Muc16/NaPi-2b Antibodies and Methods of Use

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0001] None.

REFERENCE TO RELATED APPLICATIONS

[0002] This application claim priority to U.S. Provisional Application No. 63/566,201 filed on March 15, 2024 and U.S. Provisional Application No.63/724,854 filed on November 25, 2024, the contents of each of which are incorporated herein by reference in their entireties.

SEQUENCE LISTING

[0003] This application contains a Sequence Listing which has been submitted in XML format via Patent Center and is hereby incorporated by reference in its entirety. Said XML copy, created on March 13, 2025, is named 059926-301001WO.xml and is 160 KB in size.

BACKGROUND

[0004] Antibody-drug conjugates (ADCs) represent a significant advance in cancer therapy. ADCs incorporate the specificity of monoclonal antibodies with the potency of cytotoxic drugs. By targeting cancer cells with high precision, ADCs reduce harm to healthy tissues. In an ADC, the monoclonal antibody is designed to bind to a specific antigen that is expressed on the surface of cancer cells. Once bound, the ADC is internalized into the cancer cell, where the cytotoxic agent is released to induce cell death. This targeted delivery allows for the use of highly potent drugs that might be too toxic if delivered systemically. ADCs have shown promise in treating various cancers, including breast, lung, and blood cancers. They represent a personalized and targeted cancer therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] The accompanying drawings, which are incorporated herein and form a part of the specification, illustrate exemplary embodiments and, together with the description, further serve to enable a person skilled in the pertinent art to make and use these embodiments and others that will be apparent to those skilled in the art. The invention will be more particularly described in conjunction with the following drawings wherein:

[0006] **FIGs. 1A-1C** show bi-valent formats for a bi-specific antibody. **Fig. 1A**: An asymmetric (Fab)2-Fc (IgG) antibody. **Fig. 1B**: An asymmetric Fab-scFv-Fc antibody. **Fig. 1C**: An asymmetric Diabody-Fc antibody (1C). If east-west epitope binding of paratopes are

mirror images, the antibody is symmetric, and if not, the antibody is asymmetric. Symmetry herein refers to “east-west” symmetry.

[0007] **FIG. 2** shows a tri-valent format for an antibody. This includes an asymmetric Fab1-Diabody-Fc antibody. The arrangement of Fab comprising a NaPi-2b epitope binder connected to a NaPi-2b binding portion of a diabody, and Muc16 binding epitope in the other part of the diabody is exemplary; the epitope binding sites can be placed in any arrangement. Note: Asymmetry herein refers to “east-west” asymmetry.

[0008] **FIGs. 3A-3J** show ten different formats for tetravalent antibodies. The positions of the epitopes binders of NaPi-2b and Muc16 are exemplary. The antibodies can be engineered, e.g., with common light chain variable regions, such that the paratopes directed to specific epitopes can be placed in any arrangement. All have east-west symmetry, except for **Fig. 3D** and **FIG. 3J**. **Fig. 3A**: Fab-Diabody-Fc antibody **Fig. 3B**: Diabody-Fc-Fab antibody **Fig. 3C**: Fab-Fc-scFv antibody. **Fig. 3D**: Fab/scFv-Fc-Fab/scFv antibody. **Fig. 3E**: Diabody-Fc-Diabody antibody. **Fig. 3F**: Diabody-Fc-scFv antibody. **Fig. 3G**: scFv-Fc-Diabody antibody. Fig. 3H: scFv-Fc-scFv antibody. **Fig. 3I**: Fab-Fc-Diabody antibody. **Fig. 3J**: scFv/Fab-Fc-Fab/scFv antibody. Note that if east-west epitope binding of paratopes are mirror images, the antibody is symmetric, and if not, the antibody is asymmetric.

[0009] **FIGs. 4A-4D** show four different configurations for paratopes in a bi-specific, tetravalent format, in this case, Fab-Diabody-Fc format. 4A: Symmetric antibody in which the paratope binding Epitope 1 is in the NW/NE positions, the paratope binding Epitope 2 is in the SW/SE positions. 4B: Symmetric antibody in which the paratope binding Epitope 2 is in the NW/NE positions and the paratope binding Epitope 1 is in the SW/SE positions. 4C: Asymmetric antibody in which the paratope binding Epitope 1 is in the NW/SE positions, and the paratope binding Epitope 2 is in the SW/NE positions. 4D: Asymmetric antibody in which the paratope binding epitope 1 is in the NW/SW positions, and the paratope binding epitope 2 is in the NE/SE positions.

[00010] **FIGs. 5A-5D** show four different configurations for epitope-binding paratopes in a bi-specific, tetravalent format, in this case, Diabody-Fc-Fab format. 5A: Symmetric antibody in which Epitope 1 is in the NW/NE positions, epitope 2 is in the SW/SE positions. 5B: Symmetric antibody in which Epitope 2 is in the NW/NE positions and epitope 1 is in the SW/SE positions. 5C: Asymmetric antibody in which Epitope 1 is in the NW/SE positions, and epitope 2 is in the SW/NE positions. 5D: Asymmetric antibody in which Epitope 1 is in the NW/SW positions, and epitope 2 is in the NE/SE positions.

[00011] **Figs. 6A** and **6B** show two monospecific ADCs that are homogeneous as analyzed by size exclusion chromatography (SEC) analysis. Antibodies in standard IgG

format specifically bind NaPi-2b (AB301) (Fig. 6A) and Muc16 (Fig. 6B) (AB302). Hexagons represent payload molecules conjugated via a linker, such as MC-vc-PAB linker-payloads. DAR = Drug Antibody Ratio. MMAE = monomethyl auristatin E.

[00012] **Figs. 7A-7C** show analysis of three bi-specific, tetravalent ADCs comprising binding sites for NaPi-2b and Muc16. Fig. 7A: Fab-Diabody-Fc format. Fig. 7B: Fab-Diabody-Fc format. Fig. 7C: Diabody-Fc-Fab format. The data shows that the ADCs assemble in a homogenous fashion. All ADCs exhibit uniform 4 DAR via cysteine-engineered site specific conjugation.

[00013] **Fig. 8** compares the effect of different ADCs on cell viability of OVCAR3 cells and provides inhibitory concentration 50% (IC50) values.

[00014] **Fig. 9** compares the effect of different ADCs on cell viability of OVCAR3 cells and provides IC50 values.

[00015] **Fig. 10** compares the effect of different ADCs on viability of engineered OVCAR3-AV cells. OVCAR3 cells are a cell line derived from a human ovarian carcinoma, OVCAR3-AV cells are mixtures of OVCAR3 wild type cells with OVCAR3 cells genetically modified to knock out Muc16 gene expression.

[00016] **Figs. 11A-11B** compare the effect of different ADCs on cell viability of Muc16 knock-out OVCAR3 cells (11A) and NaPi-2b knock-out OVCAR3 cells (11B) and provides a table with IC50 values and DAR.

[00017] **Figs. 12A-12D** show cell viability assays with varying ratio of OVCAR3 WT and Muc16-KO cells, which compare the effect of different ADCs on cell viability of cell mixtures. OVCAR3-AV1 cells comprise OVCAR3 Muc16 knockouts and OVCAR3 wild type in a ratio of 60:40. OVCAR3-AV2 cells comprise OVCAR3 Muc16 knockouts and OVCAR3 wild type in a ratio of 70:30. OVCAR3-AV3 cells comprise OVCAR3 Muc16 knockouts and OVCAR3 wild type in a ratio of 80:20. OVCAR3-AV4 cells comprise OVCAR3 Muc16 knockouts and OVCAR3 wild type in a ratio of 90:10. Accordingly, all the OVCAR3-AV cell mixtures have constant expression levels of NaPi-2b, and different levels of expression of Muc16. This mimics the expression of both targets to that of patient tumors. **12A**: 60% Muc16 knock-out, 40% OVCAR3 wild type. **12B**: 70% Muc16 knock-out, 30% OVCAR3 wild type. **12C**: 80% Muc16 knock-out, 20% OVCAR3 wild type. **12D**: 90% Muc16 knock-out, 10% OVCAR3 wild type. OVCAR3-AV cells have varying levels of Muc16 expression with NaPi-2b expression being constant to mimic the expression of both targets to that of patient tumors. Table shows IC50 values of different cell ratios.

[00018] **Fig. 13** compares the effect of different ADCs on cell viability of a mixture of OVCAR3-AV cells having varying different levels of expression of Muc16 and NaPi-2b (20% of the cells were expression positive for both target antigens while 60% were expression positive for only NaPi-2b, and 20% were expression positive for only Muc16).

[00019] **Fig. 14** shows manufacturability of various antibody formats. Fab-Diabody-Fc format and Diabody-Fc-Fab format demonstrate highest yields. Antibodies identified by hashtag have lowest manufacture yields.

[00020] **Fig. 15A-C** show *in vivo* efficacy of ADCs in the OVCAR3 ovarian xenograft tumor model using female CB.17 SCID mice: A. study design; Tumor growth curves (B) and Bodyweight changes (C) in mice upon treatment with ADCs. Tetravalent, bi-specific ADCs displayed superior *in vivo* efficacy compared to monospecific ADCs. No body weight changes were observed across all ADC dosing groups.

[00021] **Fig. 16** compares the effect of different ADCs on cell viability of OVCAR3 cells and provides inhibitory concentration 50% (IC50) values.

[00022] **Fig. 17A-D** show *in vivo* efficacy of ADCs (40 nmole dose comparison) in the OVCAR3 ovarian xenograft tumor model: A. study design; B. Tumor growth curves vs number of days; C. Tumor growth observed in various ADC treatment groups on day 32; D. p-values observed among treatment groups at day 32. All groups are dosed with 40 nmoles of ADC (single dose on Day1 when the tumor size was 100-150 mm^3).

[00023] **Fig. 18A-D** show *in vivo* efficacy of ADCs (AB301 vs AB309) in the OVCAR3 ovarian xenograft tumor model: A. study design; B. Tumor growth curves vs number of days; C. Tumor growth observed in various ADC treatment groups on day 32; D. p-values observed among treatment groups.

[00024] **Fig. 19A-D** show *in vivo* efficacy of ADCs (AB312 vs AB309) in the OVCAR3 ovarian xenograft tumor model: A. study design; B. Tumor growth curves vs number of days; C. Tumor growth observed in various ADC treatment groups on day 32; D. p-values observed among treatment groups.

[00025] **Fig. 20** compare bodyweight changes in mice upon treatment with ADCs.

[00026] **Fig. 21** show pharmacokinetic characterization of AB304 and AB309 antibodies in non-human primates.

[00027] **Fig. 22** compares the effect of AB304 and AB306 ADCs on cell viability of OVCAR3 cells and provides inhibitory concentration 50% (IC50) values.

[00028] **FIGs. 23A-C** shows *in vitro* toxicity of bi-specific tetravalent ADCs in OVCAR-3 wildtype and variant cells.

[00029] **FIGs. 24A-B** shows in vitro cytotoxicity of bi-specific tetravalent ADCs in models of tumor cell heterogeneity.

[00030] **FIG. 25** shows the internalization of Muc16/NaPi-2b bi-specific tetravalent antibodies evaluated by confocal microscopy.

[00031] **FIG. 26** shows the pharmacokinetic (PK) properties of the bi-specific tetravalent ADC AB309-ADC in non-human primate cynomolgus monkeys (n=3 females). Animals were administered a single dose of 3 mg/kg intravenously (IV bolus) and serum samples were collected for analysis of total antibody and total ADC concentrations while plasma samples were collected for free MMAE payload concentrations.

[00032] **FIG. 27** shows Biolayer Interferometry (BLI) analysis to confirm simultaneous binding of MUC16 and NaPi-2b antigens to AB304 and AB309 bi-specific tetravalent antibodies. Fig.27A-D where MUC16 antigen binding to AB304, AB309, AB301, AB302 was tested followed by simultaneous binding to NaPi-2b antigen. Fig. 27E-F was done in a reverse order where NaPi-2b antigen was tested followed by binding to MUC16.

SUMMARY

[00033] Provided herein are bi-specific antibodies that specifically bind to Muc16 and NaPi-2b. In certain embodiments, the antibodies are tetrameric antibodies having two paratopes that bind to Muc16 and two paratopes that bind to NaPi-2b. The paratopes can take a number of configurations, including, for example, two Fabs and a diabody. In other embodiments, the antibodies comprise two identical heavy chains and two identical light chains. The antibodies can be configured as immunoconjugates and, in particular, as antibody-drug conjugates comprising an antibody having a cytotoxic drug conjugated thereto. Such ADCs are useful for, among other things, the treatment of cancer, in particular, ovarian or endometrial cancer.

DETAILED DESCRIPTION

I. Introduction

[00034] Disclosed herein are bi-specific antibodies that specifically bind to Muc16 and NaPi-2b. As discussed herein, the antibodies can be bivalent, trivalent, tetravalent, or otherwise multivalent. Antibodies described herein may bear names with an “AB” prefix. Such antibody names may be given as “AB302 antibody.” These names do not refer to antibody drug conjugates unless specified, such as “AB302-ADC.”

[00035] In the sequences provided herein, all NaPi-2b/Muc16 antibodies contain either the lifastuzumab or sofituzumab variable domains, including their Complementarity Determining Regions ("CDR") sequences. However, it is contemplated that these CDRs can be incorporated into other framework regions and retain their binding specificity.

[00036] Furthermore, variable regions and CDRs from other antibodies that bind Muc16 or NaPi-2b also can be used in preparing the antibodies of this disclosure.

A. Muc16

[00037] "Mucin-16, cell surface associated", also referred to as Muc16 and CA125, is a member of the mucin family of proteins. These are large glycoproteins which have a role in forming mucus, which serves as a protective barrier in epithelial tissues. Muc16 is a membrane-associated mucin that possesses a single transmembrane domain. Muc16 is primarily found in the epithelial cells of the respiratory, digestive, and reproductive tracts.

[00038] Aberrant overexpression of Muc16 has been observed in several human malignancies, including ovarian, pancreatic, breast, and lung cancers. Muc16 is also thought to participate in cell-to-cell interactions that enable the metastasis of tumor cells.

[00039] Elevated levels of Muc16 in blood serum are often used as a biomarker in the diagnosis and monitoring of certain types of cancers, particularly ovarian cancer. Elevated Muc16 expression is also associated with small cell cancer of the lung and lung oat cell carcinoma.

[00040] Muc16 has the UniProt accession number Q8WXI7. The protein is encoded by the Muc16 gene, which is located on chromosome 19p13.2.

[00041] Antibodies known to specifically bind Muc16 include, for example, sofituzumab. Antibodies that specifically bind Muc16 are also described in U.S. patents US RE 47194 (Genentech 11D10); 11,453,721, 10,759,869, 10,738,130, and 7,202,346. The CDRs of any of these antibodies can be used in the bi-specific antibodies of this disclosure.

[00042] Exemplary anti-Muc16 antibodies have CDR regions including the following sequences, taken from sofituzumab:

HCDR1: GYSITNDYAWN (SEQ ID NO:1)

HCDR2: YISYSGYTT (SEQ ID NO:2)

HCDR3: WTSGLDY (SEQ ID NO:3)

LCDR1: KASDLIHNWLA (SEQ ID NO:4)

LCDR2: GATSLET (SEQ ID NO:5)

LCDR3: QQYWTTPFT (SEQ ID NO:6)

[00043] Exemplary anti-Muc16 antibodies have VH and VL regions including the following sequences, taken from sofituzumab:

[00044] Heavy chain:

EVQLVESGGGLVQPGGSLRLSCAASGYSITNDYAWNWVRQAPGKGLEWVGYISYS GYTTYNPSLKSRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARWTSGLDYWGQGT LVTVSS (SEQ ID NO:36)

[00045] Light chain:

DIQMTQSPSSLSASVGDRVTITCKASDLIHNWLAWYQQKPGKAPKLLIYGATSLE TGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYWTTPFTFGQGTKVEIK (SEQ ID NO:37)

[00046] Other exemplary anti-Muc16 antibodies have CDR regions including the following sequences, taken from AB312:

HCDR1: GFNIKDTYMH (SEQ ID NO:48)

HCDR2: RVDPANGNTK (SEQ ID NO:49)

HCDR3: DYYGHTYGFAF (SEQ ID NO:50)

LCDR1: TASSSVSSSYLH (SEQ ID NO:51)

LCDR2: STSNLAS (SEQ ID NO:52)

LCDR3: HQYHRSPYT (SEQ ID NO:53)

[00047] Exemplary anti-Muc16 antibodies have VH and VL regions including the following sequences, taken from AB312: Positions of CDRs are indicated by **bold** and asterisk. Positions in which an existing amino acid was substituted with cysteine are indicated by **bold** and hashtag.

[00048] In some embodiments, the anti-MUC 16 antibody comprises a variable heavy (VH) region comprising heavy chain (HC) complementarity determining regions: HCDR1: GYSITNDYAWN (SEQ ID NO:1), HCDR2: YISYSGYTT (SEQ ID NO:2), and HCDR3: WTSGLDY (SEQ ID NO:3). In some embodiments, the anti-MUC 16 antibody comprises a variable heavy (VH) region comprising heavy chain (HC) complementarity determining regions: HCDR1: GFNIKDTYMH (SEQ ID NO:48), HCDR2: RVDPANGNTK (SEQ ID NO:49), and HCDR3: DYYGHTYGFAF (SEQ ID NO:50). In some embodiments, the anti-MUC 16 antibody comprises a variable light (VL) region comprising light chain (LC) complementary determining regions: LCDR1: KASDLIHNWLA (SEQ ID NO:4), LCDR2:

GATSLET (SEQ ID NO:5), and [00038] LCDR3: QQYWTTPFT (SEQ ID NO:6). In some embodiments, the anti-MUC 16 antibody comprises a variable light (VL) region comprising light chain (LC) complementary determining regions: LCDR1: TASSSVSSSYLH (SEQ ID NO:51), LCDR2: STSNLAS (SEQ ID NO:52), and LCDR3: HQYHRSPYT (SEQ ID NO:53).

[00049] Heavy chain:

***HCDR1**

***HCDR2**

EVQLQQSGAELVKPGASVKLSCTAS**GFNIKDTYMH**WVKQRPEQGLEWIG**RVDPANGN**

TK

HCDR3

YDPKFQGKATLTADTSSNTAYLQLSSLTSEDTAVYFCVR**DYYGHTYGFAF**CDQGTTL

TV

#

SA**C**STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA

VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC

PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN

AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP

REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS

DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SEQ ID

NO:54)

[00050] Light chain:

HCDR1* *HCDR2*

DIVLTQSPAIMSASLGERVTMTC**TASSSVSSSYLH**WYQQKPGSSPKLWIY**STSNLAS**

GV

HCDR3

PGRFSGSGSGTSYSLTISSMEAEDAATYYC**HQYHRSPYT**FGGGTKVEIKRTVAAPSV

FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST

YSLS

#

STLTLSKADYEKHKVYACEVTHQGLSSP**C**TKSFNRGEC(SEQ ID NO:55)

1. AB302

[00051] An amino acid sequence of an antibody that binds Muc16 is provided here:

[00052] Anti-Muc16 monoclonal AB302. Variable regions are indicated by underline. Positions of CDRs are indicated by **bold** and asterisk. Cysteine substitutions are indicated by **bold** and hashtag.

[00053] Heavy chain:

HCDR1

***HCDR2*

EVQLVESGGGLVQPGGSLRLSCAAS**GYSITNDYAWN**WVRQAPGKGLEWVG**YISYSGYT**

TY

HCDR3 #

NPSLKSRFTISRDTSKNTLYLQMNSLRAEDTAVYYCAR**WTSGLDY**WGQGTLVTVSS**C**S

TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS

GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL

GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE

EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL

PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK

LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:7)

[00054] Heavy chain variable region:

*****HCDR1*****

*****HCDR2***

EVQLVESGGGLVQPGGSLRLSCAAS**GYSITNDYAWN**WVRQAPGKGLEWVG**YISYSGYT**

TY

HCDR3 #

NPSLKSRFTISRDTSKNTLYLQMNSLRAEDTAVYYCAR**WTSGLDY**WGQGTLVTVSS**C**

(SEQ ID NO:32)

[00055] Light chain:

LCDR1** *LCDR2*

DIQMTQSPSSLSASVGDRVTITC**KASDLIHNWLA**WYQQKPGKAPKLLIY**GATSLET**GV

PS

LCDR3

RFSGSGSGTDFTLTISSLQPEDFATYYC**QQYWTTPFT**FGQGTKVEIKRTVAAPSVFIF

PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS

STLT

#

LSKADYEKHKVYACEVTHQGLSSP**C**TKSFNRGEC (SEQ ID NO:8)

[00056] Light chain variable region:

LCDR1** *LCDR2*

DIQMTQSPSSLSASVGDRVTITC**KASDLIHNWLA**WYQQKPGKAPKLLIY**GATSLET**GVPS

LCDR3

RFSGSGSGTDFTLTISSLQPEDFATYYC**QQYWTTPFT**FGQGTKVEIK (SEQ ID NO:33)

B. NaPi-2b

[00057] "Sodium-dependent Phosphate Transport Protein 2B", also referred to as NaPi-2b, SLC34A2, NaPiIIb, and Npt2, is a member of the sodium/phosphate co-transporter family. It is a cell surface sodium-dependent phosphate transporter that regulates phosphate homeostasis. NaPi-2b is primarily expressed in the lung alveolar type II cells and the small intestine, where it facilitates the active transport of phosphate ions across cell membranes in conjunction with sodium ions.

[00058] NaPi-2b is a lineage marker that is expressed in 80-90% of epithelial ovarian cancers. It has been used as a target for therapeutic antibodies.

[00059] NaPi-2b has the UniProt accession number O95436. The protein is encoded by the SLC34A2 gene, which is located on chromosome 4p15.2.

[00060] Antibodies known to specifically bind NaPi-2b include, for example, lifastuzumab and Upifitamab (see, e.g., url drugs.ncats.io/substance/15OY7NA275). Antibodies that specifically bind NaPi-2b are also described in U.S. patent 11,407,825. The CDRs of any of these antibodies can be used in the bi-specific antibodies of this disclosure.

[00061] Exemplary anti-NaPi-2b antibodies have CDR regions including the following sequences, taken from lifastuzumab:

HCDR1: GFSFSDFAMS (SEQ ID NO:9)

HCDR2: TIGRVAFHTY (SEQ ID NO:10)

HCDR3: HRGFDVGHFDF (SEQ ID NO:11)

LCDR1: RSSETLVHSSGNTYLE (SEQ ID NO:12)

LCDR2: RVSNRFS (SEQ ID NO:13)

LCDR3: FQGSFNPLT (SEQ ID NO:14)

[00062] In some embodiments, the anti-NaPi-2b antibody comprises a variable heavy (VH) region comprising heavy chain (HC) complementarity determining regions: HCDR1: GFSFSDFAMS (SEQ ID NO:9), HCDR2: TIGRVAFHTY (SEQ ID NO:10), and HCDR3: HRGFDVGHFDF (SEQ ID NO:11). In some embodiments, the anti-NaPi-2b antibody comprises a variable light (VL) region comprising light chain (LC) complementary determining regions: LCDR1: RSSETLVHSSGNTYLE (SEQ ID NO:12), LCDR2: RVSNRFS (SEQ ID NO:13), and LCDR3: FQGSFNPLT (SEQ ID NO:14). In some embodiments, the anti-NaPi-2b antibody comprises a variable heavy (VH) region comprising an HCDR-1 selected from SEQ ID NOs: 60-63. In some embodiments, the anti-NaPi-2b antibody comprises a variable heavy (VH) region comprising an HCDR-2 selected from: SEQ ID NOs:64-78. In some embodiments, the anti-NaPi-2b antibody comprises a variable heavy (VH) region comprising an HCDR-3 selected from SEQ ID NOs: 80-83. In some embodiments, the anti-NaPi-2b antibody comprises a variable light (VL) region comprising an LCDR-1 selected from SEQ ID NOs: 84-97. In some embodiments, the anti-NaPi-2b antibody comprises a variable light (VL) region comprising an LCDR-2 selected from : SEQ ID NOs:98-101. In some embodiments, the anti-NaPi-2b antibody comprises a variable light (VH) region comprising an LCDR-3 selected from SEQ ID NOs: 102-104.

[00063] Further exemplary anti-NaPi-2b antibodies have CDR regions and flanking sequences including the following variant sequences, taken from U.S. Patent 8,871,911:

HCDR1:

GFTFSSYAMS (SEQ ID NO:60)

GFSFSDFAMS (SEQ ID NO:61)

SSSFSDFALS (SEQ ID NO:62)

GFNFRGFAMS (SEQ ID NO:63)

HCDR2:

SVISGDGGSTYYADSVKG (SEQ ID NO:64)

ATIGRVASHTYYPDSMKG (SEQ ID NO:65)

ATIGRVSFHTYYPVSMKG (SEQ ID NO:66)

ATIGRVAFHTYYPDSMKG (SEQ ID NO:67)

STIGRVASHTYYPVGMTG (SEQ ID NO:68)

ATIGRVWYHRYYPDSMVR (SEQ ID NO:69)

GTIGWMVSHTYYPQRLNG (SEQ ID NO:70)

ATIGRVTSRTYYPDSMKG (SEQ ID NO:71)

ATIGRVYRHTYYPTSMKG (SEQ ID NO:72)

ATIGRVPLHTYYPRSMKG (SEQ ID NO:73)

ATIGRVPLHTYYPGSMKG (SEQ ID NO:74)

ATIGRVPLHTYYPASMKG (SEQ ID NO:75)

ATIGRVEQHTYYPQSMKG (SEQ ID NO:76)

ATIGRVASHTYYPGSMKG (SEQ ID NO:77)

ATIGRVALHTYYPQSMKG (SEQ ID NO:78)

HCDR3:

ARGFDY (SEQ ID NO:79)

VRHRGFDVGHFDF (SEQ ID NO:80)

ARHRGFDVGHFVF (SEQ ID NO:81)

ARHRGWVVGHFDL (SEQ ID NO:82)

ARHRGFDVGHFDF (SEQ ID NO:83)

LCDR1:

RASQSISNYLA (SEQ ID NO:84)

RSSETLVHSNGNTYLE (SEQ ID NO:85)

RSSETLVHSSGNTYLE (SEQ ID NO:86)

RSSETLVHWSGNTYLE (SEQ ID NO:87)

RSSGTLRHWSGNTYLE (SEQ ID NO:88)

RSSGTLLHNNGNTYLE (SEQ ID NO:89)

RSSETLVHRSGNTYLE (SEQ ID NO:90)

RSSETLVHTSGNTYLE (SEQ ID NO:91)

RSSETLVHGSGNTYLE (SEQ ID NO:92)

RSSETLVHASGNTYLE (SEQ ID NO:93)

RSSETLVHNSGNTYLE (SEQ ID NO:94)

RSSETLVHKSGNTYLE (SEQ ID NO:95)

RSSRTLEHASGNTYLE (SEQ ID NO:96)

RSSQTLQHWSGNTYLE (SEQ ID NO:97)

LCDR2:

AASSLES (SEQ ID NO:98)

RVSNRFS (SEQ ID NO:99)

RVSQRFT (SEQ ID NO:100)

RVSNRFR (SEQ ID NO:101)

LCDR3:

QQYNSLPWT (SEQ ID NO:102)

FQGSHNPLT (SEQ ID NO:103)

FQGSFNPLT (SEQ ID NO:104)

[00064] Given the high sequence similarity between these CDRs, particularly the CDR-H3 sequences, one could determine with routine experimentation which combinations of six CDRs can be combined to form a functional antigen-binding domain.

[00065] Exemplary anti-NaPi-2b antibodies have VH and VL regions including the following sequences, taken from lifastuzumab:

[00066] Heavy chain:

EVQLVESGGGLVQPGGSLRLSCAASGFSFSDFAMSWVRQAPGKGLEWVATIGRVA FHTYYPDSMKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHRGFDVGHFDFW GQGTLVTVSS (SEQ ID NO:38)

[00067] Light chain:

DIQMTQSPSSLSASVGDRVTITCRSSETLVHSSGNTYLEWYQQKPGKAPKLLIYR VSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCFQGSFNPLTFGQGTKVE IK (SEQ ID NO:39)

[00068] Exemplary anti-NaPi-2b antibodies have CDR regions including the following sequences, taken from upifitamab:

HCDR1: GYTFTGYNIH (SEQ ID NO:40)

HCDR2: AIYPGNGDTS (SEQ ID NO:41)

HCDR3: GETARATFAY (SEQ ID NO:42)

LCDR1: SASQDIGNFLN (SEQ ID NO:43)

LCDR2: YTSSLYS (SEQ ID NO:44)

LCDR3: QQYSKLPLT (SEQ ID NO:45)

[00069] In some embodiments, the anti-NaPi-2b antibody comprises a variable heavy (VH) region comprising heavy chain (HC) complementarity determining regions: HCDR1: GYTFTGYNIH (SEQ ID NO:40), HCDR2: AIYPGNGDTS (SEQ ID NO:41) and HCDR3: GETARATFAY (SEQ ID NO:42). In some embodiments, the anti-NaPi-2b antibody comprises a variable light (VL) region comprising light chain (LC) complementary determining regions: LCDR1: SASQDIGNFLN (SEQ ID NO:43), LCDR2: YTSSLYS (SEQ ID NO:44), and LCDR3: QQYSKLPLT (SEQ ID NO:45).

[00070] Exemplary anti-NaPi-2b antibodies have VH and VL regions including the following sequences, taken from upifitamab:

[00071] Heavy chain:

QVQLVQSGAEVVKPGASVKMSCKASGYTFTGYNIHWVKQAPGQGLEWIGAIYPGN GDTSYKQKFRGRATLTADTSTSTVYMELSSLRSEDSAVYYCARGETARATFAYWG QGTLVTVSS (SEQ ID NO:46)

[00072] Light chain:

DIQMTQSPSSLSASVGDRVTITCSASQDIGNFLNWYQQKPGKTVKVLIYYTSSLY SGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSKLPLTFGQGTKLELK (SEQ ID NO:47)

[00073] An amino acid sequence of an antibody that binds NaPi-2b Muc16 is provided here. Variable regions are indicated by underline. Positions of CDRs are indicated by **bold** and asterisk. Cysteine substitutions are indicated by **bold** and hashtag.

1. **AB301**

[00074] Anti-NaPi-2b monoclonal AB301:

[00075] Heavy chain:

***HCDR1**

***HCDR2**

EVQLVESGGGLVQPGGSLRLSCAAS**GFSFSDFAMS**WVRQAPGKGLEWVA**TIGRVAFHT YY**

****HCDR3**

PDSMKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR**HRGFDVGHFDF**WGQGTLVTV SS

#

CSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:15)

[00076] Heavy chain variable region:

***HCDR1**

***HCDR2**

EVQLVESGGGLVQPGGSLRLSCAAS**GFSFSDFAMS**WVRQAPGKGLEWVA**TIGRVAFHT**
YY

****HCDR3**

PDSMKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR**HRGFDVGHFDF**WGQGTLVTV
SS

(SEQ ID NO:34)

[00077] Light chain:

LCDR1*****

*LCDR2

DIQMTQSPSSLSASVGDRVTITC**RSSETLVHSSGNTYLE**WYQQKPGKAPKLLIY**RVSN**
RF

* **LCDR3**

SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**FQGSFNPLT**FGQGTKVEIKRTVAAP
SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSL

#

SSTLTLSKADYEKHKVYACEVTHQGLSSP**C**TKSFNRGEC (SEQ ID NO:16)

[00078] Light chain variable region:

LCDR1*****

*LCDR2

DIQMTQSPSSLSASVGDRVTITC**RSSETLVHSSGNTYLE**WYQQKPGKAPKLLIY**RVSN**
RF

* **LCDR3**

SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**FQGSFNPLT**FGQGTKVEIK (SEQ ID NO:35)

II. Antibodies

[00079] An "antibody" is a protein or protein complex comprising a framework region from an immunoglobulin gene, which protein or protein complex binds to a target epitope. The framework region of an immunoglobulin gene refers to relatively conserved sequences within the variable region of the immunoglobulin (Ig) heavy chain and light chain. Accordingly, the term "antibody" includes the intact tetrameric antibody, which includes two heavy immunoglobulin chains and two light immunoglobulin chains. The term also includes any binding fragment of a tetrameric antibody. The term also includes engineered antibodies in which various immunoglobulin domains are rearranged, or are added to immunoglobulin chains, for example, to create antibodies with more than two binding sites, as described herein.

A. Antibody Structure

[00080] An exemplary antibody structure is an intact tetrameric antibody. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" chain (about 25 kD) and one "heavy" chain (about 50-70 kD). Antibodies can be of (i) any of the five major classes of immunoglobulins, based on the identity of their heavy-chain constant domains – alpha (IgA), delta (IgD), epsilon (IgE), gamma (IgG) and mu (IgM), or (ii) subclasses (isotypes) thereof (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2). The light chains can be either lambda or kappa. In an intact antibody, a light chain comprises, from N-terminus to C-terminus, a light chain variable (VL) region and a light chain constant (CL) region. In an intact antibody, a heavy chain comprises, from N-terminus to C-terminus, a heavy chain variable (VH) region, a heavy chain constant 1 (CH1) region, a hinge region, a heavy chain constant 2 (CH2) region, and a heavy chain constant 3 (CH3) region. Two light chains associate with the heavy chain portions comprising a VH region and a CH1 region, to form two Fab (Fragment antigen-binding) regions. The light chains and heavy chains are connected by a covalent bond (e.g., a disulfide bond) and by non-covalent interactions. The combination of a VL region and a VH region forms the antigen binding domain of the antibody, also referred to as a "paratope". The combination of the CH2 and CH3 regions are referred to collectively as the Fc ("fragment crystallizable") region.

[00081] In the formation of antibodies, different chains or portions of chains interact with each other to form higher order structures. In this way, antibody chains or portions of them

can be said to associate to form these structures. For example and without limitation, a heavy chain and light chain can associate to form a paratope. Two heavy chains can associate to form an Fc dimer. Heavy chain variable regions and light chain variable regions in an scFv molecule, or in a diabody can associate to form one or more paratopes. The association can be effected through a variety of covalent and non-covalent interactions. This includes, without limitation, disulfide bonds, which can connect heavy chains with each other and light chains with heavy chains, as well as hydrogen bonding, hydrophobic interactions, and van der Waals forces. Association between chains or portions of them can occur naturally, so that the higher order structures self-assemble.

[00082] Paratopes can be formed through the association of various light chain variable regions and heavy chain variable regions in a variety of formats, discussed in more detail herein. For example, the association of a light chain and a heavy chain variable region and a polypeptide containing a heavy chain constant 1 region form an Fab that comprises a paratope. A single chain comprising a heavy chain variable region and a light chain variable region can associate to form an scFv that comprises a paratope. Two chains, each comprising a heavy chain variable region and a light chain variable region can associate to form a diabody that comprises two paratopes. As used herein, the phrases, “an Fab comprising a paratope,” “an scFv comprising a paratope,” and “a diabody comprising two paratopes,” (and so forth) can be equivalently expressed as “a paratope comprised in an Fab,” “a paratope comprised in an scFv,” and “two paratopes comprised in a diabody.”

[00083] The variable regions of both light and heavy chains in antibodies are composed of framework regions and complementarity-determining regions (CDRs) (also called “hypervariable” regions). There are three CDRs in each variable region, labeled CDR1, CDR2, and CDR3. They typically have lengths between about seven and 25 amino acids. The CDRs play a role in antigen recognition. Framework regions intersperse between the CDRs and provide structural integrity, contributing to proper conformation of the CDRs for antigen binding.

[00084] The phrase “CDR sequence set” refers to the 3 heavy chain and/or 3 light chain CDRs of a particular antibody. A “light chain” CDR sequence set refers to the light chain CDR sequences. A “heavy chain” CDR sequence set refers to the heavy chain CDR sequences. A “full” CDR sequence set refers to both heavy chain and light chain CDR sequences. CDRs can be predicted based on, for example, IMGT sequence alignment. (The international ImMunoGeneTics information system.)

