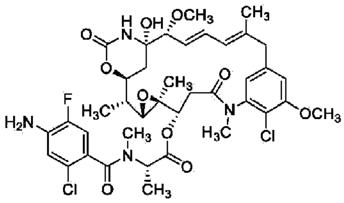

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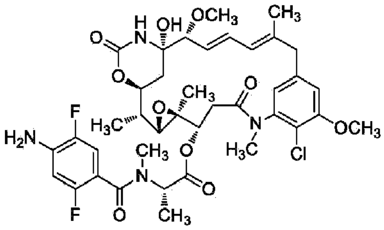

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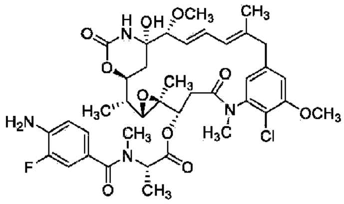

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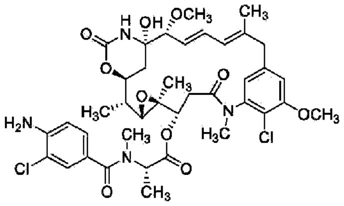

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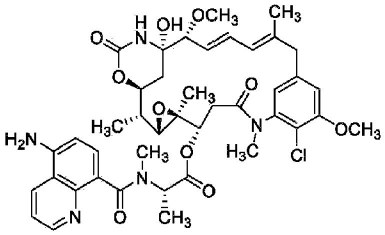

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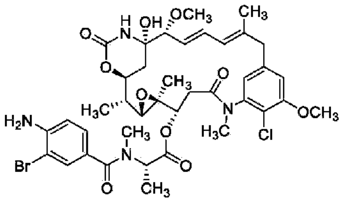

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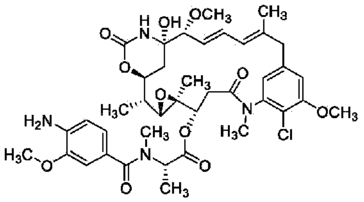

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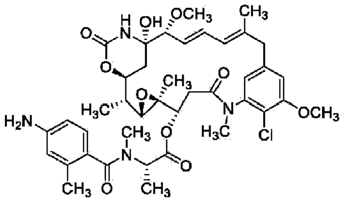

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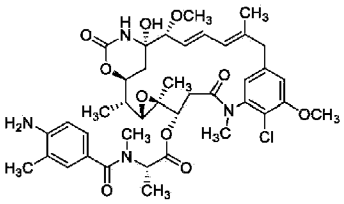

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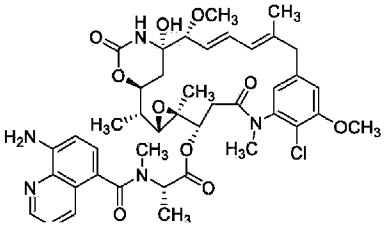

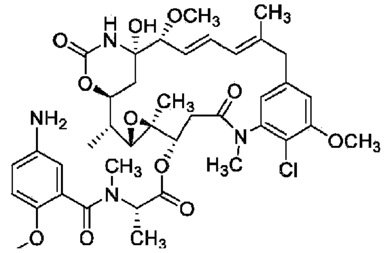

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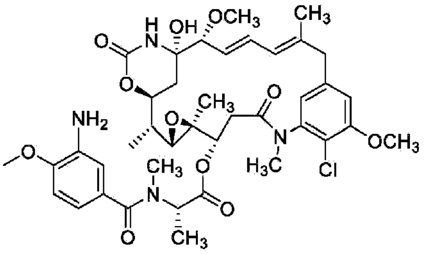

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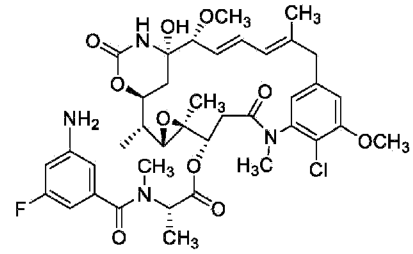

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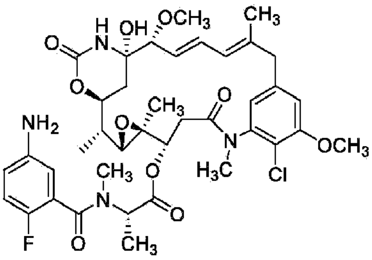

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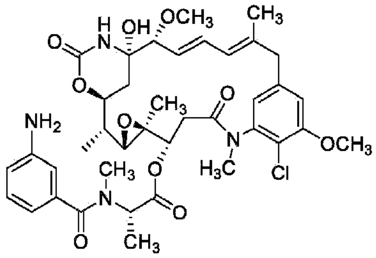

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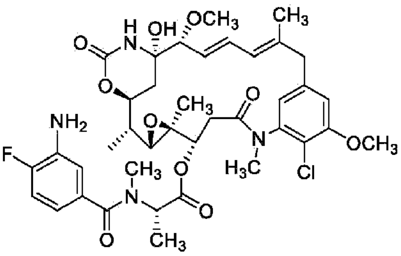

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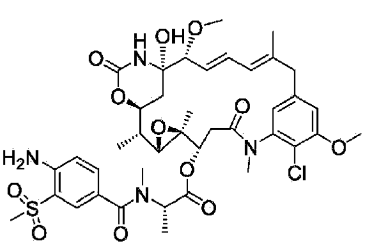

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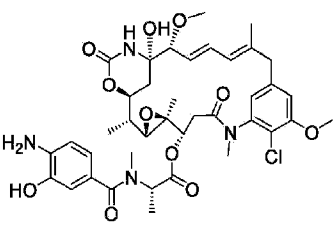

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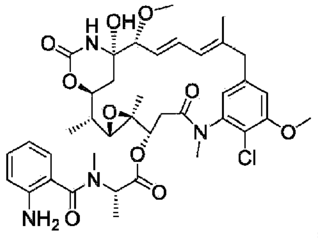

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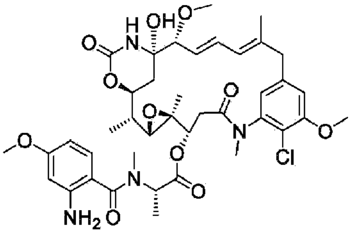

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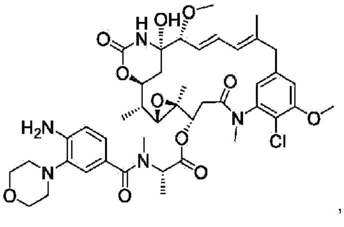

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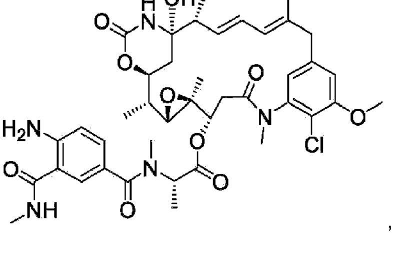

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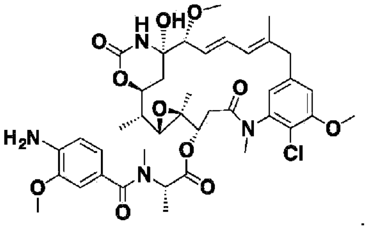
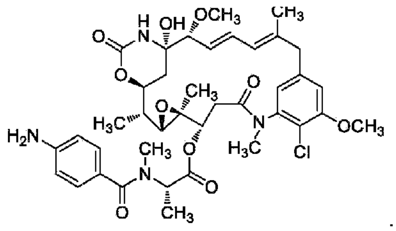


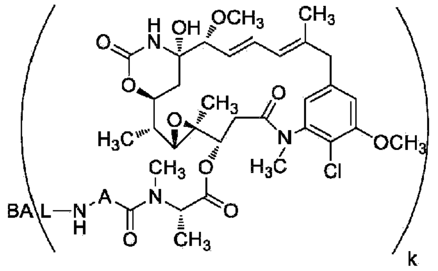
.

[0167] For instance, the compound of Formula (I) is:



.

[0168] In some instances, the maytansinoid of Formula (I) is conjugated to an anti-MUC16 antibody or antigen-binding fragment thereof via a linker, as shown in Formula (IA), below:



(Formula IA)

wherein:

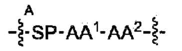
A is arylene or heteroarylene, as discussed above in connection with Formula (I);

L is a linker;

BA is an anti-MUC16 antibody or antigen-binding fragment thereof; and

k is an integer from 1 to 30.

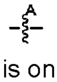
[0169] In various instances, L is:



$-\overset{A}{\wr}\text{-SP-AA}^1\text{-AA}^2\text{-}\wr\text{-}$

wherein:

SP is a spacer;



$-\overset{A}{\wr}-$

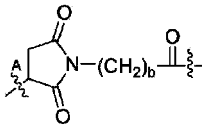
is one or more bonds to the anti-MUC16 antibody or fragment thereof;

AA^1 is an amino acid; and

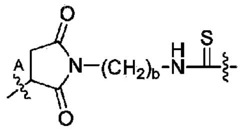
AA^2 is an amino acid.

[0170] In some instances, AA^1-AA^2 is: valine-citrulline, citrulline-valine, lysine-phenylalanine, phenylalanine-lysine, valine-asparagine, asparagine-valine, threonine-asparagine, asparagine-threonine, serine-asparagine, asparagine-serine, phenylalanine-asparagine, asparagine-phenylalanine, leucine-asparagine, asparagine-leucine, isoleucine-asparagine, asparagine-isoleucine, glycine-asparagine, asparagine-glycine, glutamic acid-asparagine, asparagine-glutamic acid, citrulline-asparagine, asparagine-citrulline, alanine-asparagine, or asparagine-alanine.

[0171] In some instances, SP is:

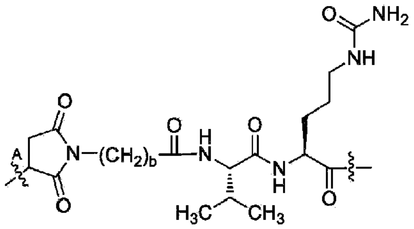


or

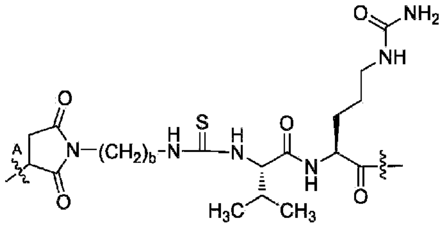


wherein:

is a bond to the anti-MUC16 antibody or fragment thereof; and
b is an integer from 2 to 8.
In other instances, L is:



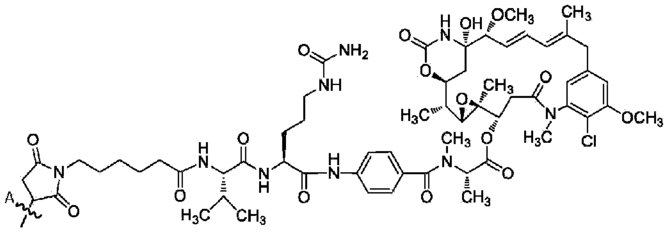
or



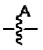
wherein:

is a bond to the anti-MUC16 antibody or fragment thereof; and
b is an integer from 2 to 8.

[0172] For instance, the compound of Formula (IA), including the linker, that is bound to the anti-MUC16 antibody or antigen-binding fragment thereof is:

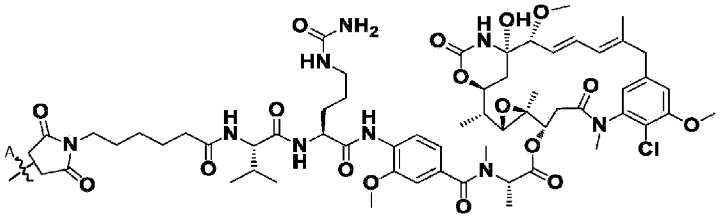


wherein



is a bond to the anti-MUC16 antibody or fragment thereof. In some instances, this moiety is referred to as "Compound 10."

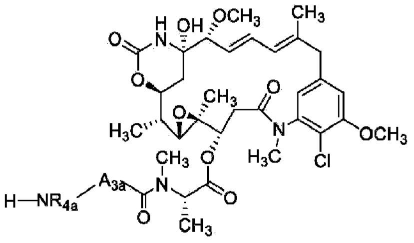
[0173] For instance, the compound of Formula (IA), including the linker, that is bound to the anti-MUC16 antibody or antigen-binding fragment thereof is:



wherein

is a bond to the anti-MUC16 antibody or fragment thereof. In some instances, this moiety is referred to as "Compound 60."

[0174] In some instances, the cytotoxic agent is a maytansinoid having the structure of Formula (II), including stereoisomers of the compounds of Formula (II):



(Formula II)

wherein:

A_{3a} is an amino acid, a peptide having 2-20 amino acids, an alkyl, an alkynyl, an alkenyl, a cycloalkyl, an aryl, a heteroaryl, a heterocyclyl, $-CR_5R_6-$, -O-, -C(=O)-,

-O-C(=O)-, -C(=O)-O-, -O-C(=O)-O-, $-C(=O)-(CH_x)_{p1}-$,$-C(=O)-O-(CH_x)_{p1}-$,

$-(CH_x)_{p1}-C(=O)-$, $-(CH_x)_{p1}-C(=O)-O-$, $-(O-(CH_2)_{p2}-)_{p3}-$,$-((CH_2)_{p2}-O-)_{p3}-$, -C(=S)-,

-C(=S)-S-, -C(=S)-NH-, -S-C(=S)-, -S-C(=S)-S-, -S-, -SO-, $-SO_2-$,

$-NR_4-$,$-N(R_4)-C(=O)-N(R_8)-$, $-N(R_4)-C(=O)O-$, $-N(R_4)-C(=O)-$, $-C(=O)-N(R_4)-$,

$-C(=O)-N(R_4)-C(=O)-$, or$-O-C(=O)-NR_4-$, wherein alkyl, alkynyl, alkenyl, cycloalkyl, aryl, heteroaryl, and heterocyclyl are optionally substituted; and

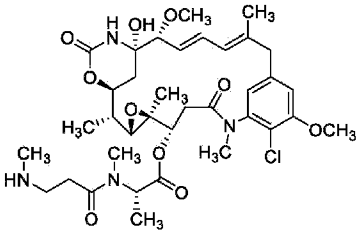
p1, p2 and p3 are each independently 0, or an integer from 1 to 100;

x is 0, 1 or 2;

R_4, R_5, R_6 and R_8 are each independently H, or a substituted or unsubstituted: alkyl, alkenyl, alkynyl, aryl, heteroaryl, or heterocyclyl; and

R_{4a} is a substituted or unsubstituted: alkyl, alkenyl, alkynyl, aryl, heteroaryl, or heterocyclyl.

[0175] In some instances, the compound of Formula (II) is selected from the group consisting of:



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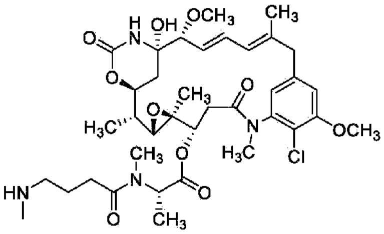

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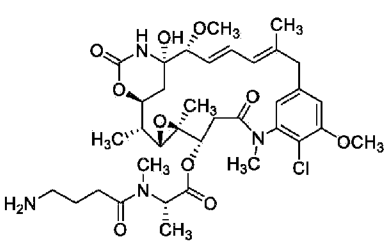

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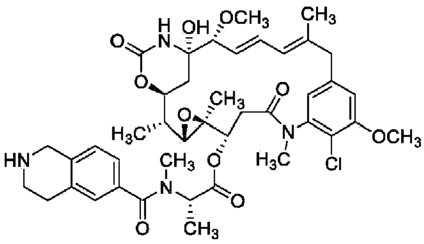
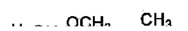
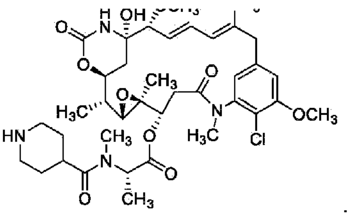


,

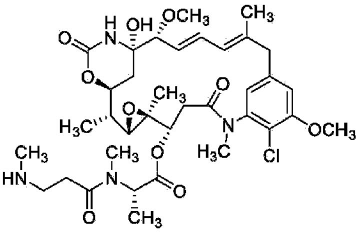
and





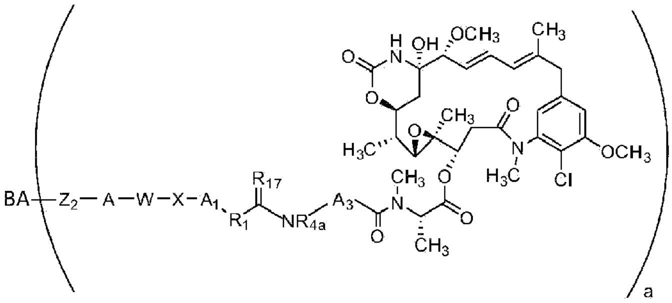
.

[0176] For instance, the compound of Formula (II) is:



.

[0177] In some instances, the maytansinoid of Formula (II) is conjugated to an anti-MUC16 antibody or antigen-binding fragment thereof via a linker, as shown in Formula (IIA), below:



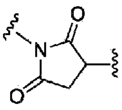
(Formula IIA)

wherein:

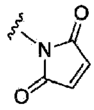
BA is an anti-MUC16 antibody or antigen-binding fragment thereof;

a is an integer from 1 to 30;

Z_2 is represented by the following structural formula: $-Z_{2A}-Z_{2B}-Z_{2C}-Z_{2D}$, wherein Z_{2A}, Z_{2B}, Z_{2C} and Z_{2D} are each independently absent, an amino acid, a peptide having 2-20 amino acids, an alkyl, an alkynyl, an alkenyl, a cycloalkyl, an aryl, a heteroaryl, a heterocyclyl, $-CR_5R_6-$, -O-, -C(=O)-, -O-C(=O)-, -C(=O)-O-,-O-C(=O)-O-, $-C(=O)-(CH_x)_{p1}$, $-C(=O)-O-(CH_x)_{p1}$, $-(CH_x)_{p1}-C(=O)-$, $-(CH_x)_{p1}-C(=O)-O-$ $-(O-(CH_2)_{p2}-)_{p3}-$, $-((CH_2)_{p2}-O-)_{p3}-$, -C(=S)-, -C(=S)-S-, -C(=S)-NH-, -S-C(=S)-, -S-C(=S)-S-, -S-, -SO-, $-SO_2-$, $-NR_4-$, $-N(R_4)-C(=O)-N(R_8)-$, $-N(R_4)-C(=O)O-$, $-N(R_4)-C(=O)-$, $-C(=O)-N(R_4)-$, $-C(=O)-N(R_4)-C(=O)-$, $-O-C(=O)-N(R_4)$, $-O-C(=S)-N(R_4)-$, $-C(=S)-N(R_4)-$, -N=C=S, -N=C=O,



or



;

A is a natural or non-natural amino acid, or a peptide comprising 2-20 amino acids;

W is -O-, -S-, $-CR_5R_6-$, or $-NR_4-$;

X is aryl, heteroaryl, cycloalkyl, or heterocyclyl, wherein aryl, heteroaryl, cycloalkyl, and heterocyclyl are optionally substituted;

wherein A_1, A_3, and R_1 are each independently an amino acid, a peptide having 2-20 amino acids, an alkyl, an alkynyl, an alkenyl, a cycloalkyl, an aryl, a heteroaryl, a heterocyclyl, $-CR_5R_6-$, -O-, -C(=O)-, -O-C(=O)-, -C(=O)-O-, -O-C(=O)-O-, $-C(=O)-(CH_x)_{p1}-$, $-C(=O)-O-(CH_x)_{p1}-$, $-(CH_x)_{p1}-C(=O)-$, $-(CH_x)_{p1}-C(=O)-O-$, $-(O-(CH_2)_{p2}-)_{p3}-$, $-((CH_2)_{p2}-O-)_{p3}-$, -C(=S)-, -C(=S)-S-, -S-C(=S)-, -C(=S)-NH-, -S-C(=S)-S-, -S-, -SO-, $-SO_2-$, $-NR_4-$, $-N(R_4)-C(=O)-N(R_8)-$, $-N(R_4)-C(=O)O-$, $-N(R_4)-C(=O)-$, $-C(=O)-N(R_4)-$, $-C(=O)-N(R_4)-C(=O)-$, or $-O-C(=O)-NR_4-$, wherein alkyl, alkynyl, alkenyl, cycloalkyl, aryl, heteroaryl, and heterocyclyl are optionally substituted;

R_{17} is selected from the group consisting of O, S, NR_{18}, and CR_5R_6 ;

R_{18} is selected from the group consisting of H, alkyl, alkynyl, alkenyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, and acyl, wherein alkyl, alkynyl, alkenyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, and acyl are optionally substituted;

R_4, R_5, R_6 and R_8 are each independently H, or a substituted or unsubstituted: alkyl, alkenyl, alkynyl, aryl, heteroaryl, or heterocyclyl;

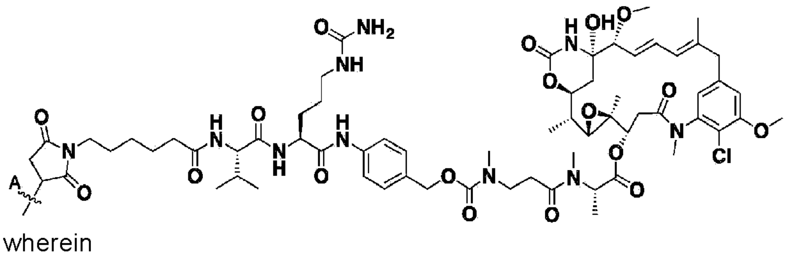
R_{4a} is a substituted or unsubstituted: alkyl, alkenyl, alkynyl, aryl, heteroaryl, or heterocyclyl;

p1, p2 and p3 are each independently 0, or an integer from 1 to 100; and

x is 0, 1 or 2.

[0178] In some instances of Formula (IIA), A is a peptide selected from the group consisting of valine-citrulline, citrulline-valine, lysine-phenylalanine, phenylalanine-lysine, valine-asparagine, asparagine-valine, threonine-asparagine, asparagine -threonine, serine-asparagine, asparagine-serine, phenylalanine-asparagine, asparagine-phenylalanine, leucine-asparagine, asparagine-leucine, isoleucine-asparagine, asparagine-isoleucine, glycine-asparagine, asparagine-glycine, glutamic acid- asparagine, asparagine-glutamic acid, citrulline-asparagine, asparagine-citrulline, alanine-asparagine, and asparagine-alanine.

