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(54) Title: ERIBULIN-BASED ANTIBODY-DRUG CONJUGATES AND METHODS OF USE

(57) Abstract: Linker toxins and antibody-drug conjugates that bind to human oncology antigen targets such as folate receptor alpha and/or provide anti-tubulin drug activity are disclosed. The linker toxins and antibody-drug conjugates comprise an eribulin drug moiety and can be internalized into target antigen-expressing cells. The disclosure further relates to methods and compositions for use in the treatment of cancer by administering the antibody-drug conjugates provided herein.

ERIBULIN-BASED ANTIBODY-DRUG CONJUGATES AND METHODS OF USE

[0001] The present application claims the benefit of priority to U.S. Provisional Patent Application No. 62/302,562, filed March 2, 2016, the entire contents of which are incorporated herein by reference.

[0002] The present disclosure relates to antibody drug conjugates (ADCs) that bind human oncology antigen targets such as folate receptor alpha and/or provide anti-tubulin drug activity. The disclosure further relates to methods and compositions useful in the treatment and diagnosis of cancers that express folate receptor alpha and/or are amenable to treatment by disrupting tubulin.

[0003] Cancer is among the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases and 8.2 million cancer-related deaths in 2012. The most common causes of cancer death are cancers of: lung (1.59 million deaths); liver (745,000 deaths); stomach (723,000 deaths); colorectal (694,000 deaths); breast (521,000 deaths); and esophagus (400,000 deaths). The number of new cancer cases is expected to rise by about 70% over the next two decades, to approximately 22 million new cancer cases per year (World Cancer Report 2014).

[0004] Microtubules are dynamic filamentous cytoskeletal proteins that are involved in a variety of cellular functions, including intracellular migration and transport, cell signaling, and the maintenance of cell shape. Microtubules also play a critical role in mitotic cell division by forming the mitotic spindle required to segregate chromosomes into two daughter cells. The biological functions of microtubules in all cells are regulated in large part by their polymerization dynamics, which occurs by the reversible, non-covalent addition of α and β tubulin dimers at both ends of microtubules. This dynamic behavior and resulting control over microtubule length is vital to the proper functioning of the mitotic spindle. Even minor alteration of microtubule dynamics can engage the spindle checkpoint, arrest cell cycle progression at mitosis, and subsequently lead to cell death (Mukhtar et al. (2014) Mol. Cancer Ther. 13:275-84). Due to their rapid cell division, cancer cells are generally more sensitive to compounds that bind to tubulin and disrupt its normal function, as compared to normal cells. For this reason, tubulin inhibitors and other microtubule-targeted agents have become a promising class of drugs for the treatment of cancer (Dumontet and Jordan (2010) Nat. Rev. Drug Discov. 9:790-803).

[0005] Folate receptor alpha (FRA) is a glycophosphatidylinositol (GPI)-linked membrane protein that binds folate. While the role of FRA in the biology of normal and cancerous tissue is not fully understood, it is highly over-expressed on a high percentage of ovarian cancers of epithelial origin (O'Shannessy et al. (2013) Int. J. Gynecol. Pathol. 32(3):258-68), as well as in a percentage of non-small cell lung carcinomas (Christoph et al. (2014) Clin. Lung Cancer 15(5):320-30). FRA also has limited expression in normal tissues. These properties make FRA an attractive target for cancer immunotherapy.

[0006] The proto-oncogene human epidermal growth factor receptor 2 (*HER2*) encodes a transmembrane tyrosine kinase receptor that belongs to the human epidermal growth factor receptor (EGFR) family (King et al. (1985) Science 229:974-6). Overexpression of HER2 enables constitutive activation of growth factor signaling pathways, such as the PI3K–AKT–mTOR pathway, and thereby serves as an oncogenic driver in several types of cancers, including approximately 20% of invasive breast carcinomas (Slamon et al. (1989) Science 244:707-12; Gajria and Chandarlapaty (2011) Expert Rev. Anticancer Ther. 11:263-75). Given that HER2 amplification mediates the transformed phenotype, HER2 is another promising target for cancer treatment.

[0007] The present disclosure provides, in part, novel compounds with biological activity against tumor cells. The compounds may inhibit tumor growth in mammals, and may be useful for treating human cancer patients.

[0008] The present disclosure more specifically relates to antibody-drug conjugate compounds that are capable of binding, internalizing, and killing tumor cells (e.g., FRA-expressing tumor cells). Antibody-drug conjugate compounds comprising a linker that attaches a drug moiety to an antibody moiety are disclosed. Antibody-drug conjugate (ADC) compounds may be represented by Formula I:

$$Ab\text{-}(L\text{-}D)_p \qquad (I)$$

wherein Ab is an internalizing antibody or an internalizing antigen-binding fragment thereof which targets a tumor cell;

D is eribulin;

L is a cleavable linker that covalently attaches Ab to D; and

p is an integer from 1 to 20.

[0009] In some embodiments, the linker is stable outside a cell, such that the ADC remains intact when present in extracellular conditions but is capable of being cleaved

on internalization in a cell, e.g., a cancer cell. In some embodiments, the eribulin drug moiety is cleaved from the antibody moiety when the ADC enters a cell that expresses an antigen specific for the antibody moiety of the ADC, and cleavage releases an unmodified form of eribulin. In some embodiments, the linker comprises a cleavable moiety that is positioned such that no part of the linker or the antibody moiety remains bound to the eribulin drug moiety upon cleavage.

[0010] In some embodiments, the cleavable moiety in the linker is a cleavable peptide moiety. In some embodiments, an ADC that comprises a cleavable peptide moiety demonstrates lower aggregation levels, improved antibody:drug ratio, increased on-target killing of cancer cells, decreased off-target killing of non-cancer cells, and/or higher drug loading (p) relative to an ADC that comprises an alternate cleavable moiety. In some embodiments, adding a cleavable moiety increases cytotoxicity and/or potency relative to a non-cleavable linker. In some embodiments, the increased potency and/or cytotoxicity is in a cancer expressing moderate levels of the antigen targeted by the antibody moiety of the ADC (e.g., moderate FRA expression). In some embodiments, the cleavable peptide moiety is cleavable by an enzyme, and the linker is an enzyme-cleavable linker. In some embodiments, the enzyme is cathepsin, and the linker is a cathepsin-cleavable linker. In certain embodiments, the enzyme-cleavable linker (e.g., the cathepsin-cleavable linker) exhibits one or more of the improved properties mentioned above, as compared to an alternate cleavage mechanism.

[0011] In some embodiments, the cleavable peptide moiety in the linker comprises an amino acid unit. In some embodiments, the amino acid unit comprises valine-citrulline (Val-Cit). In some embodiments, an ADC that comprises Val-Cit demonstrates increased stability, decreased off-target cell killing, increased on-target cell killing, lower aggregation levels, and/or higher drug loading relative to an ADC that comprises an alternate amino acid unit or alternate cleavable moiety.

[0012] In some embodiments, the linker comprises at least one spacer unit joining the antibody moiety to the cleavable moiety. In some embodiments, the spacer unit in the linker may comprise at least one polyethylene glycol (PEG) moiety. The PEG moiety may, for example, comprise $-(PEG)_m-$, wherein m is an integer from 1 to 10. In some embodiments, the spacer unit in the linker comprises $(PEG)_2$. In some embodiments, an ADC that comprises a shorter spacer unit (e.g., $(PEG)_2$) demonstrates lower aggregation

levels and/or higher drug loading relative to an ADC that comprises a longer spacer unit (e.g., $(PEG)_8$) despite the shorter linker length.

[0013] In some embodiments, the spacer unit in the linker attaches to the antibody moiety of the ADC via a maleimide moiety (Mal). In some embodiments, an ADC that comprises a linker attached to the antibody moiety via a Mal demonstrates higher drug loading relative to an ADC that comprises a linker attached to the antibody moiety via an alternate moiety. In some embodiments, the Mal in the linker is reactive with a cysteine residue on the antibody moiety. In some embodiments, the Mal in the linker is joined to the antibody moiety via a cysteine residue. In some embodiments, the Mal-spacer unit comprises a PEG moiety. In some embodiments, the linker comprises Mal-$(PEG)_m$, e.g., Mal-$(PEG)_2$. In some embodiments, the linker comprises Mal-$(PEG)_2$. In some embodiments, the Mal-spacer unit attaches the antibody moiety to the cleavable moiety in the linker. In some embodiments, the cleavable moiety in the linker is a cleavable peptide moiety, e.g., an amino acid unit. In some embodiments, the linker comprises Mal-$(PEG)_2$-Val-Cit.

[0014] In some embodiments, the cleavable moiety in the linker is directly joined to the eribulin drug moiety of the ADC, and the cleavable moiety is either directly connected to the antibody moiety or connected through a spacer unit. In some embodiments, a spacer unit also attaches the cleavable moiety in the linker to the eribulin drug moiety. In some embodiments, the spacer unit that attaches the cleavable moiety in the linker to the eribulin drug moiety is self-immolative. In some embodiments, the self-immolative spacer is capable of releasing unmodified eribulin in a target cell. In some embodiments, the self-immolative spacer unit comprises a p-aminobenzyl alcohol. In some embodiments, the self-immolative spacer unit comprises p-aminobenzyloxycarbonyl (pAB). The pAB in the linker, in some embodiments, attaches the cleavable moiety to the eribulin drug moiety. In some embodiments, the cleavable moiety is a cleavable peptide moiety, e.g., an amino acid unit. In some embodiments, the linker comprises Val-Cit-pAB. In some embodiments, the linker comprises Val-Cit-pAB and a PEG spacer unit joining the linker to the antibody moiety through a Mal.

[0015] In some embodiments, p is an integer from 1 to 6, from 2 to 5, or preferably, from 3 to 4. In the some embodiments, p is 4. In some embodiments, a pool of ADCs are provided, and the average p in the pool is about 4 (e.g., 3.5-4.5, such as about 3.8).

In some embodiments, the linker comprises Mal-$(PEG)_2$-Val-Cit-pAB. In some embodiments, the linker comprises Mal-$(PEG)_2$-Val-Cit-pAB and p is 4. In some embodiments, a pool of ADCs are provided, wherein each ADC comprises a Mal-$(PEG)_2$-Val-Cit-pAB linker, and the average p in the pool is about 4 (e.g., 3.5-4.5, such as about 3.8).

[0016] In some embodiments, the internalizing antibody or internalizing antigen-binding fragment (Ab or Ab moiety) of the ADC is an anti-folate receptor alpha (FRA) antibody or internalizing antibody fragment, and can bind FRA-expressing tumor cells (i.e., the ADC targets FRA-expressing cells). In some embodiments, the ADC comprising an anti-FRA Ab moiety and a cleavable peptide moiety demonstrates lower aggregation levels, improved antibody:drug ratio, increased on-target killing of cancer cells, decreased off-target killing of non-cancer cells, higher drug loading (p), increased cytotoxicity, and/or potency relative to a non-cleavable linker or an alternate cleavage mechanism. In some embodiments, the increased potency and/or cytotoxicity is in a cancer expressing moderate levels of the antigen targeted by the antibody moiety of the ADC (e.g., moderate FRA expression). In some embodiments, the cleavable peptide moiety is cleavable by an enzyme, and the linker is an enzyme-cleavable linker. In some embodiments, the enzyme is cathepsin, and the linker is a cathepsin-cleavable linker. In certain embodiments, the enzyme-cleavable linker (e.g., the cathepsin-cleavable linker) exhibits one or more of the improved properties mentioned above, as compared to an alternate cleavage mechanism. In some embodiments, the linker is a Mal-$(PEG)_m$-Val-Cit-pAB.

[0017] In some embodiments, the internalizing antibody or internalizing antigen-binding fragment binds to folate receptor alpha (FRA) and targets FRA-expressing tumor cells. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises three heavy chain complementarity determining regions (CDRs) and three light chain CDRs, wherein the heavy chain CDRs comprise heavy chain CDR1 consisting of SEQ ID NO:2, heavy chain CDR2 consisting of SEQ ID NO:3, and heavy chain CDR3 consisting of SEQ ID NO:4; and the three light chain CDRs comprise light chain CDR1 consisting of SEQ ID NO:7, light chain CDR2 consisting of SEQ ID NO:8, and light chain CDR3 consisting of SEQ ID NO:9, as defined by the Kabat numbering system; or wherein the heavy chain CDRs comprise heavy chain CDR1 consisting of SEQ ID NO:13, heavy chain CDR2 consisting of SEQ

ID NO:14, and heavy chain CDR3 consisting of SEQ ID NO:15; and the light chain CDRs comprise light chain CDR1 consisting of SEQ ID NO:16, light chain CDR2 consisting of SEQ ID NO:17, and light chain CDR3 consisting of SEQ ID NO:18, as defined by the IMGT numbering system. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises human framework sequences. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises a heavy chain variable domain of SEQ ID NO:23 and a light chain variable domain of SEQ ID NO:24. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises a human IgG1 heavy chain constant domain and an Ig kappa light chain constant domain. In some embodiments, the internalizing antibody or internalizing antigen-binding competes for binding and/or binds the same epitope as an antibody comprising a heavy chain variable domain of SEQ ID NO:23 and a light chain variable domain of SEQ ID NO:24. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment binds to an epitope comprising alanine-histadine-lysine-aspartic acid (AHKD) (SEQ ID NO:365) (O'Shannessy et al., (2011) Oncotarget 2:1227-43). In some embodiments, the internalizing antibody or internalizing antigen-binding fragment binds to an epitope comprising NTSQEAHKDVSYL (SEQ ID NO:366).

[0018] In some embodiments, the internalizing antibody or internalizing antigen-binding fragment is an internalizing anti-FRA antibody or internalizing antigen-binding fragment. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises three heavy chain CDRs and three light chain CDRs, wherein the heavy chain CDRs comprise heavy chain CDR1 consisting of SEQ ID NO:2, heavy chain CDR2 consisting of SEQ ID NO:3, and heavy chain CDR3 consisting of SEQ ID NO:4; and the three light chain CDRs comprise light chain CDR1 consisting of SEQ ID NO:7, light chain CDR2 consisting of SEQ ID NO:8, and light chain CDR3 consisting of SEQ ID NO:9, as defined by the Kabat numbering system; or wherein the heavy chain CDRs comprise heavy chain CDR1 consisting of SEQ ID NO:13, heavy chain CDR2 consisting of SEQ ID NO:14, and heavy chain CDR3 consisting of SEQ ID NO:15; and the light chain CDRs comprise light chain CDR1 consisting of SEQ ID NO:16, light chain CDR2 consisting of SEQ ID NO:17, and light chain CDR3 consisting of SEQ ID NO:18, as defined by the IMGT numbering system; the linker comprises Mal-$(PEG)_2$-Val-Cit-pAB; and p is 4. In some embodiments, a

pool of such ADCs are provided and *p* is about 4 (e.g., about 3.8). In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises a heavy chain variable domain of SEQ ID NO:23 and a light chain variable domain of SEQ ID NO:24. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises a human IgG1 heavy chain constant domain and an Ig kappa light chain constant domain. In some embodiments, the internalizing antibody or internalizing antigen-binding competes for binding and/or binds the same epitope as an antibody comprising a heavy chain variable domain of SEQ ID NO:23 and a light chain variable domain of SEQ ID NO:24. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment binds to an epitope comprising SEQ ID NO:365. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment binds to an epitope comprising SEQ ID NO:366.

[0019] In some embodiments, the internalizing antibody or internalizing antigen-binding fragment binds to human epidermal growth factor receptor 2 (her2) and targets her2-expressing tumor cells. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises three heavy chain complementarity determining regions (CDRs) and three light chain CDRs, wherein the heavy chain CDRs comprise heavy chain CDR1 consisting of SEQ ID NO:71 heavy chain CDR2 consisting of SEQ ID NO:72, and heavy chain CDR3 consisting of SEQ ID NO:73; and the three light chain CDRs comprise light chain CDR1 consisting of SEQ ID NO:74, light chain CDR2 consisting of SEQ ID NO:75, and light chain CDR3 consisting of SEQ ID NO:76, as defined by the Kabat numbering system; or wherein the heavy chain CDRs comprise heavy chain CDR1 consisting of SEQ ID NO:191, heavy chain CDR2 consisting of SEQ ID NO:192, and heavy chain CDR3 consisting of SEQ ID NO:193; and the light chain CDRs comprise light chain CDR1 consisting of SEQ ID NO:194, light chain CDR2 consisting of SEQ ID NO:195, and light chain CDR3 consisting of SEQ ID NO:196, as defined by the IMGT numbering system. In some embodiments, the antibody or internalizing antigen-binding fragment comprises human framework sequences. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises a heavy chain variable domain of SEQ ID NO:27 and a light chain variable domain of SEQ ID NO:28. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises a human IgG1 heavy

chain constant domain and an Ig kappa light chain constant domain. In some embodiments, the internalizing antibody or internalizing antigen-binding competes for binding and/or binds the same epitope as an antibody comprising a heavy chain variable domain of SEQ ID NO:27 and a light chain variable domain of SEQ ID NO:28.

[0020] In some embodiments, the internalizing antibody or internalizing antigen-binding fragment is an internalizing anti-her2 antibody or internalizing antigen-binding fragment. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises three heavy chain CDRs and three light chain CDRs, wherein the heavy chain CDRs comprise heavy chain CDR1 consisting of SEQ ID NO:71 heavy chain CDR2 consisting of SEQ ID NO:72, and heavy chain CDR3 consisting of SEQ ID NO:73; and the three light chain CDRs comprise light chain CDR1 consisting of SEQ ID NO:74, light chain CDR2 consisting of SEQ ID NO:75, and light chain CDR3 consisting of SEQ ID NO:76, as defined by the Kabat numbering system; or wherein the heavy chain CDRs comprise heavy chain CDR1 consisting of SEQ ID NO:191, heavy chain CDR2 consisting of SEQ ID NO:192, and heavy chain CDR3 consisting of SEQ ID NO:193; and the light chain CDRs comprise light chain CDR1 consisting of SEQ ID NO:194, light chain CDR2 consisting of SEQ ID NO:195, and light chain CDR3 consisting of SEQ ID NO:196, as defined by the IMGT numbering system; the linker comprises Mal-$(PEG)_2$-Val-Cit-pAB; and p is 4. In some embodiments, a pool of such ADCs are provided and p is about 4 (e.g., about 3.8). In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises a heavy chain variable domain of SEQ ID NO:27 and a light chain variable domain of SEQ ID NO:28. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises a human IgG1 heavy chain constant domain and an Ig kappa light chain constant domain. In some embodiments, the internalizing antibody or internalizing antigen-binding competes for binding and/or binds the same epitope as an antibody comprising a heavy chain variable domain of SEQ ID NO:27 and a light chain variable domain of SEQ ID NO:28.

[0021] In some embodiments, the internalizing antibody or internalizing antigen-binding fragment binds to mesothelin (MSLN) and targets MSLN-expressing tumor cells. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises three heavy chain complementarity determining regions (CDRs) and three light chain CDRs, wherein the heavy chain CDRs comprise heavy chain

CDR1 consisting of SEQ ID NO:65 heavy chain CDR2 consisting of SEQ ID NO:66, and heavy chain CDR3 consisting of SEQ ID NO:67; and the three light chain CDRs comprise light chain CDR1 consisting of SEQ ID NO:68, light chain CDR2 consisting of SEQ ID NO:69, and light chain CDR3 consisting of SEQ ID NO:70, as defined by the Kabat numbering system; or wherein the heavy chain CDRs comprise heavy chain CDR1 consisting of SEQ ID NO:185, heavy chain CDR2 consisting of SEQ ID NO:186, and heavy chain CDR3 consisting of SEQ ID NO:187; and the light chain CDRs comprise light chain CDR1 consisting of SEQ ID NO:188, light chain CDR2 consisting of SEQ ID NO:189, and light chain CDR3 consisting of SEQ ID NO:190, as defined by the IMGT numbering system. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises a heavy chain variable domain of SEQ ID NO:25 and a light chain variable domain of SEQ ID NO:26. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises a human IgG1 heavy chain constant domain and an Ig kappa light chain constant domain. In some embodiments, the internalizing antibody or internalizing antigen-binding competes for binding and/or binds the same epitope as an antibody comprising a heavy chain variable domain of SEQ ID NO:25 and a light chain variable domain of SEQ ID NO:26.