[00085] The constant region interacts with other immune cells of the body. Between the Fab and Fc regions of IgG, IgD, and IgA, is the hinge region that provides flexibility to articulate antigen binding.

B. Antibody Nomenclature

[00086] Antibodies can exist in natural or genetically modified forms such as humanized, human, single-chain, chimeric, synthetic, recombinant, hybrid, mutated, grafted, and *in vitro* generated antibodies.

[00087] As used herein, the term "monoclonal antibody" refers to a clonal preparation or composition of antibodies with a single binding specificity and affinity for a given epitope on an antigen ("monoclonal antibody composition"). A "polyclonal antibody" refers to a preparation or composition of antibodies that are raised against a single antigen, but with different binding specificities and affinities ("polyclonal antibody composition").

[00088] As used herein, the term "chimeric antibody" refers to an antibody having amino acid sequences derived from two or more species. In one embodiment, the variable region of both light and heavy chains correspond to the variable region of antibodies derived from one species of mammal (e.g., mouse, rat, rabbit, etc.) with the desired specificity, affinity and capability, while the constant region contain amino acid sequences derived from another species (typically in the subject receiving the therapy, e.g., human) to avoid eliciting an immune response.

[00089] As used herein, the term "humanized antibody" refers to a chimeric antibody in which the CDRs, obtained from the VH and VL regions of a non-human antibody having the desired specificity, affinity and capability are grafted to a human framework sequence. In one embodiment, the framework residues of the humanized antibody are modified to refine and optimize the antibody specificity, affinity and capability. Humanization, i.e., substitution of non-human CDR sequences for the corresponding sequences of a human antibody, can be performed following the methods described in, e.g., U.S. Patent Nos. 5,545,806; 5,569,825; 5,633,425; 5,661,016; Riechmann et al., Nature 332:323-327 (1988); Marks et al., Bio/Technology 10:779-783 (1992); Morrison, Nature 368:812-13 (1994); Fishwild et al., Nature Biotechnology 14:845-51 (1996).

[00090] As used herein, the term "human antibody" refers to an antibody produced by a human or an antibody having an amino acid sequence corresponding thereto made by any technique known in the art.

[00091] As used herein, the term "immunoassay" refers to a method for detecting an analyte by detecting binding between the analyte and an antibody that recognizes the analyte.

[00092] Amino acid sequences of antibody drugs can be found, for example, at go.drugbank.com/drugs as well as at opig.stats.ox.ac.uk/webapps/sabdab-sabpred/ and opig.stats.ox.ac.uk/webapps/sabdab-sabpred/therasabdab/, Raybould et al. *Nucleic Acids Res.* 48:D383-D388, (2020).

[00093] As used herein, the term "polypeptide" refers to a molecule having a sequence of natural and/or unnatural amino acids connected through peptide bonds. The term "peptide" refers to a short polypeptide, typically no more than 30 amino acids long. The amino acid sequence of a polypeptide is referred to as its "primary structure." The term "protein" refers to a polypeptide having a secondary, tertiary and/or quaternary structure, e.g., structures stabilized by hydrogen bonds, relationships between secondary structures and structures formed of more than one protein. Proteins can be further modified by other attached moieties such as carbohydrate (glycoproteins), lipids (lipoproteins) phosphate groups (phosphoproteins) and the like.

[00094] As used herein, an amino acid sequence "consists of" only the amino acids in that sequence.

[00095] As used herein, a first amino acid sequence is a "fragment" of a second amino acid sequence if the second amino acid sequence comprises the first amino acid sequence. In certain embodiments, a first amino acid sequence that is a fragment of a second amino acid sequence may have no more than any of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 fewer amino acids than the second amino acid sequence.

[00096] As used herein, a "functional equivalent" of a reference amino acid sequence is a sequence that is not identical to the reference sequence, but that contains minor alterations such as, for example, insertion, deletion or substitution of one or a few amino acids. A functionally equivalent sequence retains the function (*e.g.*, antigen binding, immunogenicity) of the reference sequence to which it is equivalent. If a functionally equivalent amino acid sequence contains substitution of one or more amino acids with respect to the reference sequence, these will generally be conservative amino acid substitutions.

[00097] The term "cross-linked" with respect to an antibody refers to attachment of the antibody to a solid or semisolid matrix (*e.g.*, Sepharose, beads, microtiter plate), or to another protein or antibody. For example, an antibody can be multimerized to create an antibody complex with multiple (more than 2) antigen-binding sites. The antibody can be multimerized by expressing the antibody as a high-valency isotype (*e.g.*, IgA or IgM, which

typically form complexes of 2 or 5 antibodies, respectively). Antibody multimerization can also be carried out by using a cross-linker comprising a reactive group capable of linking proteins (*e.g.*, carbodiimide, NHS esters, etc.). Methods and compositions for cross-linking an antibody to a matrix are described, *e.g.*, in the Abcam and New England Biolab catalogs and websites (available at abcam.com and neb.com). Cross-linker compounds with various reactive groups are described, *e.g.*, in Thermo Fisher Scientific catalog and website (available at piercenet.com).

C. Antibody Binding

[00098] The specificity of antibody binding can be expressed in terms of the dissociation constant (K_d) of the complex between the antibody (or other targeting moiety) and its target, as compared to the dissociation constant of a complex between the antibody and a non-target molecule. A larger (higher) K_d is a K_d that describes a lower affinity interaction. Conversely a smaller (lower) K_d is a K_d that describes a higher affinity interaction or tighter binding. By way of example only, the K_d for an antibody specifically binding to a target may be femtomolar, picomolar, nanomolar, or micromolar and the K_d for the antibody binding to unrelated material may be millimolar or higher. Binding affinity can be in the nanomole range ($K_d = 10^{-7}$ M to 10^{-9} M), picomole range ($K_d = 10^{-10}$ M to 10^{-12} M), or femtomole range $K_d = 10^{-13}$ M to 10^{-15} M).

[00099] As used herein, an antibody "specifically binds" or "is specific for" an antigen or epitope of an antigen if it binds the antigen or epitope with a Kd of less than 10^{-7} M (i.e., in the nanomolar range). This includes, for example, less than any of 1×10^{-7}M, 1×10^{-8}M, 1×10^{-9} M, 1×10^{-10} M, 1×10^{-11} M, 1×10^{-12} M. Typically, specific binding is characterized by binding the antigen with sufficient affinity that the antibody is useful as a diagnostic to detect the antigen or epitope and/or as a therapeutic agent in targeting the antigen or epitope.

[000100] The term "binds" with respect to a cell type (e.g., an antibody that binds cancer cells), typically indicates that an agent binds a majority of the cells in a pure population of those cells. For example, an antibody that binds a given cell type typically binds to at least 2/3 of the cells in a population of the indicated cells (e.g., 67, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% of the cells in the population). In some cases, binding of an antibody to a polypeptide can be assayed by comparing binding of the antibody to a cell that presents the polypeptide to binding (or lack thereof) of the antibody to a cell that does not express the polypeptide. One of skill will recognize that some variability will arise depending on the method and/or threshold of determining binding. Affinity of an antibody for a target can be determined according to methods known in the art, *e.g.*, as reviewed in Ernst *et al.*

Determination of Equilibrium Dissociation Constants, *Therapeutic Monoclonal Antibodies* (Wiley & Sons ed. 2009).

[000101] As used herein, the term “greater affinity” refers to a relative degree of antibody binding where an antibody X binds to target Y more strongly and/or with a lower dissociation constant than to target Z, and in this context antibody X has a greater affinity for target Y than for Z. Likewise, the term "lesser affinity" herein refers to a degree of antibody binding where an antibody X binds to target Y less strongly and/or with a higher dissociation constant than to target Z, and in this context antibody X has a lesser affinity for target Y than for Z. The affinity of binding between an antibody and its target antigen, can be expressed as K_A equal to $1/K_D$ where K_D is equal to k_{off} /k_{on}/. The k_{on} and k_{off} values can be measured using surface plasmon resonance technology, for example, using a Molecular Affinity Screening System (MASS-1) (Sierra Sensors GmbH, Hamburg, Germany).

[000102] An antagonist or blocking antibody is an antibody that partially or fully blocks, inhibits or neutralizes a biological activity related to the target antigen relative to the activity under similar physiological conditions when the antibody is not present. Antagonists can be competitive, non-competitive or irreversible. A competitive antagonist is a substance that binds to a natural ligand or receptor at the same site as is used in the natural ligand-receptor interaction or binds allosterically in a manner that induces a change to prevent normal binding of the natural ligand. A non-competitive antagonist binds at a different site, on either the ligand or the receptor, than is used in the natural ligand-receptor interaction, but increases the K_d of the interaction, or lowers a signal resulting from the interaction. An irreversible inhibitor causes, e.g., covalent modifications to the receptor or to the natural ligand which prevent binding of the natural ligand to the receptor.

[000103] As used herein, the term “avidity” refers to the overall stability of the binding complex between the antibody and the target antigen. It is governed by three factors, (i) the intrinsic affinity of the antibody for the antigen, (2) the valency of the antibody, and (3) the geometric arrangement of the interacting components. Affinity is the strength of the interaction between the antibody and a single target, whereas avidity is an accumulated strength of multiple affinities.

[000104] As used herein, an antibody “preferentially binds” binds a first antigen relative to a second antigen if it binds the first antigen with greater affinity than it does the second antigen. Preferential binding can be at least any of 2-fold, 5-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 500-fold or 1000-fold greater affinity.

[000105] As used herein, and antibody “blocks” or “antagonizes” the binding of a ligand to receptor when it reduces or prevents interaction of the ligand with the receptor. In

embodiments, the measured level of reduction can be at least any of 5%, 10%, 25%, 50%, 80%, 90%, 95%, 97.5%, 99%, 99.5%, or 99.9% of a control) ligand-receptor complex (i.e., a ligand-receptor complex in the absence of the antibody).

[000106] The term "captures" with respect to an antibody target (e.g., antigen, analyte, immune complex), typically indicates that an antibody binds a majority of the antibody targets in a pure population (assuming appropriate molar ratios). For example, an antibody that binds a given antibody target typically binds to at least 2/3 of the antibody targets in a solution (e.g., at least any of 67, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% of the targets). One of skill will recognize that some variability will arise depending on the method and/or threshold of determining binding.

D. Antibody Formats

[000107] The following are a non-exhaustive list of different antibody formats, all retaining antigen binding activity:

(1) whole immunoglobulins (also referred to as "intact" antibodies) (two light chains and two heavy chains, e.g., a tetramer, e.g., IgG, IgA, IgD, IgE and fragments of IgM).

(2) an immunoglobulin polypeptide (a light chain or a heavy chain).

(3) an antibody fragment, including rearranged binding regions. These include, for example:

- Fv (a monovalent or bi-valent variable region fragment, and can encompass only the variable regions (*e.g.*, V_L and/or V_H),
- Fab (V_LC_L V_HC_H),
- F(ab')2,
- Fv (V_LV_H),
- scFv (single chain Fv) (a polypeptide comprising a V_L and V_H joined by a linker, *e.g.*, a peptide linker),
- (scFv)2, (optionally bi-specific),
- sc(Fv)2 (optionally bi-specific),
- minibody (sc(Fv)2 fused to CH3 domain)
- diabody (noncovalent dimer of heavy chain variable (VH) and light chain variable (VL) regions connected by a peptide linker) (It is understood that while the VH and VL regions on a first chain can be in any order, the order of these regions on the second chain is reversed relative to these regions on the first chain relative to the order of these regions on the first chain)

- triabody: trivalent sc(Fv)3 or trispecific sc(Fv)3.
- Antibody fragments further include Fd (the portion of the heavy chain included in the Fab fragment) and single domain antibodies. A single domain antibody (sdAb) is a variable domain of either a heavy chain or a light chain, produced by recombinant methods.

(4) a multispecific antibody (an antibody comprising at least two paratopes that bind to different epitopes)

(5) a multivalent antibody (an antibody having two or more paratopes, including bivalent, trivalent and tetravalent antibodies)

(6) a fusion protein comprising a binding portion of an immunoglobulin fused to another amino acid sequence (such as a fluorescent protein).

[000108] Any of these formats can be monospecific, or multi-specific, e.g., bi-specific, tri-specific or tetra-specific.

E. Antibody Symmetry

[000109] Antibodies comprising two heavy chains, typically, can be symmetric or asymmetric. A symmetric antibody has mirror-image parts. Typically, this will involve having identical heavy chains. An asymmetric antibody is not formatted as a mirror image. In this case, typically, the heavy chains are not identical.

[000110] An asymmetric antibody typically has two non-identical heavy chains. Usually, paratopes in the same relative position will bind different epitopes. A challenge in producing such antibodies is ensuring that the two different heavy chains pair correctly and selectively without mismatching. Mismatch can produce a mixture of monospecific and bi-specific antibodies. Such chains may not stably associate with one another. Knobs-into-holes or Knob and Holes (KIHs) technology addresses this problem. KIHs involves engineering CH3 domains to create a “knob” in a first heavy chain and a “hole” in a second heavy chain to promote heterodimerization between the first and second heavy chains.

[000111] In this approach a 'knob' variant can be obtained by replacement of a small amino acid (e.g., threonine) with a larger one (e.g., tyrosine) in the CH3 domain of an antibody (e.g., T366Y). The knob is designed to insert into a 'hole' in the CH3 domain of another antibody. In one embodiment, a 'hole' is created by replacement of a large residue (e.g., tyrosine) with a smaller one (e.g., threonine), such as in Y407T.

[000112] When protein domains or subdomain interact, a knob is a bulky side chain that protrudes into the opposite (“hole”) domain where it is aligned with a small side chain that makes such invasion possible. In this approach, knob and hole variants were anticipated to

heterodimerize by virtue of the knob inserting into an appropriately designed hole on the partner CH3 domain. Knobs were constructed by replacing small side chains with the largest side chains, tyrosine or tryptophan. Holes of identical or similar size to the knobs were created by replacing large side chains with the smaller ones, in this case alanine or threonine. This way, two heavy chains that are knob variants cannot homoassociate because of side chain clashes, and the homoassociation of two hole variants is less favored because of the absence of a stabilizing side-chain interaction. Subsequently, a disulfide bond was introduced near the C-terminus of the CH3 domain to further stabilize the assembled bi-specific antibodies. See, for example, U.S. Pat. No. 7,183,076, incorporated herein by reference.

III. Multivalent Antibody Platform Architectures

[000113] "Multivalent" antibodies have more than one antigen binding site or paratope. For example, an antibody that has two binding sites is bivalent, an antibody with three binding sites is trivalent, and an antibody with four binding sites is tetravalent. Tetravalent antibodies can be referred to as "tetramabs."

[000114] Contemplated herein are a variety of formats for bi-specific, multivalent antibodies. Variables include the degree of valency, the format of the paratopes, and the arrangement of binding sites for particular epitopes.

[000115] A multivalent antibody can have a plurality of binding sites that bind the same epitope as well as one or more binding sites that bind different epitopes. Antibodies in nature, such as IgG molecules, are typically mono-specific and bivalent. An antibody is "monospecific" if all of its antigen binding sites bind to the same epitope. A multi-specific antibody has at least two different antigen binding sites which each bind to a different epitope or antigen. So, for example, if a bivalent antibody has antigen binding sites that each bind to a different epitope, then the antibody is bi-specific. A trivalent antibody can be monospecific, bi-specific or tri-specific. A tetravalent antibody can be monospecific, bi-specific, tri-specific or tetra-specific.

[000116] In describing antibodies herein, the orientation of various functional portions of a heavy chain or light chain is described from amino terminus to carboxy terminus, unless explicitly provided otherwise.

[000117] Engineered antibodies disclosed herein comprise functional portions attached to each other. This includes, for example, variable regions attached to each other in an scFv or diabody format, different paratope configurations (e.g., Fab, diabody, scFv) attached to each other, or paratopes attached to an Fc region, either on the amino side or the carboxy side. These portions can be attached through linker moieties. Linkers can be, for example 1 to 50

amino acids long. The linker can have, for example, any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acids. For example, the polypeptide linker can have between any of 1 to 50, 5 to 50, 1 to 30, 1 to 25, 5 to 25, 5 to 20, 5 to 15, 5 to 10, 15, 20, 1-10 or 1-5 amino acids. In one embodiment, the linker is a monomer or polymer of the sequence GGGGS (SEQ ID NO:19). An Fab attached to an Fc (Fab-Fc) is typically referred to as "IgG" (or other immunoglobulin format), but the Fab could be attached through a linker.

[000118] In antibodies that have more than one light chain associated with a heavy chain, there is the issue of whether the paratopes formed by these associations bind the same or a different epitope. For example, in the antibodies shown schematically in Figs. 4A and 4B, paratopes 1 and 2 bind the same epitope. However, in the antibodies shown schematically in Figs. 4C and 4D, paratopes 1 and 2 bind different epitopes. Where the paratopes bind the same epitope, and the antibody is symmetric, a single light chain and a single heavy chain expressed in a cell can assemble to form the antibody. Where the paratopes bind different epitopes, and the antibody is asymmetric, the following approaches to antibody assembly can be used. In one approach, each different light chain/heavy chain pair can be expressed in a different cell, and the antibody can be assembled *in vitro*. In another approach, each heavy chain variable region pairs with a common light chain variable region. Such approaches are well known in the art. See, for example, U.S. Patent 8,642,745 ("Method for making multispecific antibodies having heteromultimeric and common components"); U.S. Patent 11,033,009 ("Transgenic chicken for production of antibodies having a common light chain"); U.S. Patent 9,303,081 ("Recombinant production of mixtures of antibodies"); and U.S. Patent 11,325,982 ("Biparatopic and multiparatopic antibodies with common light chain and method of use"). Accordingly, contemplated herein are multivalent, bi-specific antibodies comprising a first paratope that specifically binds Muc16 and a second paratope that specifically binds NaPi-2b, wherein the first and second paratopes share common light chains or light chain variable regions.

[000119] Diabodies comprise a pair of chains that associate to form two paratopes. (See, e.g., Fig. 1C.) Each chain comprises VL and VH regions linked to each other. This combination is referred to as a "diabody region." These regions can be arranged in any order, provided the regions of each partner chain are in an order consistent with formation of the diabody. In certain antibody architectures, such as shown in Fig. 4A and 4B, both paratopes of a diabody bind the same epitope. In this configuration, each diabody region can comprise VL and VH regions directed to the same epitope. In other architectures, such as shown in Fig. 4C and 4D, the paratopes bind different epitopes. In this configuration, the

diabody region of a first chain includes a light or heavy chain variable region that binds a first epitope, and a heavy or light chain variable region (respectively) that binds a second epitope. In this way, each variable region can associate with the appropriate variable region on the diabody region of the other chain.

[000120] It is understood that references to "first," "second," "third," and "fourth" regions or portions of antibodies are for nomenclature purposes only, and do not necessarily indicate specific positions of these regions or portions in an antibody, for example, specific positions in the compass rose, or equivalence between different antibody formats.

A. Bivalent, Bi-specific Antibodies

[000121] This disclosure contemplates a variety of bivalent, bi-specific formats. Each bivalent format comprises two paratopes. Either paratope can be configured to bind Muc16 or NaPi-2b.

[000122] Referring to Fig. 1, a bi-valent antibody can have tetrameric format comprising two heavy chains and two light chains, as shown in Fig. 1A. This format is also called "(Fab)2-Fc" to refer to the fact that it has two Fab regions and an Fc region. In this case, one of the Fab paratopes binds Muc16, and the other Fab paratope binds NaPi-2b. This antibody is comprised of two heavy chains and two light chains in which each of the heavy chains has the traditional amino-carboxy configuration of VH-CH1-CH2-CH3. Both light chains also have the traditional amino-carboxy configuration of VL-CL. The bi-specific antibody is asymmetric, that is, it has two different heavy chains, attached to each other, in this case, through a knob-and-hole arrangement.

[000123] Another format, shown in Fig. 1B, is referred to as "Fab-scFv-Fc." The antibody has two paratopes, one configured as an Fab and the other configured as scFv. This antibody comprises a first heavy chain having, from amino terminus to carboxy terminus, a portion comprising VH and CH1 region, and an Fc region; a second heavy chain comprising an scFv region and an Fc region; and a light chain comprising a VL and CL region. The light chain and heavy chain VH/CH1 portion form a first paratope and the scFv forms a second paratope. In some embodiments, the antibody comprises a first heavy chain having, from amino terminus to carboxy terminus, a portion comprising VH and CH1 region, and an Fc region; a second heavy chain comprising an scFv region and an Fc region; and a light chain comprising a VL and CL region. In some embodiments, the light chain and heavy chain VH/CH1 portion form a first paratope and the scFv forms a second paratope.

1. AB303

[000124] Amino acid sequences of heavy and light chains of antibody AB303 in the Fab/scFv Fc format binding NaPi-2b (Fab) and Muc16 (scFv) are presented here (FIG. 1B):

[000125] AB303:

[000126] Heavy Chain 1:

DIQMTQSPSSLSASVGDRVTITCKASDLIHNWLAWYQQKPGKAPKLLIYGATSLETGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYWTTPFTFGQGTKVEIKGTTAASGSS
GGSSSGAEVQLVESGGGLVQPGGSLRLSCAASGYSITNDYAWNWVRQAPGKGLEWVGY
ISYSGYTTYNPSLKSRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARWTSGLDYWGQG
TLVTVSSD

#

KTHTCPPCPAPELLGGP**C**VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ
ID NO:17)

[000127] Heavy Chain 2:

EVQLVESGGGLVQPGGSLRLSCAASGFSFSDFAMSWVRQAPGKGLEWVATIGRVAFHT
YYPDSMKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHRGFDVGHFDFWGQGTLV
TVSS

#

CSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE
LLGG

#

P**C**VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
IRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:18)

[000128] Light Chain: 7: Lifastuzumab human kappa with V205C substitution (AB301 Light Chain, SEQ ID NO:16)

[000129] In some embodiments, the antibody comprises SEQ ID NO:16.

[000130] Another format, shown in Fig. 1C, is referred to as “diabody-Fc.” The antibody has two paratopes, both comprised within a diabody. It includes a first and second heavy chains, each comprising a diabody region comprising a VH region and a VL region; and an Fc region. These are typically arranged, from amino to carboxy, as VH-VL, but they can be arranged as VL-VH as well. Each VL region of one heavy chain associates with a VHL region of the other heavy chain, to form a diabody comprising first and second paratopes. In some embodiments, the antibody has two paratopes, both comprised within a diabody. In some embodiments, the antibody includes a first and second heavy chains, each comprising a diabody region comprising a VH region and a VL region; and an Fc region. In some embodiments, the antibody is arranged, from amino to carboxy, as VH-VL, but they can be arranged as VL-VH as well. In some embodiments, each VL region of one heavy chain associates with a VHL region of the other heavy chain, to form a diabody comprising first and second paratopes.

2. AB306

[000131] Amino acid sequences of heavy and light chains of antibody AB306 in the diabody-Fc format binding NaPi-2b and Muc16 are presented here (See also Fig. 1C):

[000132] AB306: (No Thiomab Mutations)

[000133] Heavy Chain 1:

EVQLVESGGGLVQPGGSLRLSCAASGYSITNDYAWNWVRQAPGKGLEWVGYISYSGYT
TYNPSLKSRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARWTSGLDYWGQGTLVTVSS
GGGGSDIQMTQSPSSLSASVGDRVTITCRSSETLVHSSGNTYLEWYQQKPGKAPKLLI
YRVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCFQGSFNPLTFGQGTKVEI
KGGGGSGGGGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK (SEQ ID NO:20)

[000134] Heavy Chain 2:

EVQLVESGGGLVQPGGSLRLSCAASGFSFSDFAMSWVRQAPGKGLEWVATIGRVAFHT
YYPDSMKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHRGFDVGHFDFWGQGTLV
TVSSGGGGSDIQMTQSPSSLSASVGDRVTITCKASDLIHNWLAWYQQKPGKAPKLLIY
GATSLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYWTTPFTFGQGTKVEIK
GGGGSGGGGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC

KVSNKALPAPIEKTISKAKGQPREPQVYTLPPIRELMTSNQVSLSCAVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK (SEQ ID NO:21)

[000135] Light Chain: No Light Chain Required.

[000136] In some embodiments, the antibody comprises SEQ ID NO:20 and SEQ ID NO:21.

3. AB311

[000137] Amino acid sequences of heavy and light chains of antibody AB311 (a thiomab version of antibody AB306) in the diabody-Fc format binding Napi-2b and Muc16 are presented here:

[000138] AB311:

[000139] Heavy Chain 1:

EVQLVESGGGLVQPGGSLRLSCAASGYSITNDYAWNWVRQAPGKGLEWVGYISYSGYT
TYNPSLKSRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARWTSGLDYWGQGTLVTVSS
GGGGSDIQMTQSPSSLSASVGDRVTITCRSSETLVHSSGNTYLEWYQQKPGKAPKLLI
YRVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCFQGSFNPLTFGQGTKVEI
KGGGGSGG
# #
GGSEPKSSDKTHTCPPCPAPELLGGP**C**VFLFPPKPKDTLMISRTPEVTCVVVD**C**SHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK (SEQ ID NO:22)

[000140] Heavy Chain 2:

EVQLVESGGGLVQPGGSLRLSCAASGFSFSDFAMSWVRQAPGKGLEWVATIGRVAFHT
YYPDSMKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHRGFDVGHFDFWGQGTLV
TVSSGGGGSDIQMTQSPSSLSASVGDRVTITCKASDLIHNWLAWYQQKPGKAPKLLIY
GATSLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYWTTPFTFGQGTKVEIK
GGGGSGGG
# #
GSEPKSSDKTHTCPPCPAPELLGGP**C**VFLFPPKPKDTLMISRTPEVTCVVVD**C**SHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQP

ENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK (SEQ ID NO:23)

[000141] In some embodiments, the antibody comprises SEQ ID NO:22 and SEQ ID NO:23.

[000142] Light Chain: No Light Chain Required.

B. Trivalent, Bi-specific Antibodies

[000143] This disclosure contemplates trivalent, bi-specific antibodies. In such a format, one paratope binds to a first epitope and two paratopes bind to a second epitope. Any of the paratopes can be configured to bind Muc16 or NaPi-2b.

[000144] In an embodiment shown in Fig. 2, the antibody comprises a first paratope configured as a Fab and second and third paratopes configured as a diabody. This format comprises a first heavy chain comprising a portion comprising a VH region and a CH1 region, a portion comprising a VH/VL diabody region, and an Fc region. It also comprises a second heavy chain comprising a portion comprising a VL/VH diabody region, and an Fc region. (The VL and VH portions of the diabody region in this and all other antibodies herein can be arranged in either orientation, that is, VL/VH or VH/VL.) It also comprises a light chain comprising a VL region and a CL region. The light chain and the heavy chain portion comprising the VH/CH1 and VL/CL regions forms a first paratope. The diabody regions combine to form a diabody producing second and third paratopes.

[000145] In some embodiments, the antibody comprises a first heavy chain comprising a portion comprising a VH region and a CH1 region, a portion comprising a VH/VL diabody region, and an Fc region. In some embodiments, the antibody further comprises a second heavy chain comprising a portion comprising a VL/VH diabody region, and an Fc region. In some embodiments, the antibody comprises a light chain comprising a VL region and a CL region. In some embodiments, the light chain and the heavy chain portion comprising the VH/CH1 and VL/CL regions forms a first paratope. In some embodiments, the diabody regions combine to form a diabody producing second and third paratopes

1. AB307

[000146] Amino acid sequences of heavy and light chains of antibody AB307 in the Fab (single)-Diabody-Fc format binding NaPi-2b (Fab) and NaPi-2b/Muc16 (diabody) are presented here (see FIG. 2):

[000147] AB307: AB306 with additional Lifastuzumab Fab arm with Knob and Hole Fc Chain

[000148] Heavy Chain 1:

EVQLVESGGGLVQPGGSLRLSCAASGYSITNDYAWNWVRQAPGKGLEWVGYISYSGYT
TYNPSLKSRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARWTSGLDYWGQGTLVTVSS
GGGGSDIQMTQSPSSLSASVGDRVTITCRSSETLVHSSGNTYLEWYQQKPGKAPKLLI
YRVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCFQGSFNPLTFGQGTKVEI
KGGGGSGG

#

GGSEPKSSDKTHTCPPCPAPELLGGP**C**VFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK (SEQ ID NO:25)

[000149] Heavy Chain 2:

EVQLVESGGGLVQPGGSLRLSCAASGFSFSDFAMSWVRQAPGKGLEWVATIGRVAFHT
YYPDSMKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHRGFDVGHFDFWGQGTLV
TVSS

#

CSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTGGGGSGGG
GSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFSFSDFAMSWVRQAPGKGLE
WVATIGRVAFHTYYPDSMKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHRGFDV
GHFDFWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCKASDLIHNWLAWYQ
QKPGKAPKLLIYGATSLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYWTTP
FTFGQGTKVEIK

#

GGGGSGGGGSEPKSSDKTHTCPPCPAPELLGGP**C**VFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPIRELMTSNQVSLSCAVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK (SEQ ID NO:26)

[000150] Light Chain: (AB301 Light Chain, SEQ ID NO:16)

[000151] In some embodiments, the antibody comprises SEQ ID NO:25, SEQ ID NO:26 and SEQ ID NO:16.

C. Tetravalent, Bi-specific Antibodies

[000152] A bi-specific, tetravalent antibody can have two paratopes directed to a first epitope, and two paratopes directed to a second epitope; or three paratopes directed to a first epitope and one paratope directed to a second epitope. The epitopes can be on different antigen molecules (that is, the antibody binds two different molecules) or the epitopes can be different epitopes on the same antigen. The paratopes can be placed in any arrangement of the four compass positions consistent with the bi-specific nature of the antibody.

[000153] In some embodiments, the bispecific, tetravalent antibody comprises two paratopes directed to a first epitope, and two paratopes directed to a second epitope. In some embodiments, the bispecific, tetravalent antibody comprises three paratopes directed to a first epitope and one paratope directed to a second epitope. In some embodiments, the epitopes are on different antigen molecules. In some embodiments, the epitopes are different epitopes on the same antigen.

[000154] The four paratopes of a tetravalent antibody can be described as being positioned on a compass rose at “northwest,” “northeast,” “southeast,” “and “southwest” positions. Antibodies typically have an asymmetric shape that can be said to include an “east” side, a “west” side, a “north” (toward the N-terminus) side, and a “south” (toward the C-terminus) side. In a tetravalent antibody, for example, binding site positions could be characterized as “northeast,” “northwest,” “southeast,” and “southwest.” Accordingly, two binding sites that bind the same epitope could be related as “northwest-northeast,” “southwest-southeast,” “northeast-southeast,” “northwest-southwest,” “northwest-southeast,” and “northeast-southwest.” See, for example, Fig. 3.

[000155] This disclosure contemplates tetravalent, bi-specific antibodies comprising at least one paratope that binds to Muc16 and at least one paratope that binds to NaPi-2b. In one such format, two paratopes bind to Muc16 and two paratopes bind to NaPi-2b. In another format, one paratope binds to either Muc16 or NaPi-2b, and three paratopes bind to NaPi-2b or Muc16, respectively.

[000156] As shown in Fig. 14, tetravalent, bi-specific antibodies including combinations of Fabs and diabodies, such as AB304, AB305 and AB309, demonstrate superior manufacturability compared with trivalent antibodies (such as AB307), or bivalent (such as AB303) or tetravalent (such as AB308) antibodies, particularly those that include scFv regions. In some embodiments, the tetravalent, bi-specific antibody is AB304. In some embodiments, the tetravalent, bi-specific antibody is AB305. In some embodiments, the tetravalent, bi-specific antibody is AB309.

1. Fab-Diabody-Fc

[000157] In one format, shown in Fig. 3A, a tetravalent, bi-specific antibody comprises first and second light chains comprising the same amino acid sequence, and first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus: a) a first pair of paratopes comprised in Fab format; b) a second pair of paratopes, comprised as a diabody; and c) an Fc region. In some embodiments, the tetravalent, bi-specific antibody comprises first and second light chains comprising the same amino acid sequence, and first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus: a) a first pair of paratopes comprised in Fab format; b) a second pair of paratopes, comprised as a diabody; and c) an Fc region.

[000158] In one embodiment, the paratopes binding NaPi-2b are positioned in the NW/NE positions and the paratopes binding Muc16 are positioned in the SW and SE positions. In embodiments the paratopes binding NaPi-2b are positioned in the SW/SE positions and the paratopes binding Muc16 are positioned in the NW and NE positions.

a) AB304

[000159] Antibody sequences of heavy and light chains of antibody AB304, in this format, are presented here:

[000160] AB304:

[000161] Heavy Chain:

EVQLVESGGGLVQPGGSLRLSCAAS**GFSFSDFAMS**WVRQAPGKGLEWVA**TIGRVAFHT**
YYPDSMKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR**HRGFDVGHFDF**WGQGTLV
TVSS
#
CSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTGGGGSGGG
GSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAAS**GYSITNDYAWN**WVRQAPGKGL
EWVG**YISYSGYTT**YNPSLKSRFTISRDTSKNTLYLQMNSLRAEDTAVYYCAR**WTSGLD**
YWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITC**KASDLIHNWLA**WYQQKPG
KAPKLLIY**GATSLET**GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQYWTTPFT**FG
QGTKVEIKGGGGSGGGGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG

FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPGK (SEQ ID NO:27)

[000162] Light Chain: (AB301 Light Chain, SEQ ID NO:16)

[000163] In one embodiment, the paratopes binding Muc16 are positioned in the NW/NE positions and the paratopes binding NaPi-2b are positioned in the SW/SE positions.

[000164] In some embodiments, the tetravalent bispecific antibody, comprising two paratopes that each specifically bind to Muc16 and two paratopes that each specifically bind to NaPi-2b, wherein the antibody comprises two heavy chains and two lights chains that assemble to form a Fab-diabody-Fc structure, wherein the heavy chains each comprise, from N-terminus to C-terminus, a variable heavy (VH) region comprising heavy chain (HC) complementarity determining regions: HCDR1: GFSFSDFAMS (SEQ ID NO:9), HCDR2: TIGRVAFHTY (SEQ ID NO:10), HCDR3: HRGFDVGHFDF (SEQ ID NO:11), a heavy chain constant 1 (CH1) region, a variable heavy (VH) region comprising heavy chain (HC) complementarity determining regions: HCDR1: GYSITNDYAWN (SEQ ID NO:1), HCDR2: YISYSGYTT (SEQ ID NO:2), HCDR3: WTSGLDY (SEQ ID NO:3), a VL region comprising light chain (LC) complementarity determining regions: LCDR1: KASDLIHNWLA (SEQ ID NO:4), LCDR2: GATSLET (SEQ ID NO:5), LCDR3: QQYWTTPFT (SEQ ID NO:6), a heavy chain constant 2 (CH2) region, a heavy chain constant 3 (CH3) region; and wherein the light chains each comprise, from N-terminus to C-terminus, variable light (VL) region comprising light chain (LC) complementarity determining regions: LCDR1: RSSETLVHSSGNTYLE (SEQ ID NO:12), LCDR2: RVSNRFS (SEQ ID NO:13), LCDR3: FQGSFNPLT (SEQ ID NO:14), and a light chain constant (CL) region.

[000165] In some embodiments, the tetravalent, bi-specific antibody is AB304.

b) AB305

[000166] Antibody sequences of heavy and light chains of antibody AB305, in this format, are presented here:

[000167] AB305:

[000168] Heavy Chain:

EVQLVESGGGLVQPGGSLRLSCAASGYSITNDYAWNWVRQAPGKGLEWVGYISYSGYT
TY

#

NPSLKSRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARWTSGLDYWGQGTLVTVSS**C**S
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTGGGGSGGGGS

GGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFSFSDFAMSWVRQAPGKGLEWV
ATIGRVAFHTYYPDSMKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHRGFDVGH
FDFWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRSSETLVHSSGNTYLE
WYQQKPGKAPKLLIYRVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCFQGS
FNPLTFGQGTKVEIKGGGGSGGGGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:28)

[000169] Light Chain: (AB302 Light Chain, SEQ ID NO:8).