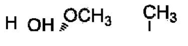
[0179] For instance, the compound of Formula (IIA) that is bound to the anti-MUC16 antibody or antigen-binding fragment thereof is:

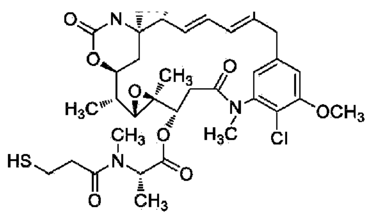


wherein

is a bond to the anti-MUC16 antibody or fragment thereof. In some instances, this moiety is referred to as "Compound 7."

[0180] In some instances, the cytotoxic agent that is conjugated to an anti-MUC16 antibody or fragment thereof is a pure, or substantially pure, diastereomer of DM1:

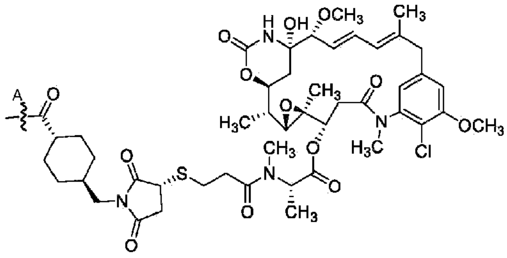




(DM1)

and y is an integer 1 to 0.

[0181] In another instance, the ADC comprises a "A - [L - P]$_y$" structure in which A is an anti-MUC16 antibody or antigen-binding fragment thereof, and [L - P] is:



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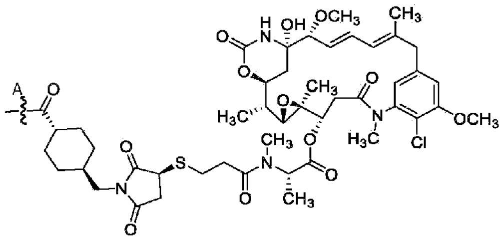

,

or

a mixture thereof, and

wherein y is an integer 1 to 30, and

is a bond to the anti-MUC16 antibody or fragment thereof.

[0182] Other maytansinoid derivatives are discussed in WO 2014/145090, WO2016/160615, and WO 2015/031396.

[0183] In some instances, the cytotoxic agent that is conjugated to an anti- MUC16 antibody or fragment thereof is MMAE or MMAF.

[0184] Other cytotoxic agents known in the art are contemplated, including, *e.g.*, protein toxins such as ricin, *C. difficile* toxin, pseudomonas exotoxin, diphtheria toxin, botulinum toxin, bryodin, saporin, pokeweed toxins (*i.e.*, phytolaccatoxin and phytolaccigenin), and others such as those set forth in Sapra et al., Pharmacol. & Therapeutics, 2013, 138:452-469.

[0185] Cytotoxic agents ("payloads") can be tethered to an anti-MUC16 antigen-binding molecule or antibody via a chemical linker that covalently binds the payload compound to the protein molecule (*i.e.* antibody). Exemplary instances of specific linkers are discussed above. More generally, and as used herein, the term "linker" refers to any divalent group or moiety that links, connects, or bonds a binding agent (*e.g.*, an antibody or an antigen-binding fragment thereof) with a payload compound set forth herein. Generally, suitable binding agent linkers for the antibody conjugates described herein are those that are sufficiently stable to exploit the circulating half-life of the antibody and, at the same time, capable of releasing its payload after antigen-mediated internalization of the conjugate. Linkers can be cleavable or non-cleavable. Cleavable linkers are linkers that are cleaved by intracellular metabolism following internalization, *e.g.*, cleavage via hydrolysis, reduction, or enzymatic reaction. Non-cleavable linkers are linkers that release an attached payload via lysosomal degradation of the antibody following internalization. Suitable linkers include, but are not limited to, acid-labile linkers, hydrolysis-labile linkers, enzymatically cleavable linkers, reduction labile linkers, self-immolative linkers, and non-cleavable linkers. Suitable linkers also include, but are not limited to, those that are or comprise peptides, glucuronides, succinimide-thioethers, polyethylene glycol (PEG) units, hydrazones, mal-caproyl units, dipeptide units, valine-citrulline units, and para-aminobenzyl (PAB) units. In some cases, the linker is capable of bonding to the antibody or antigen-binding fragment through a lysine residue or a cysteine residue (*e.g.*, via cleavage of a disulfide group of the antibody or fragment, or via a cysteine residue engineered into the antibody or fragment). In some cases, the linker is capable of bonding to the antibody or fragment through a glutamine residue, including those derived via transglutaminase-mediated conjugation.

[0186] Exemplary linkers that can be used include linkers that comprise or consist of *e.g.*, MC (6-maleimidocaproyl), MCC (maleimidomethyl cyclohexane-1-carboxylate), MP (maleimidopropanoyl), val-cit (valine-citrulline), val-ala (valine-alanine), ala-phe (alanine-phenylalanine), phe-lys (phenylalanine-lysine), dipeptide site in protease-cleavable linker, PAB (p-aminobenzyloxycarbonyl), SPP (N-Succinimidyl 4-(2-pyridylthio) pentanoate), SMCC (N-Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1 carboxylate), SIAB (N-Succinimidyl (4-iodoacetyl)aminobenzoate), and variants and combinations thereof. Additional examples of linkers that can be used are disclosed in, *e.g.*, US Pat. No. 7,754,681 and in Ducry, Bioconjugate Chem., 2010, 21:5-13, and the references cited therein,. In some cases, the linker is or contains a self-immolative spacer, such as those discussed in Jin, et al., Bioorganic & Medicinal Chemistry, 2012, 20:3465-3469, and Wu, et al., Bioorganic & Medicinal Chemistry, 2016, 24:2697-2706.

[0187] Payloads may be linked to the anti-MUC16 antibody or antigen-binding fragment via an attachment at a particular amino acid within the antibody or antigen-binding molecule. Exemplary amino acid attachments that can be used in the context of this aspect include, *e.g.*, lysine (see, e.g., U.S. Pat. No. 5,208,020; US 2010/0129314; Hollander et al., Bioconjugate Chem., 2008, 19:358-361; WO 2005/089808; U.S. Pat. No. 5,714,586; US 2013/0101546; and US 2012/0585592), cysteine (see, e.g., US 2007/0258987; WO 2013/055993; WO 2013/055990; WO 2013/053873; WO 2013/053872; WO 2011/130598; US 2013/0101546; and U.S. Pat. No. 7,750,116), selenocysteine (see, e.g., WO 2008/122039; and Hofer et al., Proc. Natl. Acad. Sci., USA, 2008, 105:12451-12456), formyl glycine (see, e.g., Carrico et al., Nat. Chem. Biol., 2007, 3:321-322; Agarwal et al., Proc. Natl. Acad. Sci., USA, 2013, 110:46-51, and Rabuka et al., Nat. Protocols, 2012, 10:1052-1067), non-natural amino acids (see, e.g., WO 2013/068874, and WO 2012/166559), and acidic amino acids (see, e.g., WO 2012/05982). Linkers can also be conjugated to an antigen-binding protein via attachment to carbohydrates (see, e.g., US 2008/0305497, and Ryan et al., Food & Agriculture Immunol., 2001, 13:127-130) and disulfide linkers (see, e.g., WO 2013/085925, WO 2010/010324, WO 2011/018611, and Shaunak et al., Nat. Chem. Biol., 2006, 2:312-313).

[0188] Drug-to-antibody ratio (DAR) is the average number of drugs conjugated to the antibody or antigen-binding fragment, which has an important effect on the efficacy, potency and pharmacokinetics of the ADC. In various instances, the DAR is from 1, 2, 3, 4, 5, 6, 7, or 8 drug molecules per antibody. In some instances, the DAR is from 1 to 4. In certain instances, the DAR is from 2 to 4. In some cases, the DAR is from 2 to 3. In certain cases, the DAR is from 3 to 4. In some instances, the DAR is from 1 to 10, 1 to 20 or 1 to 30 (*i.e.*, from 1 to 30 drug molecules per antibody or antigen-binding fragment thereof).

Therapeutic Formulation and Administration

[0189] The present disclosure provides pharmaceutical compositions comprising the antigen-binding molecules of the present disclosure. The pharmaceutical compositions 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.

[0190] 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 is used for therapeutic purposes in an adult patient, it may be advantageous to intravenously administer the bispecific antigen-binding molecule 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).

[0191] Various delivery systems are known and can be used to administer the pharmaceutical composition, *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.

[0192] A pharmaceutical composition 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. 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.

[0193] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park IL), to name only a few.

[0194] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. For instance, a pump may be used (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201). In another instance, polymeric materials can be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Florida. In yet another instance, 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.

[0195] 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.

[0196] 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

[0197] The present disclosure includes methods comprising administering to a subject in need thereof a therapeutic composition comprising an anti-MUC16 antibody or antigen-binding fragment thereof, or a bispecific antigen-binding molecule that specifically binds CD3 and MUC16. 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 MUC16 activity or a depletion of MUC16+ cells (*e.g.*, ovarian cancer cells). However, methods of therapeutic treatment are not part of the claimed method.

[0198] The antibodies and bispecific antigen-binding molecules (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 anti-MUC16 antibodies or the anti-CD3/anti-MUC16 bispecific antigen-binding molecules may be used for the treatment, prevention and/or amelioration of any disease or disorder associated with or mediated by MUC16 expression or activity or the proliferation of MUC16+ cells. The mechanism of action by which the therapeutic methods are achieved include killing of the cells expressing MUC16 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 MUC16 which can be inhibited or killed using the bispecific antigen-binding molecules include, for example, ovarian cancer cells.

[0199] The antigen-binding molecules may be used to treat a disease or disorder associates with MUC16 expression including, *e.g.*, a cancer including ovarian cancer, breast cancer, pancreatic cancer, non-small-cell lung cancer, intrahepatic cholangiocarcinoma-mass forming type, adenocarcinoma of the uterine cervix, and adenocarcinoma of the gastric tract. According to certain instances, the anti-MUC16 antibodies or anti-MUC16/anti-CD3 bispecific antibodies are useful for treating a patient afflicted with ovarian cancer. According to other related instances, methods are described comprising administering an anti-MUC16 antibody or an anti-CD3/anti-MUC16 bispecific antigen-binding molecule as disclosed herein to a patient who is afflicted with ovarian cancer. Analytic/diagnostic methods known in the art, such as tumor scanning, etc., may be used to ascertain whether a patient harbors an ovarian tumor.

[0200] The present disclosure 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.

[0201] According to certain aspects, the present disclosure provides methods for treating a disease or disorder associated with MUC16 expression (*e.g.*, ovarian cancer) comprising administering one or more of the anti-MUC16 or bispecific antigen-binding molecules described elsewhere herein to a subject after the subject has been determined to have prostate cancer. For example, the present disclosure includes methods for treating ovarian cancer comprising administering an anti-MUC16 antibody or an anti-CD3/anti-MUC16 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 hormone therapy (*e.g.*,anti-androgen therapy).

Combination Therapies and Formulations

[0202] The present disclosure 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 include, *e.g.*, 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 anagonist (*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-α inhibitor (*e.g.*, an anti-PDGFR-α antibody), a PDGFR-β inhibitor (*e.g.*, an anti-PDGFR-β 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 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 (e.g., pharmaceutical compositions comprising an anti-CD3/anti-MUC16 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).

[0203] The present disclosure 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-α, PDGFR-β, 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; $F(ab')_2$ 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 may also be administered and/or co-formulated in combination with antivirals, antibiotics, analgesics, corticosteroids and/or NSAIDs. The antigen-binding molecules may also be administered as part of a treatment regimen that also includes radiation treatment and/or conventional chemotherapy.

[0204] The additional therapeutically active component(s) may be administered just prior to, concurrent with, or shortly after the administration of an antigen-binding molecule; (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).

[0205] The present disclosure includes pharmaceutical compositions in which an antigen-binding molecule is co-formulated with one or more of the additional therapeutically active component(s) as described elsewhere herein.

Administration Regimens

[0206] According to certain instances, multiple doses of an antigen-binding molecule (e.g., an anti-MUC16 antibody or a bispecific antigen-binding molecule that specifically binds MUC16 and CD3) may be administered to a subject over a defined time course. The methods comprise sequentially administering to a subject multiple doses of an antigen-binding molecule. 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 disclosure 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.

[0207] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the antigen-binding molecule. 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 instances, 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 instances, 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").

[0208] In one exemplary instance, 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.

[0209] The methods according to this aspect may comprise administering to a patient any number of secondary and/or tertiary doses of an antigen-binding molecule (*e.g.*, an anti-MUC16 antibody or a bispecific antigen-binding molecule that specifically binds MUC16 and CD3). For example, in certain instances, only a single secondary dose is administered to the patient. In other instances, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain instances, only a single tertiary dose is administered to the patient. In other instances, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0210] In instances 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 instances 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.

Diagnostic Uses of the Antibodies

[0211] The anti-MUC16 antibodies may also be used to detect and/or measure MUC16, or MUC16-expressing cells in a sample, *e.g.*, a biological sample for diagnostic purposes. For example, an anti-MUC16 antibody, or fragment thereof, may be used to diagnose a condition or disease characterized by aberrant expression (*e.g.*, over-expression, under-expression, lack of expression, etc.) of MUC16. Exemplary diagnostic assays for MUC16 may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-MUC16 antibody, wherein the anti-MUC16 antibody is labeled with a detectable label or reporter molecule. Alternatively, an unlabeled anti-MUC16 antibody can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, beta-galactosidase, horseradish peroxidase, or luciferase. Another exemplary diagnostic use of the anti-MUC16 antibodies includes ^{89}Zr-labeled, such as ^{89}Zr-desferrioxamine-labeled, antibody for the purpose of noninvasive identification and tracking of tumor cells in a subject (*e.g.* positron emission tomography (PET) imaging). (*See, e.g.,* Tavare, R. et al. Cancer Res. 2016 Jan 1;76(1):73-82; and Azad, BB. et al. Oncotarget. 2016 Mar 15;7(11): 12344-58.) Specific exemplary assays that can be used to detect or measure MUC16 in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[0212] Samples that can be used in MUC16 diagnostic assays include any tissue or fluid sample obtainable from a patient which contains detectable quantities of MUC16 protein, or fragments thereof, under normal or pathological conditions. Generally, levels of MUC16 in a particular sample obtained from a healthy patient (*e.g.,* a patient not afflicted with a disease or condition associated with abnormal MUC16 levels or activity) will be measured to initially establish a baseline, or standard, level of MUC16. This baseline level of MUC16 can then be compared against the levels of MUC16 measured in samples obtained from individuals suspected of having a MUC16 related disease (e.g., a tumor containing MUC16-expressing cells) or condition.

Examples of tissue or fluid samples include, but are not limited to plasma, serum, ascites, ovary, uterus, cervix, liver, bladder, pancreas, stomach, small or large intestine, gall bladder, breast, lung, kidney, salivary, and lacrimal glands, or any epithelioid malignancy thereof. Additional examples of tissue or fluid samples include, but are not limited to papillary serous carcinoma of the cervix, adenocarcinoma of the endometrium, clear cell adenocarcinoma of the bladder, seminal vesicle carcinoma, gastric carcinoma, colorectal adenocarcinoma and epithelioid mesothelioma. It is envisioned that any fluid or tissue sample which contains detectable quantities of MUC16 protein, or fragments thereof, may be subjected to the detection methods described herein. The described methods may be used to monitor the development and progression of malignant diseases, or to distinguish between normal and disease conditions. As such, the described methods may be used to detect or monitor cancers, such as ovarian cancer, bladder cancer, breast cancer, pancreatic cancer, non-small-cell lung cancer, intrahepatic cholangiocarcinoma-mass forming type, adenocarcinoma of the uterine cervix, and adenocarcinoma of the gastric tract.

EXAMPLES

[0213] 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 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-MUC16 Antibodies

[0214] Anti-MUC16 antibodies were obtained by immunizing a genetically modified mouse with a human MUC16 antigen or by immunizing an engineered mouse comprising DNA encoding human immunoglobulin heavy and kappa light chain variable regions with a human MUC16 antigen.

[0215] Genetically modified mice were immunized with hMUC16.nub (a truncated format encompassing the last five SEA domains of Mucin-16 (SEQ ID: 1902)), or immunized with an hMUC16-expressing cell line, such as OVCAR-3 cells. SEQ ID NO: 1902 contains residues 13810-14451 of SEQ ID NO: 1899, as well as C-terminal tags. Following immunization, splenocytes were harvested from each mouse and either (1) fused with mouse myeloma cells to preserve their viability and form hybridoma cells and screened for MUC16 specificity, or (2) B-cell sorted (as described in US 2007/0280945A1) using a human MUC16 fragment as the sorting reagent that binds and identifies reactive antibodies (antigen-positive B cells).

[0216] Chimeric antibodies to MUC16 were initially isolated having a human variable region and a mouse constant region. The antibodies were characterized and selected for desirable characteristics, including affinity, selectivity, etc. If necessary, mouse constant regions were replaced with a desired human constant region, for example wild-type or modified IgG1 or IgG4 constant region, to generate a fully human anti-MUC16 antibody. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region. The antibody name designations such as H1H8755P and H1M7129N denote fully human antibodies "H1H" or chimeric human variable/mouse constant region antibodies "H1M". Antibodies identified by the hybridoma method are indicated with antibody ID numbers ending with "N" or "N2." Antibodies identified by the B-cell sorting method are indicated with antibody ID numbers ending with "P" or "P2".

[0217] Certain biological properties of the exemplary anti-MUC16 antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Heavy and Light Chain Variable Region Amino Acid and Nucleic Acid Sequences of anti-MUC16 antibodies

[0218] Table 1 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions and CDRs of selected anti-MUC16 antibodies. The corresponding nucleic acid sequence identifiers are set forth in Table 2.

Table 1: Amino Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
H1H8755P	2	4	6	8	10	12	14	16
H1H8767P	18	20	22	24	26	28	30	32
H1H8770P	34	36	38	40	42	44	46	48
H1H8783P	50	52	54	56	58	60	62	64
H1H8790P	66	68	70	72	74	76	78	80
H1H8794P	82	84	86	88	90	92	94	96
H1H8794P2	82	84	86	88	858	860	862	864
H1H8799P	98	100	102	104	106	108	110	112
H1H8799P2	98	100	102	104	170	172	174	176
H1H8804P	114	116	118	120	122	124	126	128
H1H8808P	130	132	134	136	138	140	142	144
H1H8810P	146	148	150	152	154	156	158	160
H1H8813P	162	164	166	168	170	172	174	176
H1M7129N	178	180	182	184	186	188	190	192
H1M7137N	194	196	198	200	394	396	398	400
H1M9519N	202	204	206	208	210	212	214	216
H1M9521N	218	220	222	224	226	228	230	232
H1M9528N	234	236	238	240	242	244	246	248
H2M7128N	250	252	254	256	1936	1938	1940	1942
H1M7130N	1944	1946	1948	1950	1952	1954	1956	1958
H2M7131N	258	260	262	264	266	268	270	272
H2M7133N	274	276	278	280	1936	1938	1940	1942
H2M7134N	282	284	286	288	290	292	294	296
H2M7135N	298	300	302	304	306	308	310	312
H2M7138N	314	316	318	320	322	324	326	328
H2M9538N	330	332	334	336	338	340	342	344
H3M9524N	346	348	350	352	354	356	358	360
H3M9525N	362	364	366	368	370	372	374	376
H3M9529N	378	380	382	384	386	388	390	392

Table 2: Nucleic Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
H1H8755P	1	3	5	7	9	11	13	15
H1H8767P	17	19	21	23	25	27	29	31
H1H8770P	33	35	37	39	41	43	45	47
H1H8783P	49	51	53	55	57	59	61	63

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H1H8790P	65	67	69	71	73	75	77	79
H1H8794P	81	83	85	87	89	91	93	95
H1H8794P2	81	83	85	87	857	859	861	863
H1H8799P	97	99	101	103	105	107	109	111
H1H8799P2	97	99	101	103	169	171	173	175
H1H8804P	113	115	117	119	121	123	125	127
H1H8808P	129	131	133	135	137	139	141	143
H1H8810P	145	147	149	151	153	155	157	159
H1H8813P	161	163	165	167	169	171	173	175
H1M7129N	177	179	181	183	185	187	189	191
H1M7137N	193	195	197	199	393	395	397	399
H1M9519N	201	203	205	207	209	211	213	215
H1M9521N	217	219	221	223	225	227	229	231
H1M9528N	233	235	237	239	241	243	245	247
H2M7128N	249	251	253	255	1935	1937	1939	1941
H1M7130N	1943	1945	1947	1949	1951	1953	1955	1957
H2M7131N	257	259	261	263	265	267	269	271
H2M7133N	273	275	277	279	1935	1937	1939	1941
H2M7134N	281	283	285	287	289	291	293	295
H2M7135N	297	299	301	303	305	307	309	311
H2M7138N	313	315	317	319	321	323	325	327
H2M9538N	329	331	333	335	337	339	341	343
H3M9524N	345	347	349	351	353	355	357	359
H3M9525N	361	363	365	367	369	371	373	375
H3M9529N	377	379	381	383	385	387	389	391

Example 2: Anti-MUC16 Antibodies Bind Specifically to Endogenously Expressed hMUC16 on OVCAR-3 Cell Line

[0219] The ability of anti-MUC16 antibodies to bind specifically to endogenously expressing MUC16 on the human ovarian carcinoma cell line (OVCAR-3) was evaluated via an electrochemiluminescence based detection assay (Meso Scale Discovery (MSD), Rockville, MD). Briefly, OVCAR-3 and a control ovarian adenocarcinoma cell line, SK-OV-3, which has no detectable hMUC16 expression, were rinsed in 1xPBS supplemented with Ca^{2+}/Mg^{2+}(Irvine Scientific, Santa Ana, CA) followed by incubation in Enzyme Free Cell Dissociation buffer (Millipore, Billerica, MA) for 10 min at 37°C. Detached cells were then washed once in 1xPBS supplemented with Ca^{2+}/Mg^{2+} and counted (Cellometer Auto T4 cell counter, Nexcelom Bioscience, Lawrence, MA). Approximately 1.0×10^4 cells were plated in MULTI-ARRAY 96-well carbon electrode plates (MSD) and incubated for 1 h at 37°C. Nonspecific binding sites were then blocked by 2% BSA (w/v) in PBS for 1 h at room temperature. Next, serial dilutions of anti-MUC16 or control antibodies (0.85pM -50nM) and no-antibody buffer controls were added to the plate-bound cells and incubated for 1 h at room temperature (RT). Plates were then washed to remove the unbound antibodies using an AquaMax2000 plate washer (MDS Analytical Technologies, Sunnyvale, CA). Plate-bound antibodies were detected with a SULFO-TAG™-conjugated anti-human kappa light chain antibody (Regeneron) or a SULFO-TAG™-conjugated anti-mouse IgG antibody (Jackson Immunoresearch, West Grove, PA) for 1h at RT. Post-wash, plates were developed with Read Buffer (MSD) according to manufacturer's protocol and luminescent signals were recorded with a SECTOR Imager 6000 (MSD) instrument.