[0022] In some embodiments, the internalizing antibody or internalizing antigen-binding fragment is an internalizing anti-MSLN antibody or internalizing antigen-binding fragment. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises three heavy chain CDRs and three light chain CDRs, wherein the heavy chain CDRs comprise heavy chain CDR1 consisting of SEQ ID NO:65 heavy chain CDR2 consisting of SEQ ID NO:66, and heavy chain CDR3 consisting of SEQ ID NO:67; and the three light chain CDRs comprise light chain CDR1 consisting of SEQ ID NO:68, light chain CDR2 consisting of SEQ ID NO:69, and light chain CDR3 consisting of SEQ ID NO:70, as defined by the Kabat numbering system; or wherein the heavy chain CDRs comprise heavy chain CDR1 consisting of SEQ ID NO:185, heavy chain CDR2 consisting of SEQ ID NO:186, and heavy chain CDR3 consisting of SEQ ID NO:187; and the light chain CDRs comprise light chain CDR1 consisting of SEQ ID NO:188, light chain CDR2 consisting of SEQ ID NO:189, and light chain CDR3 consisting of SEQ ID NO:190, as defined by the IMGT numbering system; the linker comprises Mal-$(PEG)_2$-Val-Cit-pAB; and p is 4. In some

embodiments, a pool of such ADCs are provided and *p* is about 4 (e.g., about 3.8). In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises a heavy chain variable domain of SEQ ID NO:25 and a light chain variable domain of SEQ ID NO:26. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment comprises a human IgG1 heavy chain constant domain and an Ig kappa light chain constant domain. In some embodiments, the internalizing antibody or internalizing antigen-binding competes for binding and/or binds the same epitope as an antibody comprising a heavy chain variable domain of SEQ ID NO:25 and a light chain variable domain of SEQ ID NO:26.

[0023] Also provided herein are compositions comprising multiple copies of any of the described ADCs, wherein the average drug loading (average *p*) of the ADCs in the composition is between about 3 and 4, or about 3.5 to about 4.5, or about 4. In some embodiments, the average *p* is between about 3.2 and 3.8. In some embodiments, the average *p* is between about 3.6 and 4.4.

[0024] Also provided herein are compositions comprising -L-D, wherein D is eribulin; and L is a cleavable linker that covalently attaches to D. In some embodiments, the cleavable linker covalently attaches to the C-35 amine on eribulin. In some embodiments, the cleavable linker comprises Val-Cit. In some embodiments, the cleavable linker comprises a PEG spacer unit. In some embodiments, the cleavable linker comprises Mal-$(PEG)_2$-Val-Cit-pAB.

[0025] Further provided herein are pharmaceutical compositions comprising an ADC and a pharmaceutically acceptable diluent, carrier, and/or excipient.

[0026] Another aspect of the present disclosure includes therapeutic and diagnostic uses for the described ADC compounds and compositions, e.g., in treating cancer. Another aspect includes methods of treating a cancer that expresses an antigen targeted by the antibody moiety of the ADC, such as FRA. In various embodiments, methods are provided of killing or inhibiting the proliferation of tumor cells or cancer cells by administering a therapeutically effective amount and/or regimen of any one of the described ADCs. Another aspect includes methods for detecting tumor cells or cancer cells that express FRA using the disclosed ADCs, and methods of screening for cancer patients that will be responsive to treatment with the described ADCs. In some embodiments, the cancer is a gastric cancer, a serous ovarian cancer, a clear cell ovarian cancer, a non-small cell lung cancer, a colorectal cancer, a triple negative breast cancer,

an endometrial cancer, a serous endometrial carcinoma, a lung carcinoid, or an osteosarcoma. Methods of producing the described ADCs are also disclosed.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] Figure 1 shows one of the methodologies used to prepare MORAb-003 ADCs, as disclosed in certain embodiments. In this approach, unpaired cysteines are generated through partial reduction with limited molar equivalents of the non-thiol reducing agent TCEP. This approach preferentially reduces the interchain disulfide bonds that link the light chain and heavy chain (one pair per H-L pairing) and the two heavy chains in the hinge region (two pairs per H-H pairing in the case of human IgG1), while leaving the intrachain disulfide bonds intact.

[0028] Figure 2 shows a method of synthesizing maleimide-$(PEG)_2$-Val-Cit-pAB-eribulin (mal-$(PEG)_2$-VCP-eribulin), as disclosed in certain embodiments.

[0029] Figure 3 shows an SDS-PAGE analysis of reduction conditions for MORAb-003. Lanes are indicated to the right of the figure. Lane M corresponds to protein standard; lane 1 corresponds to untreated MORAb-003; lane 2 corresponds to 5.3 mg/mL reduced in 70.6 μM TCEP; lane 3 corresponds to MORAb-003 5.3 mg/mL reduced in 141.2 μM TCEP; lane 4 corresponds to MORAb-003 1.5 mg/mL reduced in 20μM TCEP; and lane 5 corresponds to MORAb-003 1.5 mg/mL reduced in 40 μM TCEP. Identities of each band are indicated on the lower right gel. "H" indicates heavy chain. "L" indicates light chain.

[0030] Figure 4 shows an SDS-PAGE analysis of reduction conditions for MORAb-003. Lane 1 corresponds to protein standard; lane 2 corresponds to untreated MORAb-003; lane 3 corresponds to MORAb-003 treated at a ratio of MORAb-003:TCEP of 1:1; lane 4 corresponds to MORAb-003 treated at a ratio of MORAb-003:TCEP of 1:2; lane 5 corresponds to MORAb-003 treated at a ratio of MORAb-003:TCEP of 1:3; and lane 6 corresponds to MORAb-003 treated at a ratio of MORAb-003:TCEP of 1:4.

[0031] Figure 5 shows a non-reducing SDS-PAGE analysis of select MORAb-003 ADCs, including M-MMAE (lane 2), M-DM1 (lane 3), M-0026 (lane 4), M-0260 (lane 5), M-0267 (lane 6), M-0272 (lane 7), M-0285 (lane 8), M-0292 (lane 9), M-027-0381 (lane 10), and M-0284 (lane 11).

[0032] Figure 6A shows the results of a bystander cytotoxicity assay of MORAb-003-maleimido-PEG2-Val-Cit-pAB-eribulin (M3-VCP-eribulin, or "MORAb-202"). Figure

6B shows the results of a bystander cytotoxicity assay of MORAb-003-maleimido-$(CH_2)_5$-Val-Cit-pAB-ER-001150828 (M3-ER-61318). Figure 6C shows the results of a bystander cytotoxicity assay of MORAb-003-PEG-pAB-duostatin 3 (M3-027-0285). The information shown in the respective figure legends provides cell line:agent tested (cell line/cell lines cultured, seeding density of $1^{st}/2^{nd}$ cell line).

[0033] Figures 7A and 7B show drug-to-antibody ratio (DAR) distribution for ADCs MORAb-003-VCP-eribulin (Figure 7A) and MORAb-003-0285 (Figure 7B) relative to unconjugated MORAb-003, as disclosed in certain embodiments. Numbers over each peak indicate the DAR of the individual species.

[0034] Figure 8 shows the results of a cytotoxicity analysis - competition of MORAb-003-VCP-eribulin with unconjugated MORAb-003 (2 μM) in IGROV1 or SJSA-1 cells.

[0035] Figure 9 shows body weight kinetics for each group of CD-1 mice (group average and SEM) treated with a single intravenous dose of vehicle (PBS), or MORAb-202 at 10, 20, 40, or 80 mg/kg.

[0036] Figure 10 shows body weight kinetics for each group of CD-1 mice (group average and SEM) treated intravenously with PBS, or with eribulin at 0.4, 0.8, 1.6, or 3.2 mg/kg, according to a q4dx3 dosing regimen (doses administered once every four days for 3 doses total).

[0037] Figure 11 shows tumor growth kinetics for each group of CB17-SCID mice implanted with hNSCLC NCI-H2110 cells (group average and SEM) and treated with a single intravenous dose of PBS, MORAb-003-VCP-eribulin (MORAb-202) at 1, 2.5, or 5 mg/kg, or MORAb-003-0285 at 5 mg/kg.

[0038] Figure 12 shows tumor volumes of individual CB17-SCID mice implanted with hNSCLC NCI-H2110 cells, as well as group average and SEM, on day 17. Groups were treated with a single intravenous dose of PBS, MORAb-003-VCP-eribulin (MORAb-202) at 1, 2.5, or 5 mg/kg, or MORAb-003-0285 at 5 mg/kg.

[0039] Figure 13 shows body weight kinetics for each group of NCI-H2110-implanted CB17-SCID mice (group average and SEM) treated with a single intravenous dose of PBS, MORAb-003-VCP-eribulin (MORAb-202) at 1, 2.5, or 5 mg/kg, or MORAb-003-0285 at 5 mg/kg.

[0040] Figure 14 shows tumor growth kinetics for each group of NCI-H2110-implanted CB17-SCID mice (group average and SEM) treated intravenously with

vehicle (PBS), or with eribulin at 0.5, 0.2, 0.8, or 1.6 mg/kg, according to a q4dx3 dosing regimen.

[0041] Figure 15 shows tumor volumes of individual NCI-H2110-implanted CB17-SCID mice, as well as group average and SEM, on day 24. Groups were treated intravenously with vehicle (PBS), or with eribulin at 0.5, 0.2, 0.8, or 1.6 mg/kg, according to a q4dx3 dosing regimen.

[0042] Figure 16 shows body weight change kinetics for each group of NCI-H2110-implanted CB17-SCID mice (group average and SEM) treated intravenously with vehicle (PBS), or with eribulin at 0.5, 0.2, 0.8, or 1.6 mg/kg, according to a q4dx3 dosing regimen.

[0043] Figure 17 shows the potency of MORAb-003-VCP-eribulin (MORAb-202) on IGROV1, OVCAR3, NCI-H2110, A431-A3, and SJSA-1 cells, as measured by Crystal Violet cytotoxicity assay.

[0044] Figure 18 shows tumor growth kinetics for each group of NCI-H2110-implanted CB17-SCID mice (group average and SEM) treated with a single intravenous dose of PBS, or MORAb-003-VCP-eribulin (MORAb-202) at 1, 2.5, or 5 mg/kg.

[0045] Figures 19A and 19B show tumor growth kinetics (Figure 19A) and body weight change kinetics (Figure 19B) for each group of NSCLC PDx (LXFA-737) tumor-bearing mice (group average and SEM) treated with a single intravenous dose of vehicle (PBS), MORAb-003 at 5 mg/kg, or MORAb-003-VCP-eribulin (MORAb-202) at 5 mg/kg.

[0046] Figures 20A and 20B show individual tumor volume ratios (Figure 20A) and body weight change kinetics (Figure 20B) for each group of endometrial cancer PDx (Endo-12961) tumor-bearing mice (group average and SEM) treated with a single intravenous dose of PBS, eribulin at 0.1 or 3.2 mg/kg, or MORAb-003-VCP-eribulin (MORAb-202) at 5 mg/kg. Figures 20C and 20D show tumor growth kinetics (Figure 20C) and body weight change kinetics (Figure 20D) for each group of endometrial cancer PDx (Endo-10590) tumor-bearing mice (group average and SEM) treated with a single intravenous dose of PBS, eribulin at 0.1 or 3.2 mg/kg, or MORAb-003-VCP-eribulin (MORAb-202) at 5 mg/kg.

[0047] Figure 21A shows immunohistochemical (IHC) staining of tumor tissue in TNBC PDx (OD-BRE-0631) tumor-bearing mice with an anti-human IgG antibody. Tumor tissues from mice treated with a single intravenous dose of vehicle (right), or

MORAb-003-VCP-eribulin (MORAb-202) at 5 mg/kg (left), were collected and stained 5 days post-treatment. Figure 21B shows IHC staining of tumor tissue in TNBC PDx (OD-BRE-0631) tumor-bearing mice with an α-smooth muscle actin (SMA)-FITC antibody. Tumor tissues from untreated mice were collected 2 days prior to treatment (left), whereas tumor tissues from mice treated with a single intravenous dose of MORAb-003-VCP-eribulin (MORAb-202) at 5 mg/kg were collected 5 days post-treatment (right). Figure 21C shows tumor growth kinetics for each group of TNBC PDx (OD-BRE-0631) tumor-bearing mice (group average and SEM) treated with a single intravenous dose of vehicle (PBS), or MORAb-003-VCP-eribulin (MORAb-202) at 5 mg/kg.

[0048] Figure 22 shows the differentiation of human bone marrow-mesenchymal stem cells (BM-MSCs) in culture with MKN-74 cells following treatment with vehicle (PBS or ethanol), eribulin, MORAb-003, or MORAb-003-VCP-eribulin (MORAb-202), as measured by flow cytometry analysis. Stro-1$^+$/CD105$^+$, CD34$^+$/CD31$^-$, and NG2$^+$ are markers of MSCs, adipocytes, and pericytes, respectively.

[0049] Figure 23 shows the time course analysis of tumor tissues from NCI-H2110-implanted CB17-SCID mice treated with a single intravenous dose of vehicle (PBS), or MORAb-003-VCP-eribulin (MORAb-202) at 5 mg/kg, stained with an α-smooth muscle actin (SMA)-FITC antibody. Tumor tissues were collected and stained at day 0, and at days 3, 5, 7 and 9 post-treatment. Y-axis: % = [stained cells counted / total cells counted] * 100. X-axis: day (total cells counted).

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0050] The disclosed compositions and methods may be understood more readily by reference to the following detailed description taken in connection with the accompanying figures, which form a part of this disclosure. It is to be understood that the disclosed compositions and methods are not limited to the specific compositions and methods described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of the claimed compositions and methods.

[0051] Throughout this text, the descriptions refer to compositions and methods of using said compositions. Where the disclosure describes or claims a feature or embodiment associated with a composition, such a feature or embodiment is equally

applicable to the methods of using said composition. Likewise, where the disclosure describes or claims a feature or embodiment associated with a method of using a composition, such a feature or embodiment is equally applicable to the composition.

[0052] When a range of values is expressed, it includes embodiments using any particular value within the range. Further, reference to values stated in ranges includes each and every value within that range. All ranges are inclusive of their endpoints and combinable. When values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. Reference to a particular numerical value includes at least that particular value, unless the context clearly dictates otherwise. The use of "or" will mean "and/or" unless the specific context of its use dictates otherwise. All references cited herein are incorporated by reference for any purpose. Where a reference and the specification conflict, the specification will control.

[0053] It is to be appreciated that certain features of the disclosed compositions and methods, which are, for clarity, described herein in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the disclosed compositions and methods that are, for brevity, described in the context of a single embodiment, may also be provided separately or in any subcombination.

Definitions

[0054] Various terms relating to aspects of the description are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definitions provided herein.

[0055] As used herein, the singular forms "a," "an," and "the" include plural forms unless the context clearly dictates otherwise.

[0056] The terms "about" or "approximately" in the context of numerical values and ranges refers to values or ranges that approximate or are close to the recited values or ranges such that the embodiment may perform as intended, such as having a desired amount of nucleic acids or polypeptides in a reaction mixture, as is apparent to the skilled person from the teachings contained herein. This is due, at least in part, to the varying properties of nucleic acid compositions, age, race, gender, anatomical and

physiological variations and the inexactitude of biological systems. Thus, these terms encompass values beyond those resulting from systematic error.

[0057] The terms “antibody-drug conjugate,” “antibody conjugate,” “conjugate,” “immunoconjugate,” and “ADC” are used interchangeably, and refer to a compound or derivative thereof that is linked to an antibody (e.g., an anti-FRA antibody) and is defined by the generic formula: Ab-(L-D)$_p$ (Formula I), wherein Ab = an antibody moiety (i.e., antibody or antigen-binding fragment), L = a linker moiety, D = a drug moiety, and p = the number of drug moieties per antibody moiety.

[0058] The term "antibody" is used in the broadest sense to refer to an immunoglobulin molecule that recognizes and specifically binds to a target, such as a protein, polypeptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing through at least one antigen recognition site within the variable region of the immunoglobulin molecule. The heavy chain of an antibody is composed of a heavy chain variable domain (V_H) and a heavy chain constant region (C_H). The light chain is composed of a light chain variable domain (V_L) and a light chain constant domain (C_L). For the purposes of this application, the mature heavy chain and light chain variable domains each comprise three complementarity determining regions (CDR1, CDR2 and CDR3) within four framework regions (FR1, FR2, FR3 and FR4) arranged from N-terminus to C-terminus: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. An "antibody" can be naturally occurring or man-made, such as monoclonal antibodies produced by conventional hybridoma technology. The term “antibody” includes full-length monoclonal antibodies and full-length polyclonal antibodies, as well as antibody fragments such as Fab, Fab’, F(ab’)2, Fv, and single chain antibodies. An antibody can be any one of the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses thereof (e.g., isotypes IgG1, IgG2, IgG3, IgG4). The term further encompasses human antibodies, chimeric antibodies, humanized antibodies and any modified immunoglobulin molecule containing an antigen recognition site, so long as it demonstrates the desired biological activity.

[0059] The term "monoclonal antibody," as used herein, refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic epitope. In contrast,

conventional (polyclonal) antibody preparations typically include a multitude of antibodies directed against (or specific for) different epitopes. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present disclosure may be made by the hybridoma method first described by Kohler et al. (1975) Nature 256:495, or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). Monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in Clackson et al. (1991) Nature 352:624-8, and Marks et al. (1991) J. Mol. Biol. 222:581-97, for example.

[0060] The monoclonal antibodies described herein specifically include "chimeric" antibodies, in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they specifically bind the target antigen and/or exhibit the desired biological activity.

[0061] The term “human antibody,” as used herein, refers an antibody produced by a human or an antibody having an amino acid sequence of an antibody produced by a human.

[0062] The term “chimeric antibody,” as used herein, refers to antibodies wherein the amino acid sequence of the immunoglobulin molecule is derived from two or more species. In some instances, the variable regions of both heavy and light chains corresponds to the variable regions of antibodies derived from one species with the desired specificity, affinity, and activity while the constant regions are homologous to antibodies derived from another species (e.g., human) to minimize an immune response in the latter species.

[0063] As used herein, the term "humanized antibody" refers to forms of antibodies that contain sequences from non-human (e.g., murine) antibodies as well as human antibodies. Such antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. In general, the humanized antibody will

comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the framework (FR) regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. The humanized antibody can be further modified by the substitution of residues, either in the Fv framework region and/or within the replaced non-human residues to refine and optimize antibody specificity, affinity, and/or activity.

[0064] The term "antigen-binding fragment" or "antigen-binding portion" of an antibody, as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., FRA). Antigen-binding fragments preferably also retain the ability to internalize into an antigen-expressing cell. In some embodiments, antigen-binding fragments also retain immune effector activity. It has been shown that fragments of a full-length antibody can perform the antigen-binding function of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding fragment" or "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L, and C_{H1} domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_{H1} domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; (v) a dAb fragment, which comprises a single variable domain, e.g., a V_H domain (see, e.g., Ward et al. (1989) Nature 341:544-6; and Winter et al., WO 90/05144); and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv)). *See, e.g.*, Bird et al. (1988) Science 242:423-6; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-83. Such single chain antibodies are also intended to be encompassed within the term "antigen-binding fragment" or "antigen-binding portion" of an antibody, and are known in the art as an exemplary type of binding fragment that can internalize into cells upon binding. *See, e.g.*, Zhu et al. (2010) 9:2131-41; He et al. (2010) J. Nucl. Med. 51:427-32; and Fitting et al. (2015) MAbs 7:390-402. In certain embodiments,

scFv molecules may be incorporated into a fusion protein. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Holliger et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-8; and Poljak et al. (1994) Structure 2:1121-3). Antigen-binding fragments are obtained using conventional techniques known to those of skill in the art, and the binding fragments are screened for utility (e.g., binding affinity, internalization) in the same manner as are intact antibodies. Antigen-binding fragments may be prepared by cleavage of the intact protein, e.g., by protease or chemical cleavage.

[0065] "Internalizing" as used herein in reference to an antibody or antigen-binding fragment refers to an antibody or antigen-binding fragment that is capable of being taken through the cell's lipid bilayer membrane to an internal compartment (i.e., "internalized") upon binding to the cell, preferably into a degradative compartment in the cell. For example, an internalizing anti-FRA antibody is one that is capable of being taken into the cell after binding to FRA on the cell membrane.

[0066] The term "folate receptor alpha" or "FRA," as used herein, refers to any native form of human FRA. The term encompasses full-length FRA (e.g., NCBI Reference Sequence: NP_000793; SEQ ID NO: 19), as well as any form of human FRA that results from cellular processing. The term also encompasses naturally occurring variants of FRA, including but not limited to splice variants, allelic variants, and isoforms. FRA can be isolated from a human, or may be produced recombinantly or by synthetic methods.

[0067] The term "anti-FRA antibody" or "antibody that specifically binds FRA" refers to any form of antibody or fragment thereof that specifically binds FRA, and encompasses monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, and biologically functional antibody fragments so long as they specifically bind FRA. Preferably the anti-FRA antibody used in the ADCs disclosed herein is an internalizing antibody or internalizing antibody fragment. MORAb-003 is an exemplary internalizing anti-human FRA antibody. As used herein, the terms "specific," "specifically binds," and "binds specifically" refer to the selective binding of

the antibody to the target antigen epitope. Antibodies can be tested for specificity of binding by comparing binding to appropriate antigen to binding to irrelevant antigen or antigen mixture under a given set of conditions. If the antibody binds to the appropriate antigen with at least 2, 5, 7, and preferably 10 times more affinity than to irrelevant antigen or antigen mixture, then it is considered to be specific. In one embodiment, a specific antibody is one that only binds the FRA antigen, but does not bind (or exhibits minimal binding) to other antigens.