[000170] In some embodiments, the tetravalent, bi-specific antibody is AB305.

[000171] Bilaterally symmetric Fab-Diabody-Fc antibodies having identical heavy chains show superior manufacturability, as discussed above. Furthermore, in Fab-diabody-Fc antibodies, the Fab groups are positioned in the amino-terminal part of the antibody, rendering them free for binding. As such, they further show the best potency of any of the tetravalent, bi-specific antibodies described here.

2. Diabody-Fc-Fab

[000172] In one format, shown in Fig. 3B, a tetravalent, bi-specific antibody comprises first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus: a) a first pair of paratopes comprised within a diabody. B) an Fc region; and a) a second pair of paratopes comprised in Fab regions. The antibodies further comprise light chains that associate with the heavy chain Fab region. In some embodiments, the tetravalent, bi-specific antibody comprises first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus: a) a first pair of paratopes comprised within a diabody. B) an Fc region; and a) a second pair of paratopes comprised in Fab regions. In some embodiments, the tetravalent, bi-specific antibody further comprises light chains that associate with the heavy chain Fab region.

[000173] In one embodiment, the paratopes binding NaPi-2b are positioned in the NW/NE positions and the paratopes binding Muc16 are positioned in the SW and SE positions.

a) AB309

[000174] Amino acid sequences of heavy and light chains of antibody AB309 in the diabody-Fc-Fab format binding NaPi-2b (diabody) and Muc16 (Fab) are presented here (see Fig. 3B):

[000175] AB309:

[000176] Heavy Chain:

EVQLVESGGGLVQPGGSLRLSCAAS**GFSFSDFAMS**WVRQAPGKGLEWVA**TIGRVAFHT**
YYPDSMKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR**HRGFDVGHFDF**WGQGTLV
TVSSGGGGSDIQMTQSPSSLSASVGDRVTITC**RSSETLVHSSGNTYLE**WYQQKPGKAP
KLLIY**RVSNRFS**GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**FQGSFNPLT**FGQGT
KVEIKGGGGSGGGGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
HNHYTQKSLSLSPGKGGGGSGGGGSGGGTGEVQLVESGGGLVQPGGSLRLSCAASGYS
ITNDYAWNWVRQAPGKGLEWVGYISYSGYTTYNPSLKSRFTISRDTSKNTLYLQMNSL
RAEDTAVYYCARWTSGLDYW
#
GQGTLVTVSS**C**STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT
HT (SEQ ID NO:29)

[000177] Light Chain: (AB302 Light Chain, SEQ ID NO:8).

[000178] In some embodiments, the tetravalent, bi-specific antibody is AB309.

[000179] In one embodiment, the paratopes binding Muc16 are positioned in the NW/NE positions and the paratopes binding NaPi-2b are positioned in the SW/SE positions.

[000180] In some embodiments, the tetravalent bispecific antibody comprises two paratopes that each specifically bind to Muc16 and two paratopes that each specifically bind to NaPi-2b, wherein the antibody comprises two heavy chains and two light chains that assemble to form a diabody-Fc-Fab structure, wherein the heavy chains each comprise, from N-terminus to C-terminus, an anti-NaPi-2b variable heavy (VH) region comprising heavy chain (HC) complementarity determining regions: HCDR1: GFSFSDFAMS (SEQ ID NO:9), HCDR2: TIGRVAFHTY (SEQ ID NO:10), HCDR3: HRGFDVGHFDF (SEQ ID NO:11), an anti-NaPi-2b variable light (VL) region comprising light chain (LC) complementarity determining regions: LCDR1: RSSETLVHSSGNTYLE (SEQ ID NO:12), LCDR2: RVSNRFS (SEQ ID NO:13), LCDR3: FQGSFNPLT (SEQ ID NO:14), a heavy chain constant 2 (CH2) region, a heavy chain constant 3 (CH3) region, an anti-Muc16 VH region comprising heavy chain (HC) complementarity determining regions: HCDR1: GYSITNDYAWN (SEQ ID NO:1), HCDR2: YISYSGYTT (SEQ ID NO:2), HCDR3: WTSGLDY

(SEQ ID NO:3), a heavy chain constant 1 (CH1) region; and wherein the light chains each comprise, from N-terminus to C-terminus, an anti-Muc16 VL region comprising light chain (LC) complementarity determining regions: LCDR1: KASDLIHNWLA (SEQ ID NO:4), LCDR2: GATSLET (SEQ ID NO:5), LCDR3: QQYWTTPFT (SEQ ID NO:6), and a light chain constant (CL) region.

b) AB309-C

[000181] Heavy Chain:

1-EVQLVESGGG LVQPGGSLRL SCAASGFSFS DFAMSWVRQA PGKGLEWVAT
IGRVAFHTYY
61-PDSMKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARHR GFDVGHFDFW
GQGTLVTVSS
121-GGGGSDIQMT QSPSSLSASV GDRVTITCRS SETLVHSSGN TYLEWYQQKP
GKAPKLLIYR
181-VSNRFSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCFQ GSFNPLTFGQ
GTKVEIKGGG
241-GSGGGGSEPK SSDKTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE
VTCVVVDVSH
301-EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE
YKCKVSNKAL
361-PAPIEKTISK AKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA
VEWESNGQPE
421-NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ
KSLSLSPGKG
*
481-GGGSGGGG**A**G GGTGEVQLVE SGGGLVQPGG SLRLSCAASG YSITNDYAWN
WVRQAPGKGL
541-EWVGYISYSG YTTYNPSLKS RFTISRDTSK NTLYLQMNSL RAEDTAVYYC
ARWTSGLDYW
#
601-GQGTLVTVSS **C**STKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS
WNSGALTSGV
661-HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHT
(SEQ ID NO:56)

[000182] The S489A substitution is indicated by a star (*).

[000183] Light Chain: (AB302 Light Chain, SEQ ID NO:8).

[000184] In some embodiments, the tetravalent, bi-specific antibody is AB309-C.

c) AB309-D

[000185] Heavy Chain:

1-EVQLVESGGG LVQPGGSLRL SCAASGFSFS DFAMSWVRQA PGKGLEWVAT IGRVAFHTYY

61-PDSMKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARHR GFDVGHFDFW GQGTLVTVSS

121-GGGGSDIQMT QSPSSLSASV GDRVTITCRS SETLVHSSGN TYLEWYQQKP GKAPKLLIYR

181-VSNRFSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCFQ GSFNPLTFGQ GTKVEIKGGG

241-GSGGGGSEPK SSDKTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH

301-EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL

361-PAPIEKTISK AKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE

421-NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGKG

481-GGGSGGGG**G**G GGTGEVQLVE SGGGLVQPGG SLRLSCAASG YSITNDYAWN WVRQAPGKGL

541-EWVGYISYSG YTTYNPSLKS RFTISRDTSK NTLYLQMNSL RAEDTAVYYC ARWTSGLDYW

#

601-GQGTLVTVSS **C**STKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV

661-HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHT

(SEQ ID NO:57)

[000186] The S489G substitution is indicated by a star (*).

[000187] Light Chain: (AB302 Light Chain, SEQ ID NO:8).

[000188] In some embodiments, the tetravalent, bi-specific antibody is AB309-D.

d) AB309-E

[000189] Heavy Chain:

1-EVQLVESGGG LVQPGGSLRL SCAASGFSFS DFAMSWVRQA PGKGLEWVAT
IGRVAFHTYY
61-PDSMKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARHR GFDVGHFDFW
GQGTLVTVSS
121-GGGGSDIQMT QSPSSLSASV GDRVTITCRS SETLVHSSGN TYLEWYQQKP
GKAPKLLIYR
181-VSNRFSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCFQ GSFNPLTFGQ
GTKVEIKGGG
241-GSGGGGSEPK SSDKTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE
VTCVVVDVSH
301-EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE
YKCKVSNKAL
361-PAPIEKTISK AKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA
VEWESNGQPE
421-NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ
KSLSLSPGKG
* *
481-GGG**A**GGGG**A**G GGTGEVQLVE SGGGLVQPGG SLRLSCAASG YSITNDYAWN
WVRQAPGKGL
541-EWVGYISYSG YTTYNPSLKS RFTISRDTSK NTLYLQMNSL RAEDTAVYYC
ARWTSGLDYW
#
601-GQGTLVTVSS **C**STKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS
WNSGALTSGV
661-HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHT
(SEQ ID NO:58)

[000190] The S484A and the S489A substitutions are indicated by a star (*).

[000191] Light Chain: (AB302 Light Chain, SEQ ID NO:8).

[000192] In some embodiments, the tetravalent, bi-specific antibody is AB309-E.

e) AB309-F

[000193] Heavy Chain:

1-EVQLVESGGG LVQPGGSLRL SCAASGFSFS DFAMSWVRQA PGKGLEWVAT
IGRVAFHTYY
61-PDSMKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARHR GFDVGHFDFW
GQGTLVTVSS

121-GGGGSDIQMT QSPSSLSASV GDRVTITCRS SETLVHSSGN TYLEWYQQKP
GKAPKLLIYR
181-VSNRFSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCFQ GSFNPLTFGQ
GTKVEIKGGG
241-GSGGGGSEPK SSDKTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE
VTCVVVDVSH
301-EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE
YKCKVSNKAL
361-PAPIEKTISK AKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA
VEWESNGQPE
421-NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ
KSLSLSPGKG
* *
481-GGG**G**GGGG**G**G GGTGEVQLVE SGGGLVQPGG SLRLSCAASG YSITNDYAWN
WVRQAPGKGL
541-EWVGYISYSG YTTYNPSLKS RFTISRDTSK NTLYLQMNSL RAEDTAVYYC
ARWTSGLDYW
#
601-GQGTLVTVSS **C**STKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS
WNSGALTSGV
661-HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHT
(SEQ ID NO:59)

[000194] The S484G and the S489G substitutions are indicated by a star (*).

[000195] Light Chain: (AB302 Light Chain, SEQ ID NO:8).

[000196] In some embodiments, the tetravalent, bi-specific antibody is AB309-F.

Table 1: Substitutions relative to AB309

Construct	Substitution
AB309-C	S489A
AB309-D	S489G
AB309-E	S484A, S489A
AB309-F	S484G, S489G

[000197] In some embodiments, the tetravalent, bispecific antibody comprises one or more of the heavy chain substitutions as described in Table 1 relative to AB309 (SEQ ID NO:29). In some embodiments, the tetravalent, bispecific antibody comprises a S489A substitution

(relative to SEQ ID NO:29). In some embodiments, the tetravalent, bispecific antibody comprises a S89G substitution (relative to SEQ ID NO:29). In some embodiments, the tetravalent, bispecific antibody comprises S484A and S489A substitutions (relative to SEQ ID NO:29). In some embodiments, the tetravalent, bispecific antibody comprises S484G and S489G substitutions (relative to SEQ ID NO:29).

3. Fab-Fc-scFv (IgG-scFv)

[000198] In one format, shown in Fig. 3C, a tetravalent, bi-specific antibody comprises first and second light chains comprising the same amino acid sequence, and first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus: a) a first pair of paratopes comprised in Fab regions; b) an Fc region; and c) a second pair of paratopes, each comprised in an scFv portion.

[000199] In some embodiments, the tetravalent, bi-specific antibody comprises first and second light chains comprising the same amino acid sequence, and first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus: a) a first pair of paratopes comprised in Fab regions; b) an Fc region; and c) a second pair of paratopes, each comprised in an scFv portion.

a) AB308

[000200] Amino acid sequences of heavy and light chains of antibody AB308 in the Fab-Fc-scFv format are presented here (see Fig. 3C):

[000201] AB308:

[000202] Heavy Chain:

EVQLVESGGGLVQPGGSLRLSCAASGFSFSDFAMSWVRQAPGKGLEWVATIGRVAFHT
YYPDSMKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHRGFDVGHFDFWGQGTLV
TVSS
#
CSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGG
GSDIQMTQSPSSLSASVGDRVTITCKASDLIHNWLAWYQQKPGKAPKLLIYGATSLET
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYWTTPFTFGQGTKVEIKGTTAASG

SSGGSSSGAEVQLVESGGGLVQPGGSLRLSCAASGYSITNDYAWNWVRQAPGKGLEWV
GYISYSGYTTYNPSLKSRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARWTSGLDYWG
QGTLVTVSS (SEQ ID NO:24)

[000203] Light Chain: (AB301 Light Chain, SEQ ID NO:16).

[000204] In some embodiments, the tetravalent, bi-specific antibody is AB308.

4. Fab/scFv-Fc-Fab/scFv

[000205] In one format, shown in Fig. 3D, a tetravalent, bi-specific antibody comprises a first light chain and first and second heavy chains comprising different amino acid sequences. The antibody comprises, from N-terminus to C-terminus: a) a first (NW) paratope comprising a Fab and a second (NE) paratope comprising an scFv; b) an Fc region; and c) a third (SE) paratope comprising an scFv and a fourth (SW) paratope comprising an Fab.

[000206] In some embodiments, the tetravalent, bi-specific antibody comprises a first light chain and first and second heavy chains comprising different amino acid sequences. The antibody comprises, from N-terminus to C-terminus: a) a first (NW) paratope comprising a Fab and a second (NE) paratope comprising an scFv; b) an Fc region; and c) a third (SE) paratope comprising an scFv and a fourth (SW) paratope comprising an Fab.

a) AB310

[000207] Amino acid sequences of heavy and light chains of antibody AB310 in the Fab/scFv-Fc-Fab/scFv format are presented here (see Fig. 3D):

[000208] AB310:

[000209] Heavy Chain 1:

DIQMTQSPSSLSASVGDRVTITCKASDLIHNWLAWYQQKPGKAPKLLIYGATSLETGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYWTTPFTFGQGTKVEIKGTTAASGSS
GGSSSGAEVQLVESGGGLVQPGGSLRLSCAASGYSITNDYAWNWVRQAPGKGLEWVGY
ISYSGYTTYNPSLKSRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARWTSGLDYWGQG
TLVTVSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
GKGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCKASDLIHNWLAWY
QQKPGKAPKLLIYGATSLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYWTT
PFTFGQGTKVEIKGTTAASGSSGGSSSGAEVQLVESGGGLVQPGGSLRLSCAASGYSI

TNDYAWNWVRQAPGKGLEWVGYISYSGYTTYNPSLKSRFTISRDTSKNTLYLQMNSLR
AEDTAVYYCARWTSGLDYWGQGTLVTVSS (SEQ ID NO:30)

[000210] Heavy Chain 2:

EVQLVESGGGLVQPGGSLRLSCAASGFSFSDFAMSWVRQAPGKGLEWVATIGRVAFHT
YYPDSMKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHRGFDVGHFDFWGQGTLV
TVSS
#
CSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGG
GSEVQLVESGGGLVQPGGSLRLSCAASGFSFSDFAMSWVRQAPGKGLEWVATIGRVAF
HTYYPDSMKGRFTI
#
SRDNSKNTLYLQMNSLRAEDTAVYYCARHRGFDVGHFDFWGQGTLVTVSSCSTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO:31)

[000211] Light Chains: (AB301 Light Chain, SEQ ID NO:16).

[000212] In some embodiments, the tetravalent, bi-specific antibody is AB310.

5. Diabody-Fc-Diabody

[000213] In one format, shown in Fig. 3E, a tetravalent, bi-specific antibody comprises first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus: a) a first pair of paratopes comprised within a diabody; b) an Fc region; and c) a second pair of paratopes comprised within a diabody.

[000214] In some embodiments, the tetravalent, bi-specific antibody comprises first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus: a) a first pair of paratopes comprised within a diabody; b) an Fc region; and c) a second pair of paratopes comprised within a diabody.

6. Diabody-Fc-scFv

[000215] In one format, shown in Fig. 3F, a tetravalent, bi-specific antibody comprises first and second heavy chains comprising the same amino acid sequence, and comprising, from

N-terminus to C-terminus: a) a first pair of paratopes comprised within a diabody; b) an Fc region; and c) a second pair of paratopes, each comprised in an scFv region.

[000216] In some embodiments, the tetravalent, bi-specific antibody comprises first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus: a) a first pair of paratopes comprised within a diabody; b) an Fc region; and c) a second pair of paratopes, each comprised in an scFv region.

7. scFv-Fc-diabody

[000217] In one format, shown in Fig. 3G, a tetravalent, bi-specific antibody comprises first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus: a) a first pair of paratopes, each comprised in an scFv region; b) an Fc region; and c) a second pair of paratopes comprised within a diabody.

[000218] In some embodiments, the tetravalent, bi-specific antibody comprises first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus: a) a first pair of paratopes, each comprised in an scFv region; b) an Fc region; and c) a second pair of paratopes comprised within a diabody.

8. scFv-Fc-scFv

[000219] In one format, shown in Fig. 3H, a tetravalent, bi-specific antibody comprises first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus: a) a first pair of paratopes, each comprised as an scFv region; b) an Fc region; and c) a second pair of paratopes, each comprised as an scFv region.

[000220] In some embodiments, the tetravalent, bi-specific antibody comprises first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus: a) a first pair of paratopes, each comprised as an scFv region; b) an Fc region; and c) a second pair of paratopes, each comprised as an scFv region.

9. Fab-Fc-Diabody (IgG-Diabody)

[000221] In one format, shown in Fig. 3I, a tetravalent, bi-specific antibody comprises first and second light chains comprising the same amino acid sequence, and first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus: a) a first pair of paratopes comprised in Fab regions; b) an Fc region; and c) a second pair of paratopes, comprised as a diabody.

[000222] In some embodiments, the tetravalent, bi-specific antibody comprises first and second light chains comprising the same amino acid sequence, and first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-

terminus: a) a first pair of paratopes comprised in Fab regions; b) an Fc region; and c) a second pair of paratopes, comprised as a diabody.

[000223] Tetravalent antibodies as disclosed herein can have paratopes directed to a variety of epitopes and arranged in a variety of ways. Exemplary arrangements are described in FIGS. 1A-1C and FIG. 2 in which the paratopes are designated by compass position (NW, NE, SW, and SE).

[000224] Antibodies in any of these possible configurations are contemplated herein. For example, an antibody could be described as having paratopes in the NW and NE positions directed to a first epitope on a first antigen, and paratopes in the SW and SE positions directed to a second epitope on a second antigen. Or, an antibody could be described as having paratopes in the NW and NE positions directed to a first epitope on a first antigen, and paratopes in the SW and SE positions directed to a second epitope on the same first antigen. Or, an antibody could be described as having a paratope in the NW position directed to a first epitope on a first antigen, a paratope in the NE position directed to a second epitope of the same first antigen, and paratopes in the SW and SE positions directed to a third epitope on a second antigen. Or, an antibody could be described as having a paratope in the NW position directed to a first epitope on a first antigen, a paratope in the SW position directed to a second epitope on the first antigen, and paratopes in the NE and SE positions directed to a third epitope on a second antigen.

[000225] In some embodiments, the antibody comprises paratopes in the NW and NE positions directed to a first epitope on a first antigen, and paratopes in the SW and SE positions directed to a second epitope on a second antigen. In some embodiments, the antibody comprises paratopes in the NW and NE positions directed to a first epitope on a first antigen, and paratopes in the SW and SE positions directed to a second epitope on the same first antigen. In some embodiments, the antibody comprises paratope in the NW position directed to a first epitope on a first antigen, a paratope in the NE position directed to a second epitope of the same first antigen, and paratopes in the SW and SE positions directed to a third epitope on a second antigen. In some embodiments, the antibody comprises paratope in the NW position directed to a first epitope on a first antigen, a paratope in the SW position directed to a second epitope on the first antigen, and paratopes in the NE and SE positions directed to a third epitope on a second antigen.

10. scFv/Fab-Fc-Fab/scFv

[000226] In one format, shown in Fig. 3J, a tetravalent, bi-specific antibody comprises two copies of a first light chain, and first and second heavy chains comprising the different amino acid sequences. The antibody comprises, from N-terminus to C-terminus: a) a first (NW)

paratope comprising an scFv and a second (NE) paratope comprising a Fab; b) an Fc region; and c) a third (SE) paratope comprising an scFv and a fourth (SW) paratope comprising an Fab.

[000227] In some embodiments, the tetravalent, bi-specific antibody comprises two copies of a first light chain, and first and second heavy chains comprising the different amino acid sequences. In some embodiments, the antibody comprises, from N-terminus to C-terminus: a) a first (NW) paratope comprising an scFv and a second (NE) paratope comprising a Fab; b) an Fc region; and c) a third (SE) paratope comprising an scFv and a fourth (SW) paratope comprising an Fab.

D. Fab-Diabody-Fc Platform

[000228] An antibody platform having a "Fab-Fc-Diabody" configuration is presented in FIG. 3A.

[000229] In this configuration, the antibody has four paratopes, two configured as Fab regions, and two configured as diabodies. In the north-south, or N terminus-C terminus orientations, the Fab regions are positioned north of, or more toward the N terminus of, the diabody regions. Accordingly, both the Fab regions and the diabody regions are N-terminal of the Fc region. Thus, the tetravalent antibody comprises a pair of paratopes comprised in two Fab regions, a pair of paratopes comprised in a diabody, and an Fc region; wherein the antibody is arranged, from N-terminus to C-terminus: Fab regions, diabody, Fc region.

[000230] In some embodiments, the antibody has four paratopes, two configured as Fab regions, and two configured as diabodies. In some embodiments, the Fab regions are positioned north of, or more toward the N terminus of, the diabody regions. In some embodiments, both the Fab regions and the diabody regions are N-terminal of the Fc region. In some embodiments, the tetravalent antibody comprises a pair of paratopes comprised in two Fab regions, a pair of paratopes comprised in a diabody, and an Fc region; wherein the antibody is arranged, from N-terminus to C-terminus: Fab regions, diabody, Fc region.

[000231] The antibodies comprise first and second light chains, and first and second heavy chains. The first light chain can comprise, from amino terminus to carboxy terminus: (1) a first light chain variable (VL) region and (2) a first light chain constant (CL) region.

[000232] The first light chain can comprise, from amino terminus to carboxy terminus: (1) a first light chain variable (VL) region and (2) a first light chain constant (CL) region. In some embodiments, the first light chain can comprise, from amino terminus to carboxy terminus: (1) a first light chain variable (VL) region and (2) a first light chain constant (CL) region.

[000233] The second light chain can comprise, from amino terminus to carboxy terminus: (1) a second light chain variable (VL) region and (2) a second light chain constant (CL) region. In some embodiments, the second light chain can comprise, from amino terminus to carboxy terminus: (1) a second light chain variable (VL) region and (2) a second light chain constant (CL) region.

[000234] The first heavy chain can comprise, from amino terminus to carboxy terminus: (1) a first heavy chain portion comprising a first heavy chain variable (VH) region and a first heavy chain constant 1 (CH1) region; (2) a first diabody region comprising a second heavy chain variable (VH) region and a third light chain variable (VL) region (in either order); and a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region (or fragment thereof). In some embodiments, the first heavy chain can comprise, from amino terminus to carboxy terminus: (1) a first heavy chain portion comprising a first heavy chain variable (VH) region and a first heavy chain constant 1 (CH1) region; (2) a first diabody region comprising a second heavy chain variable (VH) region and a third light chain variable (VL) region (in either order); and a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region (or fragment thereof).

[000235] The second heavy chain can comprise, from amino terminus to carboxy terminus: (1) a second heavy chain portion comprising a third heavy chain variable (VH) region and a second heavy chain constant 1 (CH1) region; (2) a second diabody region comprising a fourth heavy chain variable (VH) region and a fourth light chain variable (VL) region (in either order); and a second Fc region comprising a second heavy chain constant 2 (CH2) region and a second heavy chain constant 3 (CH3) region (or fragment thereof). In some embodiments, the second heavy chain can comprise, from amino terminus to carboxy terminus: (1) a second heavy chain portion comprising a third heavy chain variable (VH) region and a second heavy chain constant 1 (CH1) region; (2) a second diabody region comprising a fourth heavy chain variable (VH) region and a fourth light chain variable (VL) region (in either order); and a second Fc region comprising a second heavy chain constant 2 (CH2) region and a second heavy chain constant 3 (CH3) region (or fragment thereof).

[000236] In this configuration the first light chain and the first heavy chain portion form a first Fab comprising a first paratope. The second light chain and the second heavy chain portion form a second Fab comprising a second paratope.

[000237] The first and second diabody regions form two paratopes, designated here paratope 3 and paratope 4. Paratope 3 can comprise the second variable heavy region and the fourth variable light region. Paratope 4 can comprise the fourth variable heavy region

and the third variable light region. In some embodiments, the first and second diabody regions form two paratopes, designated here paratope 3 and paratope 4. In some embodiments, paratope 3 comprises the second variable heavy region and the fourth variable light region. In some embodiments, paratope 4 comprises the fourth variable heavy region and the third variable light region.

[000238] In this configuration the first heavy chain Fab region can be connected with the diabody region through a peptide or polypeptide linker. Similarly, the diabody region can be connected to the Fc region through a peptide or polypeptide linker. In some embodiments, the first heavy chain region is connected with the diabody region through a peptide or polypeptide linker.

[000239] In the Fab region, light chain can be connected to the heavy chain portion through one or more disulfide bonds. The Fc regions of the heavy chain also can be connected through one or more disulfide bonds, for example, between the peptide or polypeptide linkers. In some embodiments, in the Fab region, the light chain is connected to the heavy chain portion through one or more disulfide bonds. In some embodiments, the Fc regions of the heavy chain is connected through one or more disulfide bonds, for example, between the peptide or polypeptide linkers.

[000240] This platform contemplates monospecific, bi-specific, tri-specific, and tetra-specific configurations. In one bi-specific configuration, paratope 1 and paratope 2 bind to the same epitope; and paratope 3 and paratope 4 bind the same epitope, which is different from the epitope bound by paratopes 1 and 2. In another bi-specific configuration, the two epitopes bound by paratopes 1 and 2, and paratopes 3 and 4 bind different epitopes of the same antigen. In a tri-specific configuration, two of the paratopes bind the same epitope, and the other two paratopes each bind different epitopes. In a tetra-specific configuration each paratope binds a different epitope, the two epitopes bound by paratopes 1 and 2, and paratopes 3 and 4 bind different epitopes of the same antigen. In some embodiments, two of the paratopes bind the same epitope, and the other two paratopes each bind different epitopes.

[000241] In some embodiments, the antibody is bi-specific. In some embodiments, the antibody is tri-specific. In some embodiments, the antibody is tetra-specific. In some embodiments, paratope 1 and paratope 2 bind to the same epitope; and paratope 3 and paratope 4 bind the same epitope, which is different from the epitope bound by paratopes 1 and 2. In some embodiments, each paratope binds a different epitope (the two epitopes bound by paratopes 1 and 2, and paratopes 3 and 4 bind different epitopes of the same antigen).

[000242] As disclosed herein, antibodies can be engineered in which paratopes that bind two different epitopes share a common light chain.

Diabody-Fc-Fab Platform

[000243] An antibody platform having a "Diabody-Fc-Fab" configuration is presented in FIG. 3B.

[000244] In this configuration, the antibody has four paratopes, two configured into a diabody, and two configured as Fab regions. In the north-south, or N terminus-C terminus, orientations, the diabody regions are positioned north of, or N-terminal to, the Fc region, and the Fab regions are positioned south of, or C-terminal to, the Fc region. Thus, the tetravalent antibody comprises a pair of paratopes comprised a diabody region, an Fc region, and a pair of paratopes comprised in two Fab regions; wherein the antibody is arranged, from N-terminus to C-terminus: diabody, Fc region, and Fab regions.

[000245] In some embodiments, the tetravalent antibody comprises a pair of paratopes comprised a diabody region, an Fc region, and a pair of paratopes comprised in two Fab regions; wherein the antibody is arranged, from N-terminus to C-terminus: diabody, Fc region, and Fab regions.

[000246] The antibodies comprise first and second light chains, and first and second heavy chains. In some embodiments, the antibodies comprise first and second light chains, and first and second heavy chains.

[000247] The first light chain can comprise, from amino terminus to carboxy terminus: (1) a first light chain variable (VL) region and (2) a first light chain constant (CL) region. In some embodiments, the first light chain can comprise, from amino terminus to carboxy terminus: (1) a first light chain variable (VL) region and (2) a first light chain constant (CL) region.

[000248] The second light chain can comprise, from amino terminus to carboxy terminus: (1) a second light chain variable (VL) region and (2) a second light chain constant (CL) region. In a symmetric diabody-Fc-Fab configuration, the second light chain is identical to the first. In some embodiments, the second light chain can comprise, from amino terminus to carboxy terminus: (1) a second light chain variable (VL) region and (2) a second light chain constant (CL) region. In a symmetric diabody-Fc-Fab configuration, the second light chain is identical to the first.

[000249] The first heavy chain can comprise, from amino terminus to carboxy terminus: (1) a first Diabody region comprising a first heavy chain variable (VH) region and a third light chain variable (VL) region (in either order); (2) a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region (or fragment

thereof); and (3) a first heavy chain portion comprising a second heavy chain variable (VH) region and a first heavy chain constant 1 (CH1) region. In some embodiments, the first heavy chain can comprise, from amino terminus to carboxy terminus: (1) a first Diabody region comprising a first heavy chain variable (VH) region and a third light chain variable (VL) region (in either order); (2) a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region (or fragment thereof); and (3) a first heavy chain portion comprising a second heavy chain variable (VH) region and a first heavy chain constant 1 (CH1) region.

[000250] The second heavy chain can comprise, from amino terminus to carboxy terminus: (1) a second diabody region comprising a third heavy chain variable (VH) region and a fourth light chain variable (VL) region (in either order); (2) a first Fc region comprising a second heavy chain constant 2 (CH2) region and a second heavy chain constant 3 (CH3) region what (or fragment thereof); and (3) a second heavy chain portion comprising a fourth heavy chain variable (VH) region and a second heavy chain constant 1 (CH1) region. In some embodiments, the second heavy chain can comprise, from amino terminus to carboxy terminus: (1) a second diabody region comprising a third heavy chain variable (VH) region and a fourth light chain variable (VL) region (in either order); (2) a first Fc region comprising a second heavy chain constant 2 (CH2) region and a second heavy chain constant 3 (CH3) region what (or fragment thereof); and (3) a second heavy chain portion comprising a fourth heavy chain variable (VH) region and a second heavy chain constant 1 (CH1) region.

[000251] In this configuration the first and second diabody regions form two paratopes, designated here paratope 1 and paratope 2. Paratope 1 can comprise the first variable heavy region and the fourth variable light region. Paratope 2 can comprise the third variable heavy region and the third variable light region. In some embodiments, the first and second diabody regions form two paratopes, designated here paratope 1 and paratope 2. In some embodiments, paratope 1 comprises the first variable heavy region and the fourth variable light region. In some embodiments, paratope 2 comprises the third variable heavy region and the third variable light region.

[000252] The first light chain and the second heavy chain portion form a first Fab comprising a paratope designated as paratope 3. The second light chain and the first heavy chain portion form a second Fab comprising a paratope designated paratope 4. In some embodiments, the first light chain and the second heavy chain portion form a first Fab comprising a paratope designated as paratope 3. In some embodiments, the second light chain and the first heavy chain portion form a second Fab comprising a paratope designated paratope 4.

[000253] In this configuration the diabody region can be connected to the Fc region through a peptide or polypeptide linker. Similarly, the Fc region can be connected to the Fab region through a peptide or polypeptide linker. In some embodiments, the diabody region is connected to the Fc region through a peptide or polypeptide linker. In some embodiments, the FC region is connected to the Fab region through a peptide or polypeptide linker.

[000254] In the Fab region, the light chain can be connected to the heavy chain portion through one or more disulfide bonds. The Fc regions of the heavy chain also can be connected through one or more disulfide bonds, for example, between the peptide or polypeptide linkers. In some embodiments, the light chain is connected to the heavy chain portion through one or more disulfide bonds. In some embodiments, the Fc regions of the heavy chain are connected through one or more disulfide bonds, for example, between the peptide or polypeptide linkers.

[000255] This platform contemplates monospecific, bi-specific, tri-specific, and tetra-specific configurations. In one bi-specific configuration, paratope 1 and paratope 2 bind to the same epitope; and paratope 3 and paratope 4 bind the same epitope, which is different from the epitope bound by paratopes 1 and 2. In another bi-specific configuration, the two epitopes bound by paratopes 1 and 2, and paratopes 3 and 4 bind different epitopes of the same antigen. In a tri-specific configuration, two of the paratopes bind the same epitope, and the other two paratopes each bind different epitopes. In a tetra-specific configuration each paratope binds a different epitope.

[000256] In certain embodiments the variable regions of the two light chains, or the entire two light chains, can be identical. This can be especially advantageous in an asymmetric antibody in which the first and second heavy chain variable regions are different, because, in the cell the same light chain associates with either heavy chain portion. In comparison, if different light chains are used, they may improperly associate with the incorrect heavy chain portion resulting in possibly non-functional antibodies. In some embodiments, the variable regions of the two light chains, or the entire two light chains, are identical. In some embodiments, the variable regions of the two light chains, or the entire two light chains, are not identical.

[000257] The light chain variable regions of the diabody regions can be identical, but need not be, because they will normally associate with the correct variable heavy chain portions.

E. Muc16/NaPi-2b Antibodies

[000258] Muc16 is expressed in cancers such as ovarian, breast, lung and pancreatic cancers. Sofituzumab is an antibody that binds Muc16. NaPi-2b is also expressed in ovarian, lung and breast cancers. Lifastuzumab is an antibody that binds NaPi-2b.

[000259] In one embodiment, a tetravalent antibody comprises first and second paratopes that bind the same epitope of Muc16 (e.g., in the NW/NE positions), and third and fourth paratopes that bind the same epitope of NaPi-2b (e.g., in the SW/SE positions). These arrangements can be reversed.

IV. Antibody Conjugates

[000260] An antibody conjugated to a chemical entity (i.e., "payload") is referred to herein as an "antibody conjugate" or an "immunoconjugate". Conjugation can be effected by covalent coupling or non-covalent interactions (e.g., ionic, van der Waals, electrostatic, or hydrogen bonds). The chemical entity conjugated to the antibody also can be referred to as a moiety. Chemical moieties or payloads include, without limitation, a drug, a radionuclide, a biotin, RNA, an antibiotic, a protein and a detectable moiety(e.g., a fluorophore).

[000261] As used herein, the term "antibody-drug conjugate" or "ADC" refers to an immunoconjugate wherein an antibody is conjugated to a drug, such as a cytotoxic drug, or an immune stimulator. Cytotoxic drugs include, without limitation, maytansinoid, DM-1, DM-4, auristatin, monomethyl auristatin E , monomethyl auristatin F, dolastatin, tubulysin, eribulin, cryptophycin, benzodiazepine, indolino-benzodiazepine, isoquinolidino-benzodiazepine, pyrrolo-benzodiazepine, alpha-amanitin, trichothecene, camptothecin or camptothecin derivatives (SN-38, exatecan, belotecan, DXd, topotecan, samrotecan), duocarmycin, DGN549, CC1065, calicheamicin, N-acetyl calicheamicin, an enediyne antibiotic, taxane, doxorubicin or doxorubicin derivatives (e.g., succinyl-DOX, Cis-aconityl DOX, DOX-2, dodecanoyl-doxorubicin, and TPP-DOX) anthracycline and stereoisomers, azonafide, as well as isosteres, analogs, heterodimers, homodimers or derivatives of the foregoing. Cytotoxic drugs may also comprise any of the drug "payloads" in Colombo et al. Cancer Discov (2024) 14 (11): 2089–2108, which is incorporated by reference herein in its entirety.

[000262] As used herein, the term "labeled antibody" refers to an antibody bound to a detectable label such that the presence of a target may be detected by detecting the presence of the detectable label bound to the target. As used herein, the term "detectable label" refers to a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. Examples of detectable labels are described herein and include, without limitation, colorimetric, fluorescent, chemiluminescent, enzymatic, and radioactive labels. For the purposes of the present disclosure, a detectable label can also be a moiety that does not itself produce a signal (*e.g.*, biotin), but that binds to a second moiety that is able to produce a signal (*e.g.*, labeled avidin or streptavidin).