[0220] Luminescence intensity, measured in relative light units (RLU), for the two cell lines was recorded to indicate the binding intensity of each antibody. The ratio of signal detected with 1.9nM or 16.7nM anti-MUC16 antibody binding to OVCAR-3 vs. SK-OV-3 was reported as an indication of specificity and potency of binding (Table 3).

Table 3: Cell Binding Ratios of Anti-Muc16 Antibodies to Endogenously Expressing hMUC16 OVCAR-3 vs hMUC16-negative SK-OV-3 Cell Lines

	Ratio: anti-MUC16 Binding OVCAR-3/SK-OV-3	
Antibody ID	**[Ab]: 1.9nM**	**[Ab]: 16.7nM**
Hybridoma Anti-Muc16 Antibodies (H1M, H2M, H3M)		
H2M7128N	59	31
H1M7129N	68	19
H2M7131N	86	37
H2M7133N	69	33
H2M7134N	68	29
H2M7135N	30	5
H1M7137N	77	29
H2M7138N	82	50
H3M7132N	89	38
H1M9519N	109	78
H1M9521N	132	107
H3M9524N	81	57
H3M9525N	137	51
H1M9528N	153	120
H3M9529N	143	99
H2M9538N	89	26
Human Fc anti-MUC16 Antibodies (H1H)		
H1H8755P	13	5
H1H8767P	4	NS
H1H8770P	9	3
H1H8783P	11	4
H1H8790P	8	3
H1H8794P	8	3

Human Fc anti-MUC16 Antibodies (H1H)		
H1H8794P2	5	NS
H1H8799P	10	4
H1H8799P2	10	4
H1H8804P	8	3
H1H8808P	4	NS
H1H8810P	8	3
H1H8813P	11	5
H1H9519N	41	30
H1H9521N	30	28
H1H9524N	20	48
H1H9525N	4	21
H1H9528N	41	18
H1H9529N	109	74
H1H9538N	12	13
Controls		
mIgG1 Isotype Control Antibody	NS	NS
mIgG2a Isotype Control Antibody	NS	NS
hIgG1 Isotype Control Antibody	NS	NS
NS=Non-Specific - ratio at 1.9nM or 16.7nM<2.5-fold. Isotypes: H1H: hIgG1; H1M: mIgG1; H2M: mIgG2; H3M: mIgG3 Note: variation in binding intensity ratios between the same antibody expressed with a mouse Fc and human Fc is due to the use of different SULFO-TAG secondary detect reagents.		

[0221] As the results in Table 3 show, a majority of the anti-MUC16 antibodies of this disclosure bound specifically to OVCAR-3 at both high (16.7 nM) and low (1.9 nM) antibody concentrations. mIgG1, mIgG2a and hIgG1 Isotype Control antibodies showed no specific binding to either OVCAR-3 or the SK-OV-3 cell line. Additionally, evidence exists that the method is sensitive since several antibodies that did not exhibit binding to soluble monomeric human MUC16 protein in a surface plasmon resonance binding assay (see Example 4 hereinbelow) displayed specific binding to endogenous human MUC16 expressed on OVCAR-3 cells in this cell-based binding assay.

Example 3: Generation of Bispecific Antibodies that Bind Ovarian Cell-Specific (MUC16) and CD3

[0222] The present disclosure provides bispecific antigen-binding molecules that bind CD3 and MUC16; such bispecific antigen-binding molecules are also referred to herein as "anti-MUC16/anti-CD3 or anti-MUC16xCD3 bispecific molecules." The anti-MUC16 portion of the anti-MUC16/anti-CD3 bispecific molecule is useful for targeting tumor cells that express MUC16 (also known as CA-125), and the anti-CD3 portion of the bispecific molecule is useful for activating T-cells. The simultaneous binding of MUC16 on a tumor cell and CD3 on a T-cell facilitates directed killing (cell lysis) of the targeted tumor cell by the activated T-cell.

[0223] Bispecific antibodies comprising an anti-MUC16-specific binding domain and an anti-CD3-specific binding domain were constructed using standard methodologies, wherein the anti-MUC16 antigen binding domain and the anti-CD3 antigen binding domain each comprise different, distinct HCVRs paired with a common LCVR. In exemplified bispecific antibodies, the molecules were constructed utilizing a heavy chain from an anti-CD3 antibody, a heavy chain from an anti-MUC16 antibody and a common light chain from the anti-MUC16 antibody. In other instances, the bispecific antibodies may be constructed utilizing a heavy chain from an anti-CD3 antibody, a heavy chain from an anti-MUC16 antibody and a light chain from an anti-CD3 antibody or an antibody light chain known to be promiscuous or pair effectively with a variety of heavy chain arms.

The bispecific antibodies described in the following examples consist of anti-CD3 binding arms having varying binding affinities to human soluble heterodimeric hCD3ε/δ protein (as described in Example 13 herein); and human MUC16 (see Examples 1-2 above). Exemplified bispecific antibodies were manufactured having an IgG1 Fc domain (BSMUC16/CD3-001, -002, -003, and - 004) or a modified (chimeric) IgG4 Fc domain (BSMUC16/CD3-005) as set forth in US Patent Application Publication No. US20140243504A1, published on August 28, 2014.

[0224] A summary of the component parts of the antigen-binding domains of the various anti-MUC16xCD3 bispecific antibodies constructed is set forth in Table 4.

Table 4: Summary of Component Parts of Selected Anti-MUC16xCD3 Bispecific Antibodies

Bispecific Antibody Identifier	**Anti-MUC16**	**Anti-CD3**	**Common Light Chain Variable Region**
	Antigen-Binding Domain	**Antigen-Binding Domain**	
	Heavy Chain Variable Region	***Heavy Chain Variable Region***	
BSMUC16/CD3-001	H1H8767P (SEQ ID NO:18)	CD3-VH-G (SEQ ID NO:1730)	H1H8767P (SEQ ID NO: 26)
BSMUC16/CD3-002	H1H8767P (SEQ ID NO:18)	CD3-VH-G5 (SEQ ID NO:1762)	H1H8767P (SEQ ID NO: 26)
BSMUC16/CD3-003	H1H8767P (SEQ ID NO:18)	CD3-VH-G9 (SEQ ID NO:1778)	H1H8767P (SEQ ID NO: 26)
BSMUC16/CD3-004	H1H8767P (SEQ ID NO:18)	CD3-VH-G10 (SEQ ID NO:1786)	H1H8767P (SEQ ID NO: 26)
BSMUC16/CD3-005	H1H8767P (SEQ ID NO:18)	CD3-VH-G20 (SEQ ID NO:1866)	H1H8767P (SEQ ID NO: 26)

[0225] The light chains listed in Table 4 were common to both the CD3 and MUC16 targeting arms of the bispecific antibodies. Tables 1 and 2 set out amino acid and nucleic acid sequence identifiers, respectively, for the various heavy chain variable regions, and their corresponding CDRs, of the anti-MUC16 arms of the bispecific antibodies of this Example. Table 22 and 23 set out amino acid and nucleic acid sequence identifiers, respectively, for the various heavy chain variable regions, and their corresponding CDRs, of the anti-CD3 arms of the bispecific antibodies of this Example.

Example 4: Surface Plasmon Resonance Derived Binding Affinities and Kinetic Constants of Human Monoclonal anti-MUC16 Monospecific and anti-MUC16xCD3 Bispecific antibodies

[0226] Binding affinities and kinetic constants of human anti-MUC16 antibodies were determined via real-time surface plasmon resonance (SPR; Biacore 4000 or Biacore T-200, GE Healthcare Life Sciences, Pittsburgh, PA) at 25°C. The anti-MUC16 antibodies tested in this example were bivalent monospecific binders to MUC16 (expressed with a hIgG1 (H1H), mIgG1 (H1M), mIgG2 (H2M) or mIgG3 (H3M) constant region) or bispecific antibodies comprised of an anti-MUC16 binding domain and an anti-CD3 binding domain. Antibodies were captured onto a CM4 or CM5 Biacore sensor surface (GE Healthcare Life Sciences) derivatized via amine coupling with a monoclonal anti-human Fc antibody (GE, # BR-1008-39) or a monoclonal goat anti-mouse Fc antibody (GE, # BR-1008-38). Various concentrations of soluble monomeric human MUC16, in a truncated format encompassing the last five SEA domains of Mucin-16 (hMUC16.mmh, or MUC16 "nub", SEQ ID: 1902) were injected over the anti-MUC16-antibody captured surface at a flow rate of 50uL/minute (Biacore T-200) or 30uL/minute (Biacore 4000). Antibody-reagent association was monitored for 4 min and the dissociation was monitored for 6-10 min. All binding studies were performed in HBS-ET buffer (0.01 M HEPES pH 7.4, 0.15M NaCl, 0.05% v/v Surfactant P20).

[0227] Kinetic association (k_a) and dissociation (k_d) rate constants were determined by fitting the real-time sensorgrams to a 1:1 binding model using Scrubber 2.0c curve fitting software. Binding dissociation equilibrium constants (K_D) and dissociative half-lives (t½) were calculated from the kinetic rate constants as:

$$K_D\ (\mathrm{M}) = \frac{\mathrm{kd}}{\mathrm{ka}}, \quad \text{and} \quad t½\ (\mathrm{min}) = \frac{\ln(2)}{60*\mathrm{kd}}$$

[0228] Binding kinetic parameters for the monospecific anti-MUC16 antibodies to a monomeric human MUC16 protein fragment are shown below in Tables 5A and 5B. Binding kinetic parameters for the anti-MUC16/anti-CD3 bispecific antibodies to monomeric human MUC16 protein are shown below in Table 6.

Table 5A: Biacore binding affinities of Hybridoma Anti-MUC16 antibodies (H1M, H2M and H3M) to hMUC16 fragment at 25 °C

Antibody ID	ka (1/Ms)	kd (1/s)	KD (M)	t 1/2 (min)
H2M7128N	1.89E+05	6.40E-04	3.39E-09	18
H1M7129N	5.31E+04	1.04E-04	1.97E-09	111
H2M7131N	6.47E+04	1.62E-04	2.51E-09	71
H3M7132N	2.57E+04	1.96E-04	7.62E-09	59
H2M7133N	1.67E+05	3.77E-04	2.26E-09	31
H2M7134N	6.55E+04	1.62E-04	2.47E-09	71
H2M7135N	5.10E+04	2.18E-04	4.27E-09	53
H1M7137N	5.30E+04	9.09E-05	1.72E-09	127
H2M7138N	7.41E+04	9.25E-05	1.25E-09	125
H1M9519N	NB	NB	NB	NB
H1M9521N	NB	NB	NB	NB
H3M9524N	NB	NB	NB	NB
H3M9525N	NB	NB	NB	NB
H1M9528N	NB	NB	NB	NB
H3M9529N	NB	NB	NB	NB
NB: No binding				

Table 5B: Biacore binding affinities of Human Fc anti-MUC16 antibodies (H1H) to hMUC16 fragment at 25°C

Antibody ID	ka (1/Ms)	kd (1/s)	KD (M)	$t_{1/2}$ (min)
H1H8755P	5.22E+05	1.49E-04	2.86E-10	77
H1H8767P	1.17E+05	4.18E-04	3.58E-09	28
H1H8770P	2.47E+05	3.08E-04	1.25E-09	38
H1H8783P	1.74E+05	1.07E-04	6.14E-10	108
H1H8790P	1.01E+05	7.61E-04	7.53E-09	15
H1H8794P	3.62E+05	2.79E-04	7.71E-10	41
H1H8799P	7.90E+04	3.66E-04	4.63E-09	32
H1H8799P2	7.58E+04	3.73E-04	4.92E-09	31
H1H8804P	4.94E+04	6.07E-04	1.23E-08	19
H1H8808P	4.12E+03	2.16E-04	5.24E-08	54
H1H8810P	5.77E+04	3.16E-04	5.48E-09	37
H1H8813P	5.32E+04	2.32E-04	4.35E-09	50

Table 6: Biacore binding affinitie s of anti-MUC16/ anti-CD3 Bispecific Antibodies to hMUC16 fragment at 25° C

Bispecific Antibody Identifier	ka (1/Ms)	kd (1/s)	KD (M)	t 1/2 (min)
BSMUC16/CD3-001	9.48E+04	5.86E-04	6.18E-09	20
BSMUC16/CD3-005	9.41E+04	5.64E-04	6.00E-09	21

[0229] As the results show, a majority of the anti-MUC16 antibodies of this disclosure bound to the soluble human MUC16 protein, some displaying sub-nanomolar affinity. Several antibodies (H1M9519N, H1M9521N, H3M9524N, H3M9525N, H1M9528N, H3M9529N) displayed no binding to the truncated format encompassing the last five SEA domains via surface plasmon resonance, however, displayed specific binding to endogenous human MUC16 expressed on OVCAR-3 cells in a cell-based binding assay. Anti-MUC16xCD3 bispecific antibodies of this disclosure also bound to soluble truncated human MUC16 protein exhibiting nanomolar affinity in this assay.

Example 5: Additional Binding, T-Cell Activation and Cytotoxicity Properties of Exemplified Bispecific Antibodies

[0230] In this example, the ability of MUC16xCD3 bispecific antibodies to bind to human CD3-expressing (human T cell) cell lines, compared to binding to target-specific (MUC16-specific) cell lines, via FACS was determined. Additionally, the ability of these bispecific antibodies to activate to target-specific (MUC16-specific) cell lines was also compared in a similar assay.

Binding Titration of Exemplified Bispecific Antibodies as *Measured by FACS Analysis*

[0231]

1. **A.** Briefly, flow cytometric analysis (i.e. fluorescence-activated cell sorting, or FACS) was utilized to determine binding of bispecific antibodies to Jurkat cells or cells expressing human MUC16,

followed by detection with a phycoerythrin (PE)-labeled or APC-labeled anti-human IgG antibody. Briefly, 2x 10^5 cells/well were incubated for 30 minutes at 4°C with a serial dilution ranging from 1.33E-07M to 8.03E-12M of each test antibody or isotype control (antibody of same isotype that binds a different antigen with no cross-reactivity to MUC16 or CD3). After incubation, the cells were washed twice with cold PBS containing 1% filtered FBS and a PE-conjugated or APC-conjugated anti-human secondary antibody was added to the cells and incubated for an additional 30 minutes. Wells containing no antibody or secondary only were also used as controls. After incubation, cells were washed, re-suspended in 200 µL cold PBS containing 1% filtered FBS and analyzed by flow cytometry on a BD FACS Canto II. See Table 7A.

2. **B.** In separate experiments with analogous conditions to that described above, flow cytometric analysis (or FACS) was utilized to determine binding of select bispecific antibodies to Jurkat cells, PEO-1, OVCAR3-Luc and cynomolgus T cells. For the titration analysis, serial dilution of selected MUC16xCD3 bispecific antibodies, a first isotype control antibody (a human chimeric IgG4 antibody that binds an irrelevant human antigen with no cross-reactivity to human or cynomolgus CD3) and a second isotype control antibody (a human chimeric IgG4 antibody that binds an irrelevant human antigen with no cross-reactivity to human MUC16), ranging from 66.6nM to 0.001nM. See Table 7B and Figs. 11A-11C.

[0232] 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 EC50 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. (Liu, J., et al. 2005, Biotechnol Letters 27:1821-1827). The EC50 value represents the concentration of the tested antibody where 50% of its maximal binding is observed.

Table 7A: FACS Binding on CD3 and MUC16-Specific Cell lines

Bispecific Antibody Identifier	Anti-CD3 Binding Arm	FACS binding titration EC_{50} [M]	
		Jurkat	OVCAR3 (MUC16+)
BSMUC16/CD3-001	CD3-VH-G	3.21E-09	1.20E-09
BSMUC16/CD3-002	CD3-VH-G5	Very weak	2.69E-09

Table 7B: FACS Binding on CD3 and MUC16-Specific Cell lines

Bispecific Antibody Identifier	Anti-CD3 Binding Arm	FACS binding titration EC_{50} [M]			
		PEO-1 (MUC16+)	OVCAR3-Luc (MUC16+)	Jurkat	Cynomolgus T cells
BSMUC16/CD3-001	CD3-VH-G	3.02E-09	1.32E-09	6.44E-09	1.56E-08
BSMUC16/CD3-002	CD3-VH-G5	3.13E-09	Not tested	3.01E-07	No Binding
BSMUC16/CD3-005	CD3-VH-G20	2.63E-09	1.47E-09	6.26E-08	1.17E-06

[0233] As shown in Tables 7A and 7B, the CD3 binding arms of each MUC16 bispecific antibody displayed a range of cell binding to human and monkey CD3 expressing T cells (e.g. from 3.2 nM $EC_{50}$to very weak binding). BSMUC16/CD3-001 bispecific antibody showed high measurement of binding to CD3-expressing cells (i.e. <7nM) while the BSMUC16/CD3-002 bispecific antibody showed weak-to-no binding to human and monkey CD3-expressing cells. Non-measurable binding, or no measurable binding, in the FACS assay or equivalent assay refers to a binding interaction between the antibody and its target antigen which is beyond the detection limit of the assay (e.g. at or above 1 µM). The tested bispecific antibodies displayed similar cell binding on MUC16-expressing cell lines, confirming that bispecific pairing with individual CD3 arms exhibiting high or weak (or no measurable) interactions with CD3 did not affect or diminish tumor target-specific binding (MUC16-specific binding was less than or equal to 3 nM (high binding) in these examples. The first control antibody did not bind to CD3+ cells, and the second control antibody did not bind to MUC16+ cells. See also Figs. 11A-11C.

[0234] Antibodies exhibiting weak-to-no detectable binding to human CD3 are still considered advantageous for avidity-driven bispecific pairing, and were further tested for cytotoxicity in *in vitro* (see below) and *in vivo* assays (Example 8).

T-Cell Activation and Tumor-specific Cytotoxicity Exhibited by Bispecific Antibodies* as *Measured In Vitro

[0235]

1. **A.** The specific killing of MUC16-expressing tumor target cells in the presence of CD3-based bispecific antibodies was monitored via flow cytometry. As reported previously, the bispecific antibodies displayed differential binding abilities to CD3 protein and CD3-expressing cell lines (*i.e.* very weak or strong binding). These same bispecific antibodies were tested for the ability to induce naïve human T-cells to re-direct killing toward target-expressing cells.
   Briefly, MUC16-expressing (OVCAR3) cell lines were labeled with 1µM 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 1×10^6 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 unstimulated 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. See results represented in Table 8A.
2. **B.** In analogous studies, MUC16-expressing (PEO-1 or OVCAR3-Luc) cell lines were labeled, plated and incubated overnight as described. Serial dilutions of MUC16xCD3 bispecific antibodies or isotype control were co-incubated. See results depicted in Tables 8B and 8C, and Figures 12A-12B.

[0236] For FACS analysis, cells were stained with a dead/live far red cell tracker (Invitrogen). 5×10^5 counting beads were added to each well immediately before FACS analysis. 1×10^4 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.

[0237] T cell activation was assessed by incubating cells with directly conjugated antibodies to CD2, CD69 and/or CD25, and by reporting the percent of early activated (CD69+) T cells and/or late activated (CD25+) T cells out of total T cells (CD2+).
As the results in Tables 8A-8C show, depletion of MUC16-expressing cells was observed with anti-MUC16xCD3 bispecifics. All 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 (or CD25) on CD2+ T cells, also with picomolar

(pM) EC_{50}s.

[0238] Importantly, the results of this example also demonstrate that a bispecific antibody constructed with a CD3 binding arm that displayed weak-to-no measurable 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 8A: Cytotoxicity and T-cell activation properties of selected MUC16xCD3 Bispecific Antibodies

Bispecific Antibody Identifier	Anti-CD3 Binding Arm	OVCAR3 cell depletion EC_{50} [M]	T cell activation (CD69 upregulation) EC_{50} [M]
BSMUC16/CD3-001	CD3-VH-G	2.24E-11	5.88E-12
BSMUC16/CD3-002	CD3-VH-G5	3.06E-11	1.01E-11

Table 8B: Cytotoxicity and T-cell activation properties of selected MUC16xCD3 Bispecific Antibodies

Bispecific Antibody Identifier	PEO-1 cell depletion EC50 [M]	T cells activation (CD69 upregulation) EC50 [M]	T cells activation (CD25 upregulation) EC50 [M]
BSMUC16/CD3-001	2.56E-11	8.34E-12	3.90E-11
BSMUC16/CD3-002	6.75E-11	1.34E-11	8.89E-11
BSMUC16/CD3-005	7.74E-11	1.72E-11	1.06E-10

Table 8C: Cytotoxicity and T-cell activation properties of selected MUC16xCD3 Bispecific Antibodies

Bispecific Antibody Identifier	OVCAR3-Luc cell depletion EC50 [M]	T cells activation (CD69 upregulation) EC50 [M]	T cells activation (CD25 upregulation) EC50 [M]
BSMUC16/CD3-001	1.54E-11	2.98E-12	3.06E-11
BSMUC16/CD3-005	5.16E-11	1.54E-11	1.17E-10

Example 6: Hydrogen/ Deuterium (H/D) Exchange based Epitope Mapping of Anti-Muc16 Antibodies H4sH8767P, H1H8794P2, and H1H8799P2 binding to a portion of the C-term domain of hMUC16

[0239] Experiments were conducted to determine the MUC16 amino acid residues within the C-terminal five SEA domains (SEQ ID No:1902, hereafter referred to as hMUC16.nub), with which the anti-MUC16 antibodies H4sH8767P, H1H8794P2, and H1H8799P2 interact. For this purpose, Hydrogen/Deuterium (H/D) Exchange epitope mapping with mass spectrometry (HDX-MS) was utilized. A general description of the H/D exchange method is set forth in Ehring (1999) Analytical Biochemistry 267(2):252-259; and Engen and Smith (2001) Anal. Chem. 73:256A-265A.

[0240] HDX-MS experiments were performed on an integrated Waters HDX/MS platform, consisting of a Leaptec HDX PAL system for deuterium labeling, a Waters Acquity M-Class (Auxiliary solvent manager) for sample digestion and loading, a Waters Acquity M-Class (µBinary solvent manager) for the analytical column gradient, and a Synapt G2-Si mass spectrometer for peptic peptide mass measurement.