[0068] The term "human epidermal growth factor receptor 2," "her2," or "her2/neu," as used herein, refers to any native form of human her2. The term encompasses full-length her2 (e.g., NCBI Reference Sequence: NP_004439.2; SEQ ID NO: 21), as well as any form of human her2 that results from cellular processing. The term also encompasses naturally occurring variants of her2, including but not limited to splice variants, allelic variants, and isoforms. Her2 can be isolated from human, or may be produced recombinantly or by synthetic methods.

[0069] The term "anti-her2 antibody" or "antibody that specifically binds her2" refers to any form of antibody or fragment thereof that specifically binds her2, and encompasses monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, and biologically functional antibody fragments so long as they specifically bind her2. U.S. Pat. No. 5,821,337 (incorporated herein by reference) provides exemplary her2-binding sequences, including exemplary anti-her2 antibody sequences. Preferably the anti-her2 antibody used in the ADCs disclosed herein is an internalizing antibody or internalizing antibody fragment. Trastuzumab is an exemplary internalizing anti-human her2 antibody.

[0070] The term "epitope" refers to the portion of an antigen capable of being recognized and specifically bound by an antibody. When the antigen is a polypeptide, epitopes can be formed from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of the polypeptide. The epitope bound by an antibody may be identified using any epitope mapping technique known in the art, including X-ray crystallography for epitope identification by direct visualization of the antigen-antibody complex, as well as monitoring the binding of the antibody to fragments or mutated variations of the antigen, or monitoring solvent accessibility of different parts of the antibody and the antigen. Exemplary strategies used to map antibody epitopes include, but are not limited to, array-based oligo-peptide scanning, limited proteolysis,

site-directed mutagenesis, high-throughput mutagenesis mapping, hydrogen-deuterium exchange, and mass spectrometry (see, e.g., Gershoni et al. (2007) 21:145-56; and Hager-Braun and Tomer (2005) Expert Rev. Proteomics 2:745-56).

[0071] Competitive binding and epitope binning can also be used to determine antibodies sharing identical or overlapping epitopes. Competitive binding can be evaluated using a cross-blocking assay, such as the assay described in "Antibodies, A Laboratory Manual," Cold Spring Harbor Laboratory, Harlow and Lane (1^{st} edition 1988, 2^{nd} edition 2014). In some embodiments, competitive binding is identified when a test antibody or binding protein reduces binding of a reference antibody or binding protein to a target antigen such as FRA or her2 (e.g., a binding protein comprising CDRs and/or variable domains selected from those identified in Tables 2, 4, and 6), by at least about 50% in the cross-blocking assay (e.g., 50%, 60%, 70%, 80%, 90%, 95%, 99%, 99.5%, or more, or any percentage in between), and/or vice versa. In some embodiments, competitive binding can be due to shared or similar (e.g., partially overlapping) epitopes, or due to steric hindrance where antibodies or binding proteins bind at nearby epitopes. *See, e.g.*, Tzartos, Methods in Molecular Biology (Morris, ed. (1998) vol. 66, pp. 55-66). In some embodiments, competitive binding can be used to sort groups of binding proteins that share similar epitopes, e.g., those that compete for binding can be "binned" as a group of binding proteins that have overlapping or nearby epitopes, while those that do not compete are placed in a separate group of binding proteins that do not have overlapping or nearby epitopes.

[0072] The term "k_{on}" or "k_a" refers to the on rate constant for association of an antibody to the antigen to form the antibody/antigen complex. The rate can be determined using standard assays, such as a Biacore or ELISA assay.

[0073] The term "k_{off}" or "k_d" refers to the off rate constant for dissociation of an antibody from the antibody/antigen complex. The rate can be determined using standard assays, such as a Biacore or ELISA assay.

[0074] The term "K_D" refers to the equilibrium dissociation constant of a particular antibody-antigen interaction. K_D is calculated by k_a/k_d. The rate can be determined using standard assays, such as a Biacore or ELISA assay.

[0075] The term "*p*" or "antibody:drug ratio" or "drug-to-antibody ratio" or "DAR" refers to the number of drug moieties per antibody moiety, i.e., drug loading, or the number of -L-D moieties per antibody or antigen-binding fragment (Ab) in ADCs of

Formula I. In compositions comprising multiple copies of ADCs of Formula I, "*p*" refers to the average number of -L-D moieties per antibody or antigen-binding fragment, also referred to as average drug loading.

[0076] A "linker" or "linker moiety" is any chemical moiety that is capable of covalently joining a compound, usually a drug moiety such as a chemotherapeutic agent, to another moiety such as an antibody moiety. Linkers can be susceptible to or substantially resistant to acid-induced cleavage, peptidase-induced cleavage, light-based cleavage, esterase-induced cleavage, and/or disulfide bond cleavage, at conditions under which the compound or the antibody remains active.

[0077] The term "agent" is used herein to refer to a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. The term "therapeutic agent," "drug," or "drug moiety" refers to an agent that is capable of modulating a biological process and/or has biological activity.

[0078] The term "chemotherapeutic agent" or "anti-cancer agent" is used herein to refer to all chemical compounds that are effective in treating cancer regardless of mechanism of action. Inhibition of metastasis or angiogenesis is frequently a property of a chemotherapeutic agent. Non-limiting examples of chemotherapeutic agents include alkylating agents, for example, nitrogen mustards, ethyleneimine compounds, and alkyl sulphonates; antimetabolites, for example, folic acid, purine or pyrimidine antagonists; anti-mitotic agents, for example, anti-tubulin agents such as eribulin or eribulin mesylate (Halaven™) or derivatives thereof, vinca alkaloids, and auristatins; cytotoxic antibiotics; compounds that damage or interfere with DNA expression or replication, for example, DNA minor groove binders; and growth factor receptor antagonists. In addition, chemotherapeutic agents include antibodies, biological molecules, and small molecules. A chemotherapeutic agent may be a cytotoxic or cytostatic agent. The term "cytostatic agent" refers to an agent that inhibits or suppresses cell growth and/or multiplication of cells.

[0079] The term "cytotoxic agent" refers to a substance that causes cell death primarily by interfering with a cell's expression activity and/or functioning. Examples of cytotoxic agents include, but are not limited to, anti-mitotic agents, such as eribulin, auristatins (e.g., monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF)), maytansinoids (e.g., maytansine), dolastatins, duostatins, cryptophycins, vinca alkaloids (e.g., vincristine, vinblastine), taxanes, taxols, and colchicines; anthracyclines (e.g.,

daunorubicin, doxorubicin, dihydroxyanthracindione); cytotoxic antibiotics (e.g., mitomycins, actinomycins, duocarmycins (e.g., CC-1065), auromycins, duomycins, calicheamicins, endomycins, phenomycins); alkylating agents (e.g., cisplatin); intercalating agents (e.g., ethidium bromide); topoisomerase inhibitors (e.g., etoposide, tenoposide); radioisotopes, such as At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212} or ^{213}, P^{32}, and radioactive isotopes of lutetium (e.g., Lu^{177}); and toxins of bacterial, fungal, plant or animal origin (e.g., ricin (e.g., ricin A-chain), diphtheria toxin, *Pseudomonas* exotoxin A (e.g., PE40), endotoxin, mitogellin, combrestatin, restrictocin, gelonin, alpha-sarcin, abrin (e.g., abrin A-chain), modeccin (e.g., modeccin A-chain), curicin, crotin, *Sapaonaria officinalis* inhibitor, glucocorticoid).

[0080] The term "eribulin," as used herein, refers to a synthetic analog of halichondrin B, a macrocyclic compound that was originally isolated from the marine sponge *Halichondria okadais*. The term "eribulin drug moiety" refers to the component of an ADC that has the structure of eribulin, and is attached to the linker of the ADC via its C-35 amine. Eribulin is a microtubule dynamics inhibitor, which is thought to bind tubulin and induce cell cycle arrest at the G2/M phase by inhibiting mitotic spindle assembly. The term "eribulin mesylate" refers to the mesylate salt of eribulin, which is marketed under the trade name Halaven™.

[0081] The term "homolog" refers to a molecule which exhibits homology to another molecule, by for example, having sequences of chemical residues that are the same or similar at corresponding positions.

[0082] The term "inhibit" or "inhibition of," as used herein, means to reduce by a measurable amount, and can include but does not require complete prevention or inhibition.

[0083] The term "target-negative" or "target antigen-negative" refers to the absence of target antigen expression by a cell or tissue. The term "target-positive" or "target antigen-positive" refers to the presence of target antigen expression. For example, a cell or a cell line that does not express a target antigen may be described as target-negative, whereas a cell or cell line that expresses a target antigen may be described as target-positive.

[0084] The term "bystander killing" or "bystander effect" refers to the killing of target-negative cells in the presence of target-positive cells, wherein killing of target-negative cells is not observed in the absence of target-positive cells. Cell-to-cell

contact, or at least proximity between target-positive and target-negative cells, enables bystander killing. This type of killing is distinguishable from "off-target killing," which refers to the indiscriminate killing of target-negative cells. "Off-target killing" may be observed in the absence of target-positive cells.

[0085] The term "cancer" refers to the physiological condition in mammals in which a population of cells is characterized by unregulated cell growth. Examples of cancers include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small cell lung cancer, nonsmall cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer (e.g., triple negative breast cancer), osteosarcoma, melanoma, colon cancer, colorectal cancer, endometrial (e.g., serous) or uterine cancer, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, and various types of head and neck cancers. Triple negative breast cancer refers to breast cancer that is negative for expression of the genes for estrogen receptor (ER), progesterone receptor (PR), or Her2/neu.

[0086] The terms "tumor" and "neoplasm" refer to any mass of tissue that results from excessive cell growth or proliferation, either benign or malignant, including precancerous lesions.

[0087] The terms "cancer cell" and "tumor cell" refer to individual cells or the total population of cells derived from a tumor, including both non-tumorigenic cells and cancer stem cells. As used herein, the term "tumor cell" will be modified by the term "non-tumorigenic" when referring solely to those tumor cells lacking the capacity to renew and differentiate to distinguish those tumor cells from cancer stem cells.

[0088] The terms "subject" and "patient" are used interchangeably herein to refer to any animal, such as any mammal, including but not limited to, humans, non-human primates, rodents, and the like. In some embodiments, the mammal is a mouse. In some embodiments, the mammal is a human.

[0089] The term "co-administration" or administration "in combination with" one or more therapeutic agents includes concurrent and consecutive administration in any order.

[0090] A “pharmaceutical composition” refers to a preparation which is in such form as to permit administration and subsequently provide the intended biological activity of the active ingredient(s) and/or to achieve a therapeutic effect, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. The pharmaceutical composition may be sterile.

[0091] A "pharmaceutical excipient" comprises a material such as an adjuvant, a carrier, pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservative, and the like.

[0092] "Pharmaceutically acceptable" means approved or approvable by a regulatory agency of the Federal or a state government, or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia, for use in animals, and more particularly in humans.

[0093] An “effective amount” of an ADC as disclosed herein is an amount sufficient to perform a specifically stated purpose, for example to produce a therapeutic effect after administration, such as a reduction in tumor growth rate or tumor volume, a reduction in a symptom of cancer, or some other indicia of treatment efficacy. An effective amount can be determined in a routine manner in relation to the stated purpose. The term “therapeutically effective amount” refers to an amount of an ADC effective to treat a disease or disorder in a subject. In the case of cancer, a therapeutically effective amount of ADC can reduce the number of cancer cells, reduce tumor size, inhibit (e.g., slow or stop) tumor metastasis, inhibit (e.g., slow or stop) tumor growth, and/or relieve one or more symptoms. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0094] As used herein, "to treat" or "therapeutic" and grammatically related terms, refer to any improvement of any consequence of disease, such as prolonged survival, less morbidity, and/or a lessening of side effects which are the byproducts of an alternative therapeutic modality. As is readily appreciated in the art, full eradication of disease is a preferred but albeit not a requirement for a treatment act. “Treatment” or “treat,” as used herein, refers to the administration of a described ADC to a subject, e.g., a patient. The treatment can be to cure, heal, alleviate, relieve, alter, remedy,

ameliorate, palliate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder, e.g., a cancer.

[0095] In some embodiments, a labeled ADC is used. Suitable "labels" include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like.

[0096] By "protein," as used herein, is meant at least two covalently attached amino acids. The term encompasses polypeptides, oligopeptides, and peptides. In some embodiments, the two or more covalently attached amino acids are attached by a peptide bond. The protein may be made up of naturally occurring amino acids and peptide bonds, for example when the protein is made recombinantly using expression systems and host cells. Alternatively, the protein may include synthetic amino acids (e.g., homophenylalanine, citrulline, ornithine, and norleucine), or peptidomimetic structures, i.e., "peptide or protein analogs," such as peptoids. Peptoids are an exemplary class of peptidomimetics whose side chains are appended to the nitrogen atom of the peptide backbone, rather than to the α-carbons (as they are in amino acids), and have different hydrogen bonding and conformational characteristics in comparison to peptides (see, e.g., Simon et al. (1992) Proc. Natl. Acad. Sci. USA 89:9367). As such, peptoids can be resistant to proteolysis or other physiological or storage conditions, and effective at permeating cell membranes. Such synthetic amino acids may be incorporated in particular when the antibody is synthesized *in vitro* by conventional methods well known in the art. In addition, any combination of peptidomimetic, synthetic and naturally occurring residues/structures can be used. "Amino acid" also includes imino acid residues, such as proline and hydroxyproline. The amino acid "R group" or "side chain" may be in either the (L)- or the (S)-configuration. In a specific embodiment, the amino acids are in the (L)- or (S)-configuration.

[0097] A "recombinant protein" is a protein made using recombinant techniques using any techniques and methods known in the art, i.e., through the expression of a recombinant nucleic acid. Methods and techniques for the production of recombinant proteins are well known in the art.

[0098] An "isolated" protein is unaccompanied by at least some of the material with which it is normally associated in its natural state, for example constituting at least about 5%, or at least about 50% by weight of the total protein in a given sample. It is

understood that the isolated protein may constitute from 5 to 99.9% by weight of the total protein content depending on the circumstances. For example, the protein may be made at a significantly higher concentration through the use of an inducible promoter or high expression promoter, such that the protein is made at increased concentration levels. The definition includes the production of an antibody in a wide variety of organisms and/or host cells that are known in the art.

[0099] For amino acid sequences, sequence identity and/or similarity may be determined using standard techniques known in the art, including, but not limited to, the local sequence identity algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, the sequence identity alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, the search for similarity method of Pearson and Lipman (1988) Proc. Nat. Acad. Sci. USA 85:2444, computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.), the Best Fit sequence program described by Devereux et al. (1984) Nucl. Acid Res. 12:387-95, preferably using the default settings, or by inspection. Preferably, percent identity is calculated by FastDB based upon the following parameters: mismatch penalty of 1; gap penalty of 1; gap size penalty of 0.33; and joining penalty of 30 ("Current Methods in Sequence Comparison and Analysis," Macromolecule Sequencing and Synthesis, Selected Methods and Applications, pp. 127-149 (1988), Alan R. Liss, Inc).

[00100] An example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle (1987) J. Mol. Evol. 35:351-60; the method is similar to that described by Higgins and Sharp (1989) CABIOS 5:151-3. Useful PILEUP parameters including a default gap weight of 3.00, a default gap length weight of 0.10, and weighted end gaps.

[00101] Another example of a useful algorithm is the BLAST algorithm, described in: Altschul et al. (1990) J. Mol. Biol. 215:403-10; Altschul et al. (1997) Nucleic Acids Res. 25:3389-402; and Karin et al. (1993) Proc. Natl. Acad. Sci. USA 90:5873-87. A particularly useful BLAST program is the WU-BLAST-2 program which was obtained from Altschul et al. (1996) Methods in Enzymology 266:460-80. WU-BLAST-2 uses several search parameters, most of which are set to the default values. The adjustable

parameters are set with the following values: overlap span=l, overlap fraction=0.125, word threshold (T)=II. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity.

[00102] An additional useful algorithm is gapped BLAST as reported by Altschul et al. (1993) Nucl. Acids Res. 25:3389-402. Gapped BLAST uses BLOSUM-62 substitution scores; threshold T parameter set to 9; the two-hit method to trigger ungapped extensions, charges gap lengths of k a cost of 10+k; Xu set to 16, and Xg set to 40 for database search stage and to 67 for the output stage of the algorithms. Gapped alignments are triggered by a score corresponding to about 22 bits.

[00103] Generally, the amino acid homology, similarity, or identity between proteins disclosed herein and variants thereof, including variants of FRA, variants of her2, variants of tubulin sequences, and variants of antibody variable domains (including individual variant CDRs), are at least 80% to the sequences depicted herein, and more typically with preferably increasing homologies or identities of at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and almost 100% or 100%.

[00104] In a similar manner, "percent (%) nucleic acid sequence identity" with respect to the nucleic acid sequence of the antibodies and other proteins identified herein is defined as the percentage of nucleotide residues in a candidate sequence that are identical with the nucleotide residues in the coding sequence of the antigen binding protein. A specific method utilizes the BLASTN module of WU-BLAST-2 set to the default parameters, with overlap span and overlap fraction set to 1 and 0.125, respectively.

[00105] While the site or region for introducing an amino acid sequence variation is predetermined, the mutation per se need not be predetermined. For example, in order to optimize the performance of a mutation at a given site, random mutagenesis may be conducted at the target codon or region and the expressed antigen binding protein CDR variants screened for the optimal combination of desired activity. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example, MI3 primer mutagenesis and PCR mutagenesis.

Antibody-Drug Conjugates

[00106] The compounds of the present disclosure include those with anti-cancer activity. In particular, the compounds include an antibody moiety (including an antigen-binding fragment thereof) conjugated (i.e., covalently attached by a linker) to a drug moiety, wherein the drug moiety when not conjugated to an antibody moiety has a cytotoxic or cytostatic effect. In various embodiments, the drug moiety exhibits reduced or no cytotoxicity when bound in a conjugate but resumes cytotoxicity after cleavage from the linker and antibody moiety. In various embodiments, the drug moiety exhibits reduced or no bystander killing when bound in a conjugate (e.g., using a non-cleavable linker) but exhibits increased bystander killing after cleavage from a conjugate (e.g., a conjugate having a cleavable Val-Cit cleavable moiety).

[00107] The development and production of an ADC for use as a human therapeutic agent, e.g., as an oncologic agent, may require more than the identification of an antibody capable of binding to a desired target or targets and attaching to a drug used on its own to treat cancer. Linking the antibody to the drug may have significant and unpredictable effects on the activity of one or both of the antibody and the drug, effects which will vary depending on the type of linker and/or drug chosen. In some embodiments, therefore, the components of the ADC are selected to (i) retain one or more therapeutic properties exhibited by the antibody and drug moieties in isolation, (ii) maintain the specific binding properties of the antibody moiety; (iii) optimize drug loading and drug-to-antibody ratios; (iv) allow delivery, e.g., intracellular delivery, of the drug moiety via stable attachment to the antibody moiety; (v) retain ADC stability as an intact conjugate until transport or delivery to a target site; (vi) minimize aggregation of the ADC prior to or after administration; (vii) allow for the therapeutic effect, e.g., cytotoxic effect, of the drug moiety after cleavage in the cellular environment; (viii) exhibit *in vivo* anti-cancer treatment efficacy comparable to or superior to that of the antibody and drug moieties in isolation; (ix) minimize off-target killing by the drug moiety; and/or (x) exhibit desirable pharmacokinetic and pharmacodynamics properties, formulatability, and toxicologic/immunologic profiles. Screening each of these properties may be needed to identify an improved ADC for therapeutic use (Ab et al. (2015) Mol. Cancer Ther. 14:1605-13).

[00108] In various embodiments, the ADCs disclosed herein exhibit unexpectedly favorable properties in some or each of the categories listed above. For instance, in

some embodiments, ADC constructs comprising a Mal attachment to an antibody, a PEG spacer unit (preferably a short PEG spacer unit), and/or peptide cleavable linker (e.g., a Val-Cit linker) exhibit surprisingly favorable drug loading, aggregation, and/or stability profiles, and/or preserve antibody binding function, drug activity, and/or improved bystander killing, while reducing off-target killing, as compared to ADCs using other cleavable or non-cleavable linker structures.

[00109] In some embodiments, an ADC comprising a Mal-$(PEG)_2$-Val-Cit-pAB linker joining eribulin to an antibody (e.g., an anti-FRA antibody such as MORAb-003) exhibits particularly favorable properties across the listed categories, as compared to other cleavable or non-cleavable linkers joining eribulin to an antibody moiety. In some embodiments, an ADC comprising a Mal-$(PEG)_2$-Val-Cit-pAB linker joining eribulin to an antibody (e.g., an anti-FRA antibody such as MORAb-003) exhibits particularly favorable bystander killing properties as compared to an uncleavable ADC. In some embodiments, an ADC comprising a Mal-$(PEG)_2$-Val-Cit-pAB linker joining eribulin to an antibody (e.g., an anti-FRA antibody such as MORAb-003) exhibits particularly favorable bystander killing properties as compared to an ADC using alternate cleavable linker structures.