[000263] The term "small molecule" refers to an organic or inorganic molecule having a size up to about 5000 Da, up to about 2000 Da, or up to about 1000 Da.

[000264] "Potency" refers to the ability of a compound to kill cells, e.g., in culture, or prevent their growth. Potency may be evaluated via the IC_{50} or IC_{90} of a compound or via other metrics known in the art.

[000265] Many conjugation methods are known in the art. In some embodiments, the payload is conjugated to the antibody through a linker. Methods of conjugating the linker to the antibody include, without limitation, conjugation to cysteine residues of the antibody, and conjugation to lysine, tyrosine or arginine residues of the antibody.

A. Cysteine Residue Conjugation

[000266] Cysteine residue conjugation involves reacting the thiol (-SH) groups of cysteine residues in an antibody with a payload (e.g., a linker attached to a cytotoxic drug). Thiol groups can be introduced, for example, by reducing existing disulfide bonds to expose free thiols, or by engineering additional cysteine residues into the antibody. The exposed thiol groups are reactive and can be conjugated with, for example, maleimide-functionalized drugs or labels, forming thioether bonds. Methods of maleimide-thiol coupling are well-known in the art.

B. Lysine Residue Conjugation

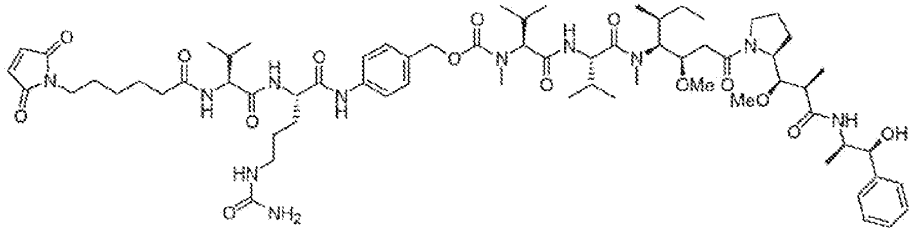
[000267] Lysine residue conjugation involves reacting the amine groups ($-NH_2$) on the side chains of lysine residues with a linker or linker-payload. This can be done, for example, by using N-hydroxysuccinimide (NHS) esters, which react with the amine groups to form amide bonds. Methods of NHS ester-amine coupling are well-known in the art.

C. Linker Chemistries

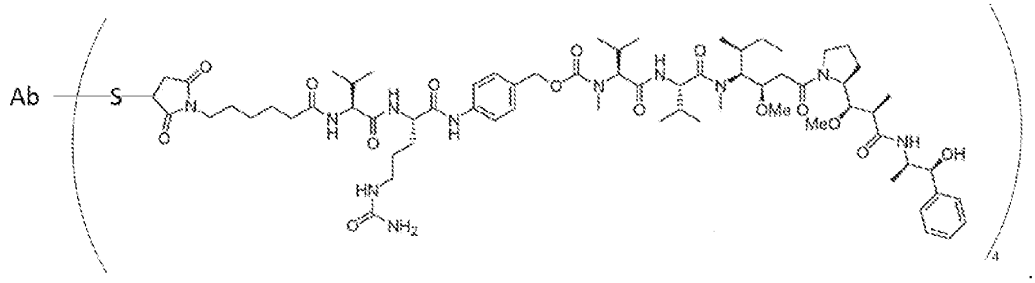
[000268] Payloads are conjugated to antibodies typically though linkers. Linkers include cleavable linkers and non-cleavable linkers. Cleavable linkers are typically sensitive to cleavage in the intracellular environment but not substantially sensitive to the extracellular environment, such that the conjugate is cleaved from the antibody when it is internalized. In some embodiments, the cleavable linker is a chemical cleavable linker. In some embodiments, the cleavable linker is an enzyme cleavable linker. Examples of cleavable linkers include linkers comprising hydrazone, disulfide, valine-citrulline, or beta-glucuronide groups.

[000269] In some embodiments, the linker is a peptidyl linker that is cleavable by an intracellular peptidase or protease enzyme. Typically, the peptidyl linker is at least two amino acids long or at least three or four amino acids long. Cleaving agents can include cathepsins

B and D and plasmin (see, e.g., Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123). Most typical are peptidyl linkers that are cleavable by enzymes that are present in certain cells. For example, a peptidyl linker that is cleavable by the thiol-dependent protease cathepsin-B, which is highly expressed in cancerous tissue, can be used (e.g., a linker comprising a Phe-Leu or a Gly- Phe-Leu-Gly peptide). Other such linkers are described, e.g., in U.S. Patent No.6,214,345. In specific embodiments, the peptidyl linker cleavable by an intracellular protease comprises a Val- Cit linker or a Phe-Lys dipeptide (see, e.g., U.S. patent 6,214,345, which describes the synthesis of doxorubicin with the Val-Cit linker). In some embodiments, the linker is a maleimidocaproyl-L-valine-L-citrulline-p-aminobenzyl linker (Mc-Val-Cit-PAB). In some embodiments, the linker-payload is Mc-VC-PAB-MMAE or vedotin:



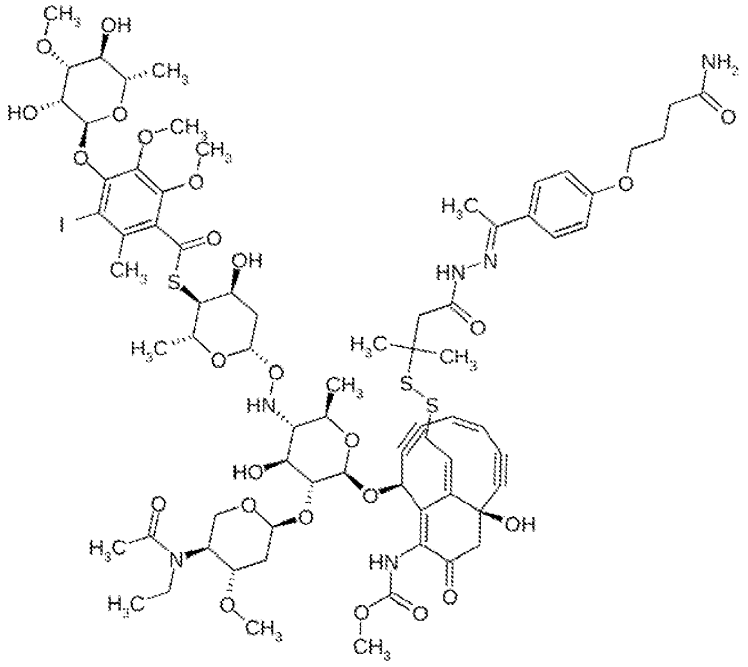
[000270] In some embodiments, the ADC is



[000271] In some embodiments, the cleavable linker is pH-sensitive, i.e., sensitive to hydrolysis at certain pH values. Typically, the pH-sensitive linker is hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (e.g., a hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, or the like) can be used. (See, e.g., U.S. Patent Nos.5,122,368; 5,824,805; 5,622,929; Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123; Neville et al, 1989, Biol. Chem.264: 14653-14661.) Such linkers are relatively stable under neutral pH conditions, such as those in the blood, but are unstable at below pH 5.5 or 5.0, the approximate pH of the lysosome. In certain embodiments, the hydrolyzable linker is a thioether linker (such as, e.g., a thioether attached to the therapeutic agent via an acylhydrazone bond (see, e.g., U.S. Patent No. 5,622,929)).

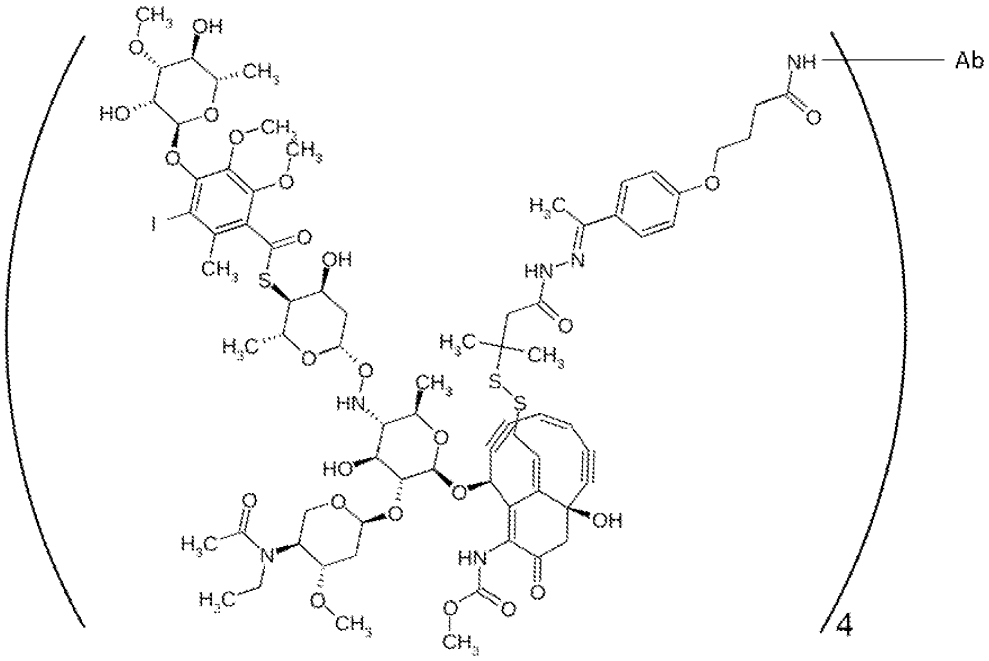
[000272] In some embodiments, the cleavable linker is cleavable under reducing conditions. Disulfide linkers include those that can be formed using SATA (N-succinimidyl-S-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyl-oxycarbonyl- alpha-methyl-alpha-(2-pyridyl-dithio)toluene), SPDB and SMPT. {See, e.g., Thorpe et al, 1987, Cancer Res.47:5924-5931; Wawrzynczak et al, In Immunoconjugates: Antibody Conjugates in Radioimagery and Therapy of Cancer (C. W. Vogel ed., Oxford U. Press, 1987. See also U.S. Patent No.4,880,935.)

[000273] In some embodiments, the linker-payload is ozogamicin. In some embodiments, the linker-payload is

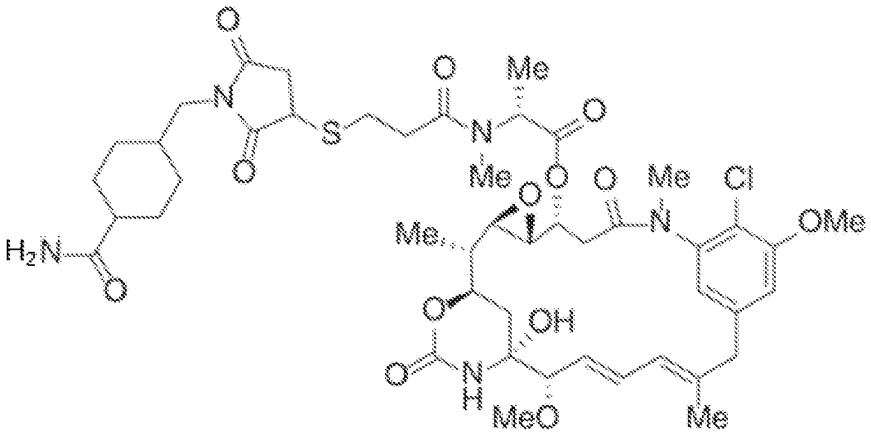


.

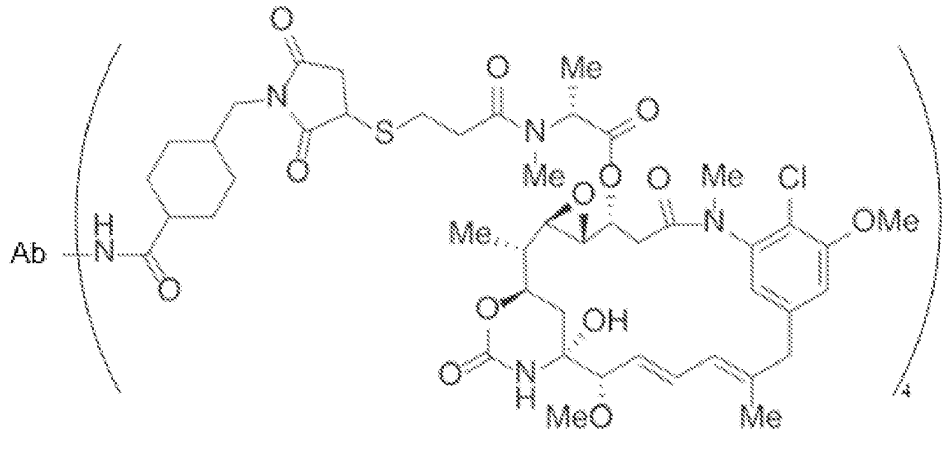
[000274] In some embodiments, the ADC is:



[000275] In some embodiments, the linker-payload is emtansine:

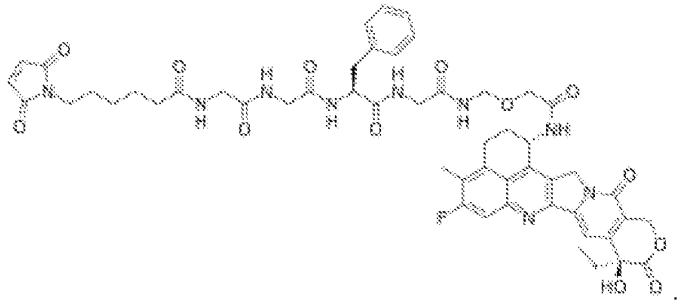


[000276] In some embodiments, the ADC is:

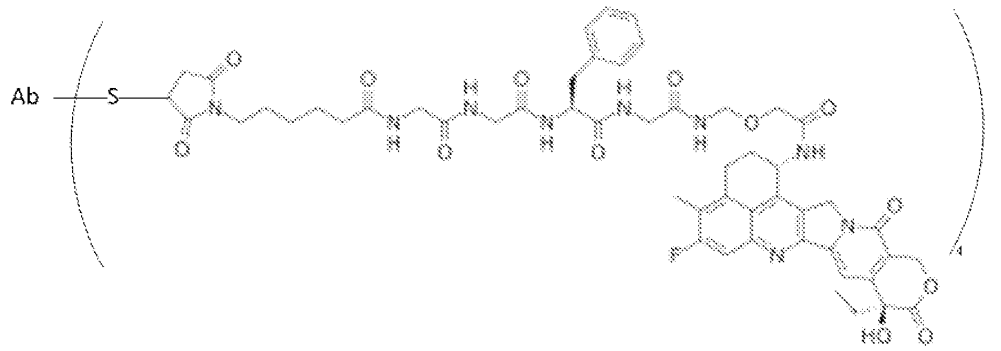


[000277] In some embodiments, the linker-payload is pasudotox. In some embodiments, the ADC comprises an antibody and four pasudotox moieties conjugated thereto.

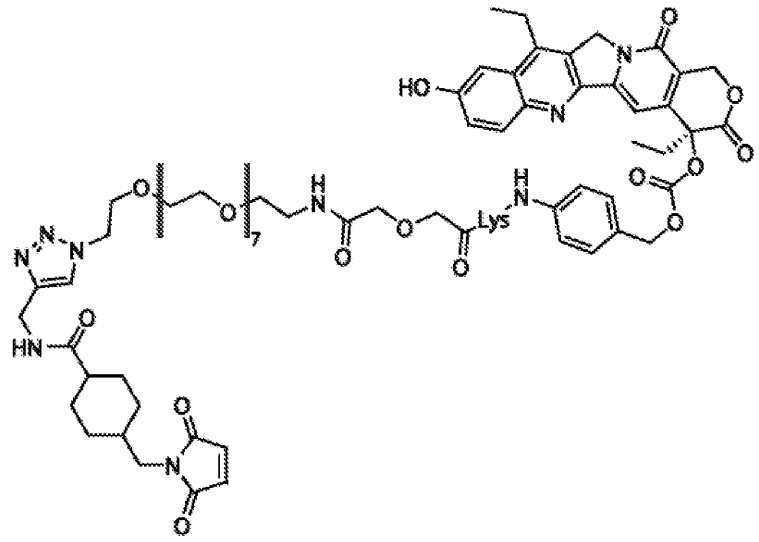
[000278] In some embodiments, the linker-payload is deruxtecan:



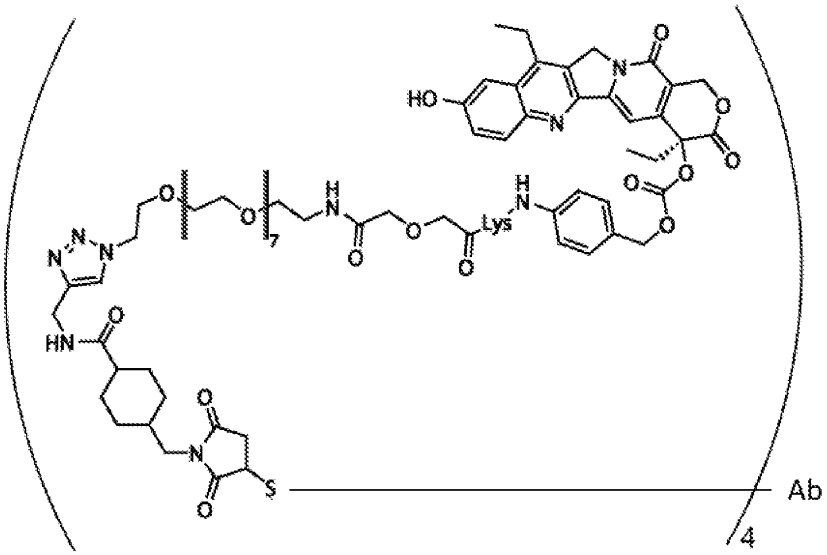
[000279] In some embodiments, the ADC is:



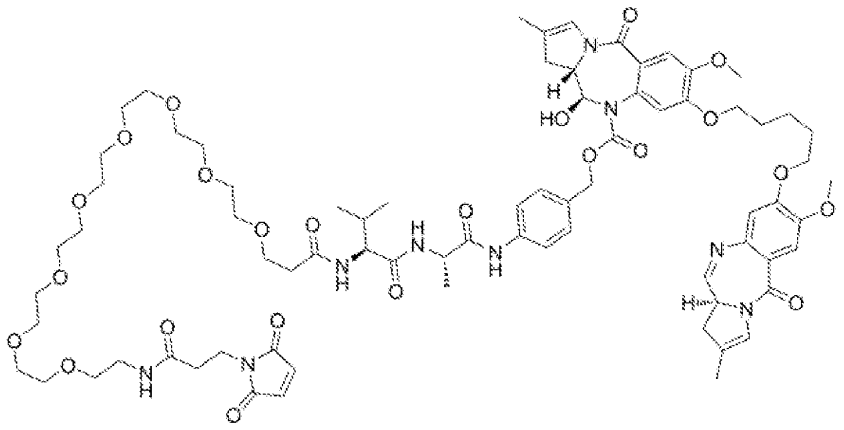
[000280] In some embodiments, the linker-payload is govitecan:



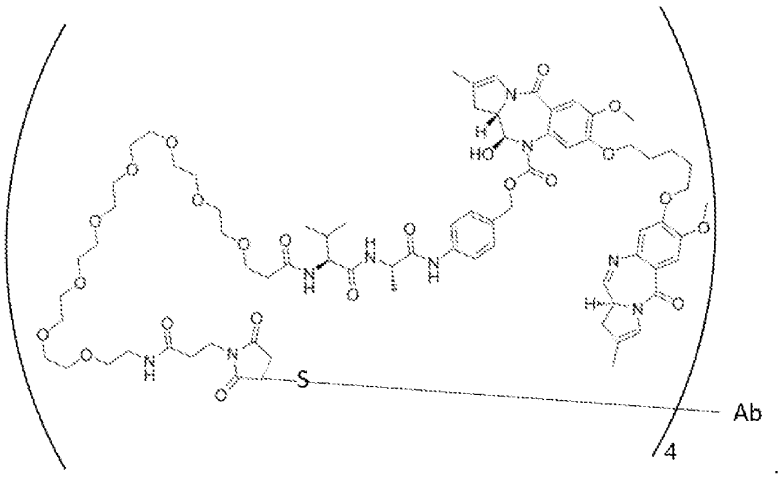
[000281] In some embodiments, the ADC is:



[000282] In some embodiments, the linker-payload is teserine:



[000283] In some embodiments, the ADC is:



[000284] The linker can also be a malonate linker (Johnson et al, 1995, Anticancer Res.15:1387-93), a maleimidobenzoyl linker (Lau et al, 1995, Bioorg-Med-Chem.3(10):1299-1304), or a 3'-N-amide analog (Lau et al, 1995, Bioorg-Med-Chem.3(10):1305-12). The linker also can be a non-cleavable linker, such as an maleimido-alkylene- or maleimide-aryl linker that is directly attached to the payload. The payload of an ADC comprising a non-cleavable linker is released by degradation of the antibody.

[000285] Linkers also can be conjugated to an antibody through a non-natural amino acid, disulfide re-bridging, a peptide tag, glycan modification, or an enzymatic modification method used to generate site-specific immunoconjugates. In some embodiments, the linker comprises a maleimide or a dibromo maleimide. In some embodiments, the linker comprises an iodoacetamide. In some embodiments, the linker comprises a pyridyl disulfide.

D. Drug-Antibody Ratio

[000286] The drug-antibody ratio, or "DAR", refers to the number of payload moieties conjugated to an antibody or the average number of payload moieties conjugated to antibodies in a composition. DAR is a function of the number of conjugatable sites on an antibody. When disulfide bonds of an antibody are reduced, producing reactive thiols, this may allow a DAR between 1 and 12. However, the introduction of cysteine residues into an immunoglobulin chain can significantly increase the possible DAR. In some embodiments, the DAR of an ADC composition can be, for example, at least any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 or more.

E. Cysteine-substituted Antibodies

[000287] A cysteine-substituted antibody is an antibody comprising at least one constant region immunoglobulin amino acid residue that has been substituted with a cysteine residue that is not present in the naturally occurring antibody. These are sometimes referred to as "THIOMABS" (Junutula et al. Nature Biotechnology, 2008:26(8):925-32, doi: 10.1038/nbt.1480). A non-naturally occurring substitution is one that is not isotypic. The thiol group(s) of the cysteine engineered antibodies can be conjugated to a drug moiety (e.g., via a linker) to form a THIOMAB™ drug conjugate (TDC). THIOMAB sites may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In one embodiment, the substituted residues in the heavy chain constant regions can include residues A118C, T155C, S157C, V266C, H285C, R301C, V303C, T307C, G316C, Y436C and L441C. These sites are based on EU numbering, as known in the art and described at www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnber.html and Edelman, G.M. et al., Proc. Natl. Acad. USA, 63, 78-85 (1969). PMID: 5257969. The substituted residues in the light chain constant region can include residue V205C (Kabat numbering; Kabat, E.A. et al., Sequences of proteins of immunological interest. 5th Edition - US Department of Health and Human Services, NIH publication no. 91-3242, pp 662,680,689 (1991)). In some embodiments, the constant region is of isotype IgG1, IgG2, IgG3 or IgG4.

[000288] Any form of antibody may be so engineered, i.e. mutated. For example, a parent monoclonal antibody may be engineered to form a "THIOMAB antibody." It should be noted a single site mutation yields two engineered cysteine residues in a THIOMAB antibody, due to the dimeric nature of the IgG antibody.

[000289] Cysteine engineered antibodies of the invention preferably retain the antigen binding capability of their wild type, parent antibody counterparts. Thus, cysteine engineered antibodies are capable of binding, preferably specifically, to antigens. Such antigens include,

for example, tumor-associated antigens (TAA), cell surface receptor proteins and other cell surface molecules.

[000290] Cysteine engineered antibodies of the present disclosure may be site-specifically and efficiently coupled with a thiol-reactive reagent. The thiol-reactive reagent may be a multifunctional linker reagent, a capture, i.e. affinity, label reagent (e.g. a biotin-linker reagent), a detection label (e.g. a fluorophore reagent), a solid phase immobilization reagent (e.g. SEPHAROSE, polystyrene, or glass), or a drug-linker intermediate. One example of a thiol-reactive reagent is N-ethyl maleimide (NEM). In an exemplary embodiment, reaction of a THIOMAB™ antibody with a biotin-linker reagent provides a biotinylated THIOMAB™ antibody by which the presence and reactivity of the engineered cysteine residue may be detected and measured. Reaction of a THIOMAB antibody with a multifunctional linker reagent provides a THIOMAB antibody with a functionalized linker which may be further reacted with a drug moiety reagent or other label. Reaction of a THIOMAB antibody with a drug-linker intermediate provides a THIOMAB antibody drug conjugate.

V. Nucleic Acids, Recombinant Cells In Methods Of Making

[000291] Provided herein are nucleic acid molecules encoding immunoglobulin chains of the antibodies of this disclosure. The antibodies of this disclosure can comprise a plurality of different polypeptides assembled into an antibody. For example, an antibody can comprise two identical heavy chains and two identical light chains. Alternatively, an antibody can comprise two different heavy chains. Further combinations of different polypeptides are possible, such as, for example, two different heavy chains and one or two different light chains. Therefore, further provided herein are nucleic acid molecules or collections of nucleic acid molecules that, as an ensemble, encode polypeptides which, when assembled, produce the antibodies of this disclosure.

A. Nucleic Acid Molecules

[000292] Antibodies of this disclosure can be made by expressing polypeptides from nucleic acid molecules that encode them. Nucleic acids comprising nucleotide sequences that encode heavy chain and light chain molecules can be incorporated into recombinant DNA molecules (such as expression vectors), transfected into cells, expressed by the cells in culture, harvested and purified. Such methods are well known in the fields of molecular biology, cell biology and biopharmaceutical manufacturing.

[000293] A nucleic acid molecule encodes a polypeptide if it comprises a nucleotide sequence that, upon transcription and/or translation, produce the polypeptide. This includes, without limitation, contiguous nucleotide sequences that encode a polypeptide and noncontiguous nucleotide sequences that encode a polypeptide. Noncontiguous nucleotide

sequences encoding a polypeptide include, for example, nucleotide sequences that include both introns and exons. Such non-coding sequences are spliced out of messenger RNAs.

[000294] Exemplary nucleic acid sequences for the tetravalent antibodies are represented by SEQ ID NOs: 106-120. In some embodiments, the tetravalent bispecific antibody comprises SEQ ID NO: 106. In some embodiments, the tetravalent bispecific antibody comprises SEQ ID NO: 107. In some embodiments, the tetravalent bispecific antibody comprises SEQ ID NO: 108. In some embodiments, the tetravalent bispecific antibody comprises SEQ ID NO: 109. In some embodiments, the tetravalent bispecific antibody comprises SEQ ID NO: 110. In some embodiments, the tetravalent bispecific antibody comprises SEQ ID NO: 111. In some embodiments, the tetravalent bispecific antibody comprises SEQ ID NO: 112. In some embodiments, the tetravalent bispecific antibody comprises SEQ ID NO: 1113. In some embodiments, the tetravalent bispecific antibody comprises SEQ ID NO: 1114. In some embodiments, the tetravalent bispecific antibody comprises SEQ ID NO: 115.

B. Expression Constructs and Vectors

[000295] The nucleic acid molecules encoding immunoglobulin molecules can be incorporated into an expression construct for expression. An expression construct is a polynucleotide comprising an expression control sequence operatively linked with a heterologous nucleotide sequence (i.e., a sequence to which the expression control sequence is not normally connected to in nature) that is to be expressed.

[000296] An expression control sequence is a nucleotide sequence that regulates transcription and/or translation of a nucleotide sequence operatively linked thereto. Expression control sequences include promoters, enhancers, repressors (transcription regulatory sequences) and ribosome binding sites (translation regulatory sequences). Suitable regulatory sequences may be derived from a variety of sources, including bacterial, fungal, viral, mammalian , or insect genes.

[000297] A nucleotide sequence is “operatively linked” with an expression control sequence when the expression control sequence functions in a cell to regulate transcription of the nucleotide sequence. This includes initiating transcription of the nucleotide sequence through an interaction between a polymerase and a transcriptional regulatory sequence such as, for example, a promoter.

[000298] Promoters typically used in eukaryotic expression systems include, without limitation, the following. CMV (Cytomegalovirus) promoter, EF-1α (Elongation Factor-1

alpha) promoter, SV40 (Simian Virus 40) promoter, PGK (Phosphoglycerate kinase) promoter, CAG promoter, AOX1 (Alcohol oxidase 1) promoter, GAP (Glyceraldehyde-3-phosphate dehydrogenase) promoter. Some promoters used in yeast, such as Saccharomyces cerevisiae, are ADH (Alcohol dehydrogenase) promoter, GAL1 and GAL10.

[000299] An expression vector is a polynucleotide comprising an expression construct and sequences sufficient for replication in a host cell or insertion into a host chromosome. A vector can comprise any intermediary vehicle for a nucleic acid molecule which enables said nucleic acid molecule, for example, to be introduced into prokaryotic and/or eukaryotic cells and/or integrated into a genome. Exemplary vectors for transfection of cells include, for example, a plasmid, a viral vector (e.g., a retrovirus, a lentivirus, an adenovirus, a papovavirus (e.g., SV40, polyoma) a parvovirus (e.g., adeno-associated virus), or a herpes simplex virus), a bacterial artificial chromosome, and a yeast artificial chromosome. The term "plasmid" as used herein generally refers to a construct of extrachromosomal genetic material, usually a circular DNA duplex, which can replicate independently of chromosomal DNA. The vector should be compatible with the host cell used. Other sequences, such as an origin of replication, DNA restriction sites, enhancers, and sequences conferring inducibility of transcription may be incorporated into the expression vector.

[000300] The recombinant expression vectors may also contain a marker gene which facilitates the selection of host cells transformed, infected or transfected with a vector for expressing an antibody described herein.

[000301] The recombinant expression vectors may also contain expression cassettes which encode a fusion moiety which provides increased expression or stability of the recombinant peptide; increased solubility of the recombinant peptide; or that aids in the purification of the target recombinant peptide by acting as a ligand in affinity purification, including for example, tags and labels described herein (e.g., a $(His)_6$ tag). A recombinant peptide and its fusion moiety are collectively termed a “fusion protein.” Further, a proteolytic cleavage site may be added to the fusion protein to allow separation of the recombinant peptide from the fusion moiety subsequent to purification of the fusion protein. Typical fusion expression vectors include pGEX (Amrad Corp., Melbourne, Australia), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the recombinant protein.

[000302] The recombinant expression vectors may also contain a signal peptide. signal peptides can be used for efficient secretion of recombinant proteins. Signal peptides are short N-terminal amino acid sequences, to direct proteins to the secretory pathway. In some

embodiments, the recombinant expression vector comprises a signal peptide. In some embodiments, the signal peptide comprises any one of SEQ ID NOs: 116-147.

C. Recombinant Cells

[000303] Antibodies as disclosed herein can be made by culturing cells engineered to express nucleic acid constructs encoding immunoglobulin polypeptides. In some embodiments, the antibodies disclosed herein are made by culturing cells engineered to express nucleic acid constructs encoding immunoglobulin polypeptides.

[000304] The recombinant host cell can be generated using any cell suitable for producing a polypeptide, for example, suitable for producing an antibody. For example, to introduce a nucleic acid (e.g., a vector) into a cell, the cell may be transfected, transformed or infected, depending upon the vector employed. In some embodiments, the recombinant host cell is generated by introducing a nucleic acid (e.g., a vector) into a cell. In some embodiments, a cell is transfected with the nucleic acid to generate the recombinant host cell. In some embodiments a cell is transformed with the nucleic acid to generate the recombinant host cell. In some embodiments a cell is infected with the nucleic acid to generate the recombinant host cell.

[000305] Methods for transfecting cells with recombinant DNA molecules are known in the art and include, for example, electroporation, liposomes and exosomes, and transfection via viral vectors.

[000306] Suitable host cells include a wide variety of prokaryotic and eukaryotic host cells. For example, the proteins described herein may be expressed in a mammalian cell (e.g., CHO, 293); an insect cell (e.g., in Sf9 cell or a high five (BTI-Tn-5B1-4) cell); a yeast cell (e.g., Pichia pastoris or and Saccharomyces cerevisiae); a bacterial cell (e.g., E. coli); or a plant cell (e.g., Arabidopsis. In some embodiments the antibody is expressed in a mammalian cell. In some embodiments, the mammalian cell is a Chinese hamster ovary (CHO) cell. In some embodiments, the mammalian cell is a human embryonic kidney-293 (HEK-293) cell. In some embodiments, the antibody is expressed. In some embodiments the antibody is expressed in an insect cell. In some embodiments, the insect cell is a Sf9 cell. In some embodiments, the insect cell is a high five (BTI-Tn-5B1-4) cell. In some embodiments the antibody is expressed in a yeast cell. In some embodiments, the yeast cell is a Pichia pastoris. In some embodiments, the yeast cell is a Saccharomyces cerevisiae. In some embodiments, the antibody is expressed in a bacterial cell. In some embodiments, the bacterial cell is an E. coli cell. In some embodiments, the antibody is expressed from a plant cell. In some embodiments, the plant cell is an Arabidopsis cell.

[000307] Mammalian cells that may be suitable include, among others: a Chinese hamster ovary (CHO) cell, an NSO cell, an SP2/0 cell, a human embryonic kidney HEK 293 cell, or a Per.C6 cell). Suitable expression vectors for directing expression in mammalian cells generally include a promoter (e.g., derived from viral genomes such as polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40), as well as other transcriptional and translational control sequences. Examples of mammalian expression vectors include pCDM8, pMT2PC and pOmni. In some embodiments, the mammalian expression vector is pCDM8. In some embodiments, the mammalian expression vector is pMT2PC.

[000308] Yeast and fungal host cells suitable for expressing an antibody include, but are not limited to Saccharomyces cerevisiae, Schizosaccharomyces pombe, the genera Pichia or Kluyveromyces and various species of the genus Aspergillus. Examples of vectors for expression in yeast S. cerevisiae include pYepSec1, pMFa, pJRY88, and pYES2 (Invitrogen Corporation, San Diego, CA). Protocols for the transformation of yeast and fungi are well known to those of ordinary skill in the art.

D. Antibody Production

[000309] Transformed/transfected cells are grown under suitable conditions that allow them to express the recombinant antibody.

[000310] The number of different immunoglobulin chains that must be expressed in a cell to produce an antibody depends on the configuration of the antibody. Generally, fewer different chains results in more effective antibody assembly.

[000311] Antibodies described herein may contain one or two different heavy chains, depending on architecture. Antibodies with the same heavy chain require only production of only one heavy chain in a cell, and they will associate naturally. If the antibody comprises different heavy chains, two polypeptides are produced, and knob-into-hole arrangements can facilitate proper assembly of antibodies, discouraging association of two “knob” chains or two “hole” chains.

[000312] Light chains also will associate with their respective heavy chains. Where the antibody comprises two of the same heavy chains, then only one light chain need also be expressed. However, in certain asymmetric antibodies, two different light chains may be needed to associate with the asymmetric heavy chains. However, in certain situations, it may be possible to produce two different paratopes that comprise different heavy chain regions and the same light chain.

[000313] For cells that secrete the recombinant antibody chains, culture medium comprising the immunoglobulin molecules is harvested and antibodies are purified from the

medium using protein A or protein G chromatography, which binds specifically to the Fc region of antibodies. In certain embodiments, recombinant cells containing light chains and/or heavy chains are lysed, and the immunoglobulin chains are purified from the lysate using standard biochemical procedures.

[000314] If desired, purified antibodies are then conjugated with payloads of the kind and by the methods of which are described herein.

VI. Pharmaceutical Compositions

[000315] Further provided herein is a composition comprising an antibody, immunoconjugate, nucleic acid molecule, vector or recombinant cell described herein, optionally with a suitable diluent, e.g., a pharmaceutically acceptable carrier. The composition can for example, comprise one or more antibodies or immunoconjugates.