[0241] The labeling solution was prepared in 10 mM PBS buffer in D_2O at pD 7.0. For deuterium labeling, 3.8 µL of hMUC16.nub (12 pmol/µL) or hMUC16.nub premixed with the anti-MUC16 antibody H4sH8767P, H1H8794P2, or H1H8799P2 in a 2:1 molar ratio was incubated with 56.2 µL. D_2O labeling solution for various time-points (e.g.: undeuterated control = 0 sec; deuterium labeling: 1 min and 20 min). The deuteration was quenched by transferring 50 µL sample to 50 µL pre-chilled quench buffer (0.2 M TCEP, 6 M guanidine chloride in 100 mM phosphate buffer, pH 2.5) and the mixed sample was incubated at 1.0 °C for two minutes. The quenched sample was then injected into a Waters HDX Manager for online pepsin/protease XIII digestion. The digested peptides were trapped onto an ACQUITY UPLC BEH C18 1.7-µm, 2.1 × 5 mm VanGuard pre-column at 0°C and eluted to an analytical column ACQUITY UPLC BEH C18 (1.7-µm, 1.0 × 50 mm) for a 9-minute gradient separation of 5%-40% B (mobile phase A: 0.1% formic acid in water, mobile phase B: 0.1% formic acid in acetonitrile). The mass spectrometer used a cone voltage of 37V, a scan time of 0.5 s, and mass/charge range of 50-1700 Th.

[0242] For the identification of the peptide residues of hMUC16.nub with which H4sH8767P, H1H8794P2, and H1H8799P2 interact, LC- MS^E data from the undeuterated sample were processed and searched against a database that included sequences for hMUC16.nub, pepsin and a randomized sequence using Waters ProteinLynx Global Server (PLGS) software. The identified peptides were imported to DynamX software and filtered by two criteria: 1) minimum products per amino acid: 0.25 and 2) replication file threshold: 2. DynamX software then automatically determined deuterium uptake of each peptide based on retention time and high mass accuracy (<10ppm) across multiple time points with 3 replicates at each time.

[0243] Using the online pepsin/protease XIII column coupled with MS^E data acquisition, a total of 109 peptides from hMUC16.nub were identified in the absence or presence of the H4sH8767P, representing 64% sequence coverage. Six peptides had significantly reduced deuteration uptake (centroid delta values > 0.5 daltons from at least one time point with p-values < 0.05) when bound to H4sH8767P and are illustrated in the Table 9A. The recorded peptide mass corresponds to the average value of the centroid MH^+ mass from three replicates. These peptides, corresponding to amino acids 428-434, 453-467, and 474-481 of hMuc16.nub, had slower deuteration rates when bound to H4sH8767P. These identified residues also correspond to residues 14237-14243, 14262-14276, and 14283-14290 of hMUC16 as defined by the Uniprot entry Q8WXI7 (MUC16_HUMAN), SEQ ID NO:1899.

Table 9A. hMUC16.nub Peptides with Altered Deuteration Rates Upon Binding H4sH8767P

Residues of SEQ ID NO: 1902		**1min Deuteration**			**20 min Deuteration**		
	Amino Acid Sequence	hMUC16. nub	hMUC16.nub + H4sH8767P	Δ	hMUC16 . nub	hMUC16.nub + H4sH8767P	Δ
428-434	LYKGSQL	809.97 ± 0.03	809.07± 0.06	**-0.26**	811.05 ± 0.16	810.17± 0.01	**-0.88**
429-434	YKGSQL	697.10 ± 0.00	696.74± 0.00	**-0.35**	698.13 ± 0.02	697.60± 0.03	**-0.52**
453-467	VTVKALFSS NLDPSL	1595.35 ± 0.08	1593.97± 0.2	**-1.38**	1596.0 1± 0.08	1595.33± 0.03	**-0.68**

Residues of SEQ ID NO: 1902		**1min Deuteration**			**20 min Deuteration**		
	Amino Acid Sequence	hMUC16. nub	hMUC16.nub + H4sH8767P	Δ	hMUC16 . nub	hMUC16.nub + H4sH8767P	Δ
459-467	FSSNLDPSL	983.19 ± 0.01	981.57± 0.08	**-1.62**	983.51 ±0.03	982.76± 0.07	**-0.75**
460,467	SSNLDPSL	835.44 ± 0.01	834.01±0.00	**-1.43**	835.89 ± 0.01	835.12± 0.15	**-0.74**
474-481	DKTLNASF	899.76 ± 0.00	899.25± 0.06	**-0.51**	900.63 ± 0.00	900.10± 0.03	**-0.54**

[0244] Using the online pepsin/protease XIII column coupled with MS^E data acquisition, a total of 109 peptides from hMUC16.nub were identified in the absence or presence of the H1H8794P2, representing 64% sequence coverage. Three peptides had significantly reduced deuteration uptake (centroid delta values > 0.5 daltons from at least one time point with p-values < 0.05) when bound to H1H8794P2 and are illustrated in the Table 9B. The recorded peptide mass corresponds to the average value of the centroid MH^+ mass from three replicates. These peptides, corresponding to amino acids 126-131, 127-131, and 132-138 of hMuc16.nub, had slower deuteration rates when bound to H1H8794P2. These identified residues also correspond to residues 13935-13940, 13936-13940, and 13941-13947 of hMUC16 as defined by the Uniprot entry Q8WXI7 (MUC16_HUMAN), SEQ ID NO:1899.

Table 9B. hMUC16.nub Peptides with Altered Deuteration Rates Upon Binding H1H8794P2

		1min Deuteration			**20 min Deuteration**		
Residues of SEQ ID NO: 1902	Amino Acid Sequence	hMUC16. nub	hMUC16.nub + H1H8794P2	Δ	hMUC16 . nub	hMUC16.nub + H1H8794P2	Δ
126-131	LRYMAD	771.41±0.01	770.60±0.04	**-0.81**	771.91 ±0.04	770.76±0.02	**-1.15**
127-131	RYMAD	658.03±0.02	657.32±0.01	**-0.71**	657.89 ±0.01	657.27±0.01	**-0.6**
132-138	MGQPGSL	692.55±0.02	691.42±0.15	**-1.13**	692.61 ±0.01	691.58±0.02	**-1.03**

[0245] Using the online pepsin/protease XIII column coupled with MS^E data acquisition, a total of 109 peptides from hMUC16.nub were identified in the absence or presence of the H1H8799P2, representing 64% sequence coverage. Four peptides had significantly reduced deuteration uptake (centroid delta values > 0.5 daltons from at least one time point with p-values < 0.05) when bound to H1H8799P2 and are illustrated in the Table 9C. The recorded peptide mass corresponds to the average value of the centroid MH^+ mass from three replicates. These peptides, corresponding to amino acids 357-369, 358-366, 358-369, and 361-369 of hMuc16.nub, had slower deuteration rates when bound to H1H8799P2. These identified residues also correspond to residues 14165-14178, 14166-14176, 14166-14178, and 14170-14178 of hMUC16 as defined by the Uniprot entry Q8WXI7 (MUC16_HUMAN), SEQ ID NO:1899.

Table 9C. hMUC16.nub Peptides with Altered Deuteration Rates Upon Binding H1H8799P2

Residues of SEQ ID NO: 1902		**1min Deuteration**			**20 min Deuteration**		
	Amino Acid Sequence	hMUC16.nub	hMUC16.nub + H1H8799P2	Δ	hMUC16.nub	hMUC16.nub + H1H8799P2	Δ
357-369	LSQLTHGVTQLGF	1404.15±0.03	1403.41±0.09	**-0.74**	1406.14±0.15	1404.26±0.02	**-2.11**
358-366	SQLTHGVTQL	972.37±0.10	972.04±0.10	**-0.33**	973.94±0.03	972.56±0.00	**-1.38**
358-369	SQLTHGVTQLGF	1291.23±0.02	1290.20±0.00	**-1.03**	1291.34±0.02	1291.05±0.06	**-2.27**
361-369	THGVTQLGF	1404.15±0.03	1403.42±0.05	-0.73	1406.14±0.14	1404.03±0.02	**-2.11**

Example 7: Pharmacokinetic Assessment of anti-MUC16 × CD3 Bispecific Antibodies

[0246] Assessment of the pharmacokinetics of anti-MUC16 × CD3 bispecific antibodies BSMUC16/CD3-001 and BSMUC16/CD3-005 and an isotype control were conducted in humanized MUC16 × CD3 mice (mice homozygous for human MUC16 and CD3 expression, MUC16 $^{hu/hu}$ × CD3 $^{hu/hu}$), CD3 humanized mice (mice homozygous for human CD3 expression, CD3 $^{hu/hu}$) 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.

[0247] 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.

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

[0248] 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).

[0249] Following i.p. administration of antibodies in CD3$^{hu/hu}$ mice, BSMUC16/CD3-001, BSMUC16/CD3-005 and isotype control had comparable C_{max} concentrations (4.6, 3.6 and 4.1 µg/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 µg/mL, which is about 28-fold less than terminal antibody concentrations determined for the isotype control (0.85 µg/mL) and 22-fold less than BSMUC16/CD3-005 (0.66 µg/mL) serum concentrations.

[0250] In MUC16 $^{hu/hu}$ × CD3 $^{hu/hu}$ double-humanized mice, the Muc16xCD3 bispecific and isotype control antibodies had comparable C_{max} concentrations (C_{max} range: 4.5-6.9 µg/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 µg/mL).

[0251] A summary of the data for total anti-MUC16 × CD3 bispecific antibodies and isotype control antibody concentrations are summarized in Table 10. Mean PK parameters are described in Tables 11A and 11B. Mean total antibody concentrations versus time are shown in Figures 1, 2, and 3. 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 10: 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 × CD3 mice

Antibody	Time (d)	Total mAb Concentration In Mouse Serum					
		WT		CD3$^{hu/hu}$		MUC16$^{hu/hu}$ × CD3$^{hu/hu}$	
		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	NO	0.07	0.02	0.06	0.05
	28.00	0.60	NO	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

Antibody	Time (d)	Total mAb Concentration In Mouse Serum					
		WT		$CD3^{hu/hu}$		$MUC16^{hu/hu}$ × $CD3^{hu/hu}$	
		Mean (µg/mL)	+/- SD	Mean (µg/mL)	+/- SD	Mean (µg/mL)	+/-SD
	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; NO = Not determined due to exclusion of mice with drug clearing anti-drug titers

Table 11A: Summary of Pharmacokinetic Parameters: $CD3^{hu/hu}$ humanized mice

Parameter	Units	**WT mice**			**$CD3^{hu/hu}$ mice**		
		Isotype Control	BSMUC16/CD3-001	BSMUC16/CD3-005	Isotype Control	BSMUC16/CD3-001	BSMUC16/CD3-005
C_{max}	µg/mL	5 ± 3	6 ± 0.4	5 ± 0.5	4.1 ± 3	4.6 ± 0.8	3.5 ± 1
$T_{1/2}$	d	11 ± 4	7 ± 3	12 ± 2	14 ± 0.5	3.9 ± 0.6	11 ± 5
AUC_{last}	d•µg/mL	35 ± 18	40 ± 11	45 ± 5	49 ± 20	16 ± 3	36 ± 13

C_{max}= Peak concentration; AUG = Area under the concentration-time curve; AUCiast = AUG computed from time zero to the time of the last positive concentration; $T_{1/2}$ = Estimated half-life observed

Table 11B: Summary of Pharmacokinetic Parameters: $MUC16^{hu/hu}$ × $CD3^{hu/hu}$ double-humanized mice

Parameter	Units	**WT mice**			**$MUC16^{hu/hu}$ x $CD3^{hu/hu}$ mice**		
		Isotype Control	BSMUC16/CD3-001	BSMUC16/CD3-005	Isotype Control	BSMUC16/CD3-001	BSMUC16/CD3-005
C_{max}	µg/mL	5 ± 3	6 ± 0.4	5 ± 0.5	6.7 ± 0.7	6.9 ± 1	4.5 ± 4
$T_{1/2}$	d	11 ± 4	7 ± 3	12 ± 2	12.9 ± 4	3.3 ± 0.8	8.2 ± 4

Parameter	Units	**WT mice**			**MUC16$^{hu/hu}$ x CD3$^{hu/hu}$ mice**		
		Isotype Control	BSMUC16 /CD3-001	BSMUC16 /CD3-005	Isotype Control	BSMUC16 /CD3-001	BSMUC16 /CD3-005
AUC$_{last}$	d•µg/mL	35 ± 18	40 ± 11	45 ± 5	46 ± 10	27 ± 3	34 ± 11

C$_{max}$= Peak concentration; AUG = Area under the concentration-time curve; AUCiast = AUG computed from time zero to the time of the last positive concentration; T$_{1/2}$ = Estimated half-life observed

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

[0252] To determine the *in vivo* efficacy of exemplary anti-MUC16/anti-CD3 bispecific antibodies identified as having weak or no detectable binding 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

[0253] 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.

[0254] Briefly, NSG mice were injected intraperitoneally (i.p.) with 5.0 × 10^6 human PBMCs. 8d later, 1.5 × 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 10µg/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 10µg/mouse (N=5/treatment group).

[0255] 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^2/steradian(sr). For the immediate-treatment group, data is shown as BLI levels 26d post tumor implantation (Table 12A). 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 12B).

[0256] 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 disclosure display potent anti-tumor efficacy in several models.

Table 12A: Efficacy of anti-MUC16/anti-CD3 Bispecific Antibodies in Immune-Compromised Xenograft Model: Immediate Dosing

Tumor Model/ Mouse Strain/ Dose	Bispecific Antibody Identifier	N	Avg Bioluminescent Radiance (photons/sec/cm^2/ steradian)
			Day 26 (mean ± SD)
OVCAR-3/Luc / *NSG*/ 10µg / 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 12B: Efficacy of anti-Muc16/anti-CD3 Bispecific Antibodies in Immune-Compromised Xenograft Model: Therapeutic Treatment

Tumor Model/ Mouse Strain/ Dose	Bispecific Antibody Identifier	N	Fold change in Avg Bioluminescent Radiance [p/s/cm^2/sr] at Day 26 relative to Day 6 (mean ± SD)
OVCAR-3/Luc / *NSG*/ 10µg / mouse	BSMUC16/CD3-001	5	2.0 ± 5.0
	BSMUC16/CD3-005	5	0.01 ± 0.02
	Isotype Control	5	21.0 ± 8.0

[0257] In further experiments, the *in vivo* efficacy of an anti-MUC16/anti-CD3 bispecific antibody was evaluated in xenogenic and syngeneic tumor models. For the first xenogenic model, NSG mice were injected intraperitoneally (IP) with OVCAR-3/Luc cells previously passaged *in vivo* (Day 0) eleven days after engraftment with human PBMCs. Mice were treated IP with 0.01, 0.1, or 0.5 mg/kg BSMUC16/CD3-001, or administered 0.5 mg/kg non-binding control or CD3-binding control on Days 6, 10, 13, 16, and 21. Tumor burden was assessed by BLI on Days 6, 14, and 20 post tumor implantation. Treatment with 0.1 or 0.5 mg/kg of BSMUC16/CD3-001 resulted in significant anti-tumor efficacy as determined by BLI measurements on Day 20, as shown in Tables 13A-C and in Figures 4-6. For the second xenogenic model, NSG mice were injected with OVCAR-3/Luc cells previously passaged *in vivo* (Day 0) thirteen days after engraftment with human PBMCs and a second batch of PBMCs were transferred on Day 4. Mice were treated intravenously (IV) with 0.1, 0.5, 1, or 5 mg/kg BSMUC16/CD3-005 or administered 5 mg/kg non-binding control or CD3-binding control on Days 5, 8, 12, 15, 19 and 22. Tumor burden was assessed by BLI on Days 4, 11, 18 and 25. Treatment with 0.5, 1, or 5 mg/kg BSMUC16/CD3-005 resulted in significant anti-tumor efficacy as shown by BLI measurements and fold changes (Tables 13D-F and Figures 7-9). To examine efficacy in an immune-competent model, the murine CD3 gene was replaced with human CD3 and a portion of the mouse MUC16 gene was replaced with the human sequence. The replacements resulted in a mouse whose T cells express human CD3 and that expresses a chimeric MUC16 molecule containing a portion of human MUC16 where the BSMUC16/CD3-001 bispecific antibody binds. For the syngeneic tumor model, ID8-VEGF cell lines engineered to express the portion of human MUC16 were used. Mice were implanted with the ID8-VEGF/huMUC16 cells subcutaneously and treated with 100 µg of BSMUC16/CD3-001 either on day of implantation or ten days after implantation when tumors were established. Treatment with 100 µg BSMUC16/CD3-001 resulted in significant anti-tumor efficacy, as shown in Table 13G and Figure 10.

[0258] *Implantation and measurement of xenograft tumors:* Ascites cells from the OVCAR-3/Luc cell line, previously passaged *in vivo,* were administered IP into the NSG mice previously engrafted with human

PBMCs. BLI was measured as a read-out for tumor growth several days after OVCAR-3/Luc implantation and at multiple times during study. After initial BLI measurement for cohorting, mice were divided into groups of 4-6 animals each and administered MUC16xCD3 bispecific or control antibodies twice per week throughout the study.

[0259] *Calculation of xenograft tumor growth and inhibition:* Bioluminescence imaging was used to measure tumor burden. Mice were injected IP with 150 mg/kg (as determined by body weights at the start of the experiment) of the luciferase substrate D-luciferin suspended in PBS. Ten minutes after dosing, BLI imaging of the mice was performed under isoflurane anesthesia using the Xenogen IVIS system. Image acquisition was carried out with the field of view at D, subject height of 1.5 cm, and medium binning level for 0.5-min exposure time. BLI signals were extracted using Living Image software. Regions of interest were drawn around each tumor mass and photon intensities were recorded as p/s/cm^2/sr. Statistical analysis was performed using GraphPad Prism software (Version 6). Statistical significance for the BLI results was determined using an unpaired nonparametric Mann-Whitney t-test. Fold changes were calculated by the formula: (Day20-Day6)/Day6 for study 1 and (Day25-Day4)/Day4 for study 2.

[0260] *Implantation and measurement of syngeneic tumors*: Mice expressing human CD3 and a human-murine chimera of MUC16 in the corresponding mouse loci were implanted with 10e6 ID8-VEGF/huMUC16 cells subcutaneously (SC). Mice were administered BSMUC16/CD3-001 or a CD3-binding control IP, twice per week throughout study. Treatment began on Day 0 or Day 10 post implantation. Tumor growth was measured with calipers twice per week. Mice were sacrificed 47 days after tumor implantation.

[0261] *Calculation of syngeneic tumor growth and inhibition*: In order to determine tumor volume by external caliper, the greatest longitudinal diameter (length) and the greatest transverse diameter (width) were determined. Tumor volume based on caliper measurements were calculated by the formula: Volume = (length × width^2)/2. Statistical significance was determined using an unpaired nonparametric Mann-Whitney t-test.

[0262] The anti-tumor efficacy of the BSMUC16/CD3-001 bispecific antibody in the xenogenic and syngeneic *in vivo* tumor models is shown in Tables 13A-D, below.

Table 13A: OVCAR-3 model study 1. Level of Bioluminescence on Day 6 post tumor implantation

Antibody (mg/kg)	**Avg Radiance [p/s/cm2^2/sr] 6 days post-implantation (median ± SEM)**
non-binding control (0.5)	8.15e+05 ± 7.88e+04
CD3-binding control (0.5)	6.39e+05 ± 8.67e+04
BSMUC16/CD3-001 (0.5)	7.64e+05 ± 1.19e+05
BSMUC16/CD3-001 (0.1)	6.31e+05 ± 1.10e+05
BSMUC16/CD3-001 (0.01)	8.77e+05 ± 7.91e+04

Table 13B: OVCAR-3 model study 1. Level of Bioluminescence on Day 20 post tumor implantation

Antibody (mg/kg)	**Avg Radiance [p/s/cm2^2/sr] 20 days post-implantation (median ± SEM)**
non-binding control (0.5)	8.63e+06 ± 1.45e+06
CD3-binding control (0.5)	9.94e+06 ± 1.08e+06
BSMUC16/CD3-001 (0.5)	9.37e+02 ± 9.62e+02
BSMUC16/CD3-001 (0.1)	2.36e+04 ± 1.28e+06

Antibody (mg/kg)	**Avg Radiance [p/s/cm2^2/sr] 20 days post-implantation (median ± SEM)**
BSMUC16/CD3-001 (0.01)	6.51e+06 ± 1.60e+06

Table 13C: OVCAR-3 model study 1. Fold Change in BLI between Day 6 and Day 20 post tumor implantation

Antibody (mg/kg)	**Fold change in Avg Radiance [p/s/cm2^2/sr] from Day 6 to D20 post-implantation (mean ± SD)**
non-binding control (0.5)	9.5 ± 1.9
CD3-binding control (0.5)	15.6 ± 6.7
BSMUC16/CD3-001 (0.5)	-1.00 ± 0.00
BSMUC16/CD3-001 (0.1)	1.2 ± 4.7
BSMUC16/CD3-001 (0.01)	5.6 ± 4.2

Table 13D: OVCAR-3 model study 2. Level of Bioluminescence on Day 4 post tumor implantation

Antibody (mg/kg)	**Avg Radiance [p/s/cm2^2/sr] 4 days post-implantation (median ± SEM)**
non-binding control (5)	1.54e+05 ± 9.93e+03
CD3-binding control (5)	1.34e+05 ± 1.55e+04
BSMUC16/CD3-005 (5)	1.54e+05 ± 1.03e+04
BSMUC16/CD3-005 (1)	1.38e+05 ± 4.65e+03
BSMUC16/CD3-005 (0.5)	1.31e+05 ± 4.03e+03
BSMUC16/CD3-005 (0.1)	1.53e+05 ± 1.93e+04

Table 13E: OVCAR-3 model study 2. Level of Bioluminescence on Day 25 post tumor implantation

Antibody (mg/kg)	**Avg Radiance [p/s/cm2^2/sr] 25 days post-implantation (median ± SEM)**
non-binding control (5)	7.20e+06 ± 8.91e+05
CD3-binding control (5)	6.15e+06 ± 7.26e+05
BSMUC16/CD3-005 (5)	1.52e+03 ± 4.86e+05
BSMUC16/CD3-005 (1)	6.99e+03 ± 6.23e+03
BSMUC16/CD3-005 (0.5)	2.23e+03 ± 2.35e+05
BSMUC16/CD3-005 (0.1)	7.63e+06 ± 1.83e+06

Table 13F: OVCAR-3 model study 2. Fold Change in BLI between Day 4 and Day 25 post tumor implantation

Antibody (mg/kg)	**Fold change in Avg Radiance [p/s/cm2^2/sr] from Day 4 to D25 post-implantation (mean ± SD)**
non-binding control (5)	46.8 ± 20.6
CD3-binding control (5)	55.0 ± 14.7
BSMUC16/CD3-005 (5)	2.5 ± 8.5
BSMUC16/CD3-005 (1)	-0.9 ± 0.1
BSMUC16/CD3-005 (0.5)	0.7 ± 3.6
BSMUC16/CD3-005 (0.1)	45.4 ± 35.7

Table 13G: ID8-VEGF/huMUC16 model. Tumor size (mm3) at Day 47

Treatment start	**Antibody (µg)**	**Tumor size (mm3) at Day 47 (mean ± SEM)**
Day 0	CD3-binding control (100)	827.5 ± 223.5
Day 0	BSMUC16/CD3-001 (100)	51.2 ± 51.2
Day 10	BSMUC16/CD3-001 (100)	273.8 ± 92.36

Reference Example 1: Conjugate Preparation and Characterization

[0263] All the monoclonal antibodies were expressed in CHO cells and purified by Protein A. An isotype control was also prepared in a similar fashion. The non-binding isotype control antibody was derived from an immunological antigen having no relation to oncology.