[00110] In some embodiments, an ADC comprising a Mal-$(PEG)_2$-Val-Cit-pAB linker joining eribulin to MORAb-003 exhibits a higher and more desirable drug:antibody ratio (i.e., a ratio of about 3-4) relative to an ADC, e.g., comprising a linker attached to the antibody via an alternate moiety (e.g., a succinimide moiety). In some embodiments, an ADC comprising a Mal-$(PEG)_2$-Val-Cit-pAB linker joining eribulin to MORAb-003 exhibits a higher and more desirable drug:antibody ratio, and/or lower aggregation levels, relative to an ADC, e.g., comprising a longer spacer unit (e.g., $(PEG)_8$). In some embodiments, an ADC comprising a Mal-$(PEG)_2$-Val-Cit-pAB linker joining eribulin to MORAb-003 demonstrates a higher and more desirable drug:antibody ratio, lower aggregation levels, increased on-target killing, and/or decreased off-target killing relative to an ADC, e.g., comprising an alternate cleavable moiety (i.e., a non-peptide cleavable moiety, such as a cleavable disulfide or sulfonamide). In some embodiments, an ADC comprising a Mal-$(PEG)_2$-Val-Cit-pAB linker joining eribulin to MORAb-003 demonstrates increased stability, increased on-target killing, decreased off-target killing, lower aggregation levels, and/or a higher and more desirable drug:antibody ratio relative to an ADC, e.g., comprising an alternate

amino acid unit (e.g., Ala-Ala-Asn) or alternate cleavable moiety (e.g., a cleavable disulfide or sulfonamide).

[00111] In some embodiments, some or all of the desirable features described above for ADCs comprising a Mal-$(PEG)_2$-Val-Cit-pAB linker joining eribulin to MORAb-003 may be observed with ADCs comprising the Mal-$(PEG)_2$-Val-Cit-pAB-eribulin linker-toxin conjugated to an anti-her2 antibody such as trastuzumab, or an anti-mesothelin antibody.

[00112] The ADC compounds of the present disclosure may selectively deliver an effective dose of a cytotoxic or cytostatic agent to cancer cells or to tumor tissue. It has been discovered that the disclosed ADCs have potent cytotoxic and/or cytostatic activity against cells expressing the respective target antigen (e.g., FRA or her2). In some embodiments, the cytotoxic and/or cytostatic activity of the ADC is dependent on the target antigen expression level in a cell. In some embodiments, the disclosed ADCs are particularly effective at killing cancer cells expressing a high level of target antigen, as compared to cancer cells expressing the same antigen at a low level. In some embodiments, the disclosed ADCs are particularly effective at killing cancer cells expressing the target antigen at a moderate level, as compared to cancer cells expressing the same antigen at a low level. Exemplary high FRA-expressing cancers include but are not limited to ovarian cancer (e.g., serous ovarian cancer, clear cell ovarian cancer), lung carcinoid, triple negative breast cancer, endometrial cancer, and nonsmall cell lung cancer (e.g., adenocarcinoma). Exemplary moderate FRA-expressing cancers include but are not limited to gastric cancer and colorectal cancer. Exemplary low FRA-expressing cancers include but are not limited to melanoma and lymphoma. Exemplary high her2-expressing cancers include but are not limited to breast cancer, gastric cancer, esophageal cancer, ovarian cancer, and endometrial cancer. Exemplary moderate her2-expressing cancers include but are not limited to lung cancer and bladder cancer.

[00113] In some embodiments, cleavage of an ADC releases eribulin from the antibody moiety and linker. In some embodiments, cleavage and release of the eribulin improves cytotoxicity of the ADC. In some embodiments, an ADC comprising a cleavable linker is particularly effective at killing cancer cells, including bystander killing, as compared to comparable treatment with an ADC comprising a non-cleavable linker. In some embodiments, an ADC comprising a cleavable linker (e.g., a Val-Cit linker) demonstrates increased on-target cell killing and/or decreased off-target cell

killing relative to an ADC comprising a non-cleavable linker (e.g., a non-cleavable $(PEG)_2$ or $(PEG)_4$ linker), particularly wherein the cells and/or cancer treated with the ADC do not express high levels of the target antigen.

[00114] In some embodiments, the disclosed ADCs also demonstrate bystander killing activity, but low off-target cytotoxicity. Without being bound by theory, the bystander killing activity of an ADC may be particularly beneficial where its penetration into a solid tumor is limited and/or target antigen expression among tumor cells is heterogeneous. In some embodiments, an ADC comprising a cleavable linker is particularly effective at bystander killing and/or demonstrates improved bystander killing activity, as compared to comparable treatment with an ADC comprising a non-cleavable linker.

[00115] Provided herein are ADC compounds comprising an antibody or antigen-binding fragment thereof (Ab) which targets a tumor cell, a drug moiety (D), and a linker moiety (L) that covalently attaches Ab to D. In certain aspects, the antibody or antigen-binding fragment is able to bind to a tumor-associated antigen (e.g., FRA or her2) with high specificity and high affinity. In certain embodiments, the antibody or antigen-binding fragment is internalized into a target cell upon binding, e.g., into a degradative compartment in the cell. Preferred ADCs are thus those that internalize upon binding to a target cell, undergo degradation, and release the drug moiety to kill cancer cells. The drug moiety may be released from the antibody and/or the linker moiety of the ADC by enzymatic action, hydrolysis, oxidation, or any other mechanism.

[00116] An exemplary ADC has Formula I:

$$Ab\text{-}(L\text{-}D)_p \qquad (I)$$

wherein Ab = antibody moiety (i.e., antibody or antigen-binding fragment), L = linker moiety, D = drug moiety, and p = the number of drug moieties per antibody moiety.

Antibodies

[00117] The antibody moiety (Ab) of Formula I includes within its scope any antibody or antigen-binding fragment that specifically binds to a target antigen on a cancer cell. The antibody or antigen-binding fragment may bind to a target antigen with a dissociation constant (K_D) of $\leq$1 mM, $\leq$100 nM or $\leq$10 nM, or any amount in between, as measured by, e.g., BIAcore® analysis. In certain embodiments, the K_D is 1 pM to

500 pM. In some embodiments, the K_D is between 500 pM to 1 μM, 1 μM to 100 nM, or 100 mM to 10 nM.

[00118] In some embodiments, the antibody moiety is a four-chain antibody (also referred to as an immunoglobulin), comprising two heavy chains and two light chains. In some embodiments the antibody moiety is a two-chain half body (one light chain and one heavy chain), or an antigen-binding fragment of an immunoglobulin.

[00119] In some embodiments, the antibody moiety is an internalizing antibody or internalizing antigen-binding fragment thereof. In some embodiments, the internalizing antibody binds to a target cancer antigen expressed on the surface of a cell and enters the cell upon binding. In some embodiments, the drug moiety of the ADC is released from the antibody moiety of the ADC after the ADC enters and is present in a cell expressing the target cancer antigen (i.e., after the ADC has been internalized).

[00120] Amino acid and nucleic acid sequences of exemplary antibodies of the present disclosure are set forth in Tables 1-9.

Table 1. Antibodies

mAb	Class/Isotype	Target
MORAb-003	humanized	human folate receptor alpha
MORAb-009	mouse-human chimeric	human mesothelin
trastuzumab	humanized	human her2/neu
33011-xi	rabbit-human chimeric	human mesothelin
33011-zu	humanized	human mesothelin
111B10-xi	rabbit-human chimeric	human mesothelin
111B10-zu	humanized	human mesothelin
201C15-xi	rabbit-human chimeric	human mesothelin
201C15-zu	humanized	human mesothelin
346C6-xi	rabbit-human chimeric	human mesothelin
346C6-zu	humanized	human mesothelin

Abbreviations: xi – chimeric; zu – humanized.

Table 2. Amino acid sequences of mAb variable regions

	mAb	IgG chain	SEQ ID NO	Amino acid sequence
1	MORAb-003	Heavy chain	23	EVQLVESGGGVVQPGRSLRLSCSASGFT FSGYGLSWVRQAPGKGLEWVAMISSGGS YTYYADSVKGRFAISRDNAKNTLFLQMD SLRPEDTGVYFCARHGDDPAWFAYWGQG TPVTVSS
2	MORAb-003	Light chain	24	DIQLTQSPSSLSASVGDRVTITCSVSSS ISSNNLHWYQQKPGKAPKPWIYGTSNLA SGVPSRFSGSGSGTDYTFTISSLQPEDI ATYYCQQWSSYPYMYTFGQGTKVEIK
3	MORAb-009	Heavy chain	25	QVQLQQSGPELEKPGASVKISCKASGYS FTGYTMNWVKQSHGKSLEWIGLITPYNG ASSYNQKFRGKATLTVDKSSSTAYMDLL SLTSEDSAVYFCARGGYDGRGFDYWGSG TPVTVSS
4	MORAb-009	Light chain	26	DIELTQSPAIMSASPGEKVTMTCSASSS VSYMHWYQQKSGTSPKRWIYDTSKLASG VPGRFSGSGSGNSYSLTISSVEAEDDAT YYCQQWSKHPLTFGSGTKVEIK
5	trastuzumab	Heavy chain	27	EVQLVESGGGLVQPGGSLRLSCAASGFN IKDTYIHWVRQAPGKGLEWVARIYPTNG YTRYADSVKGRFTISADTSKNTAYLQMN SLRAEDTAVYYCSRWGGDGFYAMDYWGQ GTLVTVSS
6	trastuzumab	Light chain	28	DIQMTQSPSSLSASVGDRVTITCRASQD VNTAVAWYQQKPGKAPKLLIYSASFLYS GVPSRFSGSRSGTDFTLTISSLQPEDFA TYYCQQHYTTPPTFGQGTKVEIK
7	33011-xi	Heavy chain	29	QSVEESGGRLVTPGTPLTLTCTVSGISL SSDAISWVRQAPGKGLEYIGIINGGGNT YYASWAKGRFTISKTSTTVDLKITSPTT EDTATYFCARGIQHGGGNSDYYYYGMDL WGPGTLVTVSS
8	33011-xi	Light chain	30	EVLMTQTPSSVSAAVGDTVTIKCQASQS ISSVLSWYQQKPGQPPKLLIYLASTLAS GVPSRFSGSRSGTEFTLTISDLECDDAA TYYCQTNYGTSSSNYGFAFGGGTEVVVK
9	33011-zu	Heavy chain	31	EVQLVESGGGLVQPGGSLRLSCAASGIS

				LSSDAISWVRQAPGKGLEYIGIINGGGN TYYASWAKGRFTISRHNSKNTLYLQMNS LRAEDTAVYYCARGIQHGGGNSDYYYYG MDLWGQGTLVTVSS
10	33011-zu	Light chain	32	DIQMTQSPSSLSASVGDRVTITCQASQS ISSVLSWYQQKPGKAPKLLIYLASTLAS GVPSRFSGSGSGTDFTLTISSLQCEDIA TYYCQTNYGTSSSNYGFAFGGGTKVEIK
11	111B10-xi	Heavy chain	33	QSVEESGGRLVTPGTPLTLTCTVSGFSL NNYAMSWVRQAPGKGLEWIGSISTGGLA FYANWAKGRFTISRTSTTVDLKMTSLTT EDTATYFCGRNGGGSYIFYYFDLWGQGT LVTVSS
12	111B10-xi	Light chain	34	AFELTQTPSSVEAAVGGTITIKCQASQS ISSYLSWYQQKPGQPPKLLIYSASTLAS GVSSRFKGSGSGTEYTLTISDLECADAA TYFCQSYYDIGTSTFGGGTEVVVK
13	111B10-zu	Heavy chain	35	EVQLVESGGGLVQPGGSLRLSCAASGFS LNNYAMSWVRQAPGKGLEWIGSISTGGL AFYANWAKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARNGGGSYIFYYFDLWG QGTLVTVSS
14	111B10-zu	Light chain	36	DIQMTQSPSSLSASVGDRVTITCQASQS ISSYLSWYQQKPGKAPKLLIYSASTLAS GVPSRFSGSGSGTDFTLTISSLQCEDAA TYYCQSYYDIGTSTFGGGTKVEIK
15	201C15-xi	Heavy chain	37	QSVKESGGRLVTPGTPLTLTCTVSGIDL SSYAMGWFRQAPGKGLEYIGTINIGGRV YYASWAKGRFTISRTSTTVDLKAPSLTA EDTATYFCARYYNGGSYDIWGPGTLVTV SL
16	201C15-xi	Light chain	38	DVVMTQTPASASEPVGGTVTIKCQASES IYRVLAWYQQKPGQPPKLLIYDTSTLAS GAPSRFKGSGYGTEFTLTISGVQCEDAA TYYCQGGYYADSYGIAFGGGTEVVVK
17	201C15-zu	Heavy chain	39	QVQLVESGGGLVQPGGSLRLSCSASGID LSSYAMGWVRQAPGKGLEYIGTINIGGR VYYASWAKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARYYNGGSYDIWGQGTL VTVSS

18	201C15-zu	Light chain	40	DIQMTQSPSTLSASVGDRVTITCQASES IYRVLAWYQQKPGKAPKLLIYDTSTLAS GVPSRFSGSGSGTEFTLTISSLQCDDAA TYYCQGGYYADSYGIAFGGGTKVEIK
19	346C6-xi	Heavy chain	41	QSVEESGGRLVKPDESLTLTCTASGFSL SSYAMIWVRQAPGEGLEWIGTISTGGIT YYASWAKGRFTISKTSTTVDLKITSPTT EDTATYFCARGGYAASSAYYLPYYFDLW GQGTLVTVSS
20	346C6-xi	Light chain	42	AAVLTQTPSPVSAAVGGTVTISCQSSQS VYNNNNLAWFQQKPGQPPKLLIYLASTL ASGVPSRFSGSGSGTQFTLTISGVQCDD AATYYCLGGCDDDADTFAFGGGTEVVVK
21	346C6-zu	Heavy chain	43	EVQLVESGGGLVQPGGSLRLSCAASGFS LSSYAMIWVRQAPGKGLEWIGTISTGGI TYYASWAKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARGGYAASSAYYLPYYF DLWGQGTLVTVSS
22	346C6-zu	Light chain	44	DIQMTQSPSSLSASVGDRVTITCQSSQS VYNNNNLAWYQQKPGKVPKLLIYLASTL ASGVPSRFSGSGSGTDFTLTISSLQCED AATYYCLGGCDDDADTFAFGGGTKVEIK

Table 3. Nucleic acid sequences encoding mAb variable regions

	mAb	IgG chain	SEQ ID NO	Nucleic acid sequence
1	MORAb-003	Heavy chain	45	GAGGTCCAACTGGTGGAGAGCGGTGGAG GTGTTGTGCAACCTGGCCGGTCCCTGCG CCTGTCCTGCTCCGCATCTGGCTTCACC TTCAGCGGCTATGGGTTGTCTTGGGTGA GACAGGCACCTGGAAAAGGTCTTGAGTG GGTTGCAATGATTAGTAGTGGTGGTAGT TATACCTACTATGCAGACAGTGTGAAGG GTAGATTTGCAATATCGCGAGACAACGC CAAGAACACATTGTTCCTGCAAATGGAC AGCCTGAGACCCGAAGACACCGGGGTCT ATTTTTGTGCAAGACATGGGGACGATCC CGCCTGGTTCGCTTATTGGGGCCAAGGG ACCCCGGTCACCGTCTCCTCA
2	MORAb-003	Light chain	46	GACATCCAGCTGACCCAGAGCCCAAGCA GCCTGAGCGCCAGCGTGGGTGACAGAGT GACCATCACCTGTAGTGTCAGCTCAAGT ATAAGTTCCAACAACTTGCACTGGTACC AGCAGAAGCCAGGTAAGGCTCCAAAGCC ATGGATCTACGGCACATCCAACCTGGCT TCTGGTGTGCCAAGCAGATTCAGCGGTA GCGGTAGCGGTACCGACTACACCTTCAC CATCAGCAGCCTCCAGCCAGAGGACATC GCCACCTACTACTGCCAACAGTGGAGTA GTTACCCGTACATGTACACGTTCGGCCA AGGGACCAAGGTGGAAATCAAA
3	MORAb-009	Heavy chain	47	CAGGTACAACTGCAGCAGTCTGGGCCTG AGCTGGAGAAGCCTGGCGCTTCAGTGAA GATATCCTGCAAGGCTTCTGGTTACTCA TTCACTGGCTACACCATGAACTGGGTGA AGCAGAGCCATGGAAAGAGCCTTGAGTG GATTGGACTTATTACTCCTTACAATGGT GCTTCTAGCTACAACCAGAAGTTCAGGG GCAAGGCCACATTAACTGTAGACAAGTC ATCCAGCACAGCCTACATGGACCTCCTC AGTCTGACATCTGAAGACTCTGCAGTCT ATTTCTGTGCAAGGGGGGGTTACGACGG GAGGGGTTTTGACTACTGGGGATCCGGG ACCCCGGTCACCGTCTCCTCA

4	MORAb-009	Light chain	48	GACATCGAGCTCACTCAGTCTCCAGCAA TCATGTCTGCATCTCCAGGGGAGAAGGT CACCATGACCTGCAGTGCCAGCTCAAGT GTAAGTTACATGCACTGGTACCAGCAGA AGTCAGGCACCTCCCCCAAAAGATGGAT TTATGACACATCCAAACTGGCTTCTGGA GTCCCAGGTCGCTTCAGTGGCAGTGGGT CTGGAAACTCTTACTCTCTCACAATCAG CAGCGTGGAGGCTGAAGATGATGCAACT TATTACTGCCAGCAGTGGAGTAAGCACC CTCTCACGTTCGGATCCGGGACCAAGGT GGAAATCAAA
5	33011-xi	Heavy chain	49	CAGTCGGTGGAGGAGTCCGGGGGTCGCC TGGTCACGCCTGGGACACCCCTGACACT CACCTGCACCGTCTCTGGAATCTCCCTC AGTAGCGATGCAATAAGCTGGGTCCGCC AGGCTCCAGGGAAGGGGCTCGAATACAT CGGAATCATTAATGGTGGTGGTAACACA TACTACGCGAGCTGGGCGAAAGGCCGAT TCACCATCTCCAAAACCTCGACCACGGT GGATCTGAAAATCACCAGTCCGACAACC GAGGACACGGCCACCTATTTCTGTGCCA GAGGCATTCAACATGGTGGTGGTAATAG TGATTATTATTATTACGGCATGGACCTC TGGGGCCCAGGCACCCTGGTCACTGTCT CTTCA
6	33011-xi	Light chain	50	GAAGTGTTGATGACCCAGACTCCATCCT CCGTGTCTGCAGCTGTGGGAGACACAGT CACCATCAAGTGCCAGGCCAGTCAGAGC ATTAGTAGTGTCTTGTCCTGGTATCAGC AGAAACCAGGGCAGCCTCCCAAGCTCCT GATCTATCTGGCATCCACTCTGGCATCT GGGGTCCCATCGCGGTTCAGCGGCAGTA GATCTGGGACAGAGTTCACTCTCACCAT CAGCGACCTGGAGTGTGACGATGCTGCC ACTTACTACTGTCAAACCAATTATGGTA CTAGTAGTAGTAATTATGGTTTTGCTTT CGGCGGAGGGACCGAGGTGGTCGTCAAA
7	33011-zu	Heavy chain	51	GAAGTCCAACTGGTGGAAAGCGGGGGAG GACTGGTGCAGCCGGGCGGATCCCTCCG

				GCTGTCATGTGCTGCATCGGGAATTTCC CTCTCCTCCGACGCGATTAGCTGGGTCA GACAGGCCCCCGGAAAGGGGCTGGAGTA CATCGGTATCATCAACGGCGGCGGAAAC ACCTACTACGCCTCCTGGGCCAAGGGCC GCTTCACCATCTCGCGGCATAATTCCAA GAACACTCTGTACTTGCAAATGAACTCC CTGAGGGCCGAGGACACCGCCGTGTACT ACTGCGCGCGCGGCATCCAGCACGGTGG TGGAAACAGCGACTACTACTACTATGGG ATGGATCTGTGGGGCCAGGGAACTCTTG TGACCGTGTCGTCA
8	33011-zu	Light chain	52	GACATTCAGATGACCCAGTCCCCAAGCT CGCTGTCCGCCTCCGTGGGCGACCGCGT GACCATCACGTGCCAGGCGTCCCAGTCA ATTAGCAGCGTGCTCTCCTGGTACCAAC AGAAGCCGGGGAAAGCACCCAAGCTGCT GATCTACTTGGCCTCCACTCTGGCCTCG GGAGTGCCTTCACGGTTCTCCGGATCGG GATCTGGTACTGATTTCACCCTCACCAT CTCGAGCCTTCAGTGCGAGGACATCGCT ACTTACTATTGTCAAACCAACTACGGAA CCTCCAGCTCCAACTACGGCTTTGCCTT CGGTGGCGGGACCAAGGTCGAAATCAAA
9	111B10-xi	Heavy chain	53	CAGTCGGTGGAGGAGTCCGGGGGTCGCC TGGTCACGCCTGGGACACCCCTGACACT CACCTGCACAGTCTCTGGATTCTCCCTC AATAACTATGCAATGAGCTGGGTCCGCC AGGCTCCAGGGAAGGGGCTGGAATGGAT CGGATCCATTAGTACTGGTGGTCTCGCA TTCTACGCGAACTGGGCAAAAGGCCGAT TCACCATCTCCAGAACCTCGACCACGGT GGATCTGAAAATGACCAGTCTGACAACC GAGGACACGGCCACCTATTTCTGTGGCA GAAATGGTGGTGGTAGTTATATTTTCTA TTATTTTGACTTGTGGGGCCAAGGCACC CTCGTCACTGTCTCTTCA
10	111B10-xi	Light chain	54	GCATTCGAATTGACCCAGACTCCATCCT CCGTGGAGGCAGCTGTGGGAGGCACAAT CACCATCAAGTGCCAGGCCAGTCAGAGC