[000316] In one embodiment, a pharmaceutical composition comprises an antibody or immunoconjugate as described herein formulated in a pharmaceutically acceptable carrier. The composition is typically formulated for intravenous injection. Accordingly, the carrier can be an aqueous carrier, such as sterilized water or saline solution. The composition can further comprise ingredients to stabilize the antibody molecules. These can include, for example, sugars and polyols, which assist in freeze-drying. Mannitol also can be used as a tonicity adjuster and bulking agent in freeze-dried formulations. Surfactants, such as polysorbate 20 or polysorbate 80 are also commonly used in monoclonal antibody formulations. Buffers, such as phosphate, histidine and citrate, are frequently used in such formulations.

[000317] In some embodiments, the composition is formulated for intradermal injection. In some embodiments, the composition is formulated for subcutaneous injection. In some embodiments, the composition is formulated for intramuscular injection. In some embodiments, the composition is formulated for intraperitoneal injection. In some embodiments, the composition is formulated for intranodal injection. In some embodiments, the composition is formulated for intrasplenic injection.

[000318] The compositions described herein can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions that can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle.

[000319] The term “pharmaceutically acceptable” refers to a carrier that is compatible with the other ingredients of a pharmaceutical composition and can be safely administered to a subject. The term is used synonymously with “physiologically acceptable” and

"pharmacologically acceptable". Pharmaceutical compositions and techniques for their preparation and use are known to those of skill in the art in light of the present disclosure. For a detailed listing of suitable pharmacological compositions and techniques for their administration one may refer to texts such as Remington's Pharmaceutical Sciences, 17th ed. 1985; Brunton et al., "Goodman and Gilman's The Pharmacological Basis of Therapeutics," McGraw-Hill, 2005; University of the Sciences in Philadelphia (eds.), "Remington: The Science and Practice of Pharmacy," Lippincott Williams & Wilkins, 2005; and University of the Sciences in Philadelphia (eds.), "Remington: The Principles of Pharmacy Practice," Lippincott Williams & Wilkins, 2008.

[000320] Pharmaceutically acceptable carriers will generally be sterile, at least for human use. A pharmaceutical composition will generally comprise agents for buffering and preservation in storage, and can include buffers and carriers for appropriate delivery, depending on the route of administration. Examples of pharmaceutically acceptable carriers include, without limitation, normal (0.9%) saline, phosphate-buffered saline (PBS) Hank's balanced salt solution (HBSS) and multiple electrolyte solutions such as PlasmaLyte ATM (Baxter).

[000321] Suitable diluents for polypeptides, including antibodies and/or cells include but are not limited to saline solutions, pH buffered solutions and glycerol solutions or other solutions suitable for freezing polypeptides and/or cells.

[000322] Suitable diluents for nucleic acids include but are not limited to water and saline solutions.

[000323] Pharmaceutical compositions include, without limitation, lyophilized powders or aqueous or non-aqueous sterile injectable solutions or suspensions, which may further contain antioxidants, buffers, bacteriostats and solutes that render the compositions substantially compatible with the tissues or the blood of an intended recipient. Other components that may be present in such compositions include water, surfactants (such as Tween), alcohols, polyols, glycerin and vegetable oils, for example. Exemplary injection solutions and suspensions may be prepared from sterile powders, granules, tablets, or concentrated solutions or suspensions. The composition may be supplied, for example, but not by way of limitation, as a lyophilized powder which is reconstituted with sterile water or saline prior to administration to the patient.

[000324] Pharmaceutical compositions may comprise a pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers include essentially chemically inert and nontoxic compositions that do not interfere with the effectiveness of the biological activity of the pharmaceutical composition. Examples of suitable pharmaceutical carriers

include, but are not limited to, water, saline solutions, glycerol solutions, ethanol, N-(1(2,3-dioleyloxy)propyl)N,N,N-trimethylammonium chloride (DOTMA), diolesylphosphotidyl-ethanolamine (DOPE), and liposomes. Such compositions should contain a therapeutically effective amount of the compound, optionally with a suitable amount of carrier so as to provide the form for direct administration to the patient. The terms “dose” and “dosage” are used interchangeably herein. A dose refers to the amount of active ingredient given to an individual at each administration. For the present invention, the dose can refer to the concentration of the antibody or associated components, e.g., the amount of therapeutic agent or dosage of radiolabel. The dose will vary depending on a number of factors, including frequency of administration; size and tolerance of the individual; severity of the condition; risk of side effects; the route of administration; and the imaging modality of the detectable label (if present). One of skill in the art will recognize that the dose can be modified depending on the above factors and/or based on therapeutic progress. The term “dosage form” refers to the particular format of the pharmaceutical, and depends on the route of administration. For example, a dosage form can be in a liquid, e.g., a saline solution for injection.

VII. Methods of Use

A. Methods of Treatment

1. Diseases

[000325] Provided herein are methods of treating a disease in a subject that comprises administering to the subject an effective amount of a nucleic acid, an antibody or an immunoconjugate of this disclosure. An “effective amount” is an amount sufficient for an antibody or immunoconjugate to accomplish a stated purpose relative to the absence of the compound. The stated purpose may be, for example, killing pathological cells, either *in vitro* or *in vivo*, or producing a therapeutic benefit to a subject. An example of an “effective amount” is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which could also be referred to as a “therapeutically effective amount.” A “reduction” of, or “reducing” a symptom means elimination of, or decreasing of the severity or frequency of, the symptom. A “prophylactically effective amount” of a substance (e.g., an antibody) is an amount of that substance, when administered to a subject, will prevent or delay the onset or reoccurrence of a disease, pathology or condition, or its symptoms, or reduce the likelihood of such. An effective amount does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, an effective amount may be administered in one or more administrations. The exact amounts will depend on the purpose of the treatment, and

will be ascertainable by one skilled in the art using known techniques (see, e.g., Lieberman, Pharmaceutical Dosage Forms (vols. 1-3, 1992); Lloyd, The Art, Science and Technology of Pharmaceutical Compounding (1999); Pickar, Dosage Calculations (1999); and Remington: The Science and Practice of Pharmacy, 20th Edition, 2003, Gennaro, Ed., Lippincott, Williams & Wilkins).

[000326] For any antibody or ADC described herein, the therapeutically effective amount can be initially estimated or determined using cell culture assays. Target concentrations will be those concentrations of antibody or ADC that are capable of a desired result (e.g., slowing cell growth), as measured using the methods described herein or known in the art. As is well known in the art, therapeutically effective amounts for use in humans can also be determined from animal models. For example, a dose for humans can be formulated to achieve a concentration that has been found to be effective in animals. The dosage in humans can be adjusted by monitoring effectiveness and adjusting the dosage upwards or downwards, as described above. Adjusting the dose to achieve maximal efficacy in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan.

[000327] In certain embodiments, the antibody comprises at least one paratope that specifically binds an epitope of Muc16, e.g., an epitope targeted by the antibody sofituzumab; and at least one paratope that binds an epitope of NaPi-2b, e.g., targeted by the antibody lifastuzumab. The paratopes can have the amino acid sequences of the variable regions, or complete light chains, of these antibodies.

[000328] Cancers that can be treated include, without limitation, solid tumors such as ovarian cancer or endometrial cancer, lung cancer (e.g., non-small cell lung cancer or NSCLC), and pancreatic cancer.

[000329] Cancer cells that express both NaPi-2b and Muc16 are expected to be present in mixed populations in which different cells express each of these biomarkers to a different degree. Without wishing to be limited by theory, bi-specific, ADC antibodies are expected to be more effective against such populations than monospecific ADC antibodies.

2. Routes of Administration

[000330] Pharmaceutical compositions comprising antibodies of this disclosure can be administered to a subject by any suitable route of administration.

[000331] Intravenous administration involves delivering the pharmaceutical composition directly into the bloodstream which facilitates rapid systemic distribution and enables immediate therapeutic action. Intravenous administration can comprise administration of a

bolus or can comprise a slow infusion depending on the desired pharmacokinetic profile in the therapeutic indication.

[000332] Intraperitoneal administration involves introducing the pharmaceutical composition into the peritoneal cavity. This mode of administration is especially useful for diseases localized within the abdominal region, as it offers direct access to the site of pathology.

[000333] The pharmaceutical composition can be administered subcutaneously, that is into the fatty tissue underneath the skin.

[000334] In intramuscular administration, a pharmaceutical composition is injected into muscle tissue. In certain cancers, the pharmaceutical composition can be administered directly into the tumor.

[000335] For diseases of the central nervous system the composition can be administered intrathecally or intraventricularly, e.g., into the spinal canal or the brain ventricles.

3. Treatment Regimen

[000336] The treatment regimen for cancers using the antibodies or ADC's of this disclosure is customized to the cancer in the patient. However, exemplary regimens for existing treatments include the following. Between 1 mg and 12 mg of antibody or ADC per kilogram of body weight is administered as a dose. After an initial dose, the drug can be administered about every week for about every three weeks for five or more cycles, e.g., until remission, sufficient reduction of symptoms, complete reduction of symptoms, or until side effects or toxicity become unmanageable or the drug ceases to have therapeutic benefit.

B. Methods of Killing Cells

[000337] The antibodies and immunoconjugates of this disclosure are useful for killing cells or labeling cells, either *in vitro* or *in vivo*. The methods involve contacting the cells with an antibody or immunoconjugate of this disclosure (e.g., by introducing said antibody or immunoconjugate into a subject in need thereof) and allowing the antibodies to bind to the target cells. Effectiveness of killing cells can be a function of ability to kill target cells, but not non-target cells in a mixed population of cells. Ability to kill cells also can be compared with that of a control, e.g., a control antibody, wherein greater ability of the test antibody compared with the control antibody to kill cells indicates that the test antibody is effective in killing those cells.

[000338] Contacting cells from cancer cell lines with the compositions disclosed herein, *in vitro*, is useful for determining toxicity of the composition on the cells.

[000339] Contacting cells *in vivo* with immunoconjugates labeled with a radioisotope is useful in imaging the location of binding and, therefore, the location of a malignant cell. Detection methods include gamma camera imaging, single photon emission coupled tomography (SPECT), and positron emission tomography (PET).

C. Methods of Detection

[000340] Antibodies of this disclosure are also useful to detect the presence of cells expressing Muc16 and/or NaPi-2b in a sample or in a subject. The methods involve contacting the sample or providing the subject with an antibody of this disclosure and detecting binding between the antibody and a cell.

[000341] Binding can be detected by providing antibodies bearing detectable labels, and detecting the label.

[000342] Methods of detection include, without limitation, enzyme-linked immunosorbent assay (ELISA), immunofluorescence (including fluorescent-activated cell sorting (FACS), surface plasmon resonance, microscopy and immunoprecipitation.

[000343] *In vivo* methods of detection include, for example, positron emission tomography (PET), in which antibodies are conjugated with a radioactive isotope; and magnetic resonance imaging (MRI), in which antibodies are conjugated with MRI contrast agents.

[000344] Such methods are also useful in the diagnosis of malignancies. The detection of cells expressing Muc16 and/or NaPi-2b in amounts above control amounts indicates the presence of malignant cells.

EXEMPLARY EMBODIMENTS I

[000345] 1. An antibody comprising:

a) first and second heavy chains, each comprising (SEQ ID NO:27); and

b) first and second light chains, each comprising (SEQ ID NO:16).

[000346] 2. An antibody comprising:

a) first and second heavy chains, each comprising (SEQ ID NO:28); and

b) first and second light chains, each comprising (SEQ ID NO:8).

[000347] 3. An antibody comprising:

a) first and second heavy chains, each comprising (SEQ ID NO:29); and

b) first and second light chains, each comprising (SEQ ID NO:8)

[000348] 4. An antibody comprising:

a) first and second heavy chains, each comprising (SEQ ID NO:56); and

b) first and second light chains, each comprising (SEQ ID NO:8)

[000349] 5. An antibody comprising:

a) first and second heavy chains, each comprising (SEQ ID NO:57); and

b) first and second light chains, each comprising (SEQ ID NO:8)

[000350] 6. An antibody comprising:

a) first and second heavy chains, each comprising (SEQ ID NO:58); and

b) first and second light chains, each comprising (SEQ ID NO:8)

[000351] 7. An antibody comprising:

a) first and second heavy chains, each comprising (SEQ ID NO:59); and

b) first and second light chains, each comprising (SEQ ID NO:8).

[000352] 8. The antibody of any of embodiments 1 to 7, further comprising one or more payloads conjugated thereto.

[000353] 9. The antibody of embodiment 8, wherein at least one payload is monomethyl auristatin E (MMAE).

[000354] 10. An antibody comprising a first paratope and a second paratope, wherein one of the paratopes specifically binds to Muc16 and another of the paratopes specifically binds to NaPi-2b.

[000355] 11. The antibody of embodiment 10, wherein the paratopes comprise a variable light chain region and a variable heavy chain portion.

[000356] 12. The antibody of embodiment 10, comprising an intact, tetrameric antibody or an antigen-binding fragment thereof.

[000357] 13. The antibody of embodiment 10, wherein either or both of the first and second paratopes comprises a fragment antigen binding (Fab), a fragment antigen-binding 2 (F(ab')2), a single chain Fab (scFab), a single chain variable fragment (scFv), a diabody, or a single domain antibody (sdAb) or a VHH antibody.

[000358] 14. The antibody of embodiment 10, wherein at least one of the paratopes is a scFv comprising a variable heavy chain (VH)-linker-variable light chain (VL) or a VL-linker-VH orientation.

[000359] 15. The antibody of any one of embodiments 10-14, wherein the paratope that binds Muc16 comprises:

a) heavy chain (HC) complementarity determining regions:

HCDR1: GYSITNDYAWN (SEQ ID NO:1)

HCDR2: YISYSGYTT (SEQ ID NO:2)

HCDR3: WTSGLDY (SEQ ID NO:3); and

b) light chain (LC) complementarity determining regions:

LCDR1: KASDLIHNWLA (SEQ ID NO:4)

LCDR2: GATSLET (SEQ ID NO:5)

LCDR3: QQYWTTPFT (SEQ ID NO:6)

[000360] 16. The antibody of any one of embodiments 10-15, wherein the paratope that binds NaPi-2b comprises:

a) heavy chain (HC) complementarity determining regions:

HCDR1: GFSFSDFAMS (SEQ ID NO:9)

HCDR2: TIGRVAFHTY (SEQ ID NO:10)

HCDR3: HRGFDVGHFDF (SEQ ID NO:11)

b) light chain (LC) complementarity determining regions:

LCDR1: RSSETLVHSSGNTYLE (SEQ ID NO:12)

LCDR2: RVSNRFS (SEQ ID NO:13)

LCDR3: FQGSFNPLT (SEQ ID NO:14)

[000361] 17. The antibody of any one of embodiments 10-15, wherein the paratope that specifically binds Muc16 comprises:

a) a heavy chain variable region sequence SEQ ID NO:7; and

b) a light chain variable region sequence SEQ ID NO:8.

[000362] 18. The antibody of any one of embodiments 10-17, wherein the paratope that specifically binds NaPi-2b comprises:

a) a heavy chain variable region sequence (SEQ ID NO:34); and

b) a light chain variable region sequence (SEQ ID NO:35).

[000363] 19. The antibody of any one of embodiments 10-18, comprising:

a) heavy chain sequences SEQ ID NO:7 and SEQ ID NO:15; and

b) light chain sequences SEQ ID NO:8 and SEQ ID NO:16; or

c) a functional variant thereof having at least 90%, at least 95%, at least 97%, at least 98% or at least 99% sequence identity thereto.

[000364] 20. The antibody of any one of embodiments 10-19, wherein the antibody is a monoclonal antibody.

[000365] 21. The antibody of embodiment 10, wherein the antibody is bi-specific and bivalent.

[000366] 22. The antibody of embodiment 21, comprising:

a) a first paratope comprised in an Fab;

b) a second paratope comprised in an Fab; and

c) an Fc region configured as a knob-into-hole.

[000367] 23. The antibody of embodiment 21, comprising:

a) a first paratope comprised in an Fab;

b) a second paratope comprised in an scFv; and

c) an Fc region configured as a knob-into-hole.

[000368] 24. The antibody of embodiment 21, comprising:

a) a first paratope and a second paratope comprised in a diabody; and

b) an Fc region configured as a knob-into-hole.

[000369] 25. The antibody of embodiment 10, which is bi-specific and trivalent.

[000370] 26. The antibody of embodiment 25, comprising two paratopes that specifically bind Muc16 and one paratope that specifically binds NaPi-2b.

[000371] 27. The antibody of embodiment 25, comprising two paratopes that specifically bind NaPi-2b and one paratope that specifically binds Muc16.

[000372] 28. The antibody of embodiment 25, comprising:

a) a first light chain comprising, from N-terminus to C-terminus, a first light chain variable (VL) region and a first light chain constant (CL) region;

b) a first heavy chain comprising, from N-terminus to C-terminus:

(i) a first heavy chain portion comprising a first heavy chain variable (VH) region and a first heavy chain constant 1 (CH1) region;

(ii) a first diabody region comprising a second VH region and a second VL region, in either order; and

(iii) a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region comprising a knob or a hole;

c) the second heavy chain comprising, from N-terminus to C-terminus:

(i) a second diabody region comprising a third VH region and a third VL region, in either order; and

(iii) a second Fc region comprising a second heavy chain constant 2 (CH2) region and a second heavy chain constant 3 (CH3) region comprising a hole or a knob, respectively;

wherein:

the two heavy chains are associated by a knob-into-hole arrangement;

the first light chain and the first heavy chain portion form a first Fab region comprising a first paratope;

the first and second diabody regions form a diabody having a second paratope comprising the third VL region and the second VH region, and a third paratope comprising the second VL region and the third VH region.

[000373] 29. The antibody of embodiment 25, comprising:

a) first heavy chain comprising from N-terminus to C-terminus:

i) a bivalent first paratope, Fab+Fab; and

ii) an Fc region with a knob or hole; and

b) second heavy chain comprising from N-terminus to C-terminus:

i) a monovalent second paratope as an scFv

ii) an Fc region with a hole or knob, respectively.

[000374] 30. The antibody of embodiment 10, wherein the antibody is bi-specific and tetravalent.

[000375] 31. The antibody of embodiment 30, comprising two paratopes that specifically bind Muc16 and two paratopes that specifically bind NaPi-2b.

[000376] 32. The antibody of embodiment 30, comprising three paratopes that specifically bind Muc16 and one paratope that specifically binds NaPi-2b.

[000377] 33. The antibody of embodiment 30, comprising three paratopes that specifically bind NaPi-2b and one paratope that specifically binds Muc16.

[000378] 34. The antibody of embodiment 30, wherein the paratopes that specifically bind Muc16 are located at NW and NE positions, and the paratopes that specifically bind NaPi-2b are located at SW and SE positions.

[000379] 35. The antibody of embodiment 30, wherein the paratopes that specifically bind NaPi-2b are located at NW and NE positions, and the paratopes that specifically bind Muc16 are located at SW and SE positions.

[000380] 36. The antibody of embodiment 30, wherein:

a) at least two paratopes are comprised in Fab regions;

b) at least two paratopes are comprised in scFvs; or

c) at least two paratopes are comprised in diabodies.

[000381] 37. The antibody of embodiment 30, comprising first and second light chains and first and second heavy chains, wherein:

a) the first light chain comprises, from N-terminus to C-terminus, a first light chain variable (VL) region and a first light chain constant (CL) region;

b) the second light chain comprises, from N-terminus to C-terminus, a second VL region and a second CL region;

wherein the first and second light chains comprise the same amino acid sequence;

c) the first heavy chain comprises, from N-terminus to C-terminus:

(i) a first heavy chain portion comprising a first heavy chain variable (VH) region and a first heavy chain constant 1 (CH1) region;

(ii) a first diabody region comprising a second VH region and a third VL region, in either order; and

(iii) a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region;

d) the second heavy chain comprises, from N-terminus to C-terminus:

(i) a second heavy chain portion comprising a third VH region and a second CH1 region;

(ii) a second diabody region comprising a fourth VH region and a fourth VL region, in either order; and

(iii) a second Fc region comprising a second CH2 region and a second CH3 region;

wherein the first and second heavy chains comprise the same amino acid sequence; and

wherein:

the two heavy chains are associated;

the first and second diabody regions form a diabody having a first paratope comprising the fourth VL region and the second VH region, and a second paratope comprising the third VL region and the fourth VH region;

the first light chain and the first heavy chain portion form a first Fab region comprising a third paratope; and

the second light chain and the second heavy chain portion form a second Fab region comprising a fourth paratope.

[000382] 38. The antibody of embodiment 30, comprising first and second light chains and first and second heavy chains, wherein:

a) the first light chain comprises, from N-terminus to C-terminus, a first light chain variable (VL) region and a first light chain constant (CL) region;

b) the second light chain comprises, from N-terminus to C-terminus, a second VL region and a second CL region;

wherein the first and second light chains comprise the same amino acid sequence;

c) the first heavy chain comprises, from N-terminus to C-terminus:

(i) a first diabody region comprising a first heavy chain variable (VH) region and a third VL region, in either order; and

(ii) a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region; and

(iii) a first heavy chain portion comprising a second VH region and a first heavy chain constant 1 (CH1) region;

d) the second heavy chain comprises, from N-terminus to C-terminus:

(i) a second diabody region comprising a third VH region and a fourth VL region, in either order; and

(ii) a second Fc region comprising a second CH2 region and a second CH3 region; and

(iii) a second heavy chain portion comprising a fourth VH region and a second CH1 region;

wherein the first and second heavy chains comprise the same amino acid sequence;

wherein:

the two heavy chains are linked;

the first and second diabody regions form a diabody having a first paratope comprising the fourth VL region and the first VH region, and a second paratope comprising the third VL region and the third VH region;

the first light chain and the first heavy chain portion form a first Fab region comprising a third paratope; and

the second light chain and the second heavy chain portion form a second Fab region comprising a fourth paratope.

[000383] 39. The antibody of embodiment 30, comprising first and second light chains and first and second heavy chains, wherein:

a) the first light chain comprises, from N-terminus to C-terminus, a first light chain variable (VL) region and a first light chain constant (CL) region;

b) the second light chain comprises, from N-terminus to C-terminus, a second VL region and a second CL region;

wherein the first and second light chains comprise the same amino acid sequence;

c) the first heavy chain comprises, from N-terminus to C-terminus:

(i) a first heavy chain portion comprising a first heavy chain variable (VH) region and a first heavy chain constant 1 (CH1) region;

(ii) a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region; and

(iii) a first scFV region comprising a second VH region and a third VL region, in either order; and

d) the second heavy chain comprises, from N-terminus to C-terminus:

(i) a second heavy chain portion comprising a third VH region and a second CH1 region;

(ii) a second Fc region comprising a second CH2 region and a second CH3 region;

(iii) a second scFV region comprising a fourth VH region and a fourth VL region, in either order; and

wherein the first and second heavy chains comprise the same amino acid sequence;

wherein:

the two heavy chains are associated;

the second VH region and a third VL region of the first scFv region assemble into a first paratope;

the fourth VH region and a fourth VL region of the second scFv region assemble into a second paratope;

the first light chain and the first heavy chain portion form a first Fab region comprising a third paratope; and

the second light chain and the second heavy chain portion form a second Fab region comprising a fourth paratope.

[000384] 40. The antibody of embodiment 30, comprising first and second light chains and first and second heavy chains, wherein:

a) the first light chain comprises, from N-terminus to C-terminus, a first light chain variable (VL) region and a first light chain constant (CL) region;

b) the second light chain comprises, from N-terminus to C-terminus, a second VL region and a second CL region;

wherein the first and second light chains comprise the same amino acid sequence;

c) the first heavy chain comprises, from N-terminus to C-terminus:

(i) a first heavy chain portion comprising a first heavy chain variable (VH) region and a first heavy chain constant 1 (CH1) region;

(ii) a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region; and

(iii) a second heavy chain portion comprising a second heavy chain variable (VH) region and a second heavy chain constant 1 (CH1) region;

d) the second heavy chain comprises, from N-terminus to C-terminus:

(i) a first scFV region comprising a third VL region and a third VH region;

(ii) a second Fc region comprising a second CH2 region and a second CH3 region;

(iii) a second scFV region comprising a fourth VH region and a fourth VL region;

wherein:

the two heavy chains are associated;

the first light chain and the first heavy chain portion form a first Fab region comprising a first paratope;

the second light chain and the second heavy chain portion form a second Fab region comprising a second paratope;

the third VH region and a third VL region of the first scFv region form a third paratope; and

the fourth VH region and a fourth VL region of the second scFv region form a fourth paratope.

[000385] 41. The antibody of embodiment 30, comprising first and second heavy chains, wherein:

a) the first heavy chain comprises, from N-terminus to C-terminus:

(i) a first diabody region comprising a first heavy chain variable (VH) region and a first VL region, in either order;

(ii) a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region; and

(iii) a second diabody region comprising a second heavy chain variable (VH) region and a second VL region, in either order;

d) the second heavy chain comprises, from N-terminus to C-terminus:

(i) a third diabody region comprising a third heavy chain variable (VH) region and a third VL region, in either order;

(ii) a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region; and

(iii) a fourth diabody region comprising a fourth heavy chain variable (VH) region and a fourth VL region, in either order;

wherein the first and second heavy chains comprise the same amino acid sequence;

wherein:

the two heavy chains are associated;

the first and third diabody regions form a diabody having a first paratope comprising the first VL region and the third VH region, and a second paratope comprising the third VL region and the first VH region;

the second and fourth diabody regions form a diabody having a third paratope comprising the fourth VL region and the second VH region, and a fourth paratope comprising the second VL region and the fourth VH region.

[000386] 42. The antibody of embodiment 30, comprising first and second heavy chains, wherein:

a) the first heavy chain comprises, from N-terminus to C-terminus:

(i) a first diabody region comprising a first heavy chain variable (VH) region and a first VL region, in either order;

(ii) a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region; and

(iii) a first scFV region comprising a second VL region and a second VH region;

b) the second heavy chain comprises, from N-terminus to C-terminus:

(i) a second diabody region comprising a third heavy chain variable (VH) region and a third VL region, in either order;

(ii) a second Fc region comprising a second heavy chain constant 2 (CH2) region and a second heavy chain constant 3 (CH3) region; and

(iii) a second scFV region comprising a fourth VH region and a fourth VL region;

wherein the first and second heavy chains comprise the same amino acid sequence;

wherein:

the two heavy chains are associated;

the first and second diabody regions form a diabody having a first paratope comprising the first VL region and the third VH region, and a second paratope comprising the third VL region and the first VH region;

the second VH region and the second VL region of the first scFv region form a third paratope; and

the fourth VH region and a fourth VL region of the second scFv region form a fourth paratope.

[000387] 43. The antibody of embodiment 30, comprising first and second heavy chains, wherein:

a) the first heavy chain comprises, from N-terminus to C-terminus:

(i) a first scFV region comprising a first VL region and a first VH region;

(ii) a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region; and

(iii) a first diabody region comprising a second heavy chain variable (VH) region and a second VL region, in either order;

d) the second heavy chain comprises, from N-terminus to C-terminus:

(i) a second scFV region comprising a third VH region and a third VL region;

(ii) a second Fc region comprising a second heavy chain constant 2 (CH2) region and a second heavy chain constant 3 (CH3) region; and

(iii) a second diabody region comprising a fourth heavy chain variable (VH) region and a fourth VL region, in either order;

wherein the first and second heavy chains comprise the same amino acid sequence; and

wherein:

the two heavy chains are associated;

the first VH region and the first VL region of the first scFv region form a first paratope;

the third VH region and a third VL region of the second scFv region form a second paratope; and

the first and second diabody regions form a diabody having a third paratope comprising the fourth VL region and the second VH region, and a fourth paratope comprising the second VL region and the fourth VH region.

[000388] 44. The antibody of embodiment 30, comprising first and second heavy chains, wherein:

a) the first heavy chain comprises, from N-terminus to C-terminus:

(i) a first scFV region comprising a first VL region and a first VH region;

(ii) a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region; and

(iii) a second scFV region comprising a second VL region and a second VH region;

b) the second heavy chain comprises, from N-terminus to C-terminus:

(i) a third scFV region comprising a third VH region and a third VL region;

(ii) a second Fc region comprising a second heavy chain constant 2 (CH2) region and a second heavy chain constant 3 (CH3) region; and

(iii) a fourth scFV region comprising a fourth VL region and a fourth VH region;

wherein the first and second heavy chains comprise the same amino acid sequence; and

wherein:

the two heavy chains are associated;

the first VH region and the first VL region of the first scFv region form a first paratope;

the second VH region and the second VL region of the second scFv region form a first paratope;

the third VH region and a third VL region of the third scFv region form a third paratope.

the fourth VH region and the fourth VL region of the fourth scFv region form a first paratope.

[000389] 45. The antibody of embodiment 30, comprising first and second light chains and first and second heavy chains, wherein:

a) the first light chain comprises, from N-terminus to C-terminus, a first light chain variable (VL) region and a first light chain constant (CL) region;

b) the second light chain comprises, from N-terminus to C-terminus, a second VL region and a second CL region;

wherein the first and second light chains comprise the same amino acid sequence;

c) the first heavy chain comprises, from N-terminus to C-terminus:

(i) a first heavy chain portion comprising a first heavy chain variable (VH) region and a first heavy chain constant 1 (CH1) region;

(ii) a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region; and

(iii) a first diabody region comprising a second VH region and a third VL region, in either order; and

d) the second heavy chain comprises, from N-terminus to C-terminus:

(i) a second heavy chain portion comprising a third heavy chain variable (VH) region and a second heavy chain constant 1 (CH1) region;

(ii) a second Fc region comprising a second heavy chain constant 2 (CH2) region and a second heavy chain constant 3 (CH3) region; and

(iii) a second diabody region comprising a fourth VH region and a fourth VL region, in either order;

wherein the first and second heavy chains comprise the same amino acid sequence and

wherein:

the two heavy chains are associated;

the first and second diabody regions form a diabody having a first paratope comprising the third VL region and the fourth VH region, and a second paratope comprising the fourth VL region and the second VH region;

the first light chain and the first heavy chain portion form a first Fab region comprising a third paratope; and

the second light chain and the second heavy chain portion form a second Fab region comprising a fourth paratope.

[000390] 46. The antibody of embodiment 30, comprising first and second light chains and first and second heavy chains, wherein:

a) the first light chain comprises, from N-terminus to C-terminus, a first light chain variable (VL) region and a first light chain constant (CL) region;

b) the second light chain comprises, from N-terminus to C-terminus, a second VL region and a second CL region;

wherein the first and second light chains comprise the same amino acid sequence;

c) the first heavy chain comprises, from N-terminus to C-terminus:

(i) a first scFV region comprising a third VL region and a first VH region;

(ii) a first Fc region comprising a first heavy chain constant 2 (CH2) region and a first heavy chain constant 3 (CH3) region; and

(iii) a first heavy chain portion comprising a second heavy chain variable (VH) region and a first heavy chain constant 1 (CH1) region;

d) the second heavy chain comprises, from N-terminus to C-terminus:

(i) a second heavy chain portion comprising a third heavy chain variable (VH) region and a second heavy chain constant 1 (CH1) region;

(ii) a second Fc region comprising a second CH2 region and a second CH3 region;

(iii) a second scFV region comprising a fourth VH region and a fourth VL region;

wherein:

the two heavy chains are associated;

the first light chain and the first heavy chain portion form a first Fab region comprising a first paratope;

the second light chain and the second heavy chain portion form a second Fab region comprising a second paratope;

the first VH region and the third VL region of the first scFv region form a third paratope; and

the fourth VH region and a fourth VL region of the second scFv region form a fourth paratope.

[000391] 47. The antibody of embodiment 30, comprising first and second light chains comprising the same amino acid sequence, and first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus:

a) a first pair of paratopes comprised in Fab regions;

b) a second pair of paratopes, comprised in a diabody; and

c) an Fc region.

[000392] 48. The antibody of embodiment 30, comprising first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus:

a) a first pair of paratopes comprised in a diabody.

b) an Fc region; and

a) a second pair of paratopes comprised in Fab regions;

[000393] 49. The antibody of embodiment 30, comprising first and second light chains comprising the same amino acid sequence, and first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus:

a) a first pair of paratopes comprised in Fab regions;

b) an Fc region; and

c) a second pair of paratopes, each comprised in an scFv region.

[000394] 50. The antibody of embodiment 30, comprising first and second light chains comprising the same amino acid sequence, and first and second heavy chains comprising different amino acid sequences, and comprising, from N-terminus to C-terminus:

a) a first pair of paratopes, one paratope comprised in an Fab region and one paratope comprised in an scFv region;

b) an Fc region; and

c) a second pair of paratopes, one paratope comprised in an Fab region and one paratope comprised in an scFv region;

wherein one of the heavy chains comprises two VH regions and two VL regions forming the scFv regions.

[000395] 51. The antibody of embodiment 30, comprising first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus:

a) a first pair of paratopes comprised within a diabody;

b) an Fc region; and

c) a second pair of paratopes comprised within a diabody.

[000396] 52. The antibody of embodiment 30, comprising first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus:

a) a first pair of paratopes comprised within a diabody;

b) an Fc region; and

c) a second pair of paratopes, each comprised in an scFv region.

[000397] 53. The antibody of embodiment 30, comprising first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus:

a) a first pair of paratopes, each comprised in an scFv region;

b) an Fc region; and

c) a second pair of paratopes comprised within a diabody.

[000398] 54. The antibody of embodiment 30, comprising first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus:

a) a first pair of paratopes, each comprised as an scFv region;

b) an Fc region; and

c) a second pair of paratopes, each comprised as an scFv region.

[000399] 55. The antibody of embodiment 30, comprising first and second light chains comprising the same amino acid sequence, and first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus:

a) a first pair of paratopes comprised in Fab regions;

b) an Fc region; and

c) a second pair of paratopes, comprised as a diabody.

[000400] 56. The antibody of embodiment 30, comprising first and second light chains comprising the same amino acid sequence, and first and second heavy chains comprising the same amino acid sequence, and comprising, from N-terminus to C-terminus:

a) a first pair of paratopes, one comprised in an Fab region and one comprised in an scFv region;

b) an Fc region; and

c) a second pair of paratopes, one comprised in an scFv region and one comprised in an Fab region.

[000401] 57. The antibody of embodiment 30, wherein all of the light chain variable regions have the same amino acid sequence.

[000402] 58. The antibody of any of embodiments 10-57, further comprising one or more payloads conjugated thereto.

[000403] 59. The antibody of embodiment 57, wherein at least one payload is selected from the group consisting of: a drug, a radionucleotide, a fluorophore, biotin, RNA, an antibiotic, a protein and a detectable moiety.

[000404] 60. The antibody of embodiment 57, wherein at least one of the payloads is selected from maytansinoid, DM-1, DM-4, auristatin, monomethyl auristatin E, monomethyl auristatin F, dolastatin, tubulysin, eribulin, cryptophycin, benzodiazepine, indolino-benzodiazepine, isoquinolidino-benzodiazepine, pyrrolo-benzodiazepine, alpha-amanitin, trichothene, camptothecin derivatives (SN-38, exatecan, belotecan, DXd, topotecan, samrotecan), duocarmycin, DGN549, CC1065, calicheamicin, N-acetyl calicheamicin,an enediyne antibiotic, taxane, doxorubicin derivatives, anthracycline and stereoisomers, azanofide, isosteres, and analogs, hetero dimer or homo dimers or derivatives thereof.

[000405] 61. The antibody of embodiment 57, wherein at least one payload is attached to the antibody via a cleavable (enzymatic or non-enzymatic) or non-cleavable or traceless linker.

[000406] 62. The antibody of embodiment 61, wherein at least one of the linkers is conjugated through:

a) a reduced disulfide bond in the antibody; or

b) an amino acid residue (e.g., cysteine or lysine) naturally present or introduced into an amino acid sequence of the antibody.

[000407] 63. The antibody of embodiment 62, comprising amino acid substitutions:

(a) HC-A118C and LC-V205C; and/or

(b) HC-S156C, HC-S239C, and HC-V266C (EU numbering).

[000408] 64. The antibody of embodiment 61, wherein at least one of the linkers is conjugated through a non-natural amino acid, disulfide re-bridging, a peptide tag, glycan modification, or an enzymatic modification method used to generate site-specific immunoconjugates.