[0264] The antibody (10 mg/ml) in 50 mM HEPES, 150 mM NaCl, pH 7.5, was treated with 1 mM dithiothreitol at 37 °C for 30 min. After gel filtration (G-25, pH 4.5 sodium acetate), one of the maleimido linker payload derivatives **Compound 7** or **Compound 10** (see Table 14) (1.2 equivalents/SH group) in DMSO (10 mg/ml) was added to the reduced antibody and the mixture adjusted to pH 7.0 with 1 M HEPES (pH 7.4). **Compound 7** and **Compound 10,** and methods of making the compounds, are described in PCT Publication No. WO2014/145090, published on September 18, 2014 and PCT Publication No. WO2016/160615, published on October 6, 2016, respectively. After 1 h the reaction was quenched with excess N-ethyl maleimide. The conjugates were purified by size exclusion chromatography and sterile filtered. Protein and linker payload concentrations were determined by UV spectral analysis. Size-exclusion HPLC established that all conjugates used were >95% monomeric.. Yields are reported in Table 14 based on protein determinations. All conjugated antibodies were analyzed by UV for linker payload loading values according to Hamblett et al, Cancer Res 2004 10 7063. The results are summarized in Table 14.

[0265] A conjugate comprising **Compound 60** can be prepared using a similar method. **Compound 60,** and methods of making the compound, is described in PCT Publication No. WO2016/160615 (Example 20), published on October 6, 2016. Compound **60** is *Maytansin-N-methyl-L-alanine-(3-methoxy-4-amino)benzamido-Cit-Val-Cap-Mal.*

Table 14: Summary of Payload (Chemotoxic Drug) and Antibody-Drug-Conjugate Parameters

Compound	**ε252 nm (cm^{-1} M^{-1})**	**ε280 nm (cm^{-1} M^{-1})**
7 *[Maytansin-3-N-methyl-L-(S)-alanine-propanamidyl-3-N-methyl-N-[4-(amino-citrulline-valine-hexanamide-6-maleimidyl)benzyl]carbamate]*	50600	8100
10 *[Maytansin-N-methyl-L-alanine-4-aminobenzamide-citrulline-valine-caprolyl-6-maleimidyl]*	45990	20600
Antibody	**ε252 nm (cm^{-1} M^{-1})**	**ε280 nm (cm^{-1} M^{-1})**
H1H9519N	83995	235280
H1H9521N	85564	232050
Isotype Control	75113	218360

Antibody	**ε252 nm (cm^{-1} M^{-1})**	**ε280 nm (cm^{-1} M^{-1})**

Antibody Conjugate	**Payload:Antibody (UV)**	**Yield %**
H1H9519N-**7**	3.5	40
H1H9521N-**7**	3.6	40
H1H9521N-**10**	3.0	40
Isotype Control-**7**	3.0	60
Isotype Control-**10**	3.4	60

Reference Example 2: Anti-MUC16 antibody drug conjugates are potent inhibitors of tumor growth in in *vivo* MUC16-positive prostate cancer xenograft models

[0266] To determine the in vivo efficacy of the anti-MUC16 antibodies conjugated to **Compound 7** and **Compound 10,** studies were performed in immunocompromised mice bearing MUC16 positive ovarian cancer xenografts.

[0267] For these studies, female SCID mice (Taconic, Hudson NY) were implanted with OVCAR3 [NIH:OVCAR-3 (OVCAR3, ATCC HTB-161)] cells transfected with luciferase (OVCAR3/luc) that endogenously express MUC16. For intraperitoneal (IP) tumors, mice were randomized into treatment groups, and dosed with either anti-MUC16 drug conjugated antibodies (see Reference Example 1), a non-binding conjugated antibody, or vehicle following detection of tumor luminescent signal. For subcutaneous (SC) xenografts, once tumors had reached an average volume of 200 mm^3 (~Day 16), mice were randomized into treatment groups, and dosed with either anti-MUC16 drug conjugated antibodies, a non-binding conjugated antibody, or vehicle. In these *in vivo* studies, antibodies were dosed and tumors were then monitored until ascites developed or an average tumor size of approximately 1200 mm^3 was attained in the cohort dosed with vehicle alone. At this point the Tumor Growth Inhibition was calculated.

[0268] In an initial intraperitoneal (IP) study, exemplary anti-MUC16 antibodies conjugated to **Compound 7** were examined for efficacy in reducing OVCAR3/luc luminescent signal. Mice received four once weekly doses of anti-MUC16 and control ADCs at 85 µg/kg of drug equivalent based on ADC drug:antibody ratios. As summarized in Table 15A, **H1H9519N-Compound 7** and H1H9521N- **Compound 7** potently inhibited ascites tumor growth. These anti-MUC16 ADCs efficiently reduced tumor size, with a 100 percent reduction in tumor luminescence relative to vehicle control. The Control ADC did not mediate any inhibition of OVCAR/luc ascites tumor cell growth.

[0269] A further study assessing the efficacy of anti-MUC16 ADCs against subcutaneous (SC) OVCAR3/luc tumor is summarized in Table 15B. Mice received four once weekly doses of anti-MUC16 and control ADCs at 85 µg/kg of drug equivalent based on ADC drug:antibody ratios. When conjugated to **Compound 7,** MUC16 ADCs H1H9519N- **Compound 7** and H1H9521N-**Compound 7** again produced significant anti-tumor effect; this time against the subcutaneous OVCAR3/luc tumors. Accordingly, these ADCs mediated a 100 % and 109 % inhibition of tumor growth respectively. Control ADC did not mediate any inhibition of OVCAR/luc subcutaneous tumor growth.

[0270] In a third study, the efficacy of the anti-MUC16 antibody H1H9521N conjugated to the linker drug **Compound 10** was assessed in the IP OVCAR3/luc tumor model. Mice received single doses of anti-MUC16 and control ADCs at 85 µg/kg, 170 µg/kg and 340 µg/kg of drug equivalent based on ADC drug:antibody ratios. As summarized in Table 15C, H1H9519N-**10** potently inhibited ascites tumor growth. The doses of H1H9519N- Compound 10 resulted in a 99-100 % inhibition of tumor luminescence relative to vehicle control. Some inhibition was observed with Control ADC using **Compound 10** although this was more moderate than that observed following anti-MUC16 H1H9519N- **Compound 10.**

Table 15A: Inhibition of OVCAR3/luc IP Tumor Growth at Day 49 in SCID mice treated with anti-MUC16 antibodies conjugated to Compound 7

Treatment Group	Final Tumor Average Radiance (mean ± SEM)	Average Tumor Growth Inhibition (%)
Vehicle	16469750 ± 10679335	0
Control- **Compound 7** 85 µg/kg	16813750 ± 4026065	-2
H1H9519N- **Compound 7** 85 µg/kg	111254 ± 187288	100
H1 H9521N- **Compound 7** 85 µg/kg	110413 ± 161353	100

Table 15B: Inhibition of OVCAR3/luc SC Tumor Growth at Day 37 in SCID mice treated with anti-MUC16 antibodies conjugated to Compound 7

Treatment Group	Final Tumor Volume (mean ± SEM)	Average Tumor Growth Inhibition (%)
Vehicle	1210 ± 426	0
Control- **Compound 7** 85 µg/kg	1737 ± 391	-51
H1H9519N- **Compound 7** 85 µg/kg	187 ± 269	100
H1H9521N- **Compound 7** 85 µg/kg	89 ± 97	109

Table 15C: Inhibition of OVCAR3/luc IP Tumor Growth at Day 49 in SCID mice treated with anti-MUC16 antibodies conjugated to Compound 10

Treatment Group	Final Tumor Radiance (mean ± SEM)	Average Tumor Growth Inhibition (%)
Vehicle	29211000 ± 23504780	0
Control- **Compound 10** 85 µg/kg	17332625 ± 14346694	41
Control- **Compound 10** 170 µg/kg	32075000 ± 15623403	-10
Control- **Compound 10** 340 µg/kg	22882350 ± 18771913	22
H1H9521N- **Compound 10** 85 µg/kg	574285 ± 306844	99
H1H9521N- **Compound 10** 170 µg/kg	236037 ± 226948	100
H1H9521N- **Compound 10** 340 µg/kg	26472 ± 25079	101

Example 9: Generation of Anti-CD3 Antibodies

[0271] Anti-CD3 antibodies were obtained by immunizing an engineered mouse comprising DNA encoding human Immunoglobulin heavy and kappa light chain variable regions with cells expressing CD3 or with DNA encoding CD3. The antibody immune response was monitored by a CD3-specific immunoassay. When a desired immune response was achieved, splenocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce CD3-specific antibodies. Using this technique several anti-CD3 chimeric antibodies (i.e., antibodies possessing human variable domains and mouse constant domains) were obtained. In addition, several fully human anti-CD3 antibodies were isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in US 2007/0280945A1.

[0272] Certain biological properties of the exemplary anti-CD3 antibodies generated in accordance with the methods of this Example are described in detail in the Examples herein.

Example 10: Heavy and Light Chain Variable Region Amino Acid and Nucleic Acid Sequences

[0273] Table 16 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions and CDRs of selected anti-CD3 antibodies of the disclosure. The corresponding nucleic acid sequence identifiers are set forth in Table 17. Methods of making the anti-CD3 antibodies disclosed herein can also be found in US publication 2014/0088295.

Table 16: Amino Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**		**HCDR3**	**LCVR**	**LCDR11**	**LCDR2**	**LCDR3**
H1H2712N	402	404	406	408	410	412	414	416
H1M2692N	418	420	422	424	426	428	430	432
H1M3542N	434	436	438	440	442	444	446	448
H1M3544N	450	452	454	456	458	460	462	464
H1M3549N	466	468	470	472	474	476	478	480
H1M3613N	482	484	486	488	490	492	494	496
H2M2689N	498	500	502	504	506	508	510	512
H2M2690N	514	516	518	520	522	524	526	528
H2M2691N	530	532	534	536	538	540	542	544
H2M2704N	546	548	550	552	554	556	558	560
H2M2705N	562	564	566	568	570	572	574	576
H2M2706N	578	580	582	584	586	588	590	592
H2M2707N	594	596	598	600	602	604	606	608
H2M2708N	610	612	614	616	618	620	622	624
H2M2709N	626	628	630	632	634	636	638	640
H2M2710N	642	644	646	648	650	652	654	656
H2M2711N	658	660	662	664	666	668	670	672
H2M2774N	674	676	678	680	682	684	686	688

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**		**HCDR3**	**LCVR**	**LCDR11**	**LCDR2**	**LCDR3**
H2M2775N	690	692	694	696	698	700	702	704
H2M2776N	706	708	710	712	714	716	718	720
H2M2777N	722	724	726	728	730	732	734	736
H2M2778N	738	740	742	744	746	748	750	752
H2M2779N	754	756	758	760	762	764	766	768
H2M2789N	770	772	774	776	778	780	782	784
H2M2862N	786	788	790	792	794	796	798	800
H2M2885N	802	804	806	808	810	812	814	816
H2M2886N	818	820	822	824	826	828	830	832
H2M3540N	834	836	838	840	842	844	846	848
H2M3541N	850	852	854	856	858	860	862	864
H2M3543N	866	868	870	872	874	876	878	880
H2M3547N	882	884	886	888	890	892	894	896
H2M3548N	898	900	902	904	906	908	910	912
H2M3563N	914	916	918	920	922	924	926	928
H1H5751P	930	932	934	936	938	940	942	944
H1H5752P	946	948	950	952	954	956	958	960
H1H5753B	962	964	966	968	970	972	974	976
H1H5754B	978	980	982	984	986	988	990	992
H1H5755B	994	996	998	1000	1002	1004	1006	1008
H1H5756B	1010	1012	1014	1016	1018	1020	1022	1024
H1H5757B	1026	1028	1030	1032	1034	1036	1038	1040
H1H5758B	1042	1044	1046	1048	1050	1052	1054	1056
H1H5761P	1058	1060	1062	1064	1066	1068	1070	1072
H1H5763P	1074	1076	1078	1080	1082	1084	1086	1088
H1H5764P	1090	1092	1094	1096	1098	1100	1102	1104
H1H5769P	1106	1108	1110	1112	1114	1116	1118	1120
H1H5771P	1122	1124	1126	1128	1130	1132	1134	1136
H1H5772P	1138	1140	1142	1144	1146	1148	1150	1152
H1H5777P	1154	1156	1158	1160	1162	1164	1166	1168
H1H5778P	1170	1172	1174	1176	1178	1180	1182	1184
H1H5780P	1186	1188	1190	1192	1194	1196	1198	1200
H1H 5781P	1202	1204	1206	1208	1210	1212	1214	1216
H1H5782P	1218	1220	1222	1224	1226	1228	1230	1232
H1H5785B	1234	1236	1238	1240	1242	1244	1246	1248
H1H5786B	1250	1252	1254	1256	1258	1260	1262	1264
H1H5788P	1266	1268	1270	1272	1274	1276	1278	1280

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**		**HCDR3**	**LCVR**	**LCDR11**	**LCDR2**	**LCDR3**
H1H5790B	*1282*	*1284*	*1286*	*1288*	*1290*	*1292*	1294	*1296*
H1H5791B	*1298*	*1300*	*1302*	*1304*	*1306*	*1308*	*1310*	*1312*
H1H5792B	*1314*	*1316*	*1318*	*1320*	*1322*	*1324*	*1326*	*1328*
H1H5793B	*1330*	*1332*	*1334*	*1336*	*1338*	*1340*	*1342*	*1344*
H1H5795B	*1346*	*1348*	*1350*	*1352*	*1354*	*1356*	*1358*	*1360*
H1H5796B	*1362*	*1364*	*1366*	*1368*	*1370*	*1372*	*1374*	*1376*
H1H5797B	*1378*	*1380*	1382	*1384*	*1386*	1388	*1390*	*1392*
H1H5798B	*1394*	*1396*	*1398*	*1400*	*1402*	*1404*	*1406*	*1408*
H1H5799P	*1410*	*1412*	*1414*	*1416*	*1418*	*1420*	1422	1424
H1H5801B	*1426*	*1428*	*1430*	*1432*	*1434*	*1436*	*1438*	*1440*
H1H7194B	1442	*1444*	*1446*	*1448*	*1634*	*1636*	*1638*	*1640*
H1H7195B	*1450*	*1452*	*1454*	*1456*	*1634*	*1636*	*1638*	*1640*
H1H7196B	*1458*	*1460*	*1462*	*1464*	*1634*	*1636*	*1638*	*1640*
H1H7198B	*1466*	*1468*	*1470*	*1472*	*1634*	*1636*	*1638*	*1640*
H1H7203B	*1474*	*1476*	*1478*	*1480*	*1634*	*1636*	*1638*	*1640*
H1H7204B	*1482*	*1484*	*1486*	*1488*	*1634*	*1636*	*1638*	*1640*
H1H7208B	*1490*	1492	*1494*	*1496*	*1634*	*1636*	*1638*	*1640*
H1H7211B	*1498*	*1500*	*1502*	*1504*	*1634*	*1636*	*1638*	*1640*
H1H7221B	*1506*	*1508*	*1510*	*1512*	*1634*	*1636*	*1638*	*1640*
H1H7223B	*1514*	*1516*	*1518*	*1520*	*1634*	*1636*	*1638*	*1640*
H1H7226B	*1522*	*1524*	*1526*	*1528*	*1634*	*1636*	*1638*	*1640*
H1H7232B	*1530*	*1532*	*1534*	*1536*	*1634*	*1636*	*1638*	*1640*
H1H7233B	*1538*	*1540*	*1542*	*1544*	*1634*	*1636*	*1638*	*1640*
H1H7241B	*1546*	*1548*	*1550*	*1552*	*1634*	*1636*	*1638*	*1640*
H1H7242B	*1554*	*1556*	*1558*	*1560*	*1634*	*1636*	*1638*	*1640*
H1H7250B	*1562*	*1564*	*1566*	*1568*	*1634*	*1636*	*1638*	*1640*
H1H7251B	*1570*	*1572*	*1574*	*1576*	*1634*	*1636*	*1638*	*1640*
H1H7254B	*1578*	*1580*	*1582*	*1584*	*1634*	*1636*	*1638*	*1640*
H1H7258B	*1586*	*1588*	*1590*	*1592*	*1634*	*1636*	*1638*	*1640*
H1H7269B	*1594*	*1596*	*1598*	*1600*	*1634*	*1636*	*1638*	*1640*
H1H7279B	*1602*	*1604*	*1606*	*1608*	*1634*	*1636*	*1638*	*1640*
H1xH7221G	*1610*	*1612*	*1614*	*1616*	*1634*	*1636*	*1638*	*1640*
H1xH7221G3	*1618*	*1620*	*1622*	*1624*	*1634*	*1636*	*1638*	*1640*
H1xH7221G5	*1626*	*1628*	*1630*	*1632*	*1634*	*1636*	*1638*	*1640*

Table 17: Nucleic Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H1H2712N	*401*	*403*	*405*	*407*	*409*	*411*	*413*	*415*
H1M2692N	*417*	*419*	*421*	*423*	*425*	*427*	*429*	*431*
H1M3542N	*433*	*435*	*437*	*439*	*441*	*443*	*445*	*447*
H1M3544N	*449*	*451*	*453*	*455*	*457*	*459*	*461*	*463*
H1M3549N	*465*	*467*	*469*	*471*	*473*	*475*	*477*	*479*
H1M3613N	*481*	*483*	*485*	*487*	*489*	*491*	*493*	*495*
H2M2689N	*497*	*499*	*501*	*503*	*505*	*507*	*509*	*511*
H2M2690N	*513*	*515*	*517*	*519*	*521*	*523*	*525*	*527*
H2M2691N	*529*	*531*	*533*	*535*	*537*	*539*	*541*	*543*
H2M2704N	*545*	*547*	*549*	*551*	*553*	*555*	*557*	*559*
H2M2705N	*561*	*563*	*565*	*567*	*569*	*571*	*573*	*575*
H2M2706N	*577*	*579*	*581*	*583*	*585*	*587*	*589*	*591*
H2M2707N	*593*	*595*	*597*	*599*	*601*	*603*	*605*	*607*
H2M2708N	*609*	*611*	*613*	*615*	*617*	*619*	*621*	*623*
H2M2709N	*625*	*627*	*629*	*631*	*633*	*635*	*637*	*639*
H2M2710N	*641*	*643*	*645*	*647*	*649*	*651*	*653*	*655*
H2M2711N	*657*	*659*	*661*	*663*	*665*	*667*	*669*	*671*
H2M2774N	*673*	*675*	*677*	*679*	*681*	*683*	*685*	*687*
H2M2775N	*689*	*691*	*693*	*695*	*697*	*699*	*701*	*703*
H2M2776N	*705*	*707*	*709*	*711*	*713*	*715*	*717*	*719*
H2M2777N	*721*	*723*	*725*	*727*	*729*	*731*	*733*	*735*
H2M2778N	*737*	*739*	*741*	*743*	*745*	*747*	*749*	*751*
H2M2779N	*753*	*755*	*757*	*759*	*761*	*763*	*765*	*767*
H2M2789N	*769*	*771*	*773*	*775*	*777*	*779*	*781*	*783*
H2M2862N	*785*	*787*	*789*	*791*	*793*	*795*	*797*	*799*
H2M2885N	*801*	*803*	*805*	*807*	*809*	*811*	*813*	*815*
H2M2886N	*817*	*819*	*821*	*823*	*825*	*827*	*829*	*831*
H2M3540N	*833*	*835*	*837*	*839*	*841*	*843*	*845*	*847*
H2M3541N	*849*	*851*	*853*	*855*	*857*	*859*	*861*	*863*
H2M3543N	*865*	*867*	*869*	*871*	*873*	*875*	*877*	*879*
H2M3547N	*881*	*883*	*885*	*887*	*889*	*891*	*893*	*895*
H2M3548N	*897*	*899*	*901*	*903*	*905*	*907*	*909*	*911*
H2M3563N	*913*	*915*	*917*	*919*	*921*	*923*	*925*	*927*
H1H5751*P*	*929*	*931*	*933*	*935*	*937*	*939*	*941*	*943*
H1H5752P	*945*	*947*	*949*	*951*	*953*	*955*	*957*	*959*
H1H5753B	*961*	*963*	*965*	*967*	*969*	*971*	*973*	*975*
H1H5754B	*977*	*979*	*981*	*983*	*985*	*987*	*989*	*991*