				ATTAGTAGTTACTTATCCTGGTATCAGC AGAAACCAGGGCAGCCTCCCAAGCTCCT GATCTATTCTGCATCCACTCTGGCATCT GGGGTCTCATCGCGGTTCAAAGGCAGTG GATCTGGGACAGAGTACACTCTCACCAT CAGCGACCTGGAGTGTGCCGATGCTGCC ACTTACTTCTGTCAAAGCTATTATGATA TTGGTACTAGTACTTTCGGCGGAGGGAC CGAGGTGGTCGTCAAA
11	111B10-zu	Heavy chain	55	GAAGTGCAGCTGGTGGAATCTGGCGGCG GACTGGTGCAGCCTGGCGGATCTCTGAG ACTGTCTTGTGCCGCCTCCGGCTTCTCC CTGAACAACTACGCCATGTCCTGGGTGC GACAGGCCCCTGGCAAAGGCCTGGAATG GATCGGCTCCATCAGCACAGGCGGCCTG GCCTTCTACGCCAATTGGGCCAAGGGCC GGTTCACCATCAGCCGGGACAACTCCAA GAACACCCTGTACCTCCAGATGAACTCC CTGCGGGCCGAGGACACCGCCGTGTACT ACTGTGCCAGAAACGGCGGAGGCTCCTA CATCTTCTACTACTTCGACCTGTGGGGC CAGGGCACCCTCGTGACAGTGTCATCT
12	111B10-zu	Light chain	56	GATATTCAGATGACCCAGTCCCCCTCCA GCCTGTCCGCTTCTGTGGGCGACAGAGT GACCATCACCTGTCAGGCCTCCCAGTCC ATCTCCTCCTACCTGTCCTGGTATCAGC AGAAGCCCGGCAAGGCCCCCAAGCTGCT GATCTACTCTGCCTCCACACTGGCCTCC GGCGTGCCCTCTAGATTCTCCGGCTCTG GCTCTGGCACCGACTTTACCCTGACCAT CAGCTCCCTCCAGTGCGAGGATGCCGCC ACCTACTACTGCCAGTCCTACTACGACA TCGGCACCTCCACCTTCGGCGGAGGCAC CAAGGTGGAAATCAAA
13	201C15-xi	Heavy chain	57	CAGTCAGTGAAGGAGTCCGGGGGTCGCC TGGTCACGCCTGGGACACCCCTGACACT CACCTGCACAGTCTCTGGAATCGACCTC AGTAGCTATGCAATGGGCTGGTTCCGCC AGGCTCCAGGGAAGGGGCTGGAATACAT CGGAACCATTAATATTGGTGGTCGCGTA

				TATTACGCGAGCTGGGCAAAAGGCCGAT TCACCATCTCCAGAACCTCGACCACGGT GGATCTGAAAGCGCCCAGTCTGACAGCC GAGGACACGGCCACCTATTTCTGTGCCA GATATTATAATGGTGGTAGTTATGACAT CTGGGGCCCAGGCACCCTGGTCACCGTC TCTTTA
14	201C15-xi	Light chain	58	GATGTTGTGATGACCCAGACTCCAGCCT CCGCGTCTGAACCTGTGGGAGGCACAGT CACCATCAAGTGCCAGGCCAGTGAGAGC ATTTATCGCGTATTGGCCTGGTATCAGC AGAAACCAGGGCAGCCTCCCAAGCTCCT GATCTATGATACATCCACTCTGGCATCT GGGGCCCCATCGCGGTTCAAAGGCAGTG GATATGGGACAGAGTTCACTCTCACCAT CAGCGGCGTGCAGTGTGAAGATGCTGCC ACTTACTACTGTCAAGGCGGTTATTATG CTGATAGTTATGGTATTGCTTTCGGCGG AGGGACCGAGGTGGTGGTCAAA
15	201C15-zu	Heavy chain	59	CAGGTGCAGCTGGTGGAATCTGGCGGAG GACTGGTGCAGCCTGGCGGCTCTCTGAG ACTGTCCTGTTCCGCCTCCGGAATCGAC CTGTCCTCCTACGCTATGGGCTGGGTGC GACAGGCTCCTGGCAAGGGCCTGGAGTA CATCGGCACCATCAACATCGGCGGCAGA GTGTACTACGCCTCCTGGGCCAAGGGCC GGTTCACCATCTCCAGAGACAACTCCAA GAACACCCTGTACCTCCAGATGAACTCC CTGCGGGCCGAGGACACCGCCGTGTACT ACTGCGCCCGGTACTACAACGGCGGCTC CTACGATATCTGGGGCCAGGGCACACTC GTGACCGTGTCCTCT
16	201C15-zu	Light chain	60	GATATCCAGATGACCCAGTCCCCCTCCA CCCTGTCTGCCTCTGTGGGCGACAGAGT GACCATCACCTGTCAGGCCTCCGAGTCC ATCTACCGGGTGCTGGCCTGGTATCAGC AGAAGCCTGGCAAGGCCCCCAAGCTGCT GATCTACGACACCAGCACACTGGCCTCC GGCGTGCCCTCTAGATTCTCCGGCTCTG GCTCTGGCACCGAGTTTACCCTGACCAT

				CTCCAGCCTCCAGTGCGACGACGCCGCC ACCTACTATTGTCAGGGCGGCTACTACG CCGACTCCTACGGAATCGCTTTCGGCGG AGGCACCAAGGTGGAAATCAAA
17	346C6-xi	Heavy chain	61	CAGTCGGTGGAGGAGTCCGGCGGTCGCC TGGTAAAGCCTGACGAATCCCTGACACT CACCTGCACAGCCTCTGGATTCTCCCTC AGTAGTTATGCAATGATCTGGGTCCGCC AGGCTCCAGGGGAGGGGCTGGAATGGAT CGGAACCATTAGTACTGGTGGTATCACA TACTACGCGAGCTGGGCGAAAGGCCGAT TCACCATCTCCAAAACCTCGACCACGGT GGATCTGAAAATCACCAGTCCGACAACC GAGGACACGGCCACCTATTTCTGTGCCA GAGGGGGATATGCTGCTAGTAGTGCTTA TTATCTCCCGTACTACTTTGACTTGTGG GGCCAAGGGACCCTGGTCACCGTCTCCT CA
18	346C6-xi	Light chain	62	GCAGCCGTGCTGACCCAGACACCATCAC CCGTGTCTGCAGCTGTGGGAGGCACAGT CACCATCAGTTGCCAGTCCAGTCAGAGT GTTTATAATAATAACAACTTAGCCTGGT TTCAGCAGAAACCCGGGCAGCCTCCCAA GCTTCTGATCTATCTGGCATCCACTCTG GCATCTGGGGTCCCATCACGGTTCAGCG GCAGTGGATCTGGGACACAGTTCACTCT CACCATCAGCGGCGTGCAGTGTGACGAT GCTGCCACTTATTACTGTCTAGGTGGTT GTGATGATGATGCTGATACTTTTGCTTT CGGCGGAGGGACTGAGGTGGTGGTCAAA
19	346C6-zu	Heavy chain	63	GAAGTGCAGCTGGTGGAATCTGGCGGCG GACTGGTGCAGCCTGGCGGATCTCTGAG ACTGTCTTGTGCCGCCTCCGGCTTCTCC CTGTCCTCCTACGCTATGATCTGGGTGC GACAGGCCCCTGGCAAGGGCCTGGAATG GATCGGCACCATCTCTACCGGCGGAATT ACCTACTACGCCTCCTGGGCCAAGGGCC GGTTCACCATCTCCAGAGACAACTCCAA GAACACCCTGTACCTCCAGATGAACTCC CTGCGGGCCGAGGACACCGCCGTGTACT

				ATTGTGCTAGAGGCGGCTACGCCGCCAG CTCCGCTTACTACCTGCCCTACTACTTC GACCTGTGGGGCCAGGGCACCCTCGTGA CAGTGTCATCT
20	346C6-zu	Light chain	64	GATATTCAGATGACCCAGTCCCCCTCCA GCCTGTCCGCTTCTGTGGGCGACAGAGT GACCATCACCTGTCAGTCCTCCCAGTCC GTGTATAACAACAACAACCTGGCCTGGT ATCAGCAGAAACCCGGCAAGGTGCCCAA GCTGCTGATCTACCTGGCCTCCACACTG GCCTCTGGCGTGCCCTCTAGATTCTCCG GCTCTGGCTCTGGCACCGACTTTACCCT GACCATCAGCTCCCTCCAGTGCGAGGAT GCCGCCACCTACTATTGCCTGGGCGGCT GCGACGACGACGCCGATACCTTTGCTTT TGGCGGAGGCACCAAGGTGGAAATCAAA

Table 4. Amino acid sequences of mAb Kabat CDRs

	mAb	IgG chain	SEQ ID NO	Amino acid sequence
1	MORAb-003	HC CDR1	2	GYGLS
2	MORAb-003	HC CDR2	3	MISSGGSYTYYADSVKG
3	MORAb-003	HC CDR3	4	HGDDPAWFAY
4	MORAb-003	LC CDR1	7	SVSSSISSNNLH
5	MORAb-003	LC CDR2	8	GTSNLAS
6	MORAb-003	LC CDR3	9	QQWSSYPYMYT
7	MORAb-009	HC CDR1	65	GYTMN
8	MORAb-009	HC CDR2	66	LITPYNGASSYNQKFRG
9	MORAb-009	HC CDR3	67	GGYDGRGFDY
10	MORAb-009	LC CDR1	68	SASSSVSYMH
11	MORAb-009	LC CDR2	69	DTSKLAS
12	MORAb-009	LC CDR3	70	QQWSKHPLT
13	trastuzumab	HC CDR1	71	DTYIH
14	trastuzumab	HC CDR2	72	RIYPTNGYTRYADSVKG
15	trastuzumab	HC CDR3	73	WGGDGFYAMDY
16	trastuzumab	LC CDR1	74	RASQDVNTAVA
17	trastuzumab	LC CDR2	75	SASFLYS
18	trastuzumab	LC CDR3	76	QQHYTTPPT
19	33011-xi	HC CDR1	77	SDAIS
20	33011-xi	HC CDR2	78	IINGGGNTYYASWAKG
21	33011-xi	HC CDR3	79	GIQHGGGNSDYYYYGMDL
22	33011-xi	LC CDR1	80	QASQSISSVLS
23	33011-xi	LC CDR2	81	LASTLAS
24	33011-xi	LC CDR3	82	QTNYGTSSSNYGFA
25	33011-zu	HC CDR1	83	SDAIS
26	33011-zu	HC CDR2	84	IINGGGNTYYASWAKG
27	33011-zu	HC CDR3	85	GIQHGGGNSDYYYYGMDL
28	33011-zu	LC CDR1	86	QASQSISSVLS
29	33011-zu	LC CDR2	87	LASTLAS

30	33011-zu	LC CDR3	88	QTNYGTSSSNYGFA
31	111B10-xi	HC CDR1	89	NYAMS
32	111B10-xi	HC CDR2	90	SISTGGLAFYANWAKG
33	111B10-xi	HC CDR3	91	NGGGSYIFYYFDL
34	111B10-xi	LC CDR1	92	QASQSISSYLS
35	111B10-xi	LC CDR2	93	SASTLAS
36	111B10-xi	LC CDR3	94	QSYYDIGTST
37	111B10-zu	HC CDR1	95	NYAMS
38	111B10-zu	HC CDR2	96	SISTGGLAFYANWAKG
39	111B10-zu	HC CDR3	97	NGGGSYIFYYFDL
40	111B10-zu	LC CDR1	98	QASQSISSYLS
41	111B10-zu	LC CDR2	99	SASTLAS
42	111B10-zu	LC CDR3	100	QSYYDIGTST
43	201C15-xi	HC CDR1	101	SYAMG
44	201C15-xi	HC CDR2	102	TINIGGRVYYASWAKG
45	201C15-xi	HC CDR3	103	YYNGGSYDI
46	201C15-xi	LC CDR1	104	QASESIYRVLA
47	201C15-xi	LC CDR2	105	DTSTLAS
48	201C15-xi	LC CDR3	106	QGGYYADSYGIA
49	201C15-zu	HC CDR1	107	SYAMG
50	201C15-zu	HC CDR2	108	TINIGGRVYYASWAKG
51	201C15-zu	HC CDR3	109	YYNGGSYDI
52	201C15-zu	LC CDR1	110	QASESIYRVLA
53	201C15-zu	LC CDR2	111	DTSTLAS
54	201C15-zu	LC CDR3	112	QGGYYADSYGIA
55	346C6-xi	HC CDR1	113	SYAMI
56	346C6-xi	HC CDR2	114	TISTGGITYYASWAKG
57	346C6-xi	HC CDR3	115	GGYAASSAYYLPYYFDL
58	346C6-xi	LC CDR1	116	QSSQSVYNNNNLA
59	346C6-xi	LC CDR2	117	LASTLAS
60	346C6-xi	LC CDR3	118	LGGCDDDADTFA

61	346C6-zu	HC CDR1	119	SYAMI
62	346C6-zu	HC CDR2	120	TISTGGITYYASWAKG
63	346C6-zu	HC CDR3	121	GGYAASSAYYLPYYFDL
64	346C6-zu	LC CDR1	122	QSSQSVYNNNNLA
65	346C6-zu	LC CDR2	123	LASTLAS
66	346C6-zu	LC CDR3	124	LGGCDDDADTFA

Table 5. Nucleic acid sequences encoding mAb Kabat CDRs

	mAb	IgG chain	SEQ ID NO	Nucleic acid sequence
1	MORAb-003	HC CDR1	125	GGCTATGGGTTGTCT
2	MORAb-003	HC CDR2	126	ATGATTAGTAGTGGTGGTAGTTATACCTACTATG CAGACAGTGTGAAGGGT
3	MORAb-003	HC CDR3	127	CATGGGGACGATCCCGCCTGGTTCGCTTAT
4	MORAb-003	LC CDR1	128	AGTGTCAGCTCAAGTATAAGTTCCAACAACTTGC AC
5	MORAb-003	LC CDR2	129	GGCACATCCAACCTGGCTTCT
6	MORAb-003	LC CDR3	130	CAACAGTGGAGTAGTTACCCGTACATGTACACG
7	MORAb-009	HC CDR1	131	GGCTACACCATGAAC
8	MORAb-009	HC CDR2	132	CTTATTACTCCTTACAATGGTGCTTCTAGCTACA ACCAGAAGTTCAGGGGC
9	MORAb-009	HC CDR3	133	GGGGGTTACGACGGGAGGGGTTTTGACTAC
10	MORAb-009	LC CDR1	134	AGTGCCAGCTCAAGTGTAAGTTACATGCAC
11	MORAb-009	LC CDR2	135	GACACATCCAAACTGGCTTCT
12	MORAb-009	LC CDR3	136	CAGCAGTGGAGTAAGCACCCTCTCACG
13	33011-xi	HC CDR1	137	AGCGATGCAATAAGC
14	33011-xi	HC CDR2	138	ATCATTAATGGTGGTGGTAACACATACTACGCGA GCTGGGCGAAAGGC
15	33011-xi	HC CDR3	139	GGCATTCAACATGGTGGTGGTAATAGTGATTATT ATTATTACGGCATGGACCTC
16	33011-xi	LC CDR1	140	CAGGCCAGTCAGAGCATTAGTAGTGTCTTGTCC
17	33011-xi	LC CDR2	141	CTGGCATCCACTCTGGCATCT
18	33011-xi	LC CDR3	142	CAAACCAATTATGGTACTAGTAGTAGTAATTATG GTTTTGCT
19	33011-zu	HC CDR1	143	TCCGACGCGATTAGC
20	33011-zu	HC CDR2	144	ATCATCAACGGCGGCGGAAACACCTACTACGCCT CCTGGGCCAAGGGC
21	33011-zu	HC CDR3	145	GGCATCCAGCACGGTGGTGGAAACAGCGACTACT ACTACTATGGGATGGATCTG
22	33011-zu	LC CDR1	146	CAGGCGTCCCAGTCAATTAGCAGCGTGCTCTCC
23	33011-zu	LC CDR2	147	TTGGCCTCCACTCTGGCCTCG
24	33011-zu	LC CDR3	148	CAAACCAACTACGGAACCTCCAGCTCCAACTACG GCTTTGCC

25	111B10-xi	HC CDR1	149	AACTATGCAATGAGC
26	111B10-xi	HC CDR2	150	TCCATTAGTACTGGTGGTCTCGCATTCTACGCGA ACTGGGCAAAAGGC
27	111B10-xi	HC CDR3	151	AATGGTGGTGGTAGTTATATTTTCTATTATTTTG ACTTG
28	111B10-xi	LC CDR1	152	CAGGCCAGTCAGAGCATTAGTAGTTACTTATCC
29	111B10-xi	LC CDR2	153	TCTGCATCCACTCTGGCATCT
30	111B10-xi	LC CDR3	154	CAAAGCTATTATGATATTGGTACTAGTACT
31	111B10-zu	HC CDR1	155	AACTACGCCATGTCC
32	111B10-zu	HC CDR2	156	TCCATCAGCACAGGCGGCCTGGCCTTCTACGCCA ATTGGGCCAAGGGC
33	111B10-zu	HC CDR3	157	AACGGCGGAGGCTCCTACATCTTCTACTACTTCG ACCTG
34	111B10-zu	LC CDR1	158	CAGGCCTCCCAGTCCATCTCCTCCTACCTGTCC
35	111B10-zu	LC CDR2	159	TCTGCCTCCACACTGGCCTCC
36	111B10-zu	LC CDR3	160	CAGTCCTACTACGACATCGGCACCTCCACC
37	201C15-xi	HC CDR1	161	AGCTATGCAATGGGC
38	201C15-xi	HC CDR2	162	ACCATTAATATTGGTGGTCGCGTATATTACGCGA GCTGGGCAAAAGGC
39	201C15-xi	HC CDR3	163	TATTATAATGGTGGTAGTTATGACATC
40	201C15-xi	LC CDR1	164	CAGGCCAGTGAGAGCATTTATCGCGTATTGGCC
41	201C15-xi	LC CDR2	165	GATACATCCACTCTGGCATCT
42	201C15-xi	LC CDR3	166	CAAGGCGGTTATTATGCTGATAGTTATGGTATTG CT
43	201C15-zu	HC CDR1	167	TCCTACGCTATGGGC
44	201C15-zu	HC CDR2	168	ACCATCAACATCGGCGGCAGAGTGTACTACGCCT CCTGGGCCAAGGGC
45	201C15-zu	HC CDR3	169	TACTACAACGGCGGCTCCTACGATATC
46	201C15-zu	LC CDR1	170	CAGGCCTCCGAGTCCATCTACCGGGTGCTGGCC
47	201C15-zu	LC CDR2	171	GACACCAGCACACTGGCCTCC
48	201C15-zu	LC CDR3	172	CAGGGCGGCTACTACGCCGACTCCTACGGAATCG CT
49	346C6-xi	HC CDR1	173	AGTTATGCAATGATC
50	346C6-xi	HC CDR2	174	ACCATTAGTACTGGTGGTATCACATACTACGCGA GCTGGGCGAAAGGC