[000409] 65. The antibody of any of embodiments 10-63, wherein, for an antibody comprising an Fab, an scFv or a diabody, the region comprising the Fab, scFv or diabody is attached to another region of the antibody through a linker.

[000410] 66. The antibody of embodiment 65, wherein the linker comprises between 1 and 50 amino acids.

[000411] 67. The antibody of embodiment 66, wherein the linker is wherein the linker is a monomer or polymer of the sequence (GGGGS)n, wherein n is any of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[000412] 68. An antibody comprising:

a) a heavy chain variable region comprising:

HCDR1: GYSITNDYAWN (SEQ ID NO:1)

HCDR2: YISYSGYTT (SEQ ID NO:2)

HCDR3: WTSGLDY (SEQ ID NO:3)

b) a light chain variable region comprising:

LCDR1: KASDLIHNWLA (SEQ ID NO:4)

LCDR2: GATSLET (SEQ ID NO:5)

LCDR3: QQYWTTPFT (SEQ ID NO:6)

c) a heavy chain variable region comprising:

HCDR1: GFSFSDFAMS (SEQ ID NO:9)

HCDR2: TIGRVAFHTY (SEQ ID NO:10)

HCDR3: HRGFDVGHFDF (SEQ ID NO:11); and

d) a light chain variable region comprising:

LCDR1: RSSETLVHSSGNTYLE (SEQ ID NO:12)

LCDR2: RVSNRFS (SEQ ID NO:13)

LCDR3: FQGSFNPLT (SEQ ID NO:14).

[000413] 69. The antibody of embodiment 68, comprising:

a) a heavy chain variable region comprising:

EVQLVESGGGLVQPGGSLRLSCAASGYSITNDYAWNWVRQAPGK GLEWVGYISYSGYTTYNPSLKSRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARWT SGLDYWGQGTLVTVSSC (SEQ ID NO:32)

b) a light chain variable region comprising:

DIQMTQSPSSLSASVGDRVTITCKASDLIHNWLAWYQQKPGKAPKL LIYGATSLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYWTTPFTFGQGTK VEIK (SEQ ID NO:33)

c) a heavy chain variable region comprising:

EVQLVESGGGLVQPGGSLRLSCAASGFSFSDFAMSWVRQAPGKGL EWVATIGRVAFHTYY

PDSMKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHRGFDVG HFDFWGQGTLVTVSS (SEQ ID NO:34); and

d) a light chain variable region comprising:

DIQMTQSPSSLSASVGDRVTITCRSSETLVHSSGNTYLEWYQQKPG KAPKLLIYRVSNRF

SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCFQGSFNPLTFGQG TKVEIK (SEQ ID NO:35).

[000414] 70. A method of making an immunoconjugate comprising:

a) providing an antibody of any of embodiments 1-69; and

b) conjugating one or more payloads to the antibody.

[000415] 71. The method of embodiment 70, wherein at least one of the payloads is conjugated to the antibody through a linker.

[000416] 72. The method of embodiment 70, wherein at least one of the payloads is conjugated to the antibody through a broken disulfide bond.

[000417] 73. The method of embodiment 70, wherein the antibody is a cysteine-substituted antibody, and at least one payload is coupled to the antibody through a substituted cysteine.

[000418] 74. The method of embodiment 70, wherein the immunoconjugate has a drug-antibody ratio (“DAR”) of at least any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12.

[000419] 75. A collection of one or more nucleic acid molecules that collectively encode paratopes that bind Muc16 and NaPi-2b.

[000420] 76. The collection of nucleic acid molecules of embodiment 75, encoding one or more antibodies of any of embodiments 1-69.

[000421] 77. The collection of nucleic acid molecules of embodiment 75, encoding one or more light chains of the antibody of any of embodiments 1-69.

[000422] 78. The collection of nucleic acid molecules of embodiment 75, encoding one or more heavy chains of the antibody of any of embodiments 1-69.

[000423] 79. The collection of nucleic acid molecules of any of embodiments 75-78, encoding one or more light chains comprising variable regions of paratopes that bind Muc16 and/or NaPi-2b.

[000424] 80. The collection of nucleic acid molecules of any of embodiments 75-78, the nucleic acid molecules further comprising one or more expression control sequences operatively linked to one or more nucleotide sequences encoding the one or more light chain variable regions and/or one or more heavy chain variable regions.

[000425] 81. The collection of nucleic acid molecules of any of embodiments 75-78, comprised within one or more vectors, e.g., selected from a plasmid, a viral vector (e.g., a retrovirus, a lentivirus, and adenovirus, and adeno-associated virus, or a herpes simplex virus), a bacterial artificial chromosome, and a yeast artificial chromosome.

[000426] 82. A cell comprising a collection of nucleic acid molecules of any of embodiments 75-81.

[000427] 83. The cell of embodiment 82, selected from a mammalian cell (e.g., a Chinese hamster ovary (CHO) cell, an NS0 cell, an SP2/0 cell, a human embryonic kidney HEK 293 cell, or a Per.C6 cell); an insect cell (e.g., an Sf9 cell or a high five (BTI-Tn-5B1-4) cell); a yeast cell (e.g., Pichia pastoris or Saccharomyces cerevisiae); a bacterial cell (e.g., E. coli); and a plant cell.

[000428] 84. A method of making an antibody comprising:

a) providing a cell or cells of any of embodiments 82-83;

b) expressing immunoglobulin molecules encoded by the nucleotide sequences in the cell or cells; and

c) recovering the expressed immunoglobulin molecules.

[000429] 85. The method of embodiment 84, wherein the antibody is an asymmetric antibody and the cell comprises one or more nucleic acid molecules encoding a heavy chain comprising a knob, a heavy chain comprising a hole, and at least one light chain.

[000430] 86. The method of embodiment 85, wherein the cell comprises nucleotide sequences encoding only one light chain, wherein the light chain is configured to associate with different heavy chain portions to produce two paratopes.

[000431] 87. The method of embodiment 85, wherein the cell comprises nucleic acids encoding two different light chains, wherein a first light chain is configured to associate with a first heavy chain portion to produce a first paratope that binds to a first epitope, and a second light chain is configured to associate with a second heavy chain portion to produce a second paratope that binds to a second, different epitope.

[000432] 88. The method of embodiment 85, further comprising producing different immunoglobulin chains in different cells, recovering the immunoglobulin chains, and contacting the immunoglobulin chains, wherein the immunoglobulin chains assemble into an antibody.

[000433] 89. The method of embodiment 85, further comprising purifying the recovered antibody, e.g. using protein A or protein G.

[000434] 90. A pharmaceutical composition comprising an antibody or immunoconjugate of any one of embodiments 1-69, and a pharmaceutically acceptable carrier, diluent, or excipient.

[000435] 91. The pharmaceutical composition of embodiment 90, formulated for intravenous injection.

[000436] 92. A method for treating cancer in a subject, comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of any of embodiments 86-87.

[000437] 93. The method of embodiment 88, wherein the cancer is selected from ovarian, breast, lung, stomach, endometrial and pancreatic.

[000438] 94. A method for detecting a cell that expresses Muc16 and/or NaPi-2b, comprising: a) contacting the cell with an antibody or immunoconjugate of any of embodiments 1-69 to allow binding between the immunoconjugate and the cell; and b) detecting the binding of the antibody to the cell.

[000439] 95. The method of embodiment 94, comprising contacting the cell with an immunoconjugate comprising an antibody conjugated to a detectable moiety; and b) detecting the detectable moiety bound to the cell.

[000440] 96. A method of killing a cell comprising contacting a cell with an antibody or immunoconjugate of any of embodiments 1-69, wherein one or more paratopes of the antibody specifically bind to an epitope of the cell.

[000441] 97. The method of embodiment 96, wherein two different paratopes of the antibody bind to two different epitopes of the cell.

[000442] 98. The method of embodiment 96 or 97, wherein the epitope is comprised in a cell surface antigen.

[000443] 99. Use of an antibody of any of embodiments 1-69 in the manufacture of a medicament.

[000444] 100. Use of an antibody of any of embodiments 1-69 to produce an immunoconjugate.

EXEMPLARY EMBODIMENTS II

[000445] 1. A tetravalent bispecific antibody, comprising two paratopes that each specifically bind to Muc16 and two paratopes that each specifically bind to NaPi-2b, wherein the antibody comprises two heavy chains and two light chains that assemble to form a diabody-Fc-Fab structure,

wherein the heavy chains each comprise, from N-terminus to C-terminus,

an anti-NaPi-2b variable heavy (VH) region comprising heavy chain (HC) complementarity determining regions:

HCDR1: GFSFSDFAMS (SEQ ID NO:9) or any one of SEQ ID NOs: 60-63

HCDR2: TIGRVAFHTY (SEQ ID NO:10) or any one of SEQ ID NOs: 64-78

HCDR3: HRGFDVGHFDF (SEQ ID NO:11) or any one of SEQ ID NOs: 79-83,

an anti-NaPi-2b variable light (VL) region comprising light chain (LC) complementarity determining regions:

LCDR1: RSSETLVHSSGNTYLE (SEQ ID NO:12) or any one of SEQ ID NOs: 84-97

LCDR2: RVSNRFS (SEQ ID NO:13) or any one of SEQ ID NOs: 98-101

LCDR3: FQGSFNPLT (SEQ ID NO:14) or any one of SEQ ID NOs: 102-104,

a heavy chain constant 2 (CH2) region,

a heavy chain constant 3 (CH3) region,

an anti-Muc16 VH region comprising heavy chain (HC) complementarity determining regions:

HCDR1: GYSITNDYAWN (SEQ ID NO:1) or SEQ ID NO:48

HCDR2: YISYSGYTT (SEQ ID NO:2) or SEQ ID NO:49

HCDR3: WTSGLDY (SEQ ID NO:3) or SEQ ID NO:50, and

a heavy chain constant 1 (CH1) region; and

wherein the light chains each comprise, from N-terminus to C-terminus,

an anti-Muc16 VL region comprising light chain (LC) complementarity determining regions:

LCDR1: KASDLIHNWLA (SEQ ID NO:4) or SEQ ID NO:51

LCDR2: GATSLET (SEQ ID NO:5) or SEQ ID NO:52

LCDR3: QQYWTTPFT (SEQ ID NO:6) or SEQ ID NO:53, and

a light chain constant (CL) region.

[000446] 2. The antibody of embodiment 1, wherein the anti-NaPi-2b VH region comprises HC complementarity determining regions:

HCDR1: GFSFSDFAMS (SEQ ID NO:9)

HCDR2: TIGRVAFHTY (SEQ ID NO:10)

HCDR3: HRGFDVGHFDF (SEQ ID NO:11),

the anti-NaPi-2b VL region comprises LC complementarity determining regions:

LCDR1: RSSETLVHSSGNTYLE (SEQ ID NO:12)

LCDR2: RVSNRFS (SEQ ID NO:13)

LCDR3: FQGSFNPLT (SEQ ID NO:14),

the anti-Muc16 VH region comprises HC complementarity determining regions:

HCDR1: GYSITNDYAWN (SEQ ID NO:1)

HCDR2: YISYSGYTT (SEQ ID NO:2)

HCDR3: WTSGLDY (SEQ ID NO:3), and

the anti-Muc16 VL region comprises LC complementarity determining regions:

LCDR1: KASDLIHNWLA (SEQ ID NO:4)

LCDR2: GATSLET (SEQ ID NO:5)

LCDR3: QQYWTTPFT (SEQ ID NO:6).

[000447] 3. The antibody of embodiment 1 or 2, wherein the lights chains each comprise a heavy chain sequence at least 90% identical to or at least 95% identical to SEQ ID NO:8.

[000448] 4. The antibody of embodiment 3, wherein the lights chains each comprise a heavy chain sequence according to SEQ ID NO:8.

[000449] 5. The antibody of any one of embodiments 1-4, wherein the heavy chains each comprise a heavy chain sequence at least 90% identical to or at least 95% identical to SEQ ID NO:29 or any one of SEQ ID NO:56-59.

[000450] 6. The antibody of embodiment 5, wherein the heavy chains each comprise a heavy chain sequence according to SEQ ID NO:29 or any one of SEQ ID NO:56-59.

[000451] 7. The antibody of any one of embodiments 1-6, wherein the heavy chains comprises a cysteine (C) at position 611 related to SEQ ID NO:29 (HC-A118C by EU numbering).

[000452] 8. The antibody of any one of embodiments 1-7, wherein the light chains comprises a cysteine (C) at position 205 related to SEQ ID NO:8 (LC-V205C by EU numbering).

[000453] 9. The antibody of any one embodiments 1-8, wherein the heavy chains comprise one or more of the following substitutions: S484A, S484G, S489A, S489G.

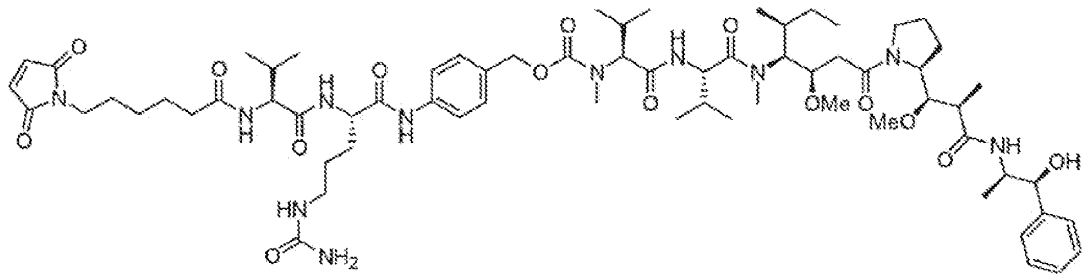
[000454] 10. The antibody of any one of embodiments 1-9, further comprising one or more payloads conjugated thereto, optionally as a linker-payload.

[000455] 11. The antibody of embodiment 10, comprising four payloads conjugated thereto (DAR = 4).

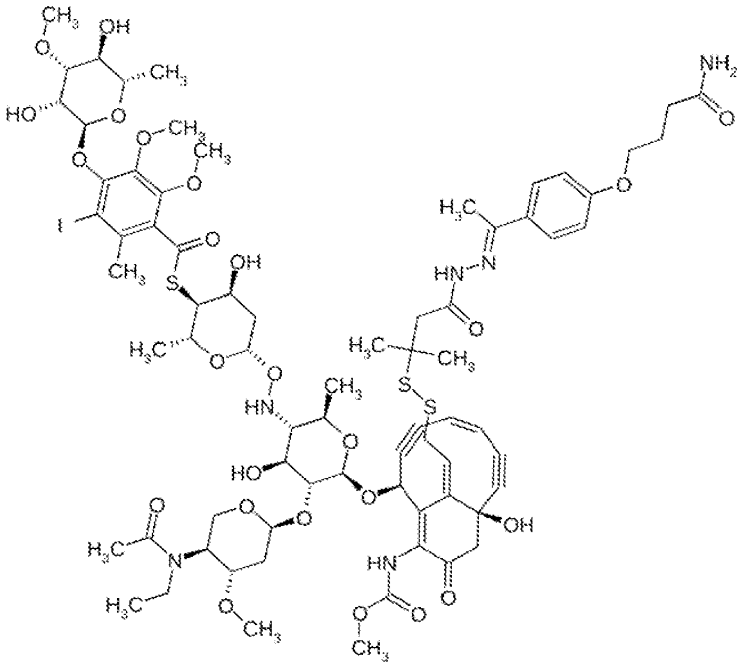
[000456] 12. The antibody of embodiment 10 or embodiment 11, wherein the payload is monomethyl auristatin E (MMAE).

[000457] 13. The antibody of embodiment 10 or embodiment 11, wherein the payload is a maytansinoid, DM-1, DM-4, an auristatin, monomethyl auristatin E, monomethyl auristatin F, dolastatin, a tubulysin, eribulin, cryptophycin, a benzodiazepine, indolino-benzodiazepine, isoquinolidino-benzodiazepine, pyrrolo-benzodiazepine, alpha-amanitin, trichothene, camptothecin, a camptothecin derivative, SN-38, exatecan, belotecan, DXd, topotecan, samrotecan, duocarmycin, DGN549, CC1065, a calicheamicin, N-acetyl calicheamicin, an enediyne antibiotic, a taxane, a doxorubicin derivative, an anthracycline or stereoisomer thereof, or azonafide, or an isostere, analogs, heterodimer, homodimer, or derivative thereof.

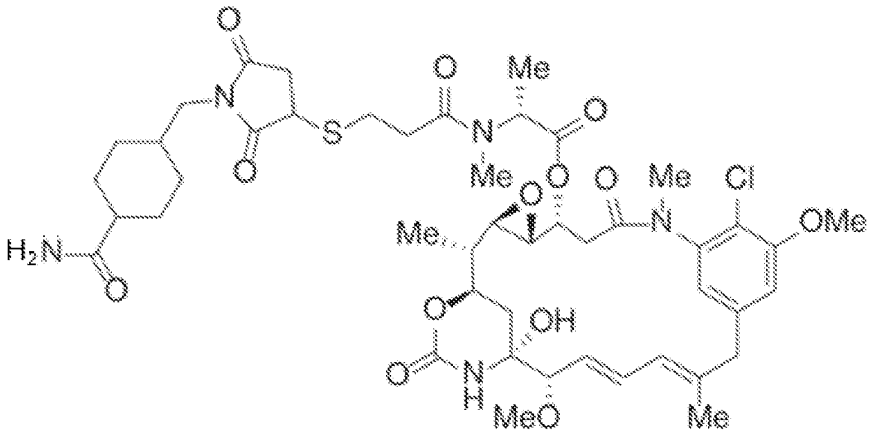
[000458] 14. The antibody of embodiment 10 or embodiment 11, wherein the linker-payload is:



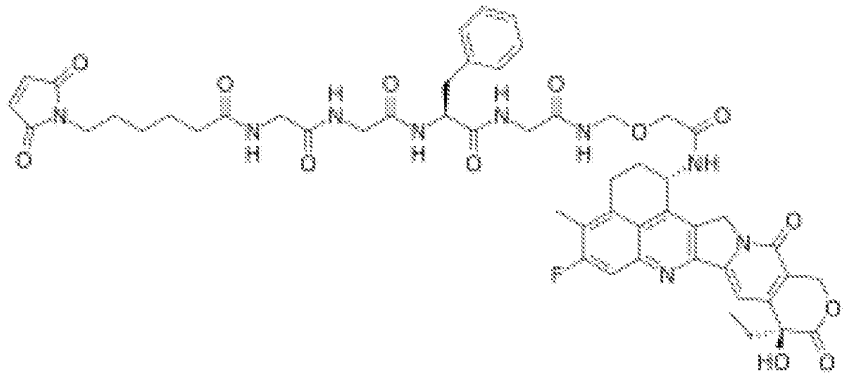
[000459] 15. The antibody of embodiment 10 or embodiment 11, wherein the linker-payload is:



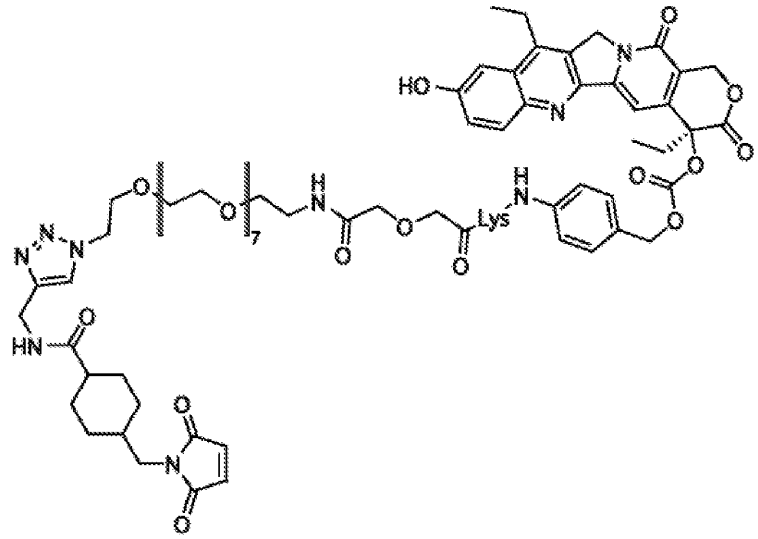
[000460] 16. The antibody of embodiment 10 or embodiment 11, wherein the linker-payload is:



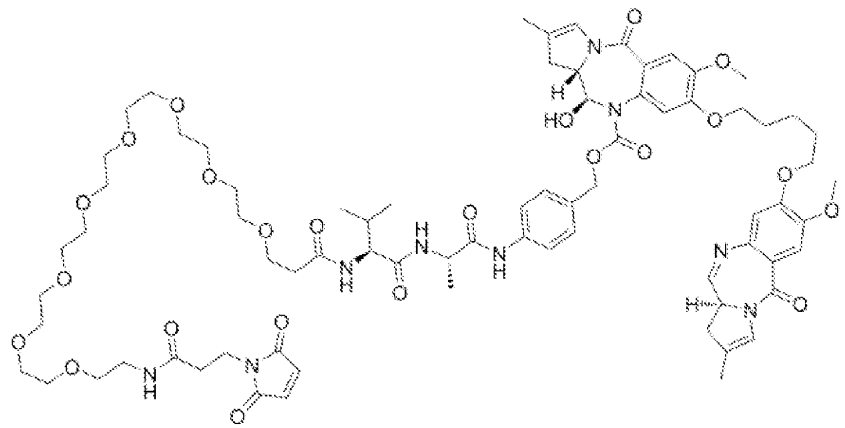
[000461] 17. The antibody of embodiment 10 or embodiment 11, wherein the linker-payload is:



[000462] 18. The antibody of embodiment 10 or embodiment 11, wherein the linker-payload is:



[000463] 19. The antibody of embodiment 10 or embodiment 11, wherein the linker-payload is:



[000464] 20. A pharmaceutical composition, comprising a therapeutically effective amount of the antibody of any one of embodiments 1-19.

[000465] 21. The pharmaceutical composition of embodiment 20, further comprising a pharmaceutically acceptable carrier, diluent or excipient.

[000466] 22. The pharmaceutical composition of embodiment 20 or 21, for use as a medicament in the treatment of cancer.

[000467] 23. A method of treating a subject with cancer, comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of any one of embodiments 20-22.

[000468] 24. The method of embodiment 23, wherein the cancer is selected from ovarian, breast, lung, stomach, endometrial and pancreatic.

[000469] 25. A method of detecting a cell that expresses Muc16 and/or NaPi-2b, comprising: a) contacting the cell with the antibody of any one of embodiments 1-19 to allow binding between the antibody and the cell; and b) detecting the binding of the antibody to the cell.

[000470] 26. The method of embodiment 25, comprising contacting the cell with the antibody conjugated to a detectable moiety; and b) detecting the detectable moiety bound to the cell.

[000471] 27. A method of killing a cell comprising contacting a cell with the antibody of any one of embodiments 1-19, wherein one or more paratopes of the antibody specifically bind to an epitope of the cell.

[000472] 28. The method of embodiment 27, wherein two different paratopes of the antibody bind to two different epitopes of the cell.

[000473] 29. The method of embodiment 27 or 28, wherein the epitope is comprised in a cell surface antigen.

[000474] 30. A polynucleotide comprising polynucleotide sequences encoding the antibody of any one of embodiments 1-19.

[000475] 31. A vector or a host cell comprising the polynucleotide of embodiment 30.

[000476] 32. A tetravalent bispecific antibody, comprising two paratopes that each specifically bind to Muc16 and two paratopes that each specifically bind to NaPi-2b, wherein the antibody comprises two heavy chains and two lights chains that assemble to form a Fab-diabody-Fc structure,

wherein the heavy chains each comprise, from N-terminus to C-terminus,

an anti-NaPi-2b variable heavy (VH) region comprising heavy chain (HC) complementarity determining regions:

HCDR1: GFSFSDFAMS (SEQ ID NO:9) or any one of SEQ ID NOs: 60-63

HCDR2: TIGRVAFHTY (SEQ ID NO:10) or any one of SEQ ID NOs: 64-78

HCDR3: HRGFDVGHFDF (SEQ ID NO:11) or any one of SEQ ID NOs: 79-83

a heavy chain constant 1 (CH1) region,

an anti-Muc16 variable heavy (VH) region comprising heavy chain (HC) complementarity determining regions:

HCDR1: GYSITNDYAWN (SEQ ID NO:1) or SEQ ID NO:48

HCDR2: YISYSGYTT (SEQ ID NO:2) or SEQ ID NO:49

HCDR3: WTSGLDY (SEQ ID NO:3) or SEQ ID NO:50,

an anti-Muc16 variable light (VL) region comprising light chain (LC) complementarity determining regions:

LCDR1: KASDLIHNWLA (SEQ ID NO:4) or SEQ ID NO:51

LCDR2: GATSLET (SEQ ID NO:5) or SEQ ID NO:52

LCDR3: QQYWTTPFT (SEQ ID NO:6) or SEQ ID NO:53

a heavy chain constant 2 (CH2) region,

a heavy chain constant 3 (CH3) region; and

wherein the light chains each comprise, from N-terminus to C-terminus,

an anti-NaPi-2b variable light (VL) region comprising light chain (LC) complementarity determining regions:

LCDR1: RSSETLVHSSGNTYLE (SEQ ID NO:12) or any one of SEQ ID NOs: 84-97

LCDR2: RVSNRFS (SEQ ID NO:13) or any one of SEQ ID NOs: 98-101

LCDR3: FQGSFNPLT (SEQ ID NO:14) or any one of SEQ ID NOs: 102-104, and

a light chain constant (CL) region.

[000477] 33. The antibody of embodiment 32, wherein the anti-NaPi-2b VH region comprises HC complementarity determining regions:

HCDR1: GFSFSDFAMS (SEQ ID NO:9)

HCDR2: TIGRVAFHTY (SEQ ID NO:10)

HCDR3: HRGFDVGHFDF (SEQ ID NO:11),

the anti-Muc16 VH region comprises HC complementarity determining regions:

HCDR1: GYSITNDYAWN (SEQ ID NO:1)

HCDR2: YISYSGYTT (SEQ ID NO:2)

HCDR3: WTSGLDY (SEQ ID NO:3),

the anti-Muc16 VL region comprises LC complementarity determining regions

LCDR1: KASDLIHNWLA (SEQ ID NO:4)

LCDR2: GATSLET (SEQ ID NO:5)

LCDR3: QQYWTTPFT (SEQ ID NO:6), and

the anti-NaPi-2b VL region comprises LC complementarity determining regions:

LCDR1: RSSETLVHSSGNTYLE (SEQ ID NO:12)

LCDR2: RVSNRFS (SEQ ID NO:13)

LCDR3: FQGSFNPLT (SEQ ID NO:14), and

the light chain constant (CL) region

[000478] 34. The antibody of embodiment 32 or 33, wherein the light chains each comprise a heavy chain sequence at least 90% identical to or at least 95% identical to SEQ ID NO:16.

[000479] 35. The antibody of embodiment 34, wherein the light chains each comprise a heavy chain sequence according to SEQ ID NO:16.

[000480] 36. The antibody of any one of embodiment 32-35, wherein the heavy chains each comprise a heavy chain sequence at least 90% identical to or at least 95% identical to SEQ ID NO:27.

[000481] 37. The antibody of embodiment 36, wherein the heavy chains each comprise a heavy chain sequence according to SEQ ID NO:27.

[000482] 38. The antibody of embodiment 32-37, wherein the heavy chains comprises a cysteine (C) at position 121 related to SEQ Id NO:27 (HC-A118C by EU numbering).

[000483] 39. The antibody of any one of embodiments 32-38, wherein the light chain comprises a cysteine (C) at position 210 related to SEQ ID NO:16 (LC-V205C by EU numbering).

[000484] 40. The antibody of any one of embodiments 32-39, further comprising one or more payloads conjugated thereto.

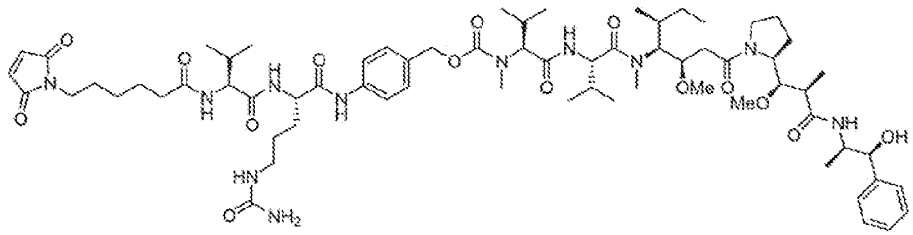
[000485] 41. The antibody of embodiment 40, comprising four payloads conjugated thereto (DAR=4).

[000486] 42. The antibody of embodiment 40 or 41, wherein the payload is monomethyl auristatin E (MMAE).

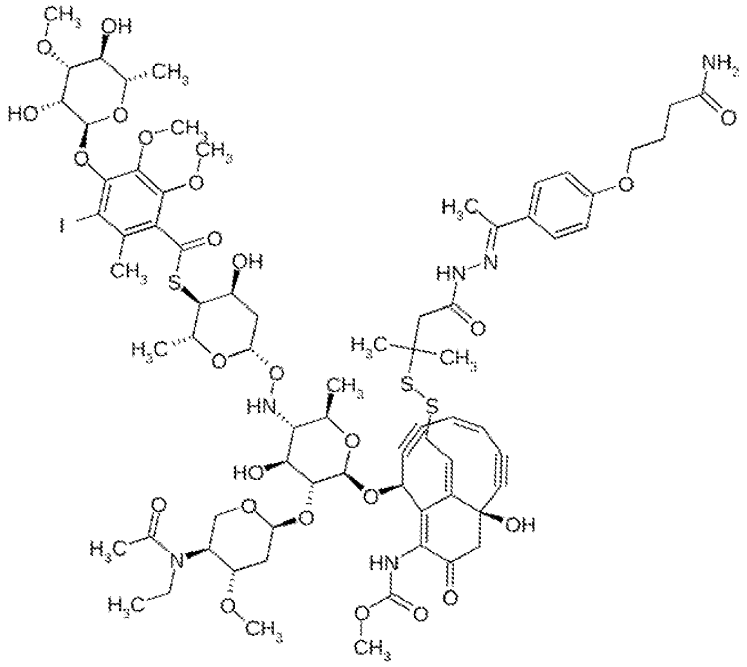
[000487] 43. The antibody of embodiment 40 or 41, wherein the payload is maytansinoid, DM-1, DM-4, auristatin, monomethyl auristatin E, monomethyl auristatin F, dolastatin, tubulysin, eribulin, cryptophycin, benzodiazepine, indolino-benzodiazepine, isoquinolidino-

benzodiazepine, pyrrolo-benzodiazepine, alpha-amanitin, trichothene, camptothecin derivatives (SN-38, exatecan, belotecan, DXd, topotecan, samrotecan), duocarmycin, DGN549, CC1065, calicheamicin, N-acetyl calicheamicin,an enediyne antibiotic, taxane, doxorubicin derivatives, anthracycline and stereoisomers, azanofide, isosteres, and analogs, hetero dimer or homo dimers or derivatives thereof.

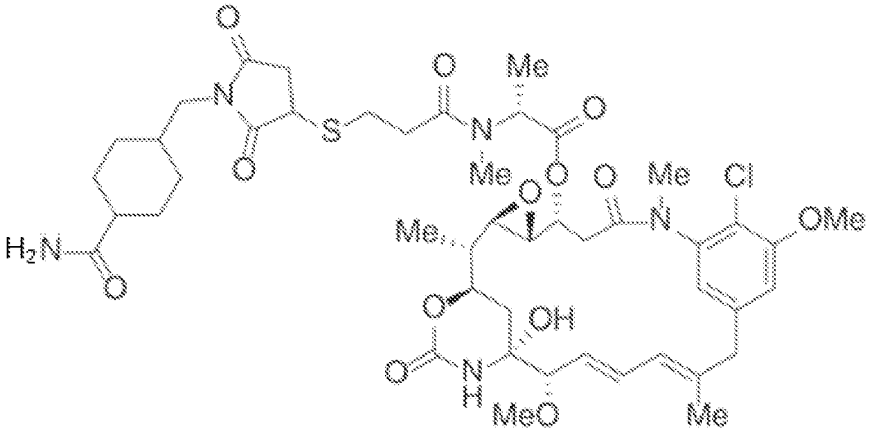
[000488] 44. The antibody of embodiment 40 or embodiment 41 wherein the linker-payload is:



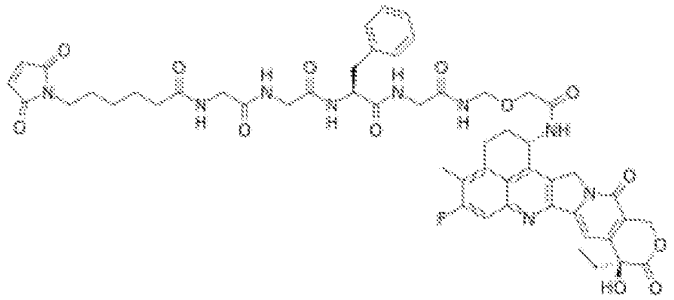
[000489] 45. The antibody of embodiment 40 or embodiment 41, wherein the linker-payload is:



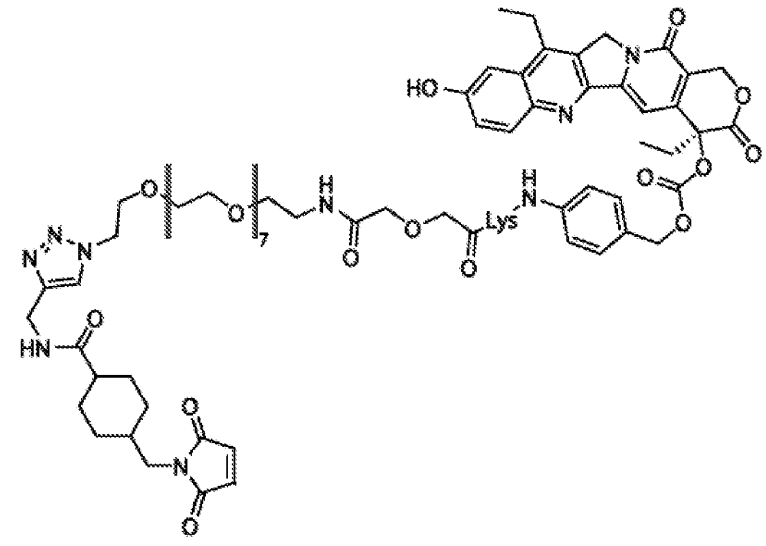
[000490] 46. The antibody of embodiment 40 or embodiment 41, wherein the linker-payload is:



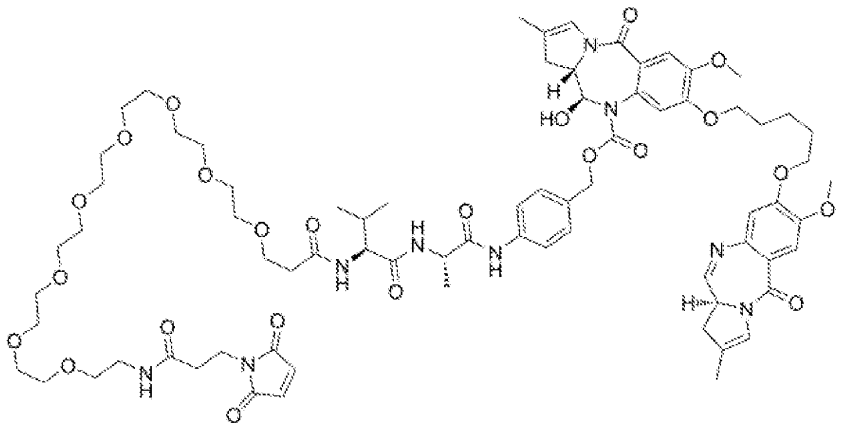
[000491] 47. The antibody of embodiment 40 or embodiment 41, wherein the linker-payload is:



[000492] 48. The antibody of embodiment 40 or embodiment 41, wherein the linker-payload is:



[000493] 49. The antibody of embodiment 40 or embodiment 41, wherein the linker-payload is:



[000494] 50. A pharmaceutical composition, comprising a therapeutically effective amount of the antibody of any one of embodiments 32-49.