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H1H5755B	993	995	997	999	1001	1003	1005	1007
H1H5756B	1009	1011	1013	1015	1017	1019	1021	1023
H1H5757B	1025	1027	1029	1031	1033	1035	1037	1039
H1H5758B	1041	1043	1045	1047	1049	1051	1053	1055
H1H5761P	1057	1059	1061	1063	1065	1067	1069	1071
H1H5763P	1073	1075	1077	1079	1081	1083	1085	1087
H1H5764P	1089	1091	1093	1095	1097	1099	1101	1103
H1H5769P	1105	1107	1109	1111	1113	1115	1117	1119
H1H5771P	1121	1123	1125	1127	1129	1131	1133	1135
H1H5772P	1137	1139	1141	1143	1145	1147	1149	1151
H1H5777P	1153	1155	1157	1159	1161	1163	1165	1167
H1H5778P	1169	1171	1173	1175	1177	1179	1181	1183
H1H5780P	1185	1187	1189	1191	1193	1195	1197	1199
H1H5781P	1201	1203	1205	1207	1209	1211	1213	1215
H1H5782P	1217	1219	1221	1223	1225	1227	1229	1231
H1H5785B	1233	1235	1237	1239	1241	1243	1245	1247
H1H5786B	1249	1251	1253	1255	1257	1259	1261	1263
H1H5788P	1265	1267	1269	1271	1273	1275	1277	1279
H1H5790B	1281	1283	1285	1287	1289	1291	1293	1295
H1H5791B	1297	1299	1301	1303	1305	1307	1309	1311
H1H5792B	1313	1315	1317	1319	1321	1323	1325	1327
H1H5793B	1329	1331	1333	1335	1337	1339	1341	1343
H1H5795B	1345	1347	1349	1351	1353	1355	1357	1359
H1H5796B	1361	1363	1365	1367	1369	1371	1373	1375
H1H5797B	1377	1379	1381	1383	1385	1387	1389	1391
H1H5798B	1393	1395	1397	1399	1401	1403	1405	1407
H1H5799P	1409	1411	1413	1415	1417	1419	1421	1423
H1H5801B	1425	1427	1429	1431	1433	1435	1437	1439
H1H7194B	1441	1443	1445	1447	1633	1635	1637	1639
H1H7195B	1449	1451	1453	1455	1633	1635	1637	1639
H1H7196B	1457	1459	1461	1463	1633	1635	1637	1639
H1H7198B	1465	1467	1469	1471	1633	1635	1637	1639
H1H7203B	1473	1475	1477	1479	1633	1635	1637	1639
H1H7204B	1481	1483	1485	1487	1633	1635	1637	1639
H1H7208B	1489	1491	1493	1495	1633	1635	1637	1639
H1H7211B	1497	1499	1501	1503	1633	1635	1637	1639
H1H7221B	1505	1507	1509	1511	1633	1635	1637	1639

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H1H7223B	*1513*	*1515*	*1517*	*1519*	*1633*	*1635*	*1637*	*1639*
H1H7226B	*1521*	*1523*	*1525*	*1527*	*1633*	*1635*	*1637*	*1639*
H1H7232B	*1529*	*1531*	*1533*	*1535*	*1633*	*1635*	*1637*	*1639*
H1H7233B	*1537*	*1539*	*1541*	*1543*	*1633*	*1635*	*1637*	*1639*
H1H7241B	*1545*	*1547*	*1549*	*1551*	*1633*	*1635*	*1637*	*1639*
H1H7242B	*1553*	*1555*	*1557*	*1559*	*1633*	*1635*	*1637*	*1639*
H1H7250B	*1561*	*1563*	*1565*	*1567*	*1633*	*1635*	*1637*	*1639*
H1H7251B	*1569*	*1571*	*1573*	*1575*	*1633*	*1635*	*1637*	*1639*
H1H7254B	*1577*	*1579*	*1581*	*1583*	*1633*	*1635*	*1637*	*1639*
H1H7258B	*1585*	*1587*	*1589*	*1591*	*1633*	*1635*	*1637*	*1639*
H1H7269B	*1593*	*1595*	*1597*	*1599*	*1633*	*1635*	*1637*	*1639*
H1H7279B	*1601*	*1603*	*1605*	*1607*	*1633*	*1635*	*1637*	*1639*
H1xH7221G	*1609*	*1611*	*1613*	*1615*	*1633*	*1635*	*1637*	*1639*
H1xH7221G3	*1617*	*1619*	*1621*	*1623*	*1633*	*1635*	*1637*	*1639*
H1xH7221G5	*1625*	*1627*	*1629*	*1631*	*1633*	*1635*	*1637*	*1639*

[0274] Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (e.g. "H1H," "H1M," "H2M," etc.), followed by a numerical identifier (*e.g.* "2712," "2692," etc., as shown in Table 1), followed by a "P," "N," or "B" suffix. Thus, according to this nomenclature, an antibody may be referred to herein as, *e.g.*, "H1H2712N," "H1M2692N," "H2M2689N," etc. The H1H, H1M and H2M prefixes on the antibody designations used herein indicate the particular Fc region isotype of the antibody. For example, an "H1H" antibody has a human IgG1 Fc, an "H1M" antibody has a mouse IgG1 Fc, and an "H2M" antibody has a mouse IgG2 Fc, (all variable regions are fully human as denoted by the first'H' in the antibody designation). As will be appreciated by a person of ordinary skill in the art, an antibody having a particular Fc isotype can be converted to an antibody with a different Fc isotype (e.g., an antibody with a mouse IgG1 Fc can be converted to an antibody with a human IgG4, etc.), but in any event, the variable domains (including the CDRs) - which are indicated by the numerical identifiers shown in Table 1 - will remain the same, and the binding properties are expected to be identical or substantially similar regardless of the nature of the Fc domain.

[0275] Tables 18 and 19 set out the amino acid sequence identifiers for heavy chain variable regions (Table 18) and light chain variable regions (Table 19), and their corresponding CDRs, of additional anti-CD3 HCVRs and LCVRs useful in anti-MUC16 × anti-CD3 bispecific antibodies of the disclosure.

Table 18 (Heavy Chain Variable Region Amino Acid Sequences)

	SEQ ID NOs			
Heavy Chain Identifier	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**
CD3-VH-AA	*1642*	*1644*	*1646*	*1648*
CD3-VH-B	*1658*	*1660*	*1662*	*1664*
CD3-VH-C	*1674*	*1676*	*1678*	*1680*
CD3-VH-D	*1690*	*1692*	*1694*	*1696*

	SEQ ID NOs			
Heavy Chain Identifier	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**
CD3-VH-E	*1706*	*1708*	*1710*	*1712*
CD3-VH-F#	*1721*	*1722*	*1723*	*1724*

Table 19 (Light Chain Variable Region Amino Acid Sequences)

	SEQ ID NOs			
Light Chain Identifier	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
CD3-VL-AA	*1650*	*1652*	*1654*	*1656*
CD3-VL-B	*1666*	*1668*	*1670*	*1672*
CD3-VL-C	*1682*	*1684*	*1686*	*1688*
CD3-VL-D	*1698*	*1700*	*1702*	*1704*
CD3-VL-E	*1714*	*1716*	*1718*	*1720*
CD3-VL-F#	*1725*	*1726*	*1727*	*1728*

[0276] The heavy and light chain variable regions of CD3-VH-F and CD3-VL-F were derived from the anti-CD3 antibody designated "L2K" as set forth in WO2004/106380.

[0277] In addition, Tables 20 and 21 set out the sequence identifiers for the nucleotide sequences encoding the heavy chain variable regions (Table 20) and light chain variable regions (Table 21), and their corresponding CDRs, of additional anti-CD3 HCVRs and LCVRs useful in anti-MUC16 × anti-CD3 bispecific antibodies of the disclosure.

Table 20 (Nucleotide Sequences Encoding Heavy Chain Variable Region Sequences)

	SEQ ID NOs			
Heavy Chain Identifier	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**
CD3-VH-AA	*1641*	*1643*	*1645*	*1647*
CD3-VH-B	*1657*	*1659*	*1661*	*1663*
CD3-VH-C	*1673*	*1675*	*1677*	*1679*
CD3-VH-D	*1689*	*1691*	*1693*	*1695*
CD3-VH-E	*1705*	*1707*	*1709*	*1711*

Table 21 (Nucleotide Sequences Encoding Light Chain Variable Region Sequences)

	SEQ ID NOs			
Light Chain Identifier	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
CD3-VL-AA	*1649*	*1651*	*1653*	*1655*
CD3-VL-B	*1665*	*1667*	*1669*	*1671*
CD3-VL-C	*1681*	*1683*	*1685*	*1687*
CD3-VL-D	*1697*	*1699*	*1701*	*1703*
CD3-VL-E	*1713*	*1715*	*1717*	*1719*

Control Constructs Used in the Following Examples

[0278] Various control constructs (anti-CD3 antibodies) were included in the following experiments for comparative purposes: "OKT-3," a mouse monoclonal antibody against human T-cell surface antigens available from the American Type Culture Collection (ATCC) under catalog no. CRL-8001; and "SP34," a commercially available mouse monoclonal antibody obtained, e.g., from Biolegend, San Diego, CA (Cat. No. 302914) or BD Pharmagen, Cat. 55052, reactive against the epsilon chain of the T3 complex on human T lymphocyte cells.

Example 11: Generation of Additional Anti-CD3 Antibodies

[0279] The following procedures were aimed at identifying antibodies that specifically recognized CD3 (T cell co-receptor) as an antigen.

[0280] A pool of anti-CD3 antibodies were derived from a genetically modified mouse. Briefly, mice 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. Antibodies described in Tables 22-25 have the same light chain sequence of VK1-39JK5 (LCVR set forth in SEQ ID NO: 1890).

[0281] Generated antibodies were tested for binding 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: 1882) was identified, amongst a few others, that were found to bind to both human and cynomolgus CD3 having an EC_{50} between 1 and 40 nM binding (or cell binding titration), as determined in a FACS titration of Jurkat cells and cynomolgus T cells, respectively. See also, e.g., FACS binding experiments outlined in Example 13 and in PCT/US2016/044732 filed July 29, 2016.

[0282] The germline amino acid residues of CD3-VH-P were subsequently identified (V-D-J rearrangement for CD3-VH-P is IGHV3-9*01, IGHJ6*02, IGHD5-12*01) 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 18.

[0283] While CD3-VH-G and some other engineered antibodies retained their binding as seen in the FACS assays, several anti-CD3 antibodies bound to human or cynomolgus CD3 *in vitro* with weak to no measurable binding, such as 40 nM EC50. 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, are described in detail in the Examples herein.

Example 12: Heavy and Light Chain Variable Regions (Amino Acid and Nucleic Acid Sequences of the CDRs)

[0284] Table 22 sets forth the amino acid sequence identifiers of the heavy chain variable regions and CDRs of selected anti-CD3 antibodies of the disclosure. The corresponding nucleic acid sequence identifiers are set forth in Table 23.

[0285] Amino acid and nucleic acid sequences were determined for each antibody heavy chain sequence. Each antibody heavy chain derived from the germline sequence (SEQ ID NO: 1910) was assigned a "G" number designation for consistent nomenclature. Table 22 sets forth the amino acid sequence identifiers of the heavy chain variable regions and CDRs of engineered anti-CD3 antibodies of the disclosure. The corresponding nucleic acid sequence identifiers are set forth in Table 23. The amino acid and nucleic acid sequence identifiers of the light chain variable region and CDRs are also identified below in Tables 24 and 25, respectively.

Table 22: Heavy Chain Amino Acid Sequence Identifiers

Antibody CD3-VH Designation	**SEQ ID NOs:**			
	HCVR	**CDR1**	**CDR2**	**CDR3**
CD3-VH-G	*1730*	*1732*	*1734*	*1736*
CD3-VH-G2	*1738*	*1740*	*1742*	*1744*
CD3-VH-G3	*1746*	*1748*	*1750*	*1752*
CD3-VH-G4	*1754*	*1756*	*1758*	*1760*
CD3-VH-G5	*1762*	*1764*	*1766*	*1768*
CD3-VH-G8	*1770*	*1772*	*1774*	*1776*
CD3-VH-G9	*1778*	*1780*	*1782*	*1784*
CD3-VH-G10	*1786*	*1788*	*1790*	1792
CD3-VH-G11	*1794*	*1796*	*1798*	*1800*
CD3-VH-G12	*1802*	*1804*	*1806*	*1808*
CD3-VH-G13	*1810*	*1812*	*1814*	*1816*
CD3-VH-G14	*1818*	*1820*	*1822*	*1824*
CD3-VH-G15	*1826*	*1828*	*1830*	*1832*
CD3-VH-G16	*1834*	*1836*	*1838*	*1840*
CD3-VH-G17	*1842*	*1844*	*1846*	*1848*
CD3-VH-G18	*1850*	*1852*	*1854*	*1856*
CD3-VH-G19	*1858*	*1860*	*1862*	*1864*
CD3-VH-G20	*1866*	*1868*	*1870*	*1872*
CD3-VH-G21	*1874*	*1876*	*1878*	*1880*
CD3-VH-P	*1882*	*1884*	*1886*	*1888*

Table 23: Heavy Chain Nucleic Acid Sequence Identifiers

Antibody CD3-VH Designation	**SEQ ID NOs:**			
	HCVR	**CDR1**	**CDR2**	**CDR3**
CD3-VH-G	*1729*	*1731*	*1733*	*1735*
CD3-VH-G2	*1737*	*1739*	*1741*	*1743*
CD3-VH-G3	*1745*	*1747*	*1749*	*1751*
CD3-VH-G4	*1753*	*1755*	*1757*	*1759*
CD3-VH-G5	*1761*	*1763*	*1765*	*1767*
CD3-VH-G8	*1769*	*1771*	*1773*	*1775*
CD3-VH-G9	*1777*	*1779*	*1781*	*1783*
CD3-VH-G10	*1785*	*1787*	*1789*	*1791*

Antibody CD3-VH Designation	SEQ ID NOs:			
	HCVR	CDR1	CDR2	CDR3
CD3-VH-G11	*1793*	*1795*	*1797*	*1799*
CD3-VH-G12	*1801*	*1803*	*1805*	*1807*
CD3-VH-G13	*1809*	*1811*	*1813*	*1815*
CD3-VH-G14	*1817*	*1819*	*1821*	*1823*
CD3-VH-G15	*1825*	*1827*	*1829*	*1831*
CD3-VH-G16	*1833*	*1835*	*1837*	*1839*
CD3-VH-G17	*1841*	*1843*	*1845*	*1847*
CD3-VH-G18	*1849*	*1851*	*1853*	*1855*
CD3-VH-G19	*1857*	*1859*	*1861*	*1863*
CD3-VH-G20	*1865*	*1867*	*1869*	*1871*
CD3-VH-G21	*1873*	*1875*	*1877*	*1879*
CD3-VH-P	*1881*	*1883*	*1885*	*1887*

Table 24: Light Chain Amino Acid Sequence Identifiers

Antibody Designation	SEQ ID NOs:			
	LCVR	CDR1	CDR2	CDR3
VK1-39JK5	1890	1892	1894	1896

Table 25: Light Chain Nucleic Acid Sequence Identifiers

Antibody Designation	SEQ ID NOs:			
	LCVR	CDR1	CDR2	CDR3
VK1-39JK5	1889	1891	1893	1895

[0286] 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).

[0287] Isotype Control Antibody, referred to in the Examples herein, is an isotype matched (modified IgG4) antibody that interacts with an irrelevant antigen, i.e. FelD1 antigen.

Example 13: In vitro and in vivo studies on Human Monoclonal Anti-CD3 Antibodies

[0288] In vivo and in vitro studies on human monoclonal anti-CD3 antibodies were done as described in US publication 2014/0088295 published March 27, 2014, and PCT/US2016/044732 filed July 29, 2016.

[0289] Some human monoclonal anti-CD3 antibodies bind soluble heterodimeric CD3 protein, in either antibody-capture or antigen-capture formats, with high affinity. Soluble heterodimeric CD3 protein (hCD3-epsilon/hCD3-delta; SEQ ID NOs:1900/1901) was prepared with either a human Fc tag (hFcΔAdp/hFc; SEQ ID NOs:1931/1932) or a mouse Fc tag (mFcΔAdp/mFc; SEQ ID NOs:1933/1934). Heterodimeric CD3 protein was purified using the method described in Davis et al. (US2010/0331527).

[0290] Some human monoclonal anti-CD3 antibodies bound human T-cells and induced T-cell proliferation. Some human monoclonal anti-CD3 antibodies bound CD2+CD4+ monkey T-cells and induced their proliferation. Some human monoclonal anti-CD3 antibodies supported redirected T-cell mediated killing via Fc/FcR interaction in a calcein based U937 killing assay. The observed killing, believed to be dependent on the antibody's Fc engagement with the Fc Receptor on U937 cells leading to clustering of CD3 on adjacent T-cells, was squelched by addition of non-specific human IgG (data not shown). A wide range of bispecific antibodies constructed with anti-CD3 arm variants described herein (particularly anti-CD3 arms based on the CD3-VH-P heavy chain derived from IGHV3-9*01, IGHJ6*02, IGHD5-12*01) activate human PBMC cells, and monkey PBMCs, and display cytotoxic activity on tumor antigen-expressing cell lines.

Reference Example 3: Screening and Identification of anti-MUC16 Monoclonal Antibodies Suitable for Immunohistochemistry (IHC) on Formalin Fixed Paraffin Embedded (FFPE) Samples.

[0291] Human tumor cell lines with known levels of expression of MUC16 were identified, fixed in 10% Neutral Buffered Formalin and embedded in paraffin. These lines were used to screen various anti-MUC16 antibodies to identify candidates for IHC studies.

[0292] Cell lines included the following MUC16 Negative cell lines:

HT29 (Colon), and PC3/ATCC parental (Prostate); Pancreatic cell lines with low to no levels of MUC16: Capan1 (Pancreatic adenocarcinoma), HPAC (Pancreatic adenocarcinoma).

Endogenously MUC16-expressing cell lines include: OVCAR3 (Ovarian) and PEO-1 (Serous ovarian carcinoma).

[0293] Transfected cell lines were engineered as follows: PC3/ATCC (parental Prostate cancer line) cells were transfected to generate PC3/MUC16 "short" and PC3/MUC16 "high" cell lines. Both constructs include the C-terminal domain of MUC16 from amino acids 13,810-14,507 (of SEQ ID NO: 1899), and this includes part of the SEA12 domain, SEA13, SEA14, SEA15, SEA16, the C-terminal non-SEA region, the transmembrane region and the cytoplasmic domain. In addition, the PC3/MUC high cells have additional N-terminal amino acids 12783-13467 (of SEQ ID NO: 1899) which include SEA5 (partial) through SEA9 and a short linker between the SEA9 domain and the start of the MUC16 short construct. This allows for differentiation between anti-MUC16 antibodies that bind in the repeat region and those that bind to the "nub" portion of MUC16 adjacent to the membrane following enzymatic cleavage and release of the repeat regions (analogous to the CA125 portion of MUC16).

[0294] All staining was performed on the Ventana Discovery XT autostainer using standard protocols. Cell pellets were deparaffinized, Heat Induced Epitope Retrieval was optimized, endogenous Biotin was blocked and Protein blocking was performed. The antibodies were applied manually at an initial concentration of 10μg/ml and were also titrated down to ensure the specificity of the signal. Comparison was made against a commercially available anti-MUC16 antibody (OC-125), which is specific for the repeat regions of CA-125 (Roche, Ventana Catalog# 760-2610), and a negative control (absence of primary antibody). Detection was with Donkey anti-mouse-Biotin followed by Streptavidin-Horseradish peroxidase. The conversion of the substrate, Di-amino Benzidine (DAB) was observed as brown staining. Samples were counterstained with hematoxylin to visualize the nuclei. The results of the staining experiments are presented in Table 26, below.

Table 26: Binding of anti-MUC16 Antibodies to MUC16-negative Cells, and Cells Expressing MUC16 or Membrane-Proximal Portions of MUC16

Cell line:	HT29	Capan1	HPAC	OVCAR3	PEO-1	PC3/ATCC parental	PC3/MUC16 Short	PC3/MUC16 High
H1M7130N	-	-	-	+++	++	-	+++	+++
H2aM7128N	-	-	+	+++	++	-	+++	+++
H2aM7131N	-	-	-	+++	++	-	+++	+++
H2aM7133N	-	-	-	+++	++	-	-	-
H2aM7138N	-	-	+	+++	++	-	+++	+++
H1M9519N	-	NT	NT	+++	+++	NT	-	+++
H3M9525N	-	NT	NT	-	-	NT	-	-
OC-125	-	-	+	+++	++	-	-	+++
Neg. Control	-	-	-	-	-	-	-	-
NT - not tested								

[0295] Four of the tested antibodies (H1M7130N, H2aM7128N, H2aM7131N and H2aM7138N) showed positive binding to cells expressing the "nub" portion of MUC16 without the repeat regions (PC3/MUC16 short). These antibodies are identified as "nub binders." One of the tested antibodies (H1M9519N) showed positive binding to cells expressing the repeat regions of MUC16 (PC3/MUC16 high), but negative binding to cells expressing only the "nub" portion of MUC16 (PC3/MUC16 short). This antibody is identified as a "repeat binder," similar to the commercially available OC-125 antibody. It is expected that the tested antibodies bind tissues or cells of different origin having the expressed proteins and/or protein fragments as described herein.

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PATENTKRAV

1. Bispecifikt antistof eller antigenbindingsfragment deraf omfattende et første antigenbindingsdomæne, der specifikt binder human CD3, og et andet antigenbindingsdomæne, der specifikt binder human MUC16, hvor det andet antigenbindingsdomæne omfatter henholdsvis HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3-domæner, omfattende aminosyresekvenserne ifølge SEQ ID NO: 20-22-24-28-30-32.

2. Bispecifikt antistof eller antigenbindingsfragment ifølge krav 1, der omfatter et variabelt område af tungkæden omfattende aminosyresekvensen ifølge SEQ ID NO: 18, og et variabelt område af letkæden omfattende aminosyresekvensen ifølge SEQ ID NO: 26.

3. Bispecifikt antistof eller antigenbindingsfragment ifølge krav 1 eller 2, hvor det første antigenbindingsdomæne, der specifikt binder human CD3, omfatter henholdsvis HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3-domæner, omfattende aminosyresekvenser valgt fra gruppen bestående af SEQ ID NO: 1732-1734-1736-28-30-32, 1764-1766-1768-28-30-32, 1780-1782-1784-28-30-32, 1788-1790-1792-28-30-32 og 1868-1870-1872-28-30-32.

4. Bispecifikt antistof eller antigenbindingsfragment ifølge krav 3, hvor det første antigenbindingsdomæne der binder human CD3, omfatter et variabelt område af tungkæden (HCVR) omfattende en aminosyresekvens valgt fra gruppen bestående af SEQ ID NO: 1730, 1762, 1778, 1786 og 1866 og et variabelt område af letkæden (LCVR) omfattende aminosyresekvensen ifølge SEQ ID NO: 26.

5. Bispecifikt antistof eller antigenbindingsfragment ifølge krav 3, hvor det første antigenbindingsdomæne omfatter henholdsvis HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3-domæner, omfattende aminosyresekvenserne ifølge SEQ ID NO: 1732-1734-1736-28-30-32 og det andet antigenbindingsdomæne omfatter henholdsvis HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3-domæner, omfattende aminosyresekvenserne ifølge SEQ ID NO: 20-22-24-28-30-32.