51	346C6-xi	HC CDR3	175	GGGGGATATGCTGCTAGTAGTGCTTATTATCTCCCGTACTACTTTGACTTG
52	346C6-xi	LC CDR1	176	CAGTCCTCCCAGTCCGTGTATAACAACAACAACCTGGCC
53	346C6-xi	LC CDR2	177	CTGGCATCCACTCTGGCATCT
54	346C6-xi	LC CDR3	178	CTAGGTGGTTGTGATGATGATGCTGATACTTTTGCT
55	346C6-zu	HC CDR1	179	TCCTACGCTATGATC
56	346C6-zu	HC CDR2	180	ACCATCTCTACCGGCGGAATTACCTACTACGCCTCCTGGGCCAAGGGC
57	346C6-zu	HC CDR3	181	GGCGGCTACGCCGCCAGCTCCGCTTACTACCTGCCCTACTACTTCGACCTG
58	346C6-zu	LC CDR1	182	CAGTCCTCCCAGTCCGTGTATAACAACAACAACCTGGCC
59	346C6-zu	LC CDR2	183	CTGGCCTCCACACTGGCCTCT
60	346C6-zu	LC CDR3	184	CTGGGCGGCTGCGACGACGACGCCGATACCTTTGCT

Table 6. Amino acid sequences of mAb IMGT CDRs

	mAb	IgG chain	SEQ ID NO	Amino acid sequence
1	MORAb-003	HC CDR1	13	GFTFSGYG
2	MORAb-003	HC CDR2	14	ISSGGSYT
3	MORAb-003	HC CDR3	15	ARHGDDPAWFAY
4	MORAb-003	LC CDR1	16	SSISSNN
5	MORAb-003	LC CDR2	17	GTS
6	MORAb-003	LC CDR3	18	QQWSSYPYMYT
7	MORAb-009	HC CDR1	185	GYSFTGYT
8	MORAb-009	HC CDR2	186	ITPYNGAS
9	MORAb-009	HC CDR3	187	ARGGYDGRGFDY
10	MORAb-009	LC CDR1	188	SSVSY
11	MORAb-009	LC CDR2	189	DTS
12	MORAb-009	LC CDR3	190	QQWSKHPLT
13	trastuzumab	HC CDR1	191	GFNIKDTY
14	trastuzumab	HC CDR2	192	IYPTNGYT
15	trastuzumab	HC CDR3	193	SRWGGDGFYAMDY
16	trastuzumab	LC CDR1	194	QDVNTA
17	trastuzumab	LC CDR2	195	SAS
18	trastuzumab	LC CDR3	196	QQHYTTPPT
19	33011-xi	HC CDR1	197	GISLSSDA
20	33011-xi	HC CDR2	198	INGGGNT
21	33011-xi	HC CDR3	199	ARGIQHGGGNSDYYYYGMDL
22	33011-xi	LC CDR1	200	QSISSV
23	33011-xi	LC CDR2	201	LAS
24	33011-xi	LC CDR3	202	QTNYGTSSSNYGFA
25	33011-zu	HC CDR1	203	GISLSSDA
26	33011-zu	HC CDR2	204	INGGGNT
27	33011-zu	HC CDR3	205	ARGIQHGGGNSDYYYYGMDL
28	33011-zu	LC CDR1	206	QSISSV
29	33011-zu	LC CDR2	207	LAS

30	33011-zu	LC CDR3	208	QTNYGTSSSNYGFA
31	111B10-xi	HC CDR1	209	GFSLNNYA
32	111B10-xi	HC CDR2	210	ISTGGLA
33	111B10-xi	HC CDR3	211	GRNGGGSYIFYYFDL
34	111B10-xi	LC CDR1	212	QSISSY
35	111B10-xi	LC CDR2	213	SAS
36	111B10-xi	LC CDR3	214	QSYYDIGTST
37	111B10-zu	HC CDR1	215	GFSLNNYA
38	111B10-zu	HC CDR2	216	ISTGGLA
39	111B10-zu	HC CDR3	217	ARNGGGSYIFYYFDL
40	111B10-zu	LC CDR1	218	QSISSY
41	111B10-zu	LC CDR2	219	SAS
42	111B10-zu	LC CDR3	220	QSYYDIGTST
43	201C15-xi	HC CDR1	221	GIDLSSYA
44	201C15-xi	HC CDR2	222	INIGGRV
45	201C15-xi	HC CDR3	223	ARYYNGGSYDI
46	201C15-xi	LC CDR1	224	ESIYRV
47	201C15-xi	LC CDR2	225	DTS
48	201C15-xi	LC CDR3	226	QGGYYADSYGIA
49	201C15-zu	HC CDR1	227	GIDLSSYA
50	201C15-zu	HC CDR2	228	INIGGRV
51	201C15-zu	HC CDR3	229	ARYYNGGSYDI
52	201C15-zu	LC CDR1	230	ESIYRV
53	201C15-zu	LC CDR2	231	DTS
54	201C15-zu	LC CDR3	232	QGGYYADSYGIA
55	346C6-xi	HC CDR1	233	GFSLSSYA
56	346C6-xi	HC CDR2	234	ISTGGIT
57	346C6-xi	HC CDR3	235	ARGGYAASSAYYLPYYFDL
58	346C6-xi	LC CDR1	236	QSVYNNNN
59	346C6-xi	LC CDR2	237	LAS
60	346C6-xi	LC CDR3	238	LGGCDDDADTFA

61	346C6-zu	HC CDR1	239	GFSLSSYA
62	346C6-zu	HC CDR2	240	ISTGGIT
63	346C6-zu	HC CDR3	241	ARGGYAASSAYYLPYYFDL
64	346C6-zu	LC CDR1	242	QSVYNNNN
65	346C6-zu	LC CDR2	243	LAS
66	346C6-zu	LC CDR3	244	LGGCDDDADTFA

Table 7. Nucleic acid sequences encoding mAb IMGT CDRs

	mAb	IgG chain	SEQ ID NO	Nucleic acid sequence
1	MORAb-003	HC CDR1	245	GGCTTCACCTTCAGCGGCTATGGG
2	MORAb-003	HC CDR2	246	ATTAGTAGTGGTGGTAGTTATACC
3	MORAb-003	HC CDR3	247	GCAAGACATGGGGACGATCCCGCCTGGTTCGCTTAT
4	MORAb-003	LC CDR1	248	TCAAGTATAAGTTCCAACAAC
5	MORAb-003	LC CDR2	249	GGCACATCC
6	MORAb-003	LC CDR3	250	CAACAGTGGAGTAGTTACCCGTACATGTACACG
7	MORAb-009	HC CDR1	251	GGTTACTCATTCACTGGCTACACC
8	MORAb-009	HC CDR2	252	ATTACTCCTTACAATGGTGCTTCT
9	MORAb-009	HC CDR3	253	GCAAGGGGGGGTTACGACGGGAGGGGTTTTGACTAC
10	MORAb-009	LC CDR1	254	TCAAGTGTAAGTTAC
11	MORAb-009	LC CDR2	255	GACACATCC
12	MORAb-009	LC CDR3	256	CAGCAGTGGAGTAAGCACCCTCTCACG
13	33011-xi	HC CDR1	257	GGAATCTCCCTCAGTAGCGATGCA
14	33011-xi	HC CDR2	258	ATTAATGGTGGTGGTAACACA
15	33011-xi	HC CDR3	259	GCCAGAGGCATTCAACATGGTGGTGGTAATAGTGATTATTATTATTACGGCATGGACCTC
16	33011-xi	LC CDR1	260	CAGAGCATTAGTAGTGTC
17	33011-xi	LC CDR2	261	CTGGCATCT
18	33011-xi	LC CDR3	262	CAAACCAATTATGGTACTAGTAGTAGTAATTATGGTTTTGCT
19	33011-zu	HC CDR1	263	GGAATTTCCCTCTCCTCCGACGCG
20	33011-zu	HC CDR2	264	ATCAACGGCGGCGGAAACACC
21	33011-zu	HC CDR3	265	GCGCGCGGCATCCAGCACGGTGGTGGAAACAGCGACTACTACTACTATGGGATGGATCTG
22	33011-zu	LC CDR1	266	CAGTCAATTAGCAGCGTG
23	33011-zu	LC CDR2	267	TTGGCCTCC
24	33011-zu	LC CDR3	268	CAAACCAACTACGGAACCTCCAGCTCCAACTACGGCTTTGCC
25	111B10-xi	HC CDR1	269	GGATTCTCCCTCAATAACTATGCA

26	111B10-xi	HC CDR2	270	ATTAGTACTGGTGGTCTCGCA
27	111B10-xi	HC CDR3	271	GGCAGAAATGGTGGTGGTAGTTATATTTTCTATTATTTTGACTTG
28	111B10-xi	LC CDR1	272	CAGAGCATTAGTAGTTAC
29	111B10-xi	LC CDR2	273	TCTGCATCC
30	111B10-xi	LC CDR3	274	CAAAGCTATTATGATATTGGTACTAGTACT
31	111B10-zu	HC CDR1	275	GGCTTCTCCCTGAACAACTACGCC
32	111B10-zu	HC CDR2	276	ATCAGCACAGGCGGCCTGGCC
33	111B10-zu	HC CDR3	277	GCCAGAAACGGCGGAGGCTCCTACATCTTCTACTACTTCGACCTG
34	111B10-zu	LC CDR1	278	CAGTCCATCTCCTCCTAC
35	111B10-zu	LC CDR2	279	TCTGCCTCC
36	111B10-zu	LC CDR3	300	CAGTCCTACTACGACATCGGCACCTCCACC
37	201C15-xi	HC CDR1	301	GGAATCGACCTCAGTAGCTATGCA
38	201C15-xi	HC CDR2	302	ATTAATATTGGTGGTCGCGTA
39	201C15-xi	HC CDR3	303	GCCAGATATTATAATGGTGGTAGTTATGACATC
40	201C15-xi	LC CDR1	304	GAGAGCATTTATCGCGTA
41	201C15-xi	LC CDR2	305	GATACATCC
42	201C15-xi	LC CDR3	306	CAAGGCGGTTATTATGCTGATAGTTATGGTATTGCT
43	201C15-zu	HC CDR1	307	GGAATCGACCTGTCCTCCTACGCT
44	201C15-zu	HC CDR2	308	ATCAACATCGGCGGCAGAGTG
45	201C15-zu	HC CDR3	309	GCCCGGTACTACAACGGCGGCTCCTACGATATC
46	201C15-zu	LC CDR1	310	GAGTCCATCTACCGGGTG
47	201C15-zu	LC CDR2	311	GACACCAGC
48	201C15-zu	LC CDR3	312	CAGGGCGGCTACTACGCCGACTCCTACGGAATCGCT
49	346C6-xi	HC CDR1	313	GGATTCTCCCTCAGTAGTTATGCA
50	346C6-xi	HC CDR2	314	ATTAGTACTGGTGGTATCACA
51	346C6-xi	HC CDR3	315	GCCAGAGGGGGATATGCTGCTAGTAGTGCTTATTATCTCCCGTACTACTTTGACTTG
52	346C6-xi	LC CDR1	316	CAGAGTGTTTATAATAATAACAAC
53	346C6-xi	LC CDR2	317	CTGGCATCC

54	346C6-xi	LC CDR3	318	CTAGGTGGTTGTGATGATGATGCTGATACTTTTGCT
55	346C6-zu	HC CDR1	319	GGCTTCTCCCTGTCCTCCTACGCT
56	346C6-zu	HC CDR2	320	ATCTCTACCGGCGGAATTACC
57	346C6-zu	HC CDR3	321	GCTAGAGGCGGCTACGCCGCCAGCTCCGCTTACTACCTGCCCTACTACTTCGACCTG
58	346C6-zu	LC CDR1	322	CAGTCCGTGTATAACAACAACAAC
59	346C6-zu	LC CDR2	323	CTGGCCTCC
60	346C6-zu	LC CDR3	324	CTGGGCGGCTGCGACGACGACGCCGATACCTTTGCT

Table 8. Amino acid sequences of full-length mAb Ig chains

	mAb	IgG chain	SEQ ID NO	Amino acid sequence
1	MORAb-003	Heavy chain	1	EVQLVESGGGVVQPGRSLRLSCSASGFTFSGY GLSWVRQAPGKGLEWVAMISSGGSYTYYADSV KGRFAISRDNAKNTLFLQMDSLRPEDTGVYFC ARHGDDPAWFAYWGQGTPVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK THTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG K
2	MORAb-003	Light chain	6	DIQLTQSPSSLSASVGDRVTITCSVSSSISSN NLHWYQQKPGKAPKPWIYGTSNLASGVPSRFS GSGSGTDYTFTISSLQPEDIATYYCQQWSSYP YMYTFGQGTKVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC
3	MORAb-009	Heavy chain	325	QVQLQQSGPELEKPGASVKISCKASGYSFTGY TMNWVKQSHGKSLEWIGLITPYNGASSYNQKF RGKATLTVDKSSSTAYMDLLSLTSEDSAVYFC ARGGYDGRGFDYWGSGTPVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK THTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

				K
4	MORAb-009	Light chain	326	DIELTQSPAIMSASPGEKVTMTCSASSSVSYM HWYQQKSGTSPKRWIYDTSKLASGVPGRFSGS GSGNSYSLTISSVEAEDDATYYCQQWSKHPLT FGSGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC
5	trastuzumab	Heavy chain	327	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDT YIHWVRQAPGKGLEWVARIYPTNGYTRYADSV KGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRWGGDGFYAMDYWGQGTLVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKKVEPPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK
6	trastuzumab	Light chain	328	DIQMTQSPSSLSASVGDRVTITCRASQDVNTA VAWYQQKPGKAPKLLIYSASFLYSGVPSRFSG SRSGTDFTLTISSLQPEDFATYYCQQHYTTPP TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
7	33011-xi	Heavy chain	329	QSVEESGGRLVTPGTPLTLTCTVSGISLSSDA ISWVRQAPGKGLEYIGIINGGGNTYYASWAKG RFTISKTSTTVDLKITSPTTEDTATYFCARGI QHGGGNSDYYYYGMDL WGPGTLVTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS

				TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
8	33011-xi	Light chain	330	EVLMTQTPSSVSAAVGDTVTIKCQASQSISSV LSWYQQKPGQPPKLLIYLASTLASGVPSRFSG SRSGTEFTLTISDLECDDAATYYCQTNYGTSS SNYGFAFGGGTEVVVKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC
9	33011-zu	Heavy chain	331	EVQLVESGGGLVQPGGSLRLSCAASGISLSSD AISWVRQAPGKGLEYIGIINGGGNTYYASWAK GRFTISRHNSKNTLYLQMNSLRAEDTAVYYCA RGIQHGGGNSDYYYYGMDLWGQGTLVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK
10	33011-zu	Light chain	332	DIQMTQSPSSLSASVGDRVTITCQASQSISSV LSWYQQKPGKAPKLLIYLASTLASGVPSRFSG SGSGTDFTLTISSLQCEDIATYYCQTNYGTSS SNYGFAFGGGTKVEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC
11	111B10-xi	Heavy chain	333	QSVEESGGRLVTPGTPLTLTCTVSGFSLNNYA MSWVRQAPGKGLEWIGSISTGGLAFYANWAKG RFTISRTSTTVDLKMTSLTTEDTATYFCGRNG GGSYIFYYFDLWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS

				SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
12	111B10-xi	Light chain	334	AFELTQTPSSVEAAVGGTITIKCQASQSISSY LSWYQQKPGQPPKLLIYSASTLASGVSSRFKG SGSGTEYTLTISDLECADAATYFCQSYYDIGT STFGGGTEVVVKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC
13	111B10-zu	Heavy chain	335	EVQLVESGGGLVQPGGSLRLSCAASGFSLNNY AMSWVRQAPGKGLEWIGSISTGGLAFYANWAK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA RNGGGSYIFYYFDLWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK
14	111B10-zu	Light chain	336	DIQMTQSPSSLSASVGDRVTITCQASQSISSY LSWYQQKPGKAPKLLIYSASTLASGVPSRFSG SGSGTDFTLTISSLQCEDAATYYCQSYYDIGT STFGGGTKVEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC
15	201C15-xi	Heavy chain	337	QSVKESGGRLVTPGTPLTLTCTVSGIDLSSYA MGWFRQAPGKGLEYIGTINIGGRVYYASWAKG RFTISRTSTTVDLKAPSLTAEDTATYFCARYY

				NGGSYDIWGPGTLVTVSLASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK
16	201C15-xi	Light chain	338	DVVMTQTPASASEPVGGTVTIKCQASESIYRV LAWYQQKPGQPPKLLIYDTSTLASGAPSRFKG SGYGTEFTLTISGVQCEDAATYYCQGGYYADS YGIAFGGGTEVVVKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC
17	201C15-zu	Heavy chain	339	QVQLVESGGGLVQPGGSLRLSCSASGIDLSSY AMGWVRQAPGKGLEYIGTINIGGRVYYASWAK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA RYYNGGSYDIWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
18	201C15-zu	Light chain	340	DIQMTQSPSTLSASVGDRVTITCQASESIYRV LAWYQQKPGKAPKLLIYDTSTLASGVPSRFSG SGSGTEFTLTISSLQCDDAATYYCQGGYYADS YGIAFGGGTKVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC
19	346C6-xi	Heavy chain	341	QSVEESGGRLVKPDESLTLTCTASGFSLSSYA

				MIWVRQAPGEGLEWIGTISTGGITYYASWAKG RFTISKTSTTVDLKITSPTTEDTATYFCARGG YAASSAYYLPYYFDLWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK
20	346C6-xi	Light chain	342	AAVLTQTPSPVSAAVGGTVTISCQSSQSVYNN NNLAWFQQKPGQPPKLLIYLASTLASGVPSRF SGSGSGTQFTLTISGVQCDDAATYYCLGGCDD DADTFAFGGGTEVVVKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC
21	346C6-zu	Heavy chain	343	EVQLVESGGGLVQPGGSLRLSCAASGFSLSSY AMIWVRQAPGKGLEWIGTISTGGITYYASWAK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA RGGYAASSAYYLPYYFDLWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK
22	346C6-zu	Light chain	344	DIQMTQSPSSLSASVGDRVTITCQSSQSVYNN NNLAWYQQKPGKVPKLLIYLASTLASGVPSRF SGSGSGTDFTLTISSLQCEDAATYYCLGGCDD DADTFAFGGGTKVEIKRTVAAPSVFIFPPSDE

				QLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC

Table 9. Nucleic acid sequences encoding full-length mAb Ig chains[+]

	mAb	IgG chain	SEQ ID NO	Nucleic acid sequence
1	MORAb-003	Heavy chain	345	GAGGTCCAACTGGTGGAGAGCGGTGGAGGTGTT GTGCAACCTGGCCGGTCCCTGCGCCTGTCCTGC TCCGCATCTGGCTTCACCTTCAGCGGCTATGGG TTGTCTTGGGTGAGACAGGCACCTGGAAAAGGT CTTGAGTGGGTTGCAATGATTAGTAGTGGTGGT AGTTATACCTACTATGCAGACAGTGTGAAGGGT AGATTTGCAATATCGCGAGACAACGCCAAGAAC ACATTGTTCCTGCAAATGGACAGCCTGAGACCC GAAGACACCGGGGTCTATTTTTGTGCAAGACAT GGGGACGATCCCGCCTGGTTCGCTTATTGGGGC CAAGGGACCCCGGTCACCGTCTCCTCAGCCTCC ACCAAGGGCCCATCGGTCTTCCCCCTGGCACCC TCCTCCAAGAGCACCTCTGGGGGCACAGCGGCC CTGGGCTGCCTGGTCAAGGACTACTTCCCCGAA CCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTA CAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG ACCTACATCTGCAACGTGAATCACAAGCCCAGC AACACCAAGGTGGACAAGAAAGTTGAGCCCAAA TCTTGTGACAAAACTCACACATGCCCACCGTGC CCAGCACCTGAACTCCTGGGGGGACCGTCAGTC TTCCTCTTCCCCCCAAAACCCAAGGACACCCTC ATGATCTCCCGGACCCCTGAGGTCACATGCGTG GTGGTGGACGTGAGCCACGAAGACCCTGAGGTC AAGTTCAACTGGTACGTGGACGGCGTGGAGGTG CATAATGCCAAGACAAAGCCGCGGGAGGAGCAG TACAACAGCACGTACCGTGTGGTCAGCGTCCTC ACCGTCCTGCACCAGGACTGGCTGAATGGCAAG GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTC CCAGCCCCCATCGAGAAAACCATCTCCAAAGCC AAAGGGCAGCCCCGAGAACCACAGGTGTACACC CTGCCCCCATCCCGGGATGAGCTGACCAAGAAC CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTC TATCCCAGCGACATCGCCGTGGAGTGGGAGAGC AATGGGCAGCCGGAGAACAACTACAAGACCACG CCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC TTATATTCAAAGCTCACCGTGGACAAGAGCAGG TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTG ATGCATGAGGCTCTGCACAACCACTACACGCAG