[000495] 51. The pharmaceutical composition of embodiment 50, further comprising a pharmaceutically acceptable carrier, diluent or excipient.

[000496] 52. The pharmaceutical composition of embodiment 50 or 51, for use as a medicament in the treatment of cancer.

[000497] 53. A method of treating a subject with cancer, comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of any one of embodiments 50-52.

[000498] 54. The method of embodiment 53, wherein the cancer is selected from ovarian, breast, lung, stomach, endometrial and pancreatic.

[000499] 55. A method of detecting a cell that expresses Muc16 and/or NaPi-2b, comprising: a) contacting the cell with the antibody of any one of embodiments 32-49 to allow binding between the antibody and the cell; and b) detecting the binding of the antibody to the cell.

[000500] 56. The method of embodiment 55, comprising contacting the cell with the antibody conjugated to a detectable moiety; and b) detecting the detectable moiety bound to the cell.

[000501] 57. A method of killing a cell comprising contacting a cell with the antibody of any one of embodiments 32-49, wherein one or more paratopes of the antibody specifically bind to an epitope of the cell.

[000502] 58. The method of embodiment 57, wherein two different paratopes of the antibody bind to two different epitopes of the cell.

[000503] 59. The method of embodiment 57 or 58, wherein the epitope is comprised in a cell surface antigen.

[000504] 60. A polynucleotide comprising polynucleotide sequences encoding the antibody of any one of embodiments 32-49.

[000505] 61. A vector or a host cell comprising the polynucleotide of embodiment 60.

[000506] 62. Use of an antibody of any of embodiments 1-19 in the manufacture of a medicament.

[000507] 63. Use of an antibody of any of embodiments 1-19 to produce an immunoconjugate.

[000508] 64. Use of an antibody of any of embodiments 32-49 in the manufacture of a medicament.

[000509] 65. Use of an antibody of any of embodiments 32-49 to produce an immunoconjugate.

[000510] 66. A method of making an immunoconjugate comprising:

a) providing an antibody of any of embodiments 1-9 or 32-39; and

b) conjugating one or more payloads to the antibody.

[000511] 67. The method of embodiment 66, wherein at least one of the payloads is conjugated to the antibody through a linker.

[000512] 68. The method of embodiment 66, wherein at least one of the payloads is conjugated to the antibody through a broken disulfide bond.

[000513] 69. The method of embodiment 66, wherein the antibody is a cysteine-substituted antibody, and at least one payload is coupled to the antibody through a substituted cysteine.

[000514] 70. The method of embodiment 66, wherein the immunoconjugate has a drug-antibody ratio (“DAR”) of at least any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12.

[000515] 71. A collection of one or more nucleic acid molecules that collectively encode paratopes that bind Muc16 and NaPi-2b.

[000516] 72. The collection of nucleic acid molecules of embodiment 71, encoding one or more antibodies of any of embodiments 1-9 or 32-39.

[000517] 73. The collection of nucleic acid molecules of embodiment 71, encoding one or more light chains of the antibody of any of embodiments 1-9 or 32-39.

[000518] 74. The collection of nucleic acid molecules of embodiment 71, encoding one or more heavy chains of the antibody of any of embodiments 1-9 or 32-39.

[000519] 75. The collection of nucleic acid molecules of any of embodiments 71-74, encoding one or more light chains comprising variable regions of paratopes that bind Muc16 and/or NaPi-2b.

[000520] 76. The collection of nucleic acid molecules of any of embodiments 71-74, the nucleic acid molecules further comprising one or more expression control sequences operatively linked to one or more nucleotide sequences encoding the one or more light chain variable regions and/or one or more heavy chain variable regions.

[000521] 77. The collection of nucleic acid molecules of any of embodiments 71-74, comprised within one or more vectors, e.g., selected from a plasmid, a viral vector (e.g., a retrovirus, a lentivirus, and adenovirus, and adeno-associated virus, or a herpes simplex virus), a bacterial artificial chromosome, and a yeast artificial chromosome.

[000522] 78. A cell comprising a collection of nucleic acid molecules of any of embodiments 71-77.

[000523] 79. The cell of embodiment 78, selected from a mammalian cell (e.g., a Chinese hamster ovary (CHO) cell, an NS0 cell, an SP2/0 cell, a human embryonic kidney HEK 293 cell, or a Per.C6 cell); an insect cell (e.g., an Sf9 cell or a high five (BTI-Tn-5B1-4) cell); a yeast cell (e.g., Pichia pastoris or Saccharomyces cerevisiae); a bacterial cell (e.g., E. coli); and a plant cell.

[000524] 80. A method of making an antibody comprising:

a) providing a cell or cells of any of embodiments 78-79;

b) expressing immunoglobulin molecules encoded by the nucleotide sequences in the cell or cells; and

c) recovering the expressed immunoglobulin molecules.

[000525] 81. The method of embodiment 81, wherein the antibody is an asymmetric antibody and the cell comprises one or more nucleic acid molecules encoding a heavy chain comprising a knob, a heavy chain comprising a hole, and at least one light chain.

[000526] 82. The method of embodiment 81, wherein the cell comprises nucleotide sequences encoding only one light chain, wherein the light chain is configured to associate with different heavy chain portions to produce two paratopes.

[000527] 83. The method of embodiment 81, wherein the cell comprises nucleic acids encoding two different light chains, wherein a first light chain is configured to associate with

a first heavy chain portion to produce a first paratope that binds to a first epitope, and a second light chain is configured to associate with a second heavy chain portion to produce a second paratope that binds to a second, different epitope.

[000528] 84. The method of embodiment 81, further comprising producing different immunoglobulin chains in different cells, recovering the immunoglobulin chains, and contacting the immunoglobulin chains, wherein the immunoglobulin chains assemble into an antibody.

[000529] 85. The method of embodiment 81, further comprising purifying the recovered antibody, e.g. using protein A or protein G.

EXAMPLES

Example 1:

[000530] **Muc16/NaPi-2b Bi-Specific Antibodies** Bi-specific, tetravalent ADCs were produced in the Fab-Diabody-Fc format and Diabody-Fc-Fab format in which a first pair of paratopes bound Muc16, and a second pair of paratopes bound NaPi-2b.

[000531] **Figs. 6A** and **6B** shows production of two monospecific antibody-drug conjugates (ADCs) in standard IgG configuration derivatized with monomethyl auristatin E (MMAE) using the following conjugation method: Antibodies are reduced with 40x molar equivalent of 10mM Tris(2carboxyethyl)phosphine (TCEP) by incubating overnight at room temperature. Reduced samples were then dialyzed using 10kDa dialysis tubing using 1x PBS buffer, pH 7.4 for 3.5h at room temperature. After dialysis, samples were re-oxidized with 30x molar equivalent of 10mM dehydroascorbic acid (DHAA) for 3.5h at room temperature to enable formation of inter-chain disulfide bonds. After re-oxidation, antibodies were conjugated with 10x molar equivalents of MC-vc-PAB-MMAE (maleimidocaproyl-valine-citrulline -p-aminobenzyl) linker-payload by incubating at room temperature for overnight and excess linker-payload is then dialyzed against ADC formulation buffer (See, e.g., Doronina SO, et al., “Development of potent monoclonal antibody auristatin conjugates for cancer therapy,” Nat Biotechnol. 2003 Jul;21(7):778-84. doi: 10.1038/nbt832. Epub 2003 Jun 1. PMID: 12778055.) Number of drugs per antibody (DAR) were quantitated by analyzing ADC samples on RP-LC/MS. Prior to such analyses, the ADC samples were deglycosylated with peptide-N-glycosidase F to remove N-lined oligosaccharides and then reduced with DTT. Both figures 6A and 6B represent size-exclusion chromatograms of AB301-ADC and AB302-ADC. AB301-ADC specifically binds NaPi-2b, and has CDR sequences from lifastuzumab. (Fig. 6A.) AB302-ADC specifically binds Muc16, and has CDR sequences from sofituzumab.

(Fig. 6B.) Both antibodies include cysteine substitutions, which allows conjugation of the cytotoxic moiety without the need to break naturally occurring disulfide bonds. Evidence indicates that these antibodies exhibit a DAR of 4. DAR was determined by mass spectrometry.

[000532] **Figs. 7A-7C** show production of three bi-specific, tetravalent ADCs, derivatized with monomethyl auristatin E (MMAE) and analyzed as described above, comprising binding sites for NaPi-2b and Muc16. The ADC of Fig. 7A (AB304-ADC; SEQ ID NO:27 and SEQ ID NO:16) is in the Fab-diabody-Fc format. It comprises paratopes that bind NaPi-2b in the NW/NE positions and paratopes that bind Muc16 in the SW/SE positions. The ADC of Fig. 7B (AB305-ADC; SEQ ID NO:28 and SEQ ID NO:8) also is in the Fab-diabody-Fc format. It comprises paratopes that bind Muc16 in the NW/NE positions and paratopes that bind NaPi-2b in the SW/SE positions. The ADC of Fig. 7C (AB309-ADC; SEQ ID NO:29 and SEQ ID NO:8) is in the diabody-Fc-Fab format. It comprises paratopes that bind NaPi-2b in the NW/NE positions and paratopes that bind Muc16 in the SW/SE positions. These ADCs comprise cysteine-substituted antibodies and have DAR of 4.

[000533] **Fig. 8** shows the inhibitory concentration 50% (IC50) of the ADCs depicted in Fig. 6 and Fig.7 against OVCAR3 cells, an ovarian cancer cell line. Cells used in assays: NIH:OVCAR-3 (OVCAR3, ATCC# HTB-161™). To determine inhibitory concentration 50% (IC_{50}) values of the ADCs, OVCAR3 cells were seeded in 96 well tissue culture clear bottom plate at 5000 cells/well in 100uL cell culture medium and incubated overnight at 37°C under 5% CO2. Serially diluted (1:3 dilution) ADC samples (50uL) were added to each well in duplicates after overnight incubation, resulting in final ADC concentrations ranging from 1000ng/mL to 0.05ng/mL. Plates were incubated at 37°C for an additional 60-72h under 5% CO2. To measure cell viability, plates were equilibrated at room temperature for 30min. 100ul of CellTiter-Glo Luminescent Solution (Promega, Cat# G7570) was added to each well and incubated a plate shaker (300rpm) for 20min. Luminescence was measured using BioTek Synergy plate reader. IC50 values were calculated using GraphPad software. AB304-ADC demonstrated best cellular potency of any of the five ADCs, because the IC_{50} value is 0.03, compared to 0.6 for either AB305-ADC or AB309-ADC, and 0.53 for the control monoclonal antibody lifastuzumab. The error bars are derived from a duplicate data set of a single experiment.

[000534] **Fig. 9** shows the percent normalized viability of OVCAR3 cells exposed to each of three different antibodies in ADC format, AB301-ADC, AB303-ADC and AB306-ADC made and analyzed as described in Figs. 6-8 above. Both bi-specific ADCs (AB303-ADC and AB306-ADC) performed better than the mono-specific ADC (AB301-ADC).

[000535] **Fig. 10** shows that a bi-specific ADC, AB304-ADC (Fab-diabody-Fc ADC; NaPi-2b NW/NE, Muc16 SW/SE), retained toxic potency against a partial Muc16-KO OVCAR3 cell line, compared with ADC AB301-ADC (anti-Muc16 IgG ADC). Here, engineered OVCAR3-AV cells were used to measure cellular potency of ADCs. OVCAR3 cells with Muc16 knockout (KO) were generated by CRISPR. To enrich for the Muc16 KO, cell sorting was performed based on the fluorescence intensity of Muc16. Initial two rounds of enrichment were performed for Muc16 to generate OVCAR3-AV cells that consists of both original OVCAR3 cells and Muc16 KO cells. The dependency of cytotoxicity of the monospecific and bi-specific ADCs on the presence of Muc16 protein on the cell surface was tested by using these mixed pool of OVCAR3 and Muc16 KO cells. As expected, the potency of the monospecific Muc16-targeting ADC, AB302-ADC, was significantly reduced in the mixed pool of Muc16 KO cells, while the potency of the monospecific ADC targeting NaPi-2b, AB301-ADC remained essentially unaffected. The potency of bispecific ADCs is slightly decreased (3-4 fold) due to lowered Muc16 expression.

[000536] **Fig. 11** shows that mono-specific ADCs lose potency against OVCAR3 cells in which their targets are knocked out. Bi-specific tetravalent ADCs, AB304-ADC and AB309-ADC, retain potency against such cells. OVCAR3 cells with Muc16 knockout (KO) and OVCAR3 cells with NaPi-2b KO were generated by CRISPR. The phenotypic KO status (impaired expression of either Muc16 or NaPi-2b proteins on the cell surface) of KO cells was validated by FACS analysis using anti- Muc16 and anti-NaPi-2b antibodies (2000 ng/mL) followed by secondary antibody (1:1000). To enrich for the Muc16 KO and the NaPi-2b KO population, cell sorting was performed based on the fluorescence intensity of Muc16 and NaPi-2b expression, respectively. Three rounds of enrichment were performed for Muc16 to generate “Muc16 KO” cells. Wild type (WT) and Muc16 KO cells were incubated with various concentrations of ADCs to assess cytotoxic potency. As expected, the cytotoxic potency of AB302-ADC (binds Muc16) and AB301-ADC (binds NaPi-2b) was greatly reduced (by over 100-fold) when incubated with Muc16 KO and NaPi-2b KO cells respectively. As a result, cytotoxic potency of all bi-specific ADCs is now comparable to monospecific ADCs when incubated with their corresponding KO cells. These data demonstrate that bi-specific ADCs can kill Muc16 expressing cells (with low/no NaPi-2b expression), NaPi-2b expressing (with low/no Muc16 expression) and dual positive (expressing both Muc16 and NaPi-2b) cells. Co-expression of both targets further boosts the potency of bi-specific ADCs. IC50 values for the ADCs in various cell types is shown.

[000537] **Fig. 12** shows that a bi-specific, tetravalent ADC, AB304-ADC, demonstrated greater potency against cell populations that are mixtures of cells with constant levels of NaPi-2b and varying levels of Muc16 expression, compared with monovalent ADCs, AB301-

ADC (NaPi-2b) and AB302-ADC (Muc16). To assess the potency of AB304-ADC towards cells expressing normal or reduced amounts of Muc16, the ADC was incubated with mixtures of WT and Muc16 KO OVCAR3 cells in various proportions. These conditions simulate the compositions of patient tumors where cells may have the tissues have varying levels of Muc16 expression but a constant level of NaPi-2b expression. However, the experiment does not necessarily address the effects on tumors with the varying antigen density levels on the cell surface. As expected, cytotoxic potency, as measured by IC50, of ADC AB301-ADC (binds NaPi-2b) and AB304-ADC (binds NaPi-2b and Muc16) remain similar under all the 4 conditions tested, whereas the potency of AB302-ADC (binds Muc16) reduces as the proportion of Muc16-expressing WT cells decreases in the cell mixture. A-D show normalized cell viability (%) at various concentrations of ADCs in various mixtures of WT and KO cells. E. IC50 values for AB301-ADC, AB302-ADC, and AB304-ADC under the four test conditions. IC50 values are derived from a duplicate set of experiments.

[000538] **Fig. 13** shows that a bi-specific, tetravalent ADC, AB304-ADC, demonstrated greater potency compared to either individual mono specific ADCs (AB301-ADC or AB302-ADC) or their combination (AB301-ADC+AB302-ADC). AB001-ADC is non-binding ADC that had no detectable activity. To investigate if the cytotoxic potency of the bi-specific ADCs AB304-ADC and AB309-ADC towards OVCAR3 cells expressing normal or reduced amounts of Muc16 was due to additive or synergistic action of binding of these ADCs to their target antigens, the potency of these individual ADCs was compared to that of a mixture the two reference monospecific ADCs binding only NaPi-2b (AB301-ADC) or only Muc16 (AB302-ADC). In the cell mixture, 20% of the cells were expression positive for both target antigens while 60% were expression positive for only NaPi-2b, and 20% were expression positive for only Muc16. This mixture was a model for a heterogenous tumor expressing variable levels of the two target antigens. Both bi-specific ADCs showed superior potency (lower IC50 values) compared to the mixture of the two control monospecific ADCs or their combination.

[000539] **Fig. 14** shows manufacturability of various antibody formats. Fab-Diabody-Fc format and Diabody-Fc-Fab format demonstrate highest yields. Antibodies identified by hashtag have lowest manufacture yields. All antibody molecules were expressed in Expi293 cells and purified from clarified culture supernatant using Protein A chromatography. Protein yield after Protein A purification was assessed and used to calculate expression titers (shown in Fig. 14 as milligrams of protein per liter of cell culture). Purified proteins were analyzed by size exclusion chromatography (SEC) to assess homogeneity. A non-specific IgG was used as an internal control in the assay.

Example 2:

[000540] **Fig. 15** shows the efficacy of various ADCs in the OVCAR3 ovarian xenograft model using female CB.17 SCID Mice. Tumor xenografts were initiated with OVCAR3 human ovarian carcinomas maintained by serial subcutaneous transplantation in athymic nude mice. On the day of tumor implant, each test mouse received a 4 mm^3 OVCAR3 fragment implanted subcutaneously in the right flank, and tumor growth was monitored as the average size approached the target range of 100 to 150 mm^3. Tumors were measured in two dimensions using calipers, and volume was calculated. Tumor weight may be estimated with the assumption that 1 mg is equivalent to 1 mm^3 of tumor volume. When palpable tumors were established, mice were randomized to a mean tumor volume of 150 mm^3 in each group (8 mice/group; range = 100–150 mm^3 and then treated intravenously once (day 1) with AB301-ADC, AB302-ADC, AB304-ADC and AB309-ADC at the 15nmole doses (**Fig. 15A**). Mean tumor volumes (± s.e.m.) are plotted over time (**Fig. 15B**). Both bi-specific ADCs (AB304-ADC and AB309-ADC) displayed superior efficacy compared to mono-specific ADCs (AB301-ADC and AB302-ADC). There were no body weight changes observed during the study duration (**Fig. 15C**), indicating that all ADCs are safe at this dose. All ADCs have same drug-to antibody (DAR) of 4.

[000541] **Fig. 16** shows *in vitro* validation of different ADCs in the OVCAR3 cell line that are intended for *in vivo* tumor model testing in OVCAR3 xenograft model. The percent normalized viability of OVCAR3 cells exposed to different ADCs: AB001-ADC (non-binding ADC control), AB301-ADC, AB302-ADC, AB301-ADC+AB302-ADC (combination), AB304-ADC and AB309-ADC or AB312-ADC (DMUC4064A(Genentech®) made and analyzed as described in **Figs. 6-8** above. Both bi-specific ADCs (AB304-ADC and AB309-ADC) performed better than the mono-specific ADCs (AB301-ADC, AB302-ADC) or their combination (AB301-ADC+AB302-ADC) or a clinical comparator (AB312-ADC). All ADCs have same drug-to antibody (DAR) of 4.

[000542] **Fig. 17** shows the efficacy of various ADCs at 40 nmole dose in the OVCAR3 ovarian xenograft model using female CB.17 SCID Mice. The study was conducted as described in the Fig. 15. All ADCs were injected intravenously on day1 with a single 40 nmole dose (**Fig. 17A**) and mean tumor volumes (± s.e.m.) are plotted over time (**Fig. 17B**). Tumor growth differences observed on day 32 among various treatment groups is shown in **Fig. 17C** and p-values between different dose groups are shown in **Fig. 17D**. Both bi-specific ADCs (AB304-ADC and AB309-ADC) displayed superior efficacy compared to mono-specific ADCs (AB301-ADC and AB302-ADC) or their combination (AB301-ADC+AB302-ADC) or the clinical comparator (AB312-ADC). All ADCs have same drug-to antibody (DAR) of 4.

[000543] **Fig. 18** shows the efficacy of AB309-ADC at different doses in comparison to AB301-ADC. The study was conducted as described in the Fig. 15. AB309-ADC was injected intravenously on day1 with a single dose of 10 nmole or 20 nmole or 40 nmole dose (**Fig. 18A**) and mean tumor volumes (± s.e.m.) are plotted over time (**Fig. 18B**) and compared to a single 40 nmole dose of AB301-ADC. AB309-ADC at 10 nmole dose showed similar efficacy to that of 40 nmole dose of AB301-ADC indicating an approximately 4-fold improvement in the efficacy for AB309-ADC. Both 20 nmole and 40 nmole doses of AB309-ADC showed statistically significant improvement in the efficacy compared to 40 nmole of AB301-ADC. All ADCs have same drug-to antibody (DAR) of 4.

[000544] **Fig. 19** shows comparison of AB309-ADC efficacy to AB312-ADC at different doses. The study was conducted as described in Fig. 15. AB309-ADC (10 nmole or 20 nmole or 40 nmole dose) or AB312-ADC (20 nmole or 40 nmole or 80 nmole dose) were injected intravenously on day1 with a single dose of (**Fig. 19A**) and mean tumor volumes (± s.e.m.) are plotted over time (Fig. 19B) AB309-ADC at 10 nmole dose or 20 nmole showed similar efficacy to that of 40 nmole dose or 80 nmole dose, respectively of AB312-ADC indicating an approximately 4-fold improvement in the efficacy for AB309-ADC. Both 20 nmole and 40 nmole doses of AB309-ADC showed statistically significant improvement in the efficacy compared to 40 nmole or 80 nmole doses of AB312-ADC. All ADCs have same drug-to antibody (DAR) of 4.

[000545] **Fig. 20** shows there were no body weight changes observed during the study duration indicating that all ADCs are safe at the dose ranges (10 nmole-80 nmole) tested.

[000546] **Fig. 21** depicts the characterization of pharmacokinetic (PK) properties of AB304 and AB309 antibodies when given via a single intravenous (IV) (slow bolus) injection, to cynomolgus monkeys. Non-human primates (NHP) were dosed at 3mg/kg and blood was drawn for serum chemistry at the indicated intervals. Serum concentrations of AB304 antibody and AB309 antibody determined using standard ELISA based bioanalytical methods and plotted against time intervals. There were no AB304 antibody or AB309 antibody -related changes in body weights. All animals survived to end of study. There were no AB304 antibody or AB309 antibody-related clinical observations. In conclusion, administration of AB304 antibody or AB309 antibody by intravenous (slow bolus) injection was tolerated at 3 mg/kg. The mean clearance (Cl) for AB304 antibody and AB309 antibody were 19.84 mL/day/kg and 13.37 mL/day/kg, respectively. These clearance rates were in the same range of reported clearance values for anti-NaPi-2b antibody (13.8 mL/day/kg) as described in Clin Cancer Res. 2015;21(22):5139-5150.

[000547] **Fig. 22** shows validation of different ADCs in the OVCAR3 cell line. The percent normalized viability of OVCAR3 cells exposed to different ADCs: AB304 (tetravalent bispecific ADC) or AB306 (bivalent bi-specific ADC) made and analyzed as described in **Figs**. **6-8** above except with the following changes. Cells are incubated with ADCs for a short time (4hrs instead of continuous exposure). After 4hrs, cells were washed and added to growth medium not containing ADCs. Tetravalent bispecific ADC (AB304) shows 4-fold improvement in the potency compared to monospecific ADC. Both ADCs have the same drug-to-antibody ratio (DAR) of 4.

[000548] As used herein, the following meanings apply unless otherwise specified. The words “can” and “may” are used in a permissive sense (i.e., meaning having the potential to), rather than the mandatory sense (i.e., meaning must). The words “include”, “including”, and “includes” and the like mean including, but not limited to. The singular forms “a,” “an,” and “the” include plural referents. Thus, for example, reference to “an element” includes a combination of two or more elements, notwithstanding use of other terms and phrases for one or more elements, such as “one or more.” The phrase “at least one” includes “one”, “one or more”, “one or a plurality”, and, therefore, contemplates the use of the term “a plurality”. The term “or” is, unless indicated otherwise, non-exclusive, i.e., encompassing both “and” and “or.” The term “any of” between a modifier and a sequence means that the modifier modifies each member of the sequence. So, for example, the phrase “at least any of 1, 2 or 3” means “at least 1, at least 2, or at least 3”. The term “about” refers to a range that is 5% plus or minus from a stated numerical value within the context of the particular usage. The term "consisting essentially of" refers to the inclusion of recited elements and other elements that do not materially affect the basic and novel characteristics of a claimed combination.

[000549] It should be understood that the description and the drawings are not intended to limit the invention to the particular form disclosed, but to the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the present invention as defined by the appended claims. Further modifications and alternative embodiments of various aspects of the invention will be apparent to those skilled in the art in view of this description. Accordingly, this description and the drawings are to be construed as illustrative only and are for the purpose of teaching those skilled in the art the general manner of carrying out the invention. It is to be understood that the forms of the invention shown and described herein are to be taken as examples of embodiments. Elements and materials may be substituted for those illustrated and described herein, parts and processes may be reversed or omitted, and certain features of the invention may be utilized independently, all as would be apparent to one skilled in the art after having the benefit of

this description of the invention. Changes may be made in the elements described herein without departing from the spirit and scope of the invention as described in the following claims.

[000550] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

Example 3:

[000551] This example evaluated the cytotoxic effects of Muc16/NaPi-2b bi-specific tetravalent ADCs in OVCAR wild-type (WT) and engineered variant cells. In vitro cytotoxicity assay was performed as described in Example 1 and the IC50 was calculated using GraphPad software. OVCAR-3 Muc16 knock-out KO and NaPi-2b KO cell lines were generated as described in Example 1. All test articles are DAR4 MMAE ADCs, except for the unconjugated AV-P138-antibody. The data is normalized to 100% and depicted as mean ± SD values from duplicate technical replicates. These results are representative of three independent experiments.

[000552] Fig. 23 shows in vitro cytotoxicity of bi-specific tetravalent ADCs in OVCAR-3 WT and variant cells. In Figure 23A, AB304-ADC and AB309ADC showed robust activity in compared to non-binding AB001-ADC and unconjugated AB309-antibody controls in OVCAR-3 WT cells, indicating target dependent, and MMAE driven activity. AB304-ADC and AB309-ADC exhibited similar or better cytotoxicity compared to AB301-ADC, AB302-ADC, the combination of both monospecific ADCs, and AB311 (Muc16/NaPi-2b bi-specific bivalent ADC). AB309-ADC also retained activity in the Muc16 KO (Fig. 23B) and NaPi-2b KO (Fig. 23C) variant cell lines, while reduced activity was observed for the monospecific AB302-ADC and AB301-ADC, respectively.

[000553] The in vitro cytotoxic effects of Muc16/NaPi-2b bi-specific tetravalent ADCs was assessed in models of tumor cell heterogeneity by mixing OVCAR-3 WT, Muc16 KO, and NaPi-2b KO cells. These models represent ovarian tumor heterogeneity in patients, with the expected estimated prevalence based on publicly available immunohistochemistry data. All test articles are DAR4 MMAE ADCs. The data is normalized to 100% and depicted as mean ± SD values from duplicate technical replicates. These results are representative of three independent experiments.

[000554] Fig. 24 shows in vitro cytotoxicity of bi-specific tetravalent ADCs in models of tumor cell heterogeneity. Analysis revealed that NaPi-2b is more predominantly expressed

over Muc16 in at least 50% of patients (Fig. 24A, NaPi-2b high and Muc16 low). Mimicking these relative expression levels was achieved by mixing OVCAR3 cells that are 80% NaPi-2b positive (Muc16KO), 10% Muc16 positive (NaPi-2b KO), and 10% dual positive (WT). Less common at approximately 15% are tumors with intermediate levels of NaPi-2b and Muc16 (Fig. 24B). Cell mixtures mimicking these relative expression levels consisted of OVCAR3 cells that are 45% NaPi-2b positive (Muc16KO), 45% Muc16 positive (NaPi-2b KO), and 10% dual positive (WT). It should be noted that WT OVCAR-3 cells which have high levels of both targets (Fig. 24A)represent 25% of ovarian patient tumors.

[000555] In all three in vitro models representing diverse NaPi-2b and Muc16 expression levels described in Fig. 23A and 24A-B, bi-specific tetravalentAB304-ADC and AB309-ADC enhanced cytotoxicity compared to a single or combination of monospecific AB302-ADC and AB301-ADC controls. As expected, the nontargeting AB001-ADC showed the least activity. These results demonstrate that dual targeting by bi-specific tetravalent ADCs can improve in vitro activity and overcome tumor cell heterogeneity.

[000556] Fig. 25 shows the internalization of Muc16/NaPi-2b bi-specific tetravalent antibodies evaluated by confocal microscopy. Briefly, OVCAR-3 WT cells were with treated with 50 nM of fluorescently labeled antibodies. Cells were incubated at 37 ◦C then stained and fixed at time 0, 5, and 20 hours post-treatment. Images were acquired using Nikon AXR microscope with a 40x water objective and data were analyzed using CellProfiler software.

[000557] Fig. 25 show differences in internalization as early as 5 hours post-treatment suggesting faster internalization of bi-specific tetravalent antibodies (AB304-antibody and AB309-antibody) versus monospecific antibodies (AB301-antibody and AB302-antibody) or a bi-specific bivalent antibody (AB311-antibody). At 20 hours post-treatment, the fluorescence signal from cells treated with AB304-antibody or AB309-antibody had at least 2-fold higher fluorescence indicating more internalized antibody compared to AB301-antibody, AB302-antibody, and AB311-antibody. No signal was detected for the nontargeting control AB001-antibody. These results demonstrate that dual targeting bi-specific tetravalent antibodies showed overall improved internalization compared to monospecific or bi-specific bivalent antibody controls.

Example 4

[000558] The pharmacokinetic (PK) properties of the bi-specific tetravalent AB309-ADC was assessed in non-human primate cynomolgus monkeys (n=3 females). Animals were administered a single dose of 3 mg/kg intravenously (IV bolus) and serum samples were collected for analysis of total antibody and total ADC concentrations while plasma samples were collected for free MMAE payload concentrations. Pharmacokinetic parameters were

calculated using the non-compartmental analysis (Phoenix Software V 8.3) using the concentration-time data for each analyte. Table 2 presents mean pharmacokinetic parameters following dosing of AB309-ADC for each of the analytes and the concentration-time profiles are shown in Fig. 26.

Table 2. Summary (Mean ± SD) Pharmacokinetic Parameters Following IV Administration of AB309-ADC in Female Cynomolgus Monkeys

Analyte	**C_{max} (ng/mL)**	**$AUC_{0\text{-}1008hr}$ (hr*ng/mL)**	**Half-life (hr)**	**CL (mL/Day/kg)**
Total Antibody	74500 ±5800	5650000 ±834000	127 ±2.62	12.86 ±1.92
Total ADC	76100 ±9980	4360000 ±472000	123 ±19.5	16.54 ±1.82
Free MMAE	0.131 ±0.014	7.660 ±1.210	80.7 ±NA	8640000 ±NA

Cmax: Maximum plasma or serum concentration; AUC: Area under curve; Half-life: Terminal half-life; CL: Systemic Clearance (for ADC and total antibody) or apparent clearance (for Free MMAE); NA: Not applicable

[000559] In general, the AB309-ADC PK profile is similar to the AB309-antibody alone (12.9vs 13.3 mL/Day/kg CL, respectively) and comparable to reported IgG therapeutics cited in Fig. 21. The overlapping concentration time profile of the total antibody and total ADC following IV dosing of AB309-ADC indicates minimal deconjugation and suggests good ADC stability (Fig. 26). This is consistent with the lower AUC for free MMAE observed following AB309-ADC administration compared to other ADCs with random conjugation as reported in Molecular Cancer Therapeutics 2024; 23(10):1483-1493.

Example 5

[000560] The ability of bi-specific tetravalent ADCS to bind both antigens simultaneously was demonstrated by biolayer interferometry (BLI) experiments using both antigens as analytes. In these experiments, bi-specific tetravalent ADC variants and monospecific ADC controls were subjected to two consecutive binding cycles in which the immobilized ADCs were associated with recombinant Muc16 in the first cycle, followed by association with recombinant NaPi-2b (Fig 27 A-D). The experiment was also performed wherein the order of the analytes were reversed (Fig. 27 E-H). Representative results from three independent experiments are shown in Fig. 27.

[000561] Initial binding signals were generated upon specific binding of Muc16, with further signal increased through binding of NaPi-2b for the bi-specific tetravalent ADCs AB304 (Fig. 27A) and AB309 (Fig. 27B). Monospecific ADCs AB301 and AB302 only showed signal

increase when binding to only NaPi-2b (Fig 27C) or Muc16 (Fig. 27D), respectively, but did not display any additional binding to the other target. Similar results are observed when the order of the analytes were exchanged with recombinant NaPi-2b in the first cycle, followed by recombinant Muc16 as shown in Fig. 27 E-H. These results indicate that the bi-specific tetravalent ADCs are able to bind to both targets (Muc16 and NaPi-2b) simultaneously.