6. Bispecifikt antistof eller antigenbindingsfragment ifølge krav 3, hvor det første antigenbindingsdomæne omfatter henholdsvis HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3-domæner, omfattende aminosyresekvenserne ifølge SEQ

ID NO: 1868-1870-1872-28-30-32 og det andet antigenbindingsdomæne omfatter henholdsvis HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3-domæner, omfattende aminosyresekvenserne ifølge SEQ ID NO: 20-22-24-28-30-32.

7. Bispecifikt antistof ifølge krav 5, hvor det første antigenbindingsdomæne omfatter et HCVR omfattende aminosyresekvensen ifølge SEQ ID NO: 1730 og et LCVR omfattende aminosyresekvensen ifølge SEQ ID NO: 26, og hvor det andet antigenbindingsdomæne omfatter et HCVR omfattende aminosyresekvensen ifølge SEQ ID NO: 18 og et LCVR omfattende aminosyresekvensen ifølge SEQ ID NO: 26.

8. Bispecifikt antistof ifølge krav 6, hvor det første antigenbindingsdomæne omfatter et HCVR omfattende aminosyresekvensen ifølge SEQ ID NO: 1866 og et LCVR omfattende aminosyresekvensen ifølge SEQ ID NO: 26, og hvor det andet antigenbindingsdomæne omfatter et HCVR omfattende aminosyresekvensen ifølge SEQ ID NO: 18 og et LCVR omfattende aminosyresekvensen ifølge SEQ ID NO: 26.

9. Bispecifikt antistof ifølge krav 7, der omfatter en første bindingsarm omfattende en tungkæde omfattende aminosyresekvensen ifølge SEQ ID NO: 1961 og en letkæde omfattende aminosyresekvensen ifølge SEQ ID NO: 1960 og en anden bindingsarm omfattende en tungkæde omfattende aminosyresekvensen ifølge SEQ ID NO: 1959 og en letkæde omfattende aminosyresekvensen ifølge SEQ ID NO: 1960.

10. Bispecifikt antistof ifølge krav 8, der omfatter en første bindingsarm omfattende en tungkæde omfattende aminosyresekvensen ifølge SEQ ID NO: 1962 og en letkæde omfattende aminosyresekvensen ifølge SEQ ID NO: 1960 og en anden bindingsarm omfattende en tungkæde omfattende aminosyresekvensen ifølge SEQ ID NO: 1959 og en letkæde omfattende aminosyresekvensen ifølge SEQ ID NO: 1960.

11. Farmaceutisk sammensætning, der omfatter det bispecifikke antistof eller antigenbindingsfragmentet ifølge et hvilket som helst af kravene 1-10, og en farmaceutisk acceptabel bærer eller fortynder.

12. Bispecifikt antistof eller antigenbindingsfragment ifølge et hvilket som helst af kravene 1-10, eller farmaceutiske sammensætning ifølge krav 11 til anvendelse i en fremgangsmåde til behandling af en MUC16-udtrykkende cancer.

13. Bispecifikt antistof eller antigenbindingsfragment, eller farmaceutisk sammensætning, til anvendelse ifølge krav 12, hvor canceren er valgt fra gruppen bestående af ovariecancer, brystcancer, pancreascancer, ikke-småcellet lungecancer, intrahepatisk cholangiokarcinommassedannende type, adenokarcinom i livmoderhals og adenokarcinom i mave-tarmkanalen.

14. Bispecifikt antistof eller antigenbindingsfragment, eller farmaceutisk sammensætning, til anvendelse ifølge krav 12, hvor canceren er ovariecancer.

DRAWINGS

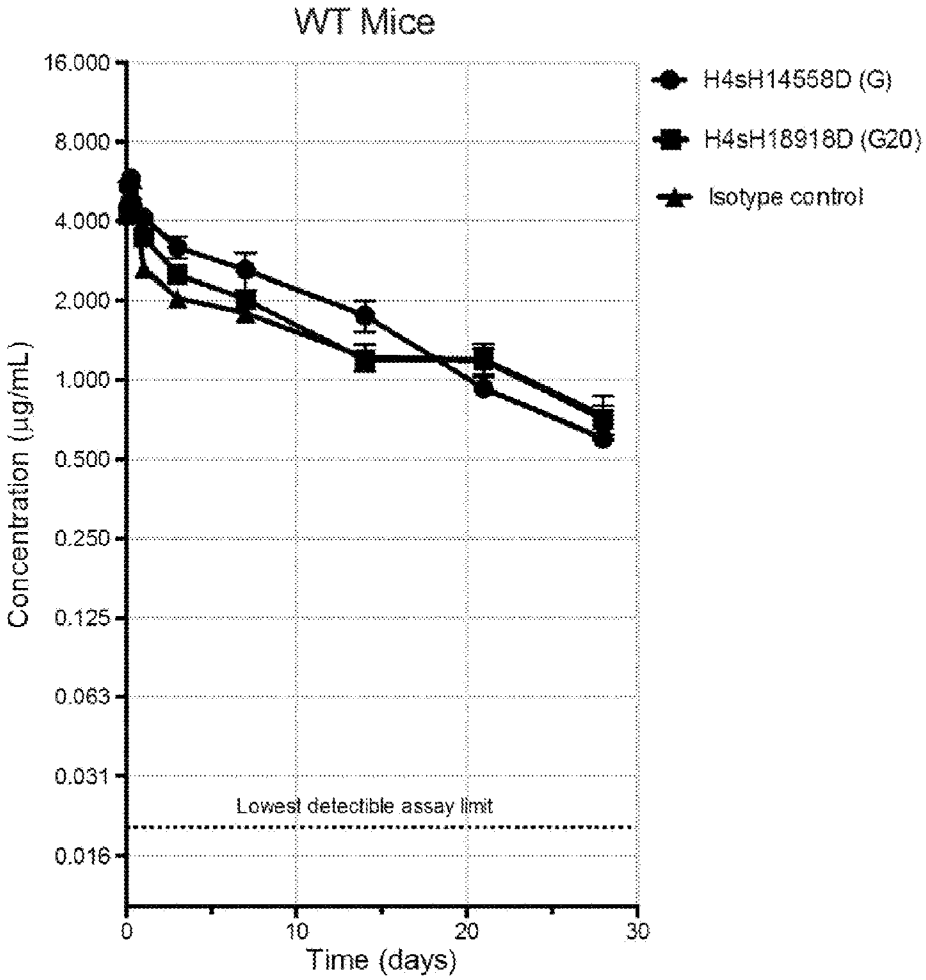

FIG. 1

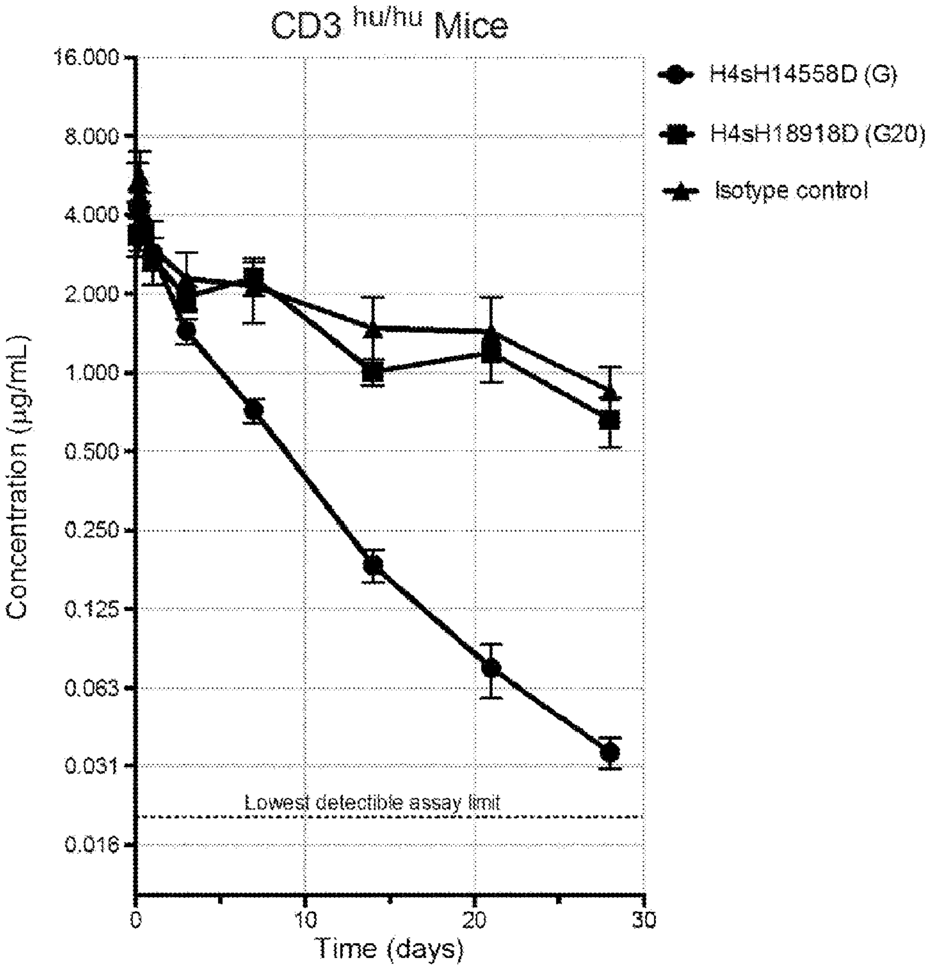
CD3 hu/hu Mice
Concentration (µg/mL)
16.000
8.000
4.000
2.000
1.000
0.500
0.250
0.125
0.063
0.031
0.016
Lowest detectible assay limit
0
10
20
30
Time (days)
H4sH14558D (G)
H4sH18918D (G20)
Isotype control

FIG. 2

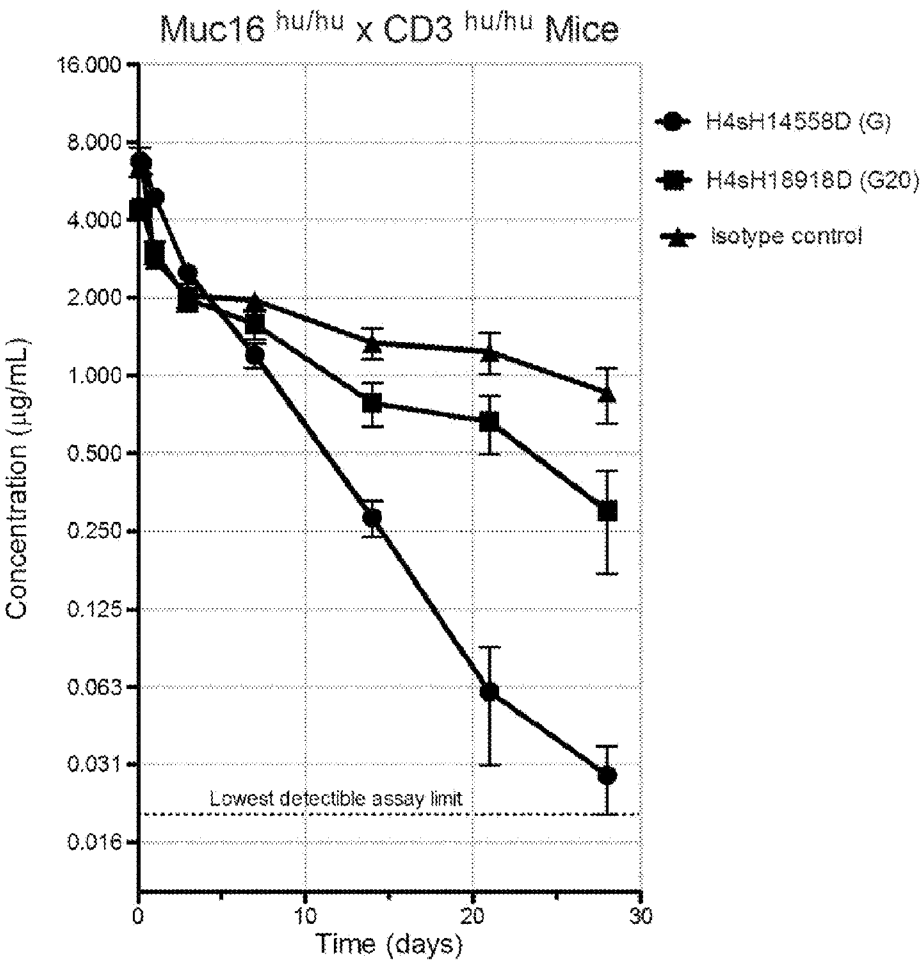
Muc16 hu/hu x CD3 hu/hu Mice
16.000
8.000
4.000
2.000
1.000
0.500
0.250
0.125
0.063
0.031
0.016
Concentration (µg/mL)
0
10
20
30
Time (days)
Lowest detectible assay limit
H4sH14558D (G)
H4sH18918D (G20)
Isotype control

FIG. 3

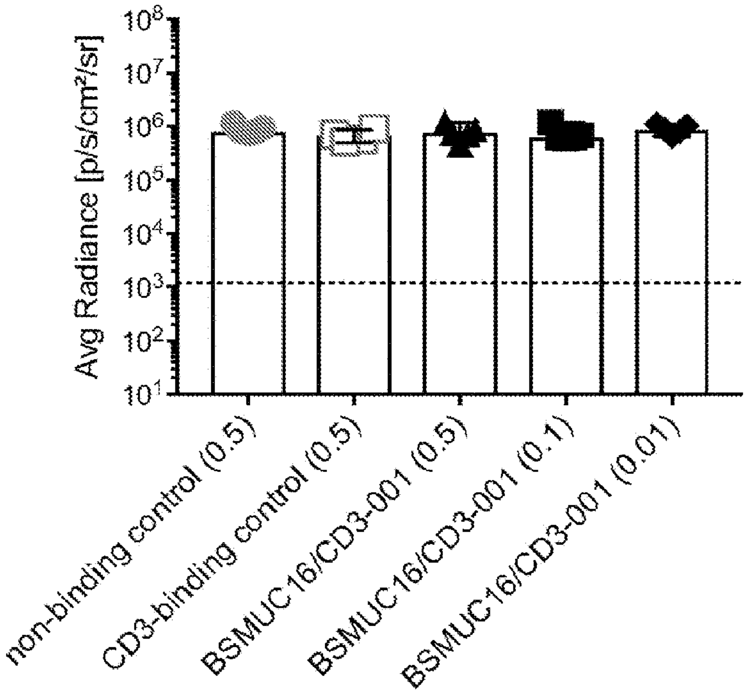
Avg Radiance [p/s/cm²/sr]
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
non-binding control (0.5)
CD3-binding control (0.5)
BSMUC16/CD3-001 (0.5)
BSMUC16/CD3-001 (0.1)
BSMUC16/CD3-001 (0.01)

FIG. 4

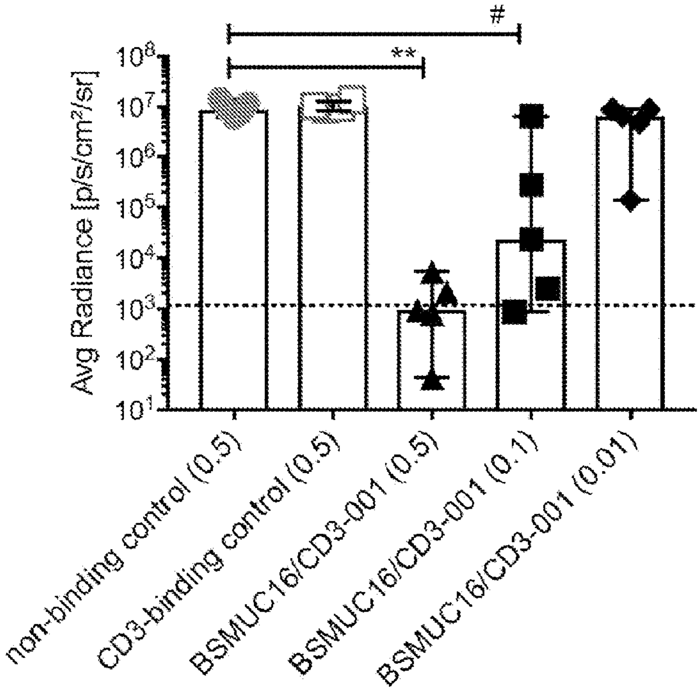
#
**
Avg Radiance [p/s/cm²/sr]
10⁸
10⁷
10⁶
10⁵
10⁴
10³
10²
10¹
non-binding control (0.5)
CD3-binding control (0.5)
BSMUC16/CD3-001 (0.5)
BSMUC16/CD3-001 (0.1)
BSMUC16/CD3-001 (0.01)

FIG. 5

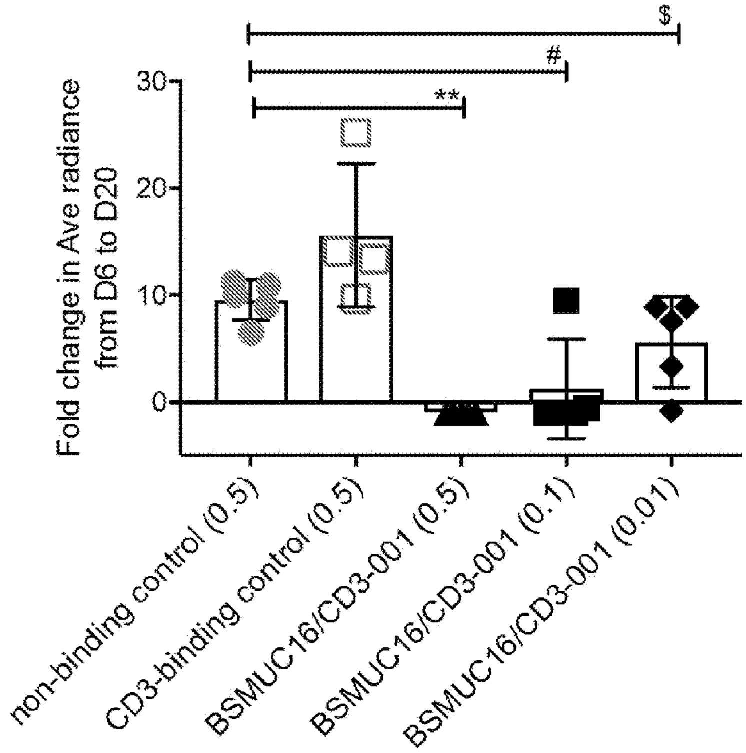
Fold change in Ave radiance
from D6 to D20
30
20
10
0
$
#
**
non-binding control (0.5)
CD3-binding control (0.5)
BSMUC16/CD3-001 (0.5)
BSMUC16/CD3-001 (0.1)
BSMUC16/CD3-001 (0.01)

FIG. 6

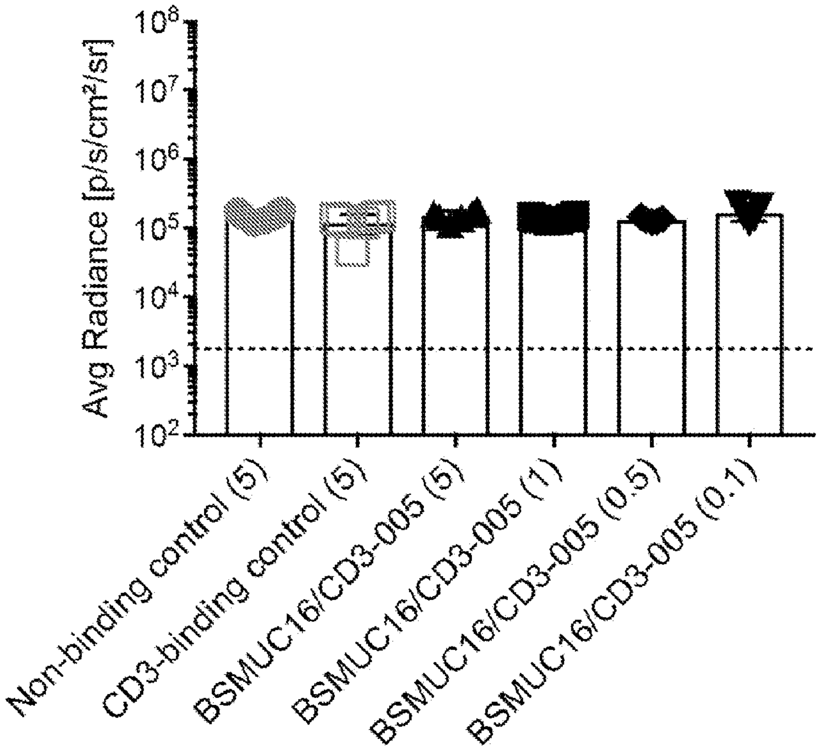
Avg Radiance [p/s/cm²/sr]
10^8
10^7
10^6
10^5
10^4
10^3
10^2
Non-binding control (5)
CD3-binding control (5)
BSMUC16/CD3-005 (5)
BSMUC16/CD3-005 (1)
BSMUC16/CD3-005 (0.5)
BSMUC16/CD3-005 (0.1)

FIG. 7

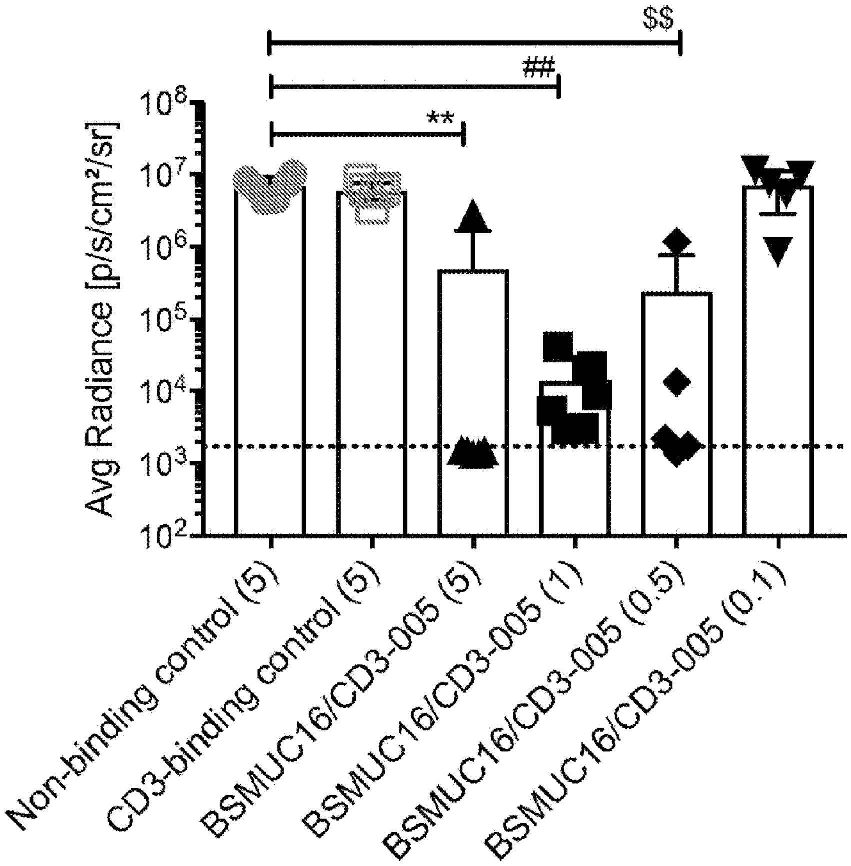
$$
**
##
Avg Radiance [p/s/cm²/sr]
10⁸
10⁷
10⁶
10⁵
10⁴
10³
10²
Non-binding control (5)
CD3-binding control (5)
BSMUC16/CD3-005 (5)
BSMUC16/CD3-005 (1)
BSMUC16/CD3-005 (0.5)
BSMUC16/CD3-005 (0.1)