				AAGAGCCTCTCCCTGTCTCCCGGGAAATGA
2	MORAb-003	Light chain	346	GACATCCAGCTGACCCAGAGCCCAAGCAGCCTG AGCGCCAGCGTGGGTGACAGAGTGACCATCACC TGTAGTGTCAGCTCAAGTATAAGTTCCAACAAC TTGCACTGGTACCAGCAGAAGCCAGGTAAGGCT CCAAAGCCATGGATCTACGGCACATCCAACCTG GCTTCTGGTGTGCCAAGCAGATTCAGCGGTAGC GGTAGCGGTACCGACTACACCTTCACCATCAGC AGCCTCCAGCCAGAGGACATCGCCACCTACTAC TGCCAACAGTGGAGTAGTTACCCGTACATGTAC ACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA CGAACTGTGGCTGCACCATCTGTCTTCATCTTC CCGCCATCTGATGAGCAGTTGAAATCTGGAACT GCCTCTGTTGTGTGCCTGCTGAATAACTTCTAT CCCAGAGAGGCCAAAGTACAGTGGAAGGTGGAT AACGCCCTCCAATCGGGTAACTCCCAGGAGAGT GTCACAGAGCAGGACAGCAAGGACAGCACCTAC AGCCTCAGCAGCACCCTGACGCTGAGCAAAGCA GACTACGAGAAACACAAAGTCTACGCCTGCGAA GTCACCCATCAGGGCCTGAGCTCGCCCGTCACA AAGAGCTTCAACAGGGGAGAGTGTTAA
3	MORAb-009	Heavy chain	347	CAGGTACAACTGCAGCAGTCTGGGCCTGAGCTG GAGAAGCCTGGCGCTTCAGTGAAGATATCCTGC AAGGCTTCTGGTTACTCATTCACTGGCTACACC ATGAACTGGGTGAAGCAGAGCCATGGAAAGAGC CTTGAGTGGATTGGACTTATTACTCCTTACAAT GGTGCTTCTAGCTACAACCAGAAGTTCAGGGGC AAGGCCACATTAACTGTAGACAAGTCATCCAGC ACAGCCTACATGGACCTCCTCAGTCTGACATCT GAAGACTCTGCAGTCTATTTCTGTGCAAGGGGG GGTTACGACGGGAGGGGTTTTGACTACTGGGGA TCCGGGACCCCGGTCACCGTCTCCTCAGCCTCC ACCAAGGGCCCATCGGTCTTCCCCCTGGCACCC TCCTCCAAGAGCACCTCTGGGGGCACAGCGGCC CTGGGCTGCCTGGTCAAGGACTACTTCCCCGAA CCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTA CAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG ACCTACATCTGCAACGTGAATCACAAGCCCAGC AACACCAAGGTGGACAAGAAAGTTGAGCCCAAA TCTTGTGACAAAACTCACACATGCCCACCGTGC CCAGCACCTGAACTCCTGGGGGGACCGTCAGTC TTCCTCTTCCCCCCAAAACCCAAGGACACCCTC ATGATCTCCCGGACCCCTGAGGTCACATGCGTG

				GTGGTGGACGTGAGCCACGAAGACCCTGAGGTC AAGTTCAACTGGTACGTGGACGGCGTGGAGGTG CATAATGCCAAGACAAAGCCGCGGGAGGAGCAG TACAACAGCACGTACCGTGTGGTCAGCGTCCTC ACCGTCCTGCACCAGGACTGGCTGAATGGCAAG GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTC CCAGCCCCCATCGAGAAAACCATCTCCAAAGCC AAAGGGCAGCCCCGAGAACCACAGGTGTACACC CTGCCCCCATCCCGGGATGAGCTGACCAAGAAC CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTC TATCCCAGCGACATCGCCGTGGAGTGGGAGAGC AATGGGCAGCCGGAGAACAACTACAAGACCACG CCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC CTCTACAGCAAGCTCACCGTGGACAAGAGCAGG TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTG ATGCATGAGGCTCTGCACAACCACTACACGCAG AAGAGCCTCTCCCTGTCTCCCGGGAAATGA
4	MORAb-009	Light chain	348	GACATCGAGCTCACTCAGTCTCCAGCAATCATG TCTGCATCTCCAGGGGAGAAGGTCACCATGACC TGCAGTGCCAGCTCAAGTGTAAGTTACATGCAC TGGTACCAGCAGAAGTCAGGCACCTCCCCCAAA AGATGGATTTATGACACATCCAAACTGGCTTCT GGAGTCCCAGGTCGCTTCAGTGGCAGTGGGTCT GGAAACTCTTACTCTCTCACAATCAGCAGCGTG GAGGCTGAAGATGATGCAACTTATTACTGCCAG CAGTGGAGTAAGCACCCTCTCACGTTCGGATCC GGGACCAAGGTGGAAATCAAACGAACTGTGGCT GCACCATCTGTCTTCATCTTCCCGCCATCTGAT GAGCAGTTGAAATCTGGAACTGCCTCTGTTGTG TGCCTGCTGAATAACTTCTATCCCAGAGAGGCC AAAGTACAGTGGAAGGTGGATAACGCCCTCCAA TCGGGTAACTCCCAGGAGAGTGTCACAGAGCAG GACAGCAAGGACAGCACCTACAGCCTCAGCAGC ACCCTGACGCTGAGCAAAGCAGACTACGAGAAA CACAAAGTCTACGCCTGCGAAGTCACCCATCAG GGCCTGAGCTCGCCCGTCACAAAGAGCTTCAAC AGGGGAGAGTGTTAA
5	33011-xi	Heavy chain	349	CAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTC ACGCCTGGGACACCCCTGACACTCACCTGCACC GTCTCTGGAATCTCCCTCAGTAGCGATGCAATA AGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTC GAATACATCGGAATCATTAATGGTGGTGGTAAC

				ACATACTACGCGAGCTGGGCGAAAGGCCGATTC ACCATCTCCAAAACCTCGACCACGGTGGATCTG AAAATCACCAGTCCGACAACCGAGGACACGGCC ACCTATTTCTGTGCCAGAGGCATTCAACATGGT GGTGGTAATAGTGATTATTATTATTACGGCATG GACCTCTGGGGCCCAGGCACCCTGGTCACTGTC TCTTCAGCATCCACCAAGGGCCCATCGGTCTTC CCCCTGGCACCCTCCTCCAAGAGCACCTCTGGG GGCACAGCGGCCCTGGGCTGCCTGGTCAAGGAC TACTTCCCCGAACCGGTGACGGTGTCGTGGAAC TCAGGCGCCCTGACCAGCGGCGTGCACACCTTC CCGGCTGTCCTACAGTCCTCAGGACTCTACTCC CTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGC TTGGGCACCCAGACCTACATCTGCAACGTGAAT CACAAGCCCAGCAACACCAAGGTGGACAAGAAA GTTGAGCCCAAATCTTGTGACAAAACTCACACA TGCCCACCGTGCCCAGCACCTGAACTCCTGGGG GGACCGTCAGTCTTCCTCTTCCCCCCAAAACCC AAGGACACCCTCATGATCTCCCGGACCCCTGAG GTCACATGCGTGGTGGTGGACGTGAGCCACGAA GACCCTGAGGTCAAGTTCAACTGGTACGTGGAC GGCGTGGAGGTGCATAATGCCAAGACAAAGCCG CGGGAGGAGCAGTACAACAGCACGTACCGTGTG GTCAGCGTCCTCACCGTCCTGCACCAGGACTGG CTGAATGGCAAGGAGTACAAGTGCAAGGTCTCC AACAAAGCCCTCCCAGCCCCCATCGAGAAAACC ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCA CAGGTGTACACCCTGCCCCCATCCCGGGATGAG CTGACCAAGAACCAGGTCAGCCTGACCTGCCTG GTCAAAGGCTTCTATCCCAGCGACATCGCCGTG GAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC TACAAGACCACGCCTCCCGTGCTGGACTCCGAC GGCTCCTTCTTCTTATATTCAAAGCTCACCGTG GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTC TCATGCTCCGTGATGCATGAGGCTCTGCACAAC CACTACACGCAGAAGAGCCTCTCCCTGTCTCCC GGGAAATGA
6	33011-xi	Light chain	350	GAAGTGTTGATGACCCAGACTCCATCCTCCGTG TCTGCAGCTGTGGGAGACACAGTCACCATCAAG TGCCAGGCCAGTCAGAGCATTAGTAGTGTCTTG

				TCCTGGTATCAGCAGAAACCAGGGCAGCCTCCC AAGCTCCTGATCTATCTGGCATCCACTCTGGCA TCTGGGGTCCCATCGCGGTTCAGCGGCAGTAGA TCTGGGACAGAGTTCACTCTCACCATCAGCGAC CTGGAGTGTGACGATGCTGCCACTTACTACTGT CAAACCAATTATGGTACTAGTAGTAGTAATTAT GGTTTTGCTTTCGGCGGAGGGACCGAGGTGGTC GTCAAACGAACTGTGGCTGCACCATCTGTCTTC ATCTTCCCGCCATCTGATGAGCAGTTGAAATCT GGAACTGCCTCTGTTGTGTGCCTGCTGAATAAC TTCTATCCCAGAGAGGCCAAAGTACAGTGGAAG GTGGATAACGCCCTCCAATCGGGTAACTCCCAG GAGAGTGTCACAGAGCAGGACAGCAAGGACAGC ACCTACAGCCTCAGCAGCACCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTCTACGCC TGCGAAGTCACCCATCAGGGCCTGAGCTCGCCC GTCACAAAGAGCTTCAACAGGGGAGAGTGTTGA
7	33011-zu	Heavy chain	351	GAAGTCCAACTGGTGGAAAGCGGGGGAGGACTG GTGCAGCCGGGCGGATCCCTCCGGCTGTCATGT GCTGCATCGGGAATTTCCCTCTCCTCCGACGCG ATTAGCTGGGTCAGACAGGCCCCCGGAAAGGGG CTGGAGTACATCGGTATCATCAACGGCGGCGGA AACACCTACTACGCCTCCTGGGCCAAGGGCCGC TTCACCATCTCGCGGCATAATTCCAAGAACACT CTGTACTTGCAAATGAACTCCCTGAGGGCCGAG GACACCGCCGTGTACTACTGCGCGCGCGGCATC CAGCACGGTGGTGGAAACAGCGACTACTACTAC TATGGGATGGATCTGTGGGGCCAGGGAACTCTT GTGACCGTGTCGTCAGCATCCACCAAGGGCCCA TCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGC ACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTG GTCAAGGACTACTTCCCCGAACCGGTGACGGTG TCGTGGAACTCAGGCGCCCTGACCAGCGGCGTG CACACCTTCCCGGCTGTCCTACAGTCCTCAGGA CTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC TCCAGCAGCTTGGGCACCCAGACCTACATCTGC AACGTGAATCACAAGCCCAGCAACACCAAGGTG GACAAGAAAGTTGAGCCCAAATCTTGTGACAAA ACTCACACATGCCCACCGTGCCCAGCACCTGAA CTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCC

				CCAAAACCCAAGGACACCCTCATGATCTCCCGG ACCCCTGAGGTCACATGCGTGGTGGTGGACGTG AGCCACGAAGACCCTGAGGTCAAGTTCAACTGG TACGTGGACGGCGTGGAGGTGCATAATGCCAAG ACAAAGCCGCGGGAGGAGCAGTACAACAGCACG TACCGTGTGGTCAGCGTCCTCACCGTCCTGCAC CAGGACTGGCTGAATGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATC GAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC CGAGAACCACAGGTGTACACCCTGCCCCCATCC CGGGATGAGCTGACCAAGAACCAGGTCAGCCTG ACCTGCCTGGTCAAAGGCTTCTATCCCAGCGAC ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCG GAGAACAACTACAAGACCACGCCTCCCGTGCTG GACTCCGACGGCTCCTTCTTCTTATATTCAAAG CTCACCGTGGACAAGAGCAGGTGGCAGCAGGGG AACGTCTTCTCATGCTCCGTGATGCATGAGGCT CTGCACAACCACTACACGCAGAAGAGCCTCTCC CTGTCTCCCGGGAAATGA
8	33011-zu	Light chain	352	GACATTCAGATGACCCAGTCCCCAAGCTCGCTG TCCGCCTCCGTGGGCGACCGCGTGACCATCACG TGCCAGGCGTCCCAGTCAATTAGCAGCGTGCTC TCCTGGTACCAACAGAAGCCGGGGAAAGCACCC AAGCTGCTGATCTACTTGGCCTCCACTCTGGCC TCGGGAGTGCCTTCACGGTTCTCCGGATCGGGA TCTGGTACTGATTTCACCCTCACCATCTCGAGC CTTCAGTGCGAGGACATCGCTACTTACTATTGT CAAACCAACTACGGAACCTCCAGCTCCAACTAC GGCTTTGCCTTCGGTGGCGGGACCAAGGTCGAA ATCAAACGAACTGTGGCTGCACCATCTGTCTTC ATCTTCCCGCCATCTGATGAGCAGTTGAAATCT GGAACTGCCTCTGTTGTGTGCCTGCTGAATAAC TTCTATCCCAGAGAGGCCAAAGTACAGTGGAAG GTGGATAACGCCCTCCAATCGGGTAACTCCCAG GAGAGTGTCACAGAGCAGGACAGCAAGGACAGC ACCTACAGCCTCAGCAGCACCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTCTACGCC TGCGAAGTCACCCATCAGGGCCTGAGCTCGCCC GTCACAAAGAGCTTCAACAGGGGAGAGTGTTGA
9	111B10-xi	Heavy chain	353	CAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTC

				ACGCCTGGGACACCCCTGACACTCACCTGCACA
				GTCTCTGGATTCTCCCTCAATAACTATGCAATG
				AGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTG
				GAATGGATCGGATCCATTAGTACTGGTGGTCTC
				GCATTCTACGCGAACTGGGCAAAAGGCCGATTC
				ACCATCTCCAGAACCTCGACCACGGTGGATCTG
				AAAATGACCAGTCTGACAACCGAGGACACGGCC
				ACCTATTTCTGTGGCAGAAATGGTGGTGGTAGT
				TATATTTTCTATTATTTTGACTTGTGGGGCCAA
				GGCACCCTCGTCACTGTCTCTTCAGCATCCACC
				AAGGGCCCATCGGTCTTCCCCCTGGCACCCTCC
				TCCAAGAGCACCTCTGGGGGCACAGCGGCCCTG
				GGCTGCCTGGTCAAGGACTACTTCCCCGAACCG
				GTGACGGTGTCGTGGAACTCAGGCGCCCTGACC
				AGCGGCGTGCACACCTTCCCGGCTGTCCTACAG
				TCCTCAGGACTCTACTCCCTCAGCAGCGTGGTG
				ACCGTGCCCTCCAGCAGCTTGGGCACCCAGACC
				TACATCTGCAACGTGAATCACAAGCCCAGCAAC
				ACCAAGGTGGACAAGAAAGTTGAGCCCAAATCT
				TGTGACAAAACTCACACATGCCCACCGTGCCCA
				GCACCTGAACTCCTGGGGGGACCGTCAGTCTTC
				CTCTTCCCCCCAAAACCCAAGGACACCCTCATG
				ATCTCCCGGACCCCTGAGGTCACATGCGTGGTG
				GTGGACGTGAGCCACGAAGACCCTGAGGTCAAG
				TTCAACTGGTACGTGGACGGCGTGGAGGTGCAT
				AATGCCAAGACAAAGCCGCGGGAGGAGCAGTAC
				AACAGCACGTACCGTGTGGTCAGCGTCCTCACC
				GTCCTGCACCAGGACTGGCTGAATGGCAAGGAG
				TACAAGTGCAAGGTCTCCAACAAAGCCCTCCCA
				GCCCCCATCGAGAAAACCATCTCCAAAGCCAAA
				GGGCAGCCCCGAGAACCACAGGTGTACACCCTG
				CCCCCATCCCGGGATGAGCTGACCAAGAACCAG
				GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTAT
				CCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT
				GGGCAGCCGGAGAACAACTACAAGACCACGCCT
				CCCGTGCTGGACTCCGACGGCTCCTTCTTCTTA
				TATTCAAAGCTCACCGTGGACAAGAGCAGGTGG
				CAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
				CATGAGGCTCTGCACAACCACTACACGCAGAAG
				AGCCTCTCCCTGTCTCCCGGGAAATGA

10	111B10-xi	Light chain	354	GCATTCGAATTGACCCAGACTCCATCCTCCGTG GAGGCAGCTGTGGGAGGCACAATCACCATCAAG TGCCAGGCCAGTCAGAGCATTAGTAGTTACTTA TCCTGGTATCAGCAGAAACCAGGGCAGCCTCCC AAGCTCCTGATCTATTCTGCATCCACTCTGGCA TCTGGGGTCTCATCGCGGTTCAAAGGCAGTGGA TCTGGGACAGAGTACACTCTCACCATCAGCGAC CTGGAGTGTGCCGATGCTGCCACTTACTTCTGT CAAAGCTATTATGATATTGGTACTAGTACTTTC GGCGGAGGGACCGAGGTGGTCGTCAAACGAACT GTGGCTGCACCATCTGTCTTCATCTTCCCGCCA TCTGATGAGCAGTTGAAATCTGGAACTGCCTCT GTTGTGTGCCTGCTGAATAACTTCTATCCCAGA GAGGCCAAAGTACAGTGGAAGGTGGATAACGCC CTCCAATCGGGTAACTCCCAGGAGAGTGTCACA GAGCAGGACAGCAAGGACAGCACCTACAGCCTC AGCAGCACCCTGACGCTGAGCAAAGCAGACTAC GAGAAACACAAAGTCTACGCCTGCGAAGTCACC CATCAGGGCCTGAGCTCGCCCGTCACAAAGAGC TTCAACAGGGGAGAGTGTTGA
11	111B10-zu	Heavy chain	355	GAAGTGCAGCTGGTGGAATCTGGCGGCGGACTG GTGCAGCCTGGCGGATCTCTGAGACTGTCTTGT GCCGCCTCCGGCTTCTCCCTGAACAACTACGCC ATGTCCTGGGTGCGACAGGCCCCTGGCAAAGGC CTGGAATGGATCGGCTCCATCAGCACAGGCGGC CTGGCCTTCTACGCCAATTGGGCCAAGGGCCGG TTCACCATCAGCCGGGACAACTCCAAGAACACC CTGTACCTCCAGATGAACTCCCTGCGGGCCGAG GACACCGCCGTGTACTACTGTGCCAGAAACGGC GGAGGCTCCTACATCTTCTACTACTTCGACCTG TGGGGCCAGGGCACCCTCGTGACAGTGTCATCT GCATCCACCAAGGGCCCATCGGTCTTCCCCCTG GCACCCTCCTCCAAGAGCACCTCTGGGGGCACA GCGGCCCTGGGCTGCCTGGTCAAGGACTACTTC CCCGAACCGGTGACGGTGTCGTGGAACTCAGGC GCCCTGACCAGCGGCGTGCACACCTTCCCGGCT GTCCTACAGTCCTCAGGACTCTACTCCCTCAGC AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGC ACCCAGACCTACATCTGCAACGTGAATCACAAG CCCAGCAACACCAAGGTGGACAAGAAAGTTGAG

				CCCAAATCTTGTGACAAAACTCACACATGCCCA CCGTGCCCAGCACCTGAACTCCTGGGGGGACCG TCAGTCTTCCTCTTCCCCCCAAAACCCAAGGAC ACCCTCATGATCTCCCGGACCCCTGAGGTCACA TGCGTGGTGGTGGACGTGAGCCACGAAGACCCT GAGGTCAAGTTCAACTGGTACGTGGACGGCGTG GAGGTGCATAATGCCAAGACAAAGCCGCGGGAG GAGCAGTACAACAGCACGTACCGTGTGGTCAGC GTCCTCACCGTCCTGCACCAGGACTGGCTGAAT GGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA GCCCTCCCAGCCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTG TACACCCTGCCCCCATCCCGGGATGAGCTGACC AAGAACCAGGTCAGCCTGACCTGCCTGGTCAAA GGCTTCTATCCCAGCGACATCGCCGTGGAGTGG GAGAGCAATGGGCAGCCGGAGAACAACTACAAG ACCACGCCTCCCGTGCTGGACTCCGACGGCTCC TTCTTCTTATATTCAAAGCTCACCGTGGACAAG AGCAGGTGGCAGCAGGGGAACGTCTTCTCATGC TCCGTGATGCATGAGGCTCTGCACAACCACTAC ACGCAGAAGAGCCTCTCCCTGTCTCCCGGGAAA TGA
12	111B10-zu	Light chain	356	GATATTCAGATGACCCAGTCCCCCTCCAGCCTG TCCGCTTCTGTGGGCGACAGAGTGACCATCACC TGTCAGGCCTCCCAGTCCATCTCCTCCTACCTG TCCTGGTATCAGCAGAAGCCCGGCAAGGCCCCC AAGCTGCTGATCTACTCTGCCTCCACACTGGCC TCCGGCGTGCCCTCTAGATTCTCCGGCTCTGGC TCTGGCACCGACTTTACCCTGACCATCAGCTCC CTCCAGTGCGAGGATGCCGCCACCTACTACTGC CAGTCCTACTACGACATCGGCACCTCCACCTTC GGCGGAGGCACCAAGGTGGAAATCAAACGAACT GTGGCTGCACCATCTGTCTTCATCTTCCCGCCA TCTGATGAGCAGTTGAAATCTGGAACTGCCTCT GTTGTGTGCCTGCTGAATAACTTCTATCCCAGA GAGGCCAAAGTACAGTGGAAGGTGGATAACGCC CTCCAATCGGGTAACTCCCAGGAGAGTGTCACA GAGCAGGACAGCAAGGACAGCACCTACAGCCTC AGCAGCACCCTGACGCTGAGCAAAGCAGACTAC GAGAAACACAAAGTCTACGCCTGCGAAGTCACC