SEQUENCE LISTING

SEQ ID NO:1: Muc16 HCDR1

SEQ ID NO:2: Muc16 HCDR2

SEQ ID NO:3: Muc16 HCDR3

SEQ ID NO:4: Muc16 LCDR1

SEQ ID NO:5: Muc16 LCDR2

SEQ ID NO:6: Muc16 LCDR3

SEQ ID NO:7: Muc16 AB302 Heavy chain

SEQ ID NO:8: Muc16 AB302 Light chain

SEQ ID NO:9: NaPi-2b HCDR1

SEQ ID NO:10: NaPi-2b HCDR2

SEQ ID NO:11: NaPi-2b HCDR3

SEQ ID NO:12: NaPi-2b LCDR1

SEQ ID NO:13: NaPi-2b LCDR2

SEQ ID NO:14: NaPi-2b LCDR3

SEQ ID NO:15: NaPi-2b AB301 Heavy Chain

SEQ ID NO:16: NaPi-2b AB301 Light Chain

SEQ ID NO:17: AB303 Heavy Chain 1

SEQ ID NO:18: AB303 Heavy Chain 2

SEQ ID NO:19: Linker Sequence

SEQ ID NO:20: AB306 Heavy Chain 1

SEQ ID NO:21: AB306 Heavy Chain 2

SEQ ID NO:22: AB311 Heavy Chain 1

SEQ ID NO:23: AB311 Heavy Chain 2

SEQ ID NO:24: AB308 Heavy Chain

SEQ ID NO:25: AB307 Heavy Chain 1

SEQ ID NO:26: AB307 Heavy Chain 2

SEQ ID NO:27: AB304 Heavy Chain

SEQ ID NO:28: AB305 Heavy Chain

SEQ ID NO:29: AB309 Heavy Chain

SEQ ID NO:30: AB310 Heavy Chain 1

SEQ ID NO:31: AB310 Heavy Chain 2

SEQ ID NO:32: AB302 Heavy Chain variable region (Muc16)

SEQ ID NO:33: AB302 Light Chain variable region (Muc16)

SEQ ID NO:34: AB301 Heavy Chain variable region (NaPi-2b)

SEQ ID NO:35: AB301 Light Chain variable region (NaPi-2b)

SEQ ID NO:36: sofituzumab Heavy chain variable region

SEQ ID NO:37: sofituzumab Light chain variable region

SEQ ID NO:38: lifastuzumab Heavy chain variable region

SEQ ID NO:39: lifastuzumab Light chain variable region

SEQ ID NO:40: upifitamab HCDR1

SEQ ID NO:41: upifitamab HCDR2

SEQ ID NO:42: upifitamab HCDR3

SEQ ID NO:43: upifitamab LCDR1

SEQ ID NO:44: upifitamab LCDR2

SEQ ID NO:45: upifitamab LCDR3

SEQ ID NO:46: upifitamab Heavy chain variable region

SEQ ID NO:47: upifitamab Light chain variable region

SEQ ID NO:48: AB312 HCDR1

SEQ ID NO:49: AB312 HCDR2

SEQ ID NO:50: AB312 HCDR3

SEQ ID NO:51: AB312 LCDR1

SEQ ID NO:52: AB312 LCDR2

SEQ ID NO:53: AB312 LCDR3

SEQ ID NO:54: AB312 Heavy chain

SEQ ID NO:55: AB312 Light chain

SEQ ID NO:56: AB309-C Heavy chain

SEQ ID NO:57: AB309-D Heavy chain

SEQ ID NO:58: AB309-E Heavy chain

SEQ ID NO:59: AB309-F Heavy chain

SEQ ID NO:60: anti-NaPi-2b HCDR1

SEQ ID NO:61: anti-NaPi-2b HCDR1

SEQ ID NO:62: anti-NaPi-2b HCDR1

SEQ ID NO:63: anti-NaPi-2b HCDR1

SEQ ID NO:64: anti-NaPi-2b HCDR2

SEQ ID NO:65: anti-NaPi-2b HCDR2

SEQ ID NO:66: anti-NaPi-2b HCDR2

SEQ ID NO:67: anti-NaPi-2b HCDR2

SEQ ID NO:68: anti-NaPi-2b HCDR2

SEQ ID NO:69: anti-NaPi-2b HCDR2

SEQ ID NO:70: anti-NaPi-2b HCDR2

SEQ ID NO:71: anti-NaPi-2b HCDR2

SEQ ID NO:72: anti-NaPi-2b HCDR2

SEQ ID NO:73: anti-NaPi-2b HCDR2

SEQ ID NO:74: anti-NaPi-2b HCDR2

SEQ ID NO:75: anti-NaPi-2b HCDR2

SEQ ID NO:76: anti-NaPi-2b HCDR2

SEQ ID NO:77: anti-NaPi-2b HCDR2

SEQ ID NO:78: anti-NaPi-2b HCDR2

SEQ ID NO:79: anti-NaPi-2b HCDR3

SEQ ID NO:80: anti-NaPi-2b HCDR3

SEQ ID NO:81: anti-NaPi-2b HCDR3

SEQ ID NO:82: anti-NaPi-2b HCDR3

SEQ ID NO:83: anti-NaPi-2b HCDR3

SEQ ID NO:84: anti-NaPi-2b LCDR1

SEQ ID NO:85: anti-NaPi-2b LCDR1

SEQ ID NO:86: anti-NaPi-2b LCDR1

SEQ ID NO:87: anti-NaPi-2b LCDR1

SEQ ID NO:88: anti-NaPi-2b LCDR1

SEQ ID NO:89: anti-NaPi-2b LCDR1

SEQ ID NO:90: anti-NaPi-2b LCDR1

SEQ ID NO:91: anti-NaPi-2b LCDR1

SEQ ID NO:92: anti-NaPi-2b LCDR1

SEQ ID NO:93: anti-NaPi-2b LCDR1

SEQ ID NO:94: anti-NaPi-2b LCDR1

SEQ ID NO:95: anti-NaPi-2b LCDR1

SEQ ID NO:96: anti-NaPi-2b LCDR1

SEQ ID NO:97: anti-NaPi-2b LCDR1

SEQ ID NO:98: anti-NaPi-2b LCDR2

SEQ ID NO:99: anti-NaPi-2b LCDR2

SEQ ID NO:100: anti-NaPi-2b LCDR2

SEQ ID NO:101: anti-NaPi-2b LCDR2

SEQ ID NO:102: anti-NaPi-2b LCDR3

SEQ ID NO:103: anti-NaPi-2b LCDR3

SEQ ID NO:104: anti-NaPi-2b LCDR3

WHAT IS CLAIMED IS:

1. A tetravalent bispecific antibody, comprising two paratopes that each specifically bind to Muc16 and two paratopes that each specifically bind to NaPi-2b, wherein the antibody comprises two heavy chains and two light chains that assemble to form a diabody-Fc-Fab structure,

wherein the heavy chains each comprise, from N-terminus to C-terminus,

an anti-NaPi-2b variable heavy (VH) region comprising heavy chain (HC) complementarity determining regions:

HCDR1: GFSFSDFAMS (SEQ ID NO:9) or any one of SEQ ID NOs: 60-63

HCDR2: TIGRVAFHTY (SEQ ID NO:10) or any one of SEQ ID NOs: 64-78

HCDR3: HRGFDVGHFDF (SEQ ID NO:11) or any one of SEQ ID NOs: 79-83,

an anti-NaPi-2b variable light (VL) region comprising light chain (LC) complementarity determining regions:

LCDR1: RSSETLVHSSGNTYLE (SEQ ID NO:12) or any one of SEQ ID NOs: 84-97

LCDR2: RVSNRFS (SEQ ID NO:13) or any one of SEQ ID NOs: 98-101

LCDR3: FQGSFNPLT (SEQ ID NO:14) or any one of SEQ ID NOs: 102-104,

a heavy chain constant 2 (CH2) region,

a heavy chain constant 3 (CH3) region,

an anti-Muc16 VH region comprising heavy chain (HC) complementarity determining regions:

HCDR1: GYSITNDYAWN (SEQ ID NO:1) or SEQ ID NO:48

HCDR2: YISYSGYTT (SEQ ID NO:2) or SEQ ID NO:49

HCDR3: WTSGLDY (SEQ ID NO:3) or SEQ ID NO:50, and

a heavy chain constant 1 (CH1) region; and

wherein the light chains each comprise, from N-terminus to C-terminus,

an anti-Muc16 VL region comprising light chain (LC) complementarity determining regions:

LCDR1: KASDLIHNWLA (SEQ ID NO:4) or SEQ ID NO:51

LCDR2: GATSLET (SEQ ID NO:5) or SEQ ID NO:52

LCDR3: QQYWTTPFT (SEQ ID NO:6) or SEQ ID NO:53, and

a light chain constant (CL) region.

2. The antibody of claim 1, wherein the anti-NaPi-2b VH region comprises HC complementarity determining regions:

HCDR1: GFSFSDFAMS (SEQ ID NO:9)

HCDR2: TIGRVAFHTY (SEQ ID NO:10)
HCDR3: HRGFDVGHFDF (SEQ ID NO:11),
the anti-NaPi-2b VL region comprises LC complementarity determining regions:
LCDR1: RSSETLVHSSGNTYLE (SEQ ID NO:12)
LCDR2: RVSNRFS (SEQ ID NO:13)
LCDR3: FQGSFNPLT (SEQ ID NO:14),
the anti-Muc16 VH region comprises HC complementarity determining regions:
HCDR1: GYSITNDYAWN (SEQ ID NO:1)
HCDR2: YISYSGYTT (SEQ ID NO:2)
HCDR3: WTSGLDY (SEQ ID NO:3), and
the anti-Muc16 VL region comprises LC complementarity determining regions:
LCDR1: KASDLIHNWLA (SEQ ID NO:4)
LCDR2: GATSLET (SEQ ID NO:5)
LCDR3: QQYWTTPFT (SEQ ID NO:6).

3. The antibody of claim 1 or 2, wherein the lights chains each comprise a heavy chain sequence at least 90% identical to or at least 95% identical to SEQ ID NO:8.

4. The antibody of claim 3, wherein the lights chains each comprise a heavy chain sequence according to SEQ ID NO:8.

5. The antibody of any one of claims 1-4, wherein the heavy chains each comprise a heavy chain sequence at least 90% identical to or at least 95% identical to SEQ ID NO:29 or any one of SEQ ID NO:56-59.

6. The antibody of claim 5, wherein the heavy chains each comprise a heavy chain sequence according to SEQ ID NO:29 or any one of SEQ ID NO:56-59.

7. The antibody of any one of claims 1-6, wherein the heavy chains comprises a cysteine (C) at position 611 related to SEQ ID NO:29 (HC-A118C by EU numbering).

8. The antibody of any one of claims 1-7, wherein the light chains comprises a cysteine (C) at position 205 related to SEQ ID NO:8 (LC-V205C by EU numbering).

9. The antibody of any one claims 1-8, wherein the heavy chains comprise one or more of the following substitutions: S484A, S484G, S489A, S489G.

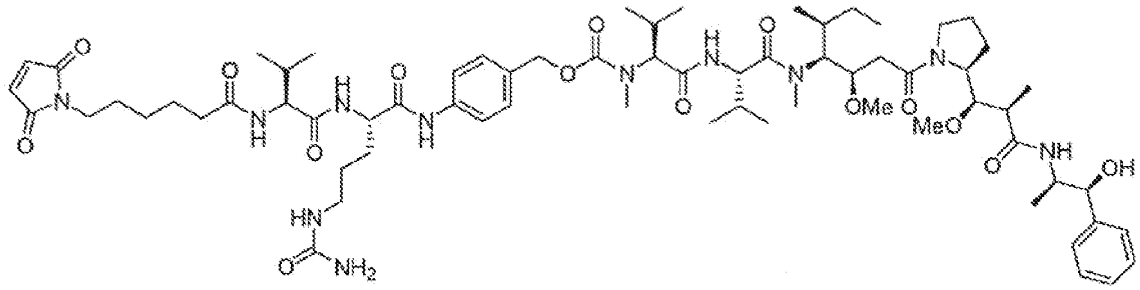
10. The antibody of any one of claims 1-9, further comprising one or more payloads conjugated thereto, optionally as a linker-payload.

11. The antibody of claim 10, comprising four payloads conjugated thereto (DAR = 4).

12. The antibody of claim 10 or claim 11, wherein the payload is monomethyl auristatin E (MMAE).

13. The antibody of claim 10 or claim 11, wherein the payload is a maytansinoid, DM-1, DM-4, an auristatin, monomethyl auristatin E, monomethyl auristatin F, dolastatin, a tubulysin, eribulin, cryptophycin, a benzodiazepine, indolino-benzodiazepine, isoquinolidino-benzodiazepine, pyrrolo-benzodiazepine, alpha-amanitin, trichothene, camptothecin, a camptothecin derivative, SN-38, exatecan, belotecan, DXd, topotecan, samrotecan, duocarmycin, DGN549, CC1065, a calicheamicin, N-acetyl calicheamicin, an enediyne antibiotic, a taxane, a doxorubicin derivative, an anthracycline or stereoisomer thereof, or azonafide, or an isostere, analogs, heterodimer, homodimer, or derivative thereof.

14. The antibody of claim 10 or claim 11, wherein the linker-payload is:



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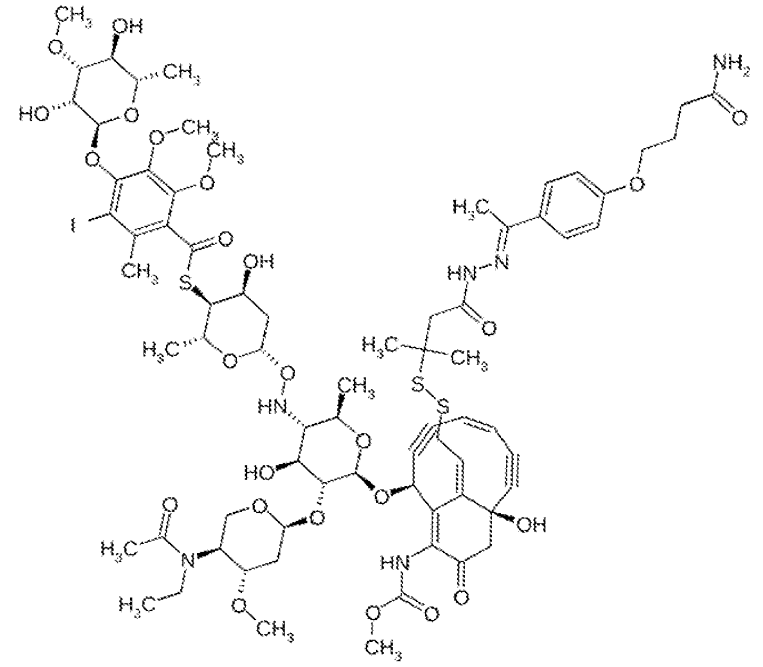
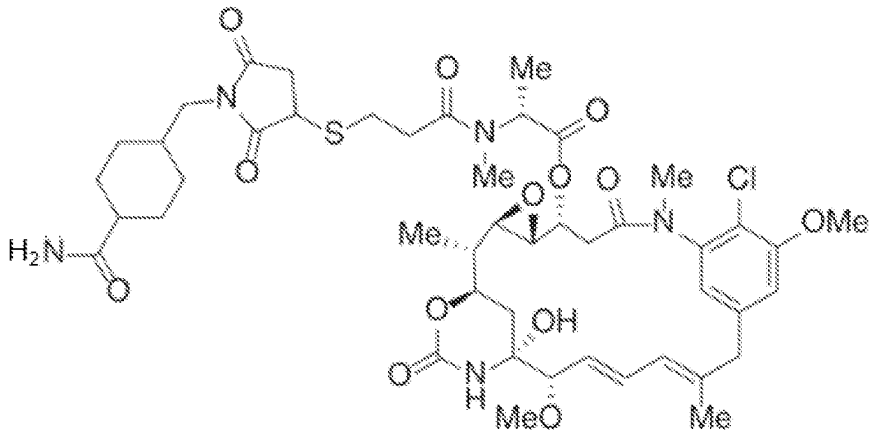
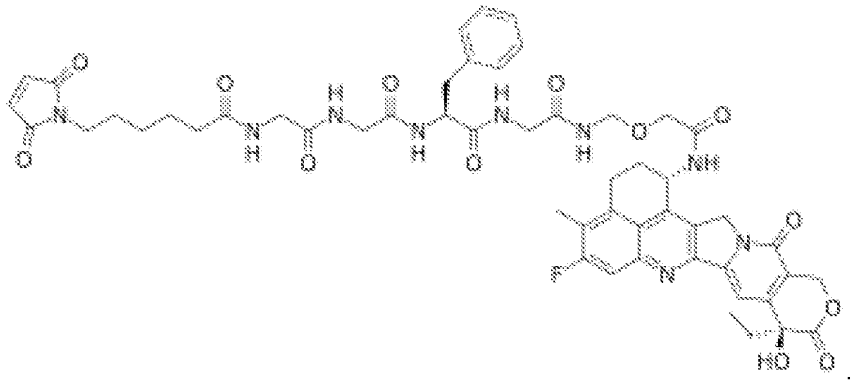
15. The antibody of claim 10 or claim 11, wherein the linker-payload is:



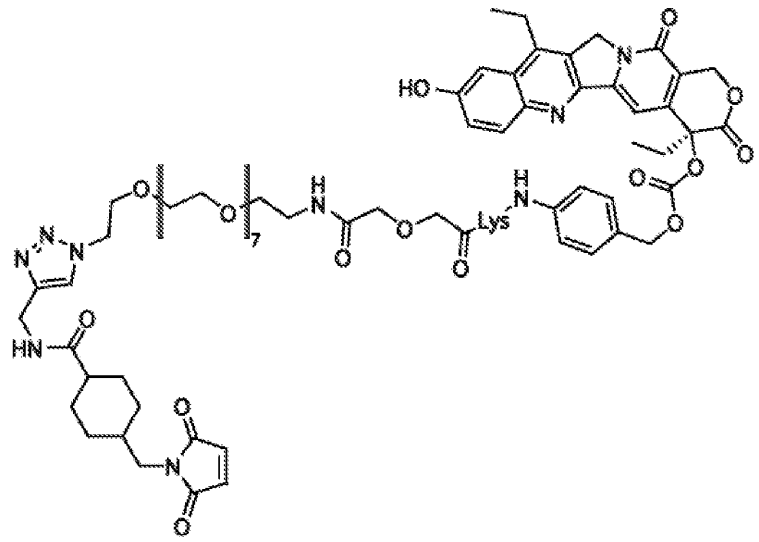
16. The antibody of claim 10 or claim 11, wherein the linker-payload is:



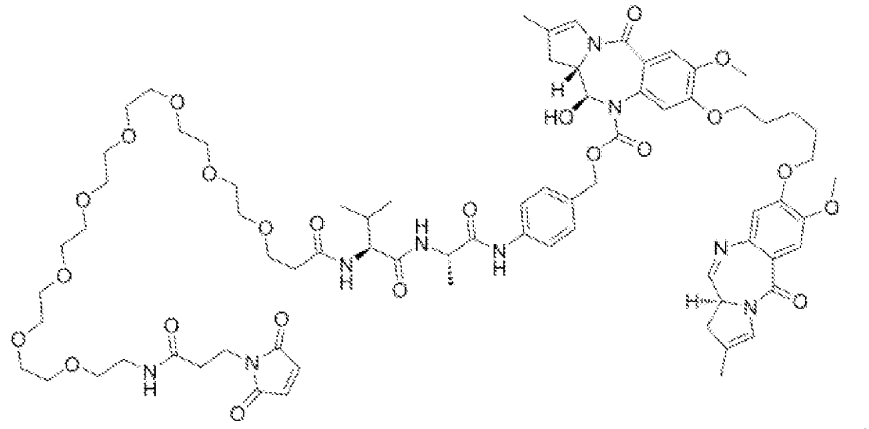
17. The antibody of claim 10 or claim 11, wherein the linker-payload is:



18. The antibody of claim 10 or claim 11, wherein the linker-payload is:



19. The antibody of claim 10 or claim 11, wherein the linker-payload is:



20. A pharmaceutical composition, comprising a therapeutically effective amount of the antibody of any one of claims 1-19.

21. The pharmaceutical composition of claim 20, further comprising a pharmaceutically acceptable carrier, diluent or excipient.

22. The pharmaceutical composition of claim 20 or 21, for use as a medicament in the treatment of cancer.

23. A method of treating a subject with cancer, comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of any one of claims 20-22.

24. The method of claim 23, wherein the cancer is selected from ovarian, breast, lung, stomach, endometrial and pancreatic.

25. A method of detecting a cell that expresses Muc16 and/or NaPi-2b, comprising: a) contacting the cell with the antibody of any one of claims 1-19 to allow binding between the antibody and the cell; and b) detecting the binding of the antibody to the cell.

26. The method of claim 25, comprising contacting the cell with the antibody conjugated to a detectable moiety; and b) detecting the detectable moiety bound to the cell.

27. A method of killing a cell comprising contacting a cell with the antibody of any one of claims 1-19, wherein one or more paratopes of the antibody specifically bind to an epitope of the cell.

28. The method of claim 27, wherein two different paratopes of the antibody bind to two different epitopes of the cell.

29. The method of claim 27 or 28, wherein the epitope is comprised in a cell surface antigen.

30. A polynucleotide comprising polynucleotide sequences encoding the antibody of any one of claims 1-19.

31. A vector or a host cell comprising the polynucleotide of claim 30.

32. A tetravalent bispecific antibody, comprising two paratopes that each specifically bind to Muc16 and two paratopes that each specifically bind to NaPi-2b, wherein the

antibody comprises two heavy chains and two lights chains that assemble to form a Fab-diabody-Fc structure,

wherein the heavy chains each comprise, from N-terminus to C-terminus,

an anti-NaPi-2b variable heavy (VH) region comprising heavy chain (HC) complementarity determining regions:

HCDR1: GFSFSDFAMS (SEQ ID NO:9) or any one of SEQ ID NOs: 60-63

HCDR2: TIGRVAFHTY (SEQ ID NO:10) or any one of SEQ ID NOs: 64-78

HCDR3: HRGFDVGHFDF (SEQ ID NO:11) or any one of SEQ ID NOs: 79-83

a heavy chain constant 1 (CH1) region,

an anti-Muc16 variable heavy (VH) region comprising heavy chain (HC) complementarity determining regions:

HCDR1: GYSITNDYAWN (SEQ ID NO:1) or SEQ ID NO:48

HCDR2: YISYSGYTT (SEQ ID NO:2) or SEQ ID NO:49

HCDR3: WTSGLDY (SEQ ID NO:3) or SEQ ID NO:50,

an anti-Muc16 variable light (VL) region comprising light chain (LC) complementarity determining regions:

LCDR1: KASDLIHNWLA (SEQ ID NO:4) or SEQ ID NO:51

LCDR2: GATSLET (SEQ ID NO:5) or SEQ ID NO:52

LCDR3: QQYWTTPFT (SEQ ID NO:6) or SEQ ID NO:53

a heavy chain constant 2 (CH2) region,

a heavy chain constant 3 (CH3) region; and

wherein the light chains each comprise, from N-terminus to C-terminus,

an anti-NaPi-2b variable light (VL) region comprising light chain (LC) complementarity determining regions:

LCDR1: RSSETLVHSSGNTYLE (SEQ ID NO:12) or any one of SEQ ID NOs: 84-97

LCDR2: RVSNRFS (SEQ ID NO:13) or any one of SEQ ID NOs: 98-101

LCDR3: FQGSFNPLT (SEQ ID NO:14) or any one of SEQ ID NOs: 102-104, and

a light chain constant (CL) region.

33. The antibody of claim 32, wherein the anti-NaPi-2b VH region comprises HC complementarity determining regions:

HCDR1: GFSFSDFAMS (SEQ ID NO:9)

HCDR2: TIGRVAFHTY (SEQ ID NO:10)

HCDR3: HRGFDVGHFDF (SEQ ID NO:11),

the anti-Muc16 VH region comprises HC complementarity determining regions:

HCDR1: GYSITNDYAWN (SEQ ID NO:1)
HCDR2: YISYSGYTT (SEQ ID NO:2)
HCDR3: WTSGLDY (SEQ ID NO:3),
the anti-Muc16 VL region comprises LC complementarity determining regions
LCDR1: KASDLIHNWLA (SEQ ID NO:4)
LCDR2: GATSLET (SEQ ID NO:5)
LCDR3: QQYWTTPFT (SEQ ID NO:6), and
the anti-NaPi-2b VL region comprises LC complementarity determining regions:
LCDR1: RSSETLVHSSGNTYLE (SEQ ID NO:12)
LCDR2: RVSNRFS (SEQ ID NO:13)
LCDR3: FQGSFNPLT (SEQ ID NO:14), and
the light chain constant (CL) region.

34. The antibody of claim 32 or 33, wherein the light chains each comprise a heavy chain sequence at least 90% identical to or at least 95% identical to SEQ ID NO:16.

35. The antibody of claim 34, wherein the light chains each comprise a heavy chain sequence according to SEQ ID NO:16.

36. The antibody of any one of claim 32-35, wherein the heavy chains each comprise a heavy chain sequence at least 90% identical to or at least 95% identical to SEQ ID NO:27.

37. The antibody of claim 36, wherein the heavy chains each comprise a heavy chain sequence according to SEQ ID NO:27.

38. The antibody of claim 32-37, wherein the heavy chains comprises a cysteine (C) at position 121 related to SEQ Id NO:27 (HC-A118C by EU numbering).

39. The antibody of any one of claims 32-38, wherein the light chain comprises a cysteine (C) at position 210 related to SEQ ID NO:16 (LC-V205C by EU numbering).

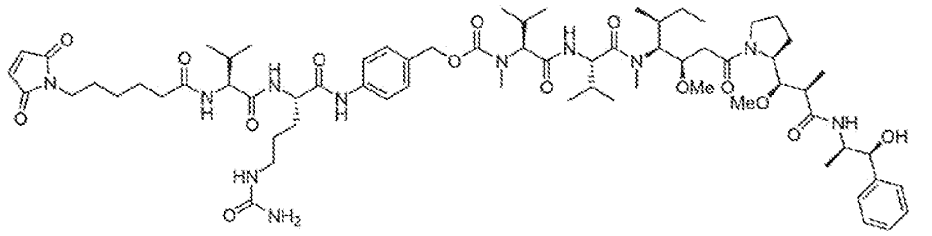
40. The antibody of any one of claims 32-39, further comprising one or more payloads conjugated thereto.

41. The antibody of claim 40, comprising four payloads conjugated thereto (DAR=4).

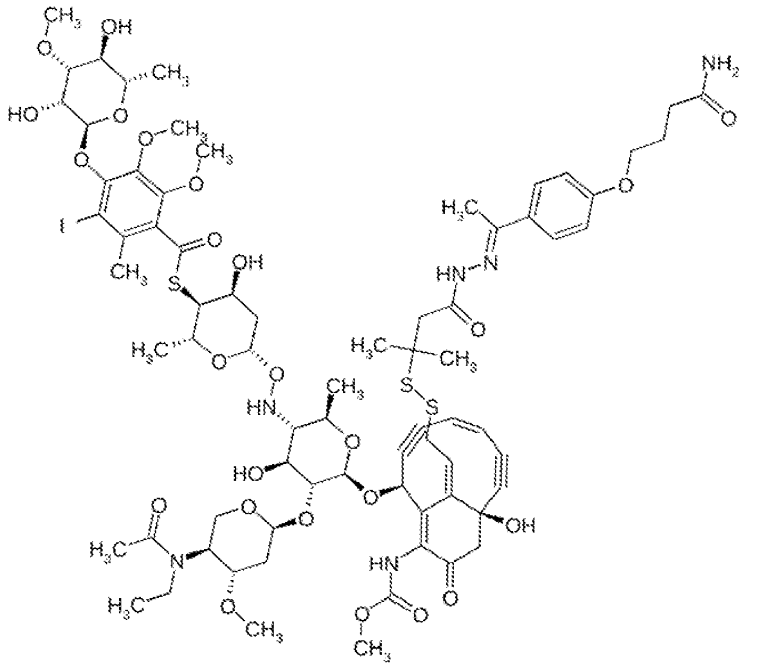
42. The antibody of claim 40 or 41, wherein the payload is monomethyl auristatin E (MMAE).

43. The antibody of claim 40 or 41, wherein the payload is maytansinoid, DM-1, DM-4, auristatin, monomethyl auristatin E, monomethyl auristatin F, dolastatin, tubulysin, eribulin, cryptophycin, benzodiazepine, indolino-benzodiazepine, isoquinolidino-benzodiazepine, pyrrolo-benzodiazepine, alpha-amanitin, trichothene, camptothecin derivatives (SN-38, exatecan, belotecan, DXd, topotecan, samrotecan), duocarmycin, DGN549, CC1065, calicheamicin, N-acetyl calicheamicin,an enediyne antibiotic, taxane, doxorubicin derivatives, anthracycline and stereoisomers, azanofide, isosteres, and analogs, hetero dimer or homo dimers or derivatives thereof.

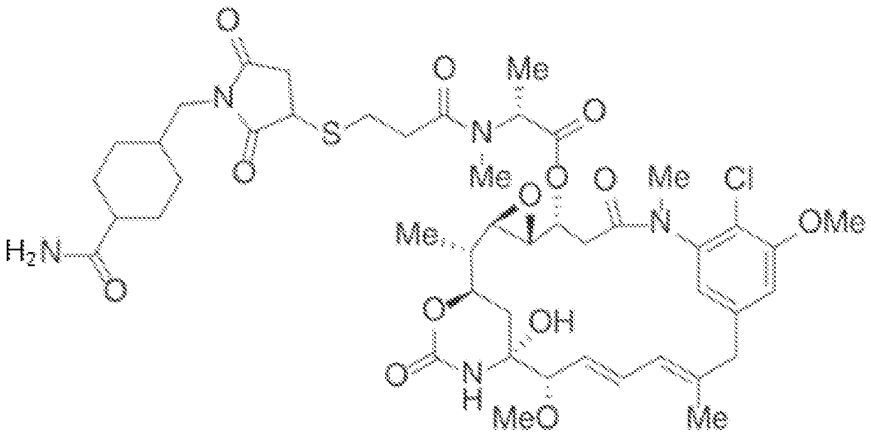
44. The antibody of claim 40 or claim 41 wherein the linker-payload is:



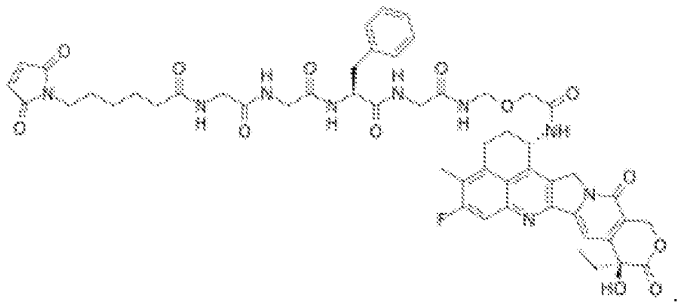
45. The antibody of claim 40 or claim 41, wherein the linker-payload is:



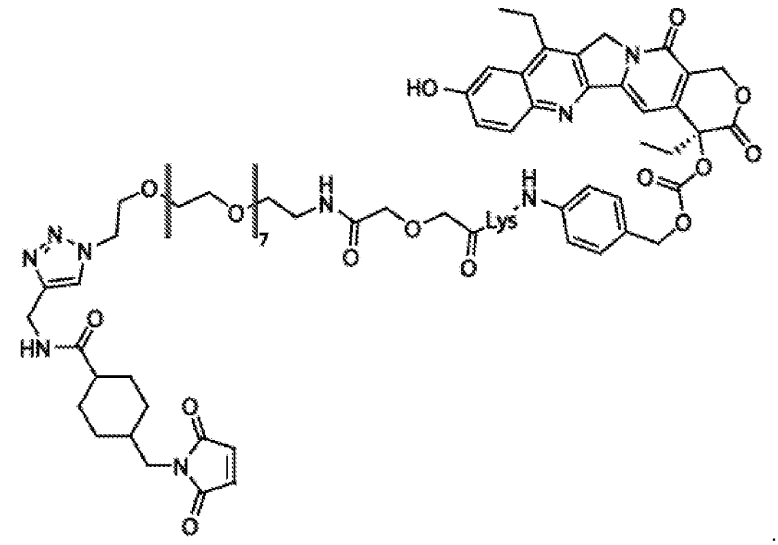
46. The antibody of claim 40 or claim 41, wherein the linker-payload is:



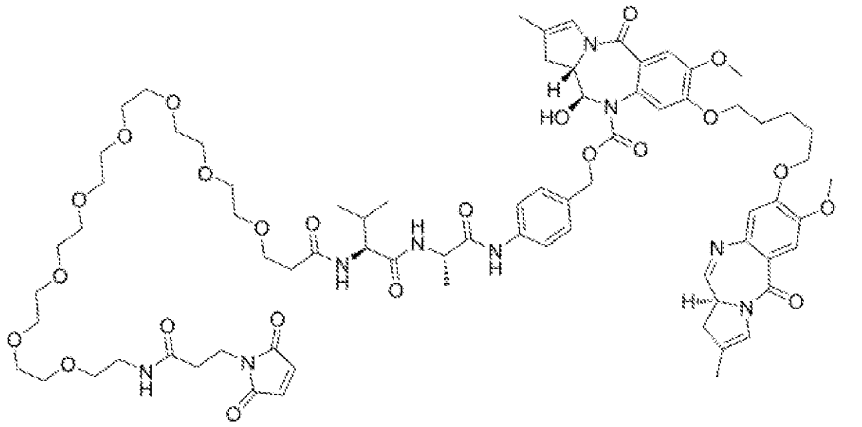
47. The antibody of claim 40 or claim 41, wherein the linker-payload is:



48. The antibody of claim 40 or claim 41, wherein the linker-payload is:



49. The antibody of claim 40 or claim 41, wherein the linker-payload is:



50. A pharmaceutical composition, comprising a therapeutically effective amount of the antibody of any one of claims 32-49.

51. The pharmaceutical composition of claim 50, further comprising a pharmaceutically acceptable carrier, diluent or excipient.

52. The pharmaceutical composition of claim 50 or 51, for use as a medicament in the treatment of cancer.

53. A method of treating a subject with cancer, comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of any one of claims 50-52.

54. The method of claim 53, wherein the cancer is selected from ovarian, breast, lung, stomach, endometrial and pancreatic.

55. A method of detecting a cell that expresses Muc16 and/or NaPi-2b, comprising: a) contacting the cell with the antibody of any one of claims 32-49 to allow binding between the antibody and the cell; and b) detecting the binding of the antibody to the cell.

56. The method of claim 55, comprising contacting the cell with the antibody conjugated to a detectable moiety; and b) detecting the detectable moiety bound to the cell.

57. A method of killing a cell comprising contacting a cell with the antibody of any one of claims 32-49, wherein one or more paratopes of the antibody specifically bind to an epitope of the cell.

58. The method of claim 57, wherein two different paratopes of the antibody bind to two different epitopes of the cell.

59. The method of claim 57 or 58, wherein the epitope is comprised in a cell surface antigen.

60. A polynucleotide comprising polynucleotide sequences encoding the antibody of any one of claims 32-49.

61. A vector or a host cell comprising the polynucleotide of claim 60.

62. Use of an antibody of any of claims 1-19 in the manufacture of a medicament.

63. Use of an antibody of any of claims 1-19 to produce an immunoconjugate.

64. Use of an antibody of any of claims 32-49 in the manufacture of a medicament.

65. Use of an antibody of any of claims 32-49 to produce an immunoconjugate.

66. A method of making an immunoconjugate comprising:

a) providing an antibody of any of claims 1-9 or 32-39; and

b) conjugating one or more payloads to the antibody.

67. The method of claim 66, wherein at least one of the payloads is conjugated to the antibody through a linker.

68. The method of claim 66, wherein at least one of the payloads is conjugated to the antibody through a broken disulfide bond.

69. The method of claim 66, wherein the antibody is a cysteine-substituted antibody, and at least one payload is coupled to the antibody through a substituted cysteine.

70. The method of claim 66, wherein the immunoconjugate has a drug-antibody ratio ("DAR") of at least any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12.

71. A collection of one or more nucleic acid molecules that collectively encode paratopes that bind Muc16 and NaPi-2b.

72. The collection of nucleic acid molecules of claim 71, encoding one or more antibodies of any of claims 1-9 or 32-39.

73. The collection of nucleic acid molecules of claim 71, encoding one or more light chains of the antibody of any of claims 1-9 or 32-39.

74. The collection of nucleic acid molecules of claim 71, encoding one or more heavy chains of the antibody of any of claims 1-9 or 32-39.

75. The collection of nucleic acid molecules of any of claims 71-74, encoding one or more light chains comprising variable regions of paratopes that bind Muc16 and/or NaPi-2b.

76. The collection of nucleic acid molecules of any of claims 71-74, the nucleic acid molecules further comprising one or more expression control sequences operatively linked to one or more nucleotide sequences encoding the one or more light chain variable regions and/or one or more heavy chain variable regions.

77. The collection of nucleic acid molecules of any of claims 71-74, comprised within one or more vectors, e.g., selected from a plasmid, a viral vector (e.g., a retrovirus, a lentivirus, and adenovirus, and adeno-associated virus, or a herpes simplex virus), a bacterial artificial chromosome, and a yeast artificial chromosome.

78. A cell comprising a collection of nucleic acid molecules of any of claims 71-77.

79. The cell of claim 78, selected from a mammalian cell (e.g., a Chinese hamster ovary (CHO) cell, an NS0 cell, an SP2/0 cell, a human embryonic kidney HEK 293 cell, or a Per.C6 cell); an insect cell (e.g., an Sf9 cell or a high five (BTI-Tn-5B1-4) cell); a yeast cell (e.g., Pichia pastoris or Saccharomyces cerevisiae); a bacterial cell (e.g., E. coli); and a plant cell.

80. A method of making an antibody comprising:

a) providing a cell or cells of any of claims 78-79;

b) expressing immunoglobulin molecules encoded by the nucleotide sequences in the cell or cells; and

c) recovering the expressed immunoglobulin molecules.

81. The method of claim 81, wherein the antibody is an asymmetric antibody and the cell comprises one or more nucleic acid molecules encoding a heavy chain comprising a knob, a heavy chain comprising a hole, and at least one light chain.

82. The method of claim 81, wherein the cell comprises nucleotide sequences encoding only one light chain, wherein the light chain is configured to associate with different heavy chain portions to produce two paratopes.

83. The method of claim 81, wherein the cell comprises nucleic acids encoding two different light chains, wherein a first light chain is configured to associate with a first heavy chain portion to produce a first paratope that binds to a first epitope, and a second light chain is configured to associate with a second heavy chain portion to produce a second paratope that binds to a second, different epitope.

84. The method of claim 81, further comprising producing different immunoglobulin chains in different cells, recovering the immunoglobulin chains, and contacting the immunoglobulin chains, wherein the immunoglobulin chains assemble into an antibody.

85. The method of claim 81, further comprising purifying the recovered antibody, e.g. using protein A or protein G.