FIG. 8

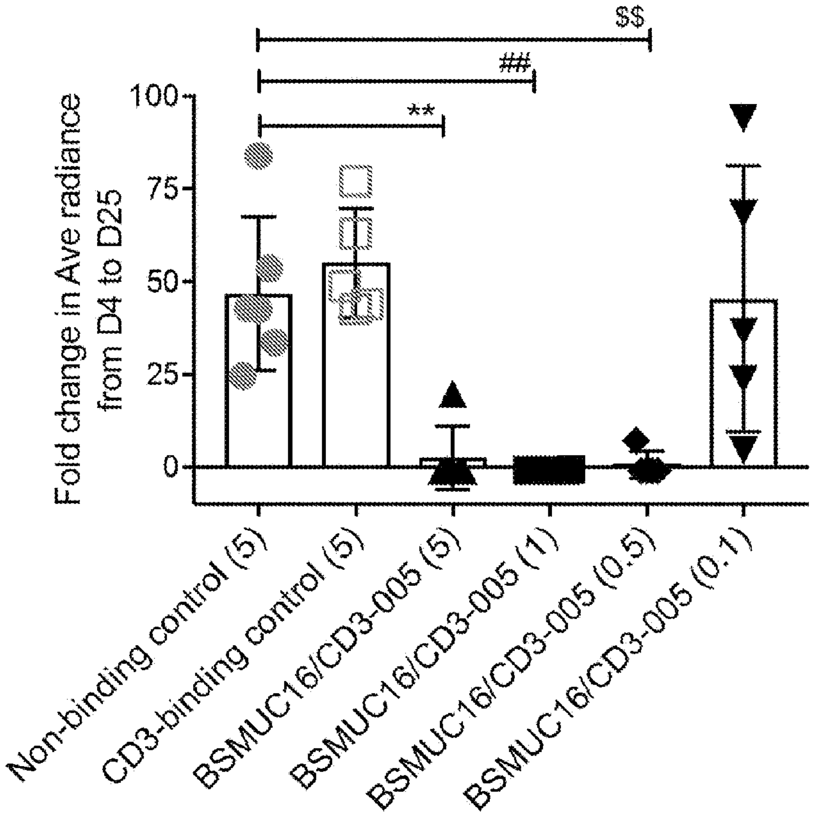
Fold change in Ave radiance from D4 to D25
100
75
50
25
0
$$
##
**
Non-binding control (5)
CD3-binding control (5)
BSMUC16/CD3-005 (5)
BSMUC16/CD3-005 (1)
BSMUC16/CD3-005 (0.5)
BSMUC16/CD3-005 (0.1)

FIG. 9

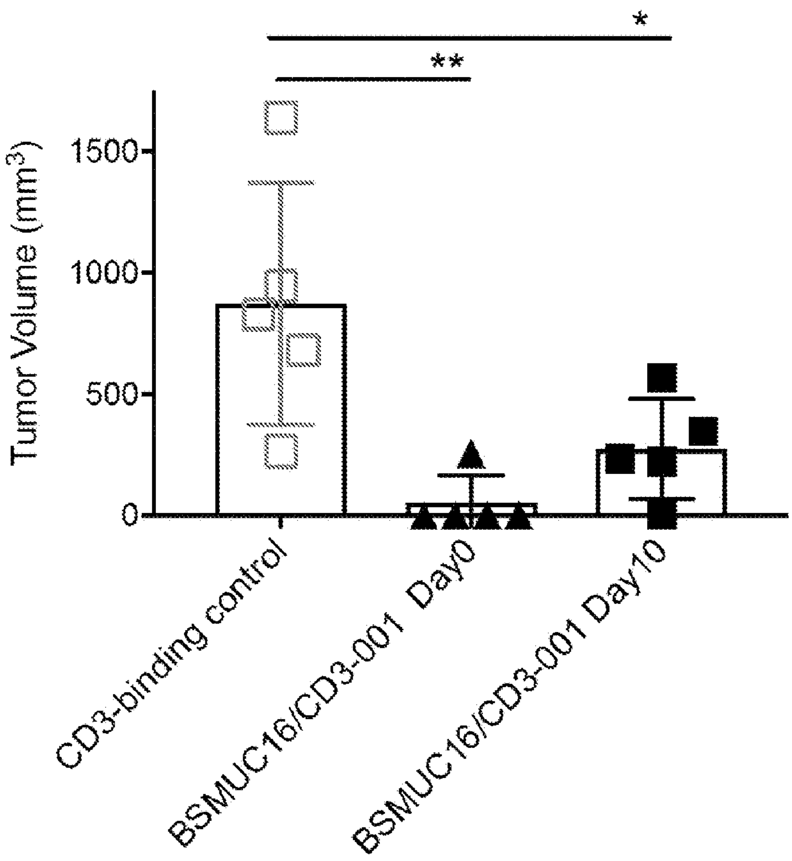
*
**
Tumor Volume (mm^3)
1500
1000
500
0
CD3-binding control
BSMUC16/CD3-001 Day0
BSMUC16/CD3-001 Day10

FIG. 10

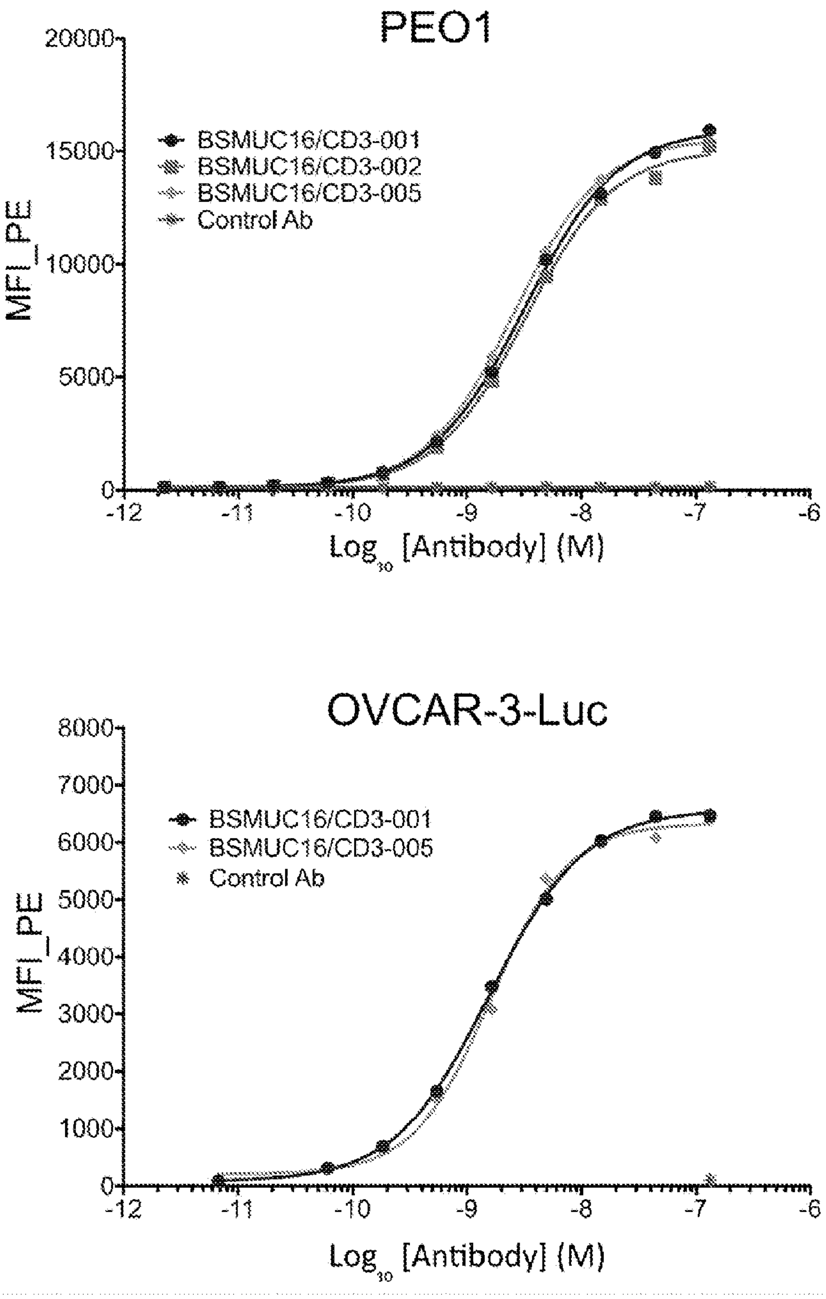
PEO1
BSMUC16/CD3-001
BSMUC16/CD3-002
BSMUC16/CD3-005
Control Ab
MFI_PE
20000
15000
10000
5000
0
-12
-11
-10
-9
-8
-7
-6
Log$_{10}$ [Antibody] (M)
OVCAR-3-Luc
BSMUC16/CD3-001
BSMUC16/CD3-005
Control Ab
MFI_PE
8000
7000
6000
5000
4000
3000
2000
1000
0
-12
-11
-10
-9
-8
-7
-6
Log$_{10}$ [Antibody] (M)

FIG. 11A

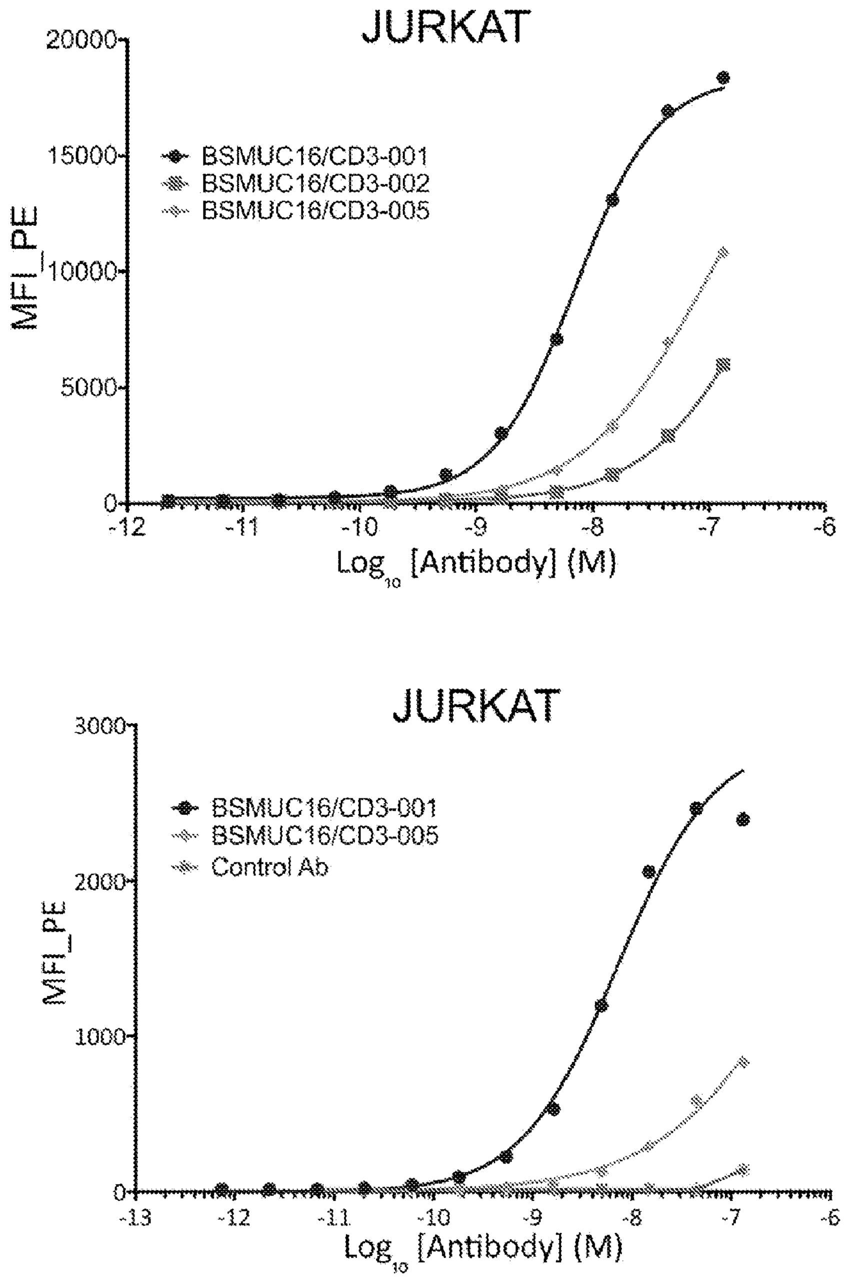
JURKAT
20000
15000
10000
5000
0
MFI_PE
BSMUC16/CD3-001
BSMUC16/CD3-002
BSMUC16/CD3-005
-12
-11
-10
-9
-8
-7
-6
Log$_{10}$ [Antibody] (M)
JURKAT
3000
2000
1000
0
MFI_PE
BSMUC16/CD3-001
BSMUC16/CD3-005
Control Ab
-13
-12
-11
-10
-9
-8
-7
-6
Log$_{10}$ [Antibody] (M)

FIG. 11B

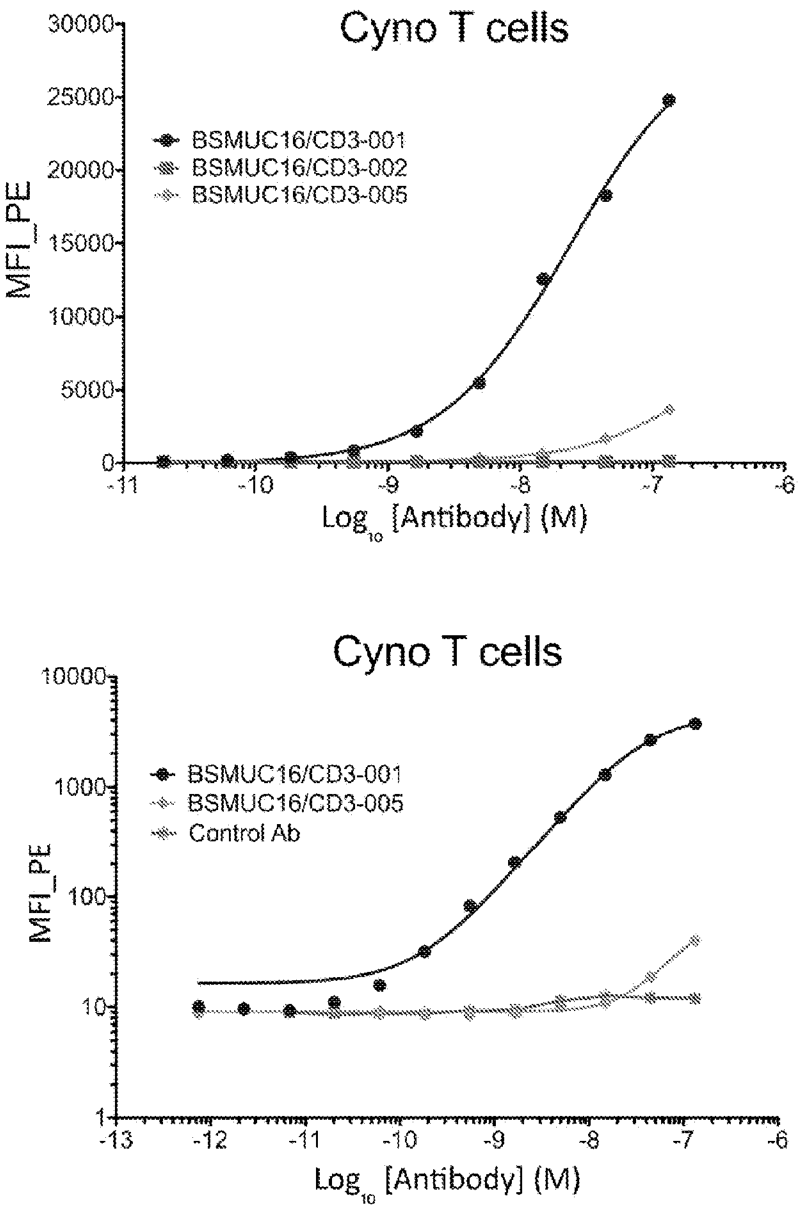
Cyno T cells
MFI_PE
30000
25000
20000
15000
10000
5000
0
BSMUC16/CD3-001
BSMUC16/CD3-002
BSMUC16/CD3-005
-11
-10
-9
-8
-7
-6
Log10 [Antibody] (M)
Cyno T cells
MFI_PE
10000
1000
100
10
1
BSMUC16/CD3-001
BSMUC16/CD3-005
Control Ab
-13
-12
-11
-10
-9
-8
-7
-6
Log10 [Antibody] (M)

FIG. 11C

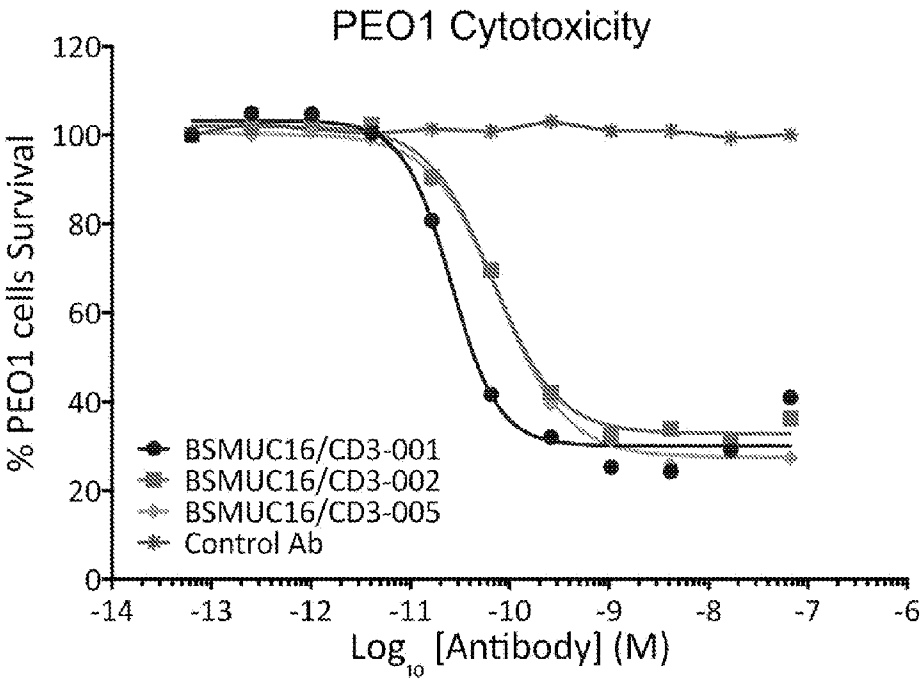
PEO1 Cytotoxicity
% PEO1 cells Survival
120
100
80
60
40
20
0
-14
-13
-12
-11
-10
-9
-8
-7
-6
Log$_{10}$ [Antibody] (M)
BSMUC16/CD3-001
BSMUC16/CD3-002
BSMUC16/CD3-005
Control Ab

FIG. 12A

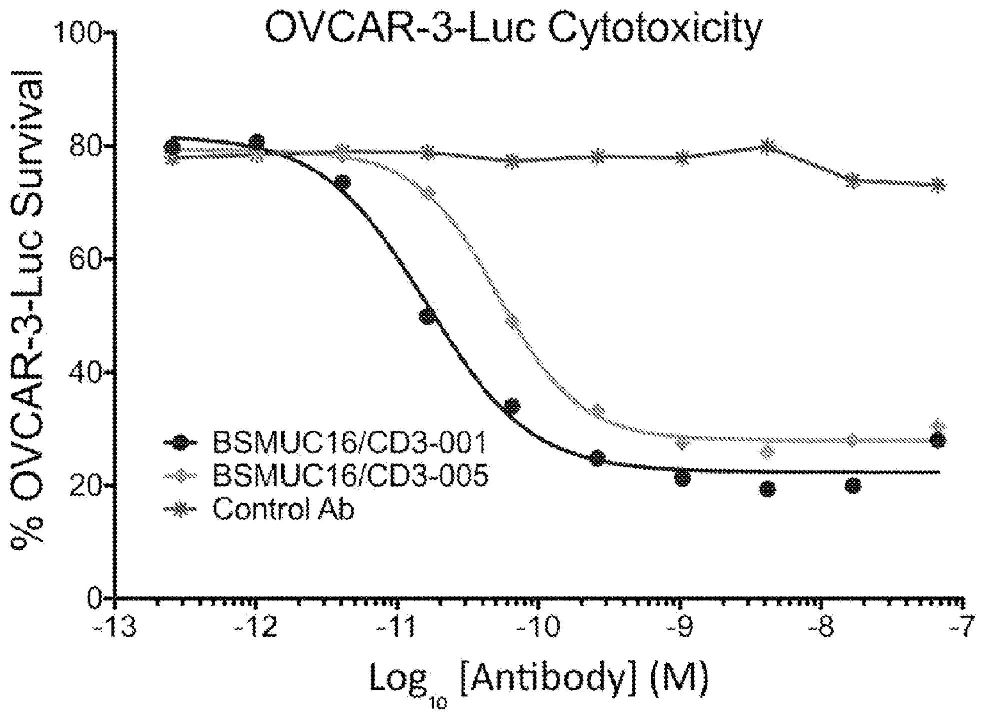
OVCAR-3-Luc Cytotoxicity
% OVCAR-3-Luc Survival
100
80
60
40
20
0
-13
-12
-11
-10
-9
-8
-7
Log10 [Antibody] (M)
BSMUC16/CD3-001
BSMUC16/CD3-005
Control Ab

FIG. 12B

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.