				CATCAGGGCCTGAGCTCGCCCGTCACAAAGAGC TTCAACAGGGGAGAGTGTTGA
13	201C15-xi	Heavy chain	357	CAGTCAGTGAAGGAGTCCGGGGGTCGCCTGGTC ACGCCTGGGACACCCCTGACACTCACCTGCACA GTCTCTGGAATCGACCTCAGTAGCTATGCAATG GGCTGGTTCCGCCAGGCTCCAGGGAAGGGGCTG GAATACATCGGAACCATTAATATTGGTGGTCGC GTATATTACGCGAGCTGGGCAAAAGGCCGATTC ACCATCTCCAGAACCTCGACCACGGTGGATCTG AAAGCGCCCAGTCTGACAGCCGAGGACACGGCC ACCTATTTCTGTGCCAGATATTATAATGGTGGT AGTTATGACATCTGGGGCCCAGGCACCCTGGTC ACCGTCTCTTTAGCATCCACCAAGGGCCCATCG GTCTTCCCCCTGGCACCCTCCTCCAAGAGCACC TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTC AAGGACTACTTCCCCGAACCGGTGACGGTGTCG TGGAACTCAGGCGCCCTGACCAGCGGCGTGCAC ACCTTCCCGGCTGTCCTACAGTCCTCAGGACTC TACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAAC GTGAATCACAAGCCCAGCAACACCAAGGTGGAC AAGAAAGTTGAGCCCAAATCTTGTGACAAAACT CACACATGCCCACCGTGCCCAGCACCTGAACTC CTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCA AAACCCAAGGACACCCTCATGATCTCCCGGACC CCTGAGGTCACATGCGTGGTGGTGGACGTGAGC CACGAAGACCCTGAGGTCAAGTTCAACTGGTAC GTGGACGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTACAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAATGGCAAGGAGTACAAGTGCAAG GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAACCACAGGTGTACACCCTGCCCCCATCCCGG GATGAGCTGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTATCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCTTATATTCAAAGCTC ACCGTGGACAAGAGCAGGTGGCAGCAGGGGAAC

				GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACGCAGAAGAGCCTCTCCCTG TCTCCCGGGAAATGA
14	201C15-xi	Light chain	358	GATGTTGTGATGACCCAGACTCCAGCCTCCGCG TCTGAACCTGTGGGAGGCACAGTCACCATCAAG TGCCAGGCCAGTGAGAGCATTTATCGCGTATTG GCCTGGTATCAGCAGAAACCAGGGCAGCCTCCC AAGCTCCTGATCTATGATACATCCACTCTGGCA TCTGGGGCCCCATCGCGGTTCAAAGGCAGTGGA TATGGGACAGAGTTCACTCTCACCATCAGCGGC GTGCAGTGTGAAGATGCTGCCACTTACTACTGT CAAGGCGGTTATTATGCTGATAGTTATGGTATT GCTTTCGGCGGAGGGACCGAGGTGGTGGTCAAA CGAACTGTGGCTGCACCATCTGTCTTCATCTTC CCGCCATCTGATGAGCAGTTGAAATCTGGAACT GCCTCTGTTGTGTGCCTGCTGAATAACTTCTAT CCCAGAGAGGCCAAAGTACAGTGGAAGGTGGAT AACGCCCTCCAATCGGGTAACTCCCAGGAGAGT GTCACAGAGCAGGACAGCAAGGACAGCACCTAC AGCCTCAGCAGCACCCTGACGCTGAGCAAAGCA GACTACGAGAAACACAAAGTCTACGCCTGCGAA GTCACCCATCAGGGCCTGAGCTCGCCCGTCACA AAGAGCTTCAACAGGGGAGAGTGTTGA
15	201C15-zu	Heavy chain	359	CAGGTGCAGCTGGTGGAATCTGGCGGAGGACTG GTGCAGCCTGGCGGCTCTCTGAGACTGTCCTGT TCCGCCTCCGGAATCGACCTGTCCTCCTACGCT ATGGGCTGGGTGCGACAGGCTCCTGGCAAGGGC CTGGAGTACATCGGCACCATCAACATCGGCGGC AGAGTGTACTACGCCTCCTGGGCCAAGGGCCGG TTCACCATCTCCAGAGACAACTCCAAGAACACC CTGTACCTCCAGATGAACTCCCTGCGGGCCGAG GACACCGCCGTGTACTACTGCGCCCGGTACTAC AACGGCGGCTCCTACGATATCTGGGGCCAGGGC ACACTCGTGACCGTGTCCTCTGCATCCACCAAG GGCCCATCGGTCTTCCCCCTGGCACCCTCCTCC AAGAGCACCTCTGGGGGCACAGCGGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC

				GTGCCCTCCAGCAGCTTGGGCACCCAGACCTAC ATCTGCAACGTGAATCACAAGCCCAGCAACACC AAGGTGGACAAGAAAGTTGAGCCCAAATCTTGT GACAAAACTCACACATGCCCACCGTGCCCAGCA CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC TTCCCCCCAAAACCCAAGGACACCCTCATGATC TCCCGGACCCCTGAGGTCACATGCGTGGTGGTG GACGTGAGCCACGAAGACCCTGAGGTCAAGTTC AACTGGTACGTGGACGGCGTGGAGGTGCATAAT GCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC AGCACGTACCGTGTGGTCAGCGTCCTCACCGTC CTGCACCAGGACTGGCTGAATGGCAAGGAGTAC AAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCC CCCATCGAGAAAACCATCTCCAAAGCCAAAGGG CAGCCCCGAGAACCACAGGTGTACACCCTGCCC CCATCCCGGGATGAGCTGACCAAGAACCAGGTC AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCC AGCGACATCGCCGTGGAGTGGGAGAGCAATGGG CAGCCGGAGAACAACTACAAGACCACGCCTCCC GTGCTGGACTCCGACGGCTCCTTCTTCTTATAT TCAAAGCTCACCGTGGACAAGAGCAGGTGGCAG CAGGGGAACGTCTTCTCATGCTCCGTGATGCAT GAGGCTCTGCACAACCACTACACGCAGAAGAGC CTCTCCCTGTCTCCCGGGAAATGA
16	201C15-zu	Light chain	360	GATATCCAGATGACCCAGTCCCCCTCCACCCTG TCTGCCTCTGTGGGCGACAGAGTGACCATCACC TGTCAGGCCTCCGAGTCCATCTACCGGGTGCTG GCCTGGTATCAGCAGAAGCCTGGCAAGGCCCCC AAGCTGCTGATCTACGACACCAGCACACTGGCC TCCGGCGTGCCCTCTAGATTCTCCGGCTCTGGC TCTGGCACCGAGTTTACCCTGACCATCTCCAGC CTCCAGTGCGACGACGCCGCCACCTACTATTGT CAGGGCGGCTACTACGCCGACTCCTACGGAATC GCTTTCGGCGGAGGCACCAAGGTGGAAATCAAA CGAACTGTGGCTGCACCATCTGTCTTCATCTTC CCGCCATCTGATGAGCAGTTGAAATCTGGAACT GCCTCTGTTGTGTGCCTGCTGAATAACTTCTAT CCCAGAGAGGCCAAAGTACAGTGGAAGGTGGAT AACGCCCTCCAATCGGGTAACTCCCAGGAGAGT GTCACAGAGCAGGACAGCAAGGACAGCACCTAC

				AGCCTCAGCAGCACCCTGACGCTGAGCAAAGCA GACTACGAGAAACACAAAGTCTACGCCTGCGAA GTCACCCATCAGGGCCTGAGCTCGCCCGTCACA AAGAGCTTCAACAGGGGAGAGTGTTGA
17	346C6-xi	Heavy chain	361	CAGTCGGTGGAGGAGTCCGGCGGTCGCCTGGTA AAGCCTGACGAATCCCTGACACTCACCTGCACA GCCTCTGGATTCTCCCTCAGTAGTTATGCAATG ATCTGGGTCCGCCAGGCTCCAGGGGAGGGGCTG GAATGGATCGGAACCATTAGTACTGGTGGTATC ACATACTACGCGAGCTGGGCGAAAGGCCGATTC ACCATCTCCAAAACCTCGACCACGGTGGATCTG AAAATCACCAGTCCGACAACCGAGGACACGGCC ACCTATTTCTGTGCCAGAGGGGGATATGCTGCT AGTAGTGCTTATTATCTCCCGTACTACTTTGAC TTGTGGGGCCAAGGGACCCTGGTCACCGTCTCC TCAGCATCCACCAAGGGCCCATCGGTCTTCCCC CTGGCACCCTCCTCCAAGAGCACCTCTGGGGGC ACAGCGGCCCTGGGCTGCCTGGTCAAGGACTAC TTCCCCGAACCGGTGACGGTGTCGTGGAACTCA GGCGCCCTGACCAGCGGCGTGCACACCTTCCCG GCTGTCCTACAGTCCTCAGGACTCTACTCCCTC AGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTG GGCACCCAGACCTACATCTGCAACGTGAATCAC AAGCCCAGCAACACCAAGGTGGACAAGAAAGTT GAGCCCAAATCTTGTGACAAAACTCACACATGC CCACCGTGCCCAGCACCTGAACTCCTGGGGGGA CCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAG GACACCCTCATGATCTCCCGGACCCCTGAGGTC ACATGCGTGGTGGTGGACGTGAGCCACGAAGAC CCTGAGGTCAAGTTCAACTGGTACGTGGACGGC GTGGAGGTGCATAATGCCAAGACAAAGCCGCGG GAGGAGCAGTACAACAGCACGTACCGTGTGGTC AGCGTCCTCACCGTCCTGCACCAGGACTGGCTG AATGGCAAGGAGTACAAGTGCAAGGTCTCCAAC AAAGCCCTCCCAGCCCCCATCGAGAAAACCATC TCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG GTGTACACCCTGCCCCCATCCCGGGATGAGCTG ACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC AAAGGCTTCTATCCCAGCGACATCGCCGTGGAG TGGGAGAGCAATGGGCAGCCGGAGAACAACTAC

				AAGACCACGCCTCCCGTGCTGGACTCCGACGGC TCCTTCTTCTTATATTCAAAGCTCACCGTGGAC AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCA TGCTCCGTGATGCATGAGGCTCTGCACAACCAC TACACGCAGAAGAGCCTCTCCCTGTCTCCCGGG AAATGA
18	346C6-xi	Light chain	362	GCAGCCGTGCTGACCCAGACACCATCACCCGTG TCTGCAGCTGTGGGAGGCACAGTCACCATCAGT TGCCAGTCCAGTCAGAGTGTTTATAATAATAAC AACTTAGCCTGGTTTCAGCAGAAACCCGGGCAG CCTCCCAAGCTTCTGATCTATCTGGCATCCACT CTGGCATCTGGGGTCCCATCACGGTTCAGCGGC AGTGGATCTGGGACACAGTTCACTCTCACCATC AGCGGCGTGCAGTGTGACGATGCTGCCACTTAT TACTGTCTAGGTGGTTGTGATGATGATGCTGAT ACTTTTGCTTTCGGCGGAGGGACTGAGGTGGTG GTCAAACGAACTGTGGCTGCACCATCTGTCTTC ATCTTCCCGCCATCTGATGAGCAGTTGAAATCT GGAACTGCCTCTGTTGTGTGCCTGCTGAATAAC TTCTATCCCAGAGAGGCCAAAGTACAGTGGAAG GTGGATAACGCCCTCCAATCGGGTAACTCCCAG GAGAGTGTCACAGAGCAGGACAGCAAGGACAGC ACCTACAGCCTCAGCAGCACCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTCTACGCC TGCGAAGTCACCCATCAGGGCCTGAGCTCGCCC GTCACAAAGAGCTTCAACAGGGGAGAGTGTTGA
19	346C6-zu	Heavy chain	363	GAAGTGCAGCTGGTGGAATCTGGCGGCGGACTG GTGCAGCCTGGCGGATCTCTGAGACTGTCTTGT GCCGCCTCCGGCTTCTCCCTGTCCTCCTACGCT ATGATCTGGGTGCGACAGGCCCCTGGCAAGGGC CTGGAATGGATCGGCACCATCTCTACCGGCGGA ATTACCTACTACGCCTCCTGGGCCAAGGGCCGG TTCACCATCTCCAGAGACAACTCCAAGAACACC CTGTACCTCCAGATGAACTCCCTGCGGGCCGAG GACACCGCCGTGTACTATTGTGCTAGAGGCGGC TACGCCGCCAGCTCCGCTTACTACCTGCCCTAC TACTTCGACCTGTGGGGCCAGGGCACCCTCGTG ACAGTGTCATCTGCATCCACCAAGGGCCCATCG GTCTTCCCCCTGGCACCCTCCTCCAAGAGCACC TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTC

				AAGGACTACTTCCCCGAACCGGTGACGGTGTCG TGGAACTCAGGCGCCCTGACCAGCGGCGTGCAC ACCTTCCCGGCTGTCCTACAGTCCTCAGGACTC TACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAAC GTGAATCACAAGCCCAGCAACACCAAGGTGGAC AAGAAAGTTGAGCCCAAATCTTGTGACAAAACT CACACATGCCCACCGTGCCCAGCACCTGAACTC CTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCA AAACCCAAGGACACCCTCATGATCTCCCGGACC CCTGAGGTCACATGCGTGGTGGTGGACGTGAGC CACGAAGACCCTGAGGTCAAGTTCAACTGGTAC GTGGACGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTACAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAATGGCAAGGAGTACAAGTGCAAG GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAACCACAGGTGTACACCCTGCCCCCATCCCGG GATGAGCTGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTATCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCTTATATTCAAAGCTC ACCGTGGACAAGAGCAGGTGGCAGCAGGGGAAC GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACGCAGAAGAGCCTCTCCCTG TCTCCCGGGAAATGA
20	346C6-zu	Light chain	364	GATATTCAGATGACCCAGTCCCCCTCCAGCCTG TCCGCTTCTGTGGGCGACAGAGTGACCATCACC TGTCAGTCCTCCCAGTCCGTGTATAACAACAAC AACCTGGCCTGGTATCAGCAGAAACCCGGCAAG GTGCCCAAGCTGCTGATCTACCTGGCCTCCACA CTGGCCTCTGGCGTGCCCTCTAGATTCTCCGGC TCTGGCTCTGGCACCGACTTTACCCTGACCATC AGCTCCCTCCAGTGCGAGGATGCCGCCACCTAC TATTGCCTGGGCGGCTGCGACGACGACGCCGAT ACCTTTGCTTTTGGCGGAGGCACCAAGGTGGAA ATCAAACGAACTGTGGCTGCACCATCTGTCTTC ATCTTCCCGCCATCTGATGAGCAGTTGAAATCT

				GGAACTGCCTCTGTTGTGTGCCTGCTGAATAAC TTCTATCCCAGAGAGGCCAAAGTACAGTGGAAG GTGGATAACGCCCTCCAATCGGGTAACTCCCAG GAGAGTGTCACAGAGCAGGACAGCAAGGACAGC ACCTACAGCCTCAGCAGCACCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTCTACGCC TGCGAAGTCACCCATCAGGGCCTGAGCTCGCCC GTCACAAAGAGCTTCAACAGGGGAGAGTGTTGA

[+] Nucleic acid sequences listed do not include leader sequences.

[00121] In various embodiments, an ADC disclosed herein may comprise any set of heavy and light chain variable domains listed in the tables above (e.g., MORAb-003 heavy and light chain variable domains, or trastuzumab heavy and light chain variable domains), or the set of six CDR sequences from the heavy and light chain set. In some embodiments, the ADC further comprises human heavy and light chain constant domains or fragments thereof. For instance, the ADC may comprise a human IgG heavy chain constant domain (such as an IgG1) and a human kappa or lambda light chain constant domain. In various embodiments, the antibody moiety of the described ADCs comprises a human immunoglobulin G subtype 1 (IgG1) heavy chain constant domain with a human Ig kappa light chain constant domain.

[00122] In various embodiments, the target cancer antigen for an ADC is folate receptor alpha ("FRA").

[00123] In various embodiments, the anti-FRA antibody or antigen-binding fragment thereof comprises three heavy chain CDRs and three light chain CDRs as follows: heavy chain CDR1 (HCDR1) consisting of SEQ ID NO:2, heavy chain CDR2 (HCDR2) consisting of SEQ ID NO:3, heavy chain CDR3 (HCDR3) consisting of SEQ ID NO:4; light chain CDR1 (LCDR1) consisting of SEQ ID NO:7, light chain CDR2 (LCDR2) consisting of SEQ ID NO:8, and light chain CDR3 (LCDR3) consisting of SEQ ID NO:9, as defined by the Kabat numbering system (Kabat, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991))).

[00124] In some embodiments, the anti-FRA antibody or antigen-binding fragment thereof comprises three heavy chain CDRs and three light chain CDRs as follows: heavy chain CDR1 consisting of SEQ ID NO:13, heavy chain CDR2 consisting of SEQ ID NO:14, heavy chain CDR3 consisting of SEQ ID NO:15; light chain CDR1

consisting of SEQ ID NO:16, light chain CDR2 consisting of SEQ ID NO:17, and light chain CDR3 consisting of SEQ ID NO:18, as defined by the IMGT numbering system (International ImMunoGeneTics Information System (IMGT®)).

[00125] In various embodiments, the anti-FRA antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:24. In some embodiments, the anti-FRA antibody or antigen-binding fragment thereof comprises the heavy chain variable region amino acid sequence of SEQ ID NO:23 and the light chain variable region amino acid sequence of SEQ ID NO:24, or sequences that are at least 95% identical to the above-mentioned sequences. In some embodiments, the anti-FRA antibody or antigen-binding fragment thereof has a heavy chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:23 and a light chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:24.

[00126] In various embodiments, the anti-FRA antibody comprises a human IgG1 heavy chain constant domain with a human Ig kappa light chain constant domain.

[00127] In various embodiments, the anti-FRA antibody comprises the heavy chain amino acid sequence of SEQ ID NO:1 or a sequence that is at least 95% identical to SEQ ID NO:1, and the light chain amino acid sequence of SEQ ID NO:6 or a sequence that is at least 95% identical to SEQ ID NO:6. In particular embodiments, the antibody comprises the heavy chain amino acid sequence of SEQ ID NO:1 and the light chain amino acid sequence of SEQ ID NO:6, or sequences that are at least 95% identical to the above-mentioned sequences. In some embodiments, the anti-FRA antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:1 and/or a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:6. In some embodiments, the anti-FRA antibody comprises a heavy chain encoded by the nucleotide sequence of SEQ ID NO:11 (with the nucleotides encoding the leader sequence), or SEQ ID NO:345 (without the nucleotides encoding the leader sequence); and a light chain encoded by the nucleotide sequence of SEQ ID NO:12 (with the nucleotides encoding the leader sequence), or SEQ ID NO:346 (without the nucleotides encoding the leader sequence). In some embodiments, the heavy chain amino acid

sequence lacks the C-terminal lysine. In various embodiments, the anti-FRA antibody has the amino acid sequence of the antibody produced by a cell line deposited under terms in accordance with the Budapest Treaty with the American Type Culture Collection (ATCC, 10801 University Blvd., Manassas, Va. 20110-2209) on Apr. 24, 2006, under the Accession No. PTA-7552, or such sequences lacking the heavy chain C-terminal lysine. In various embodiments, the anti-FRA antibody is MORAb-003 (USAN name: farletuzumab) (Ebel et al. (2007) Cancer Immunity 7:6), or an antigen-binding fragment thereof.

[00128] In various other embodiments, the target cancer antigen for an ADC is human epidermal growth factor receptor 2 ("her2").

[00129] In various embodiments, the anti-her2 antibody or antigen-binding fragment thereof comprises three heavy chain CDRs and three light chain CDRs as follows: heavy chain CDR1 (HCDR1) consisting of SEQ ID NO:71, heavy chain CDR2 (HCDR2) consisting of SEQ ID NO:72, heavy chain CDR3 (HCDR3) consisting of SEQ ID NO:73; light chain CDR1 (LCDR1) consisting of SEQ ID NO:74, light chain CDR2 (LCDR2) consisting of SEQ ID NO:75, and light chain CDR3 (LCDR3) consisting of SEQ ID NO:76, as defined by the Kabat numbering system.

[00130] In some embodiments, the anti-her2 antibody or antigen-binding fragment thereof comprises three heavy chain CDRs and three light chain CDRs as follows: heavy chain CDR1 consisting of SEQ ID NO:191, heavy chain CDR2 consisting of SEQ ID NO:192, heavy chain CDR3 consisting of SEQ ID NO:193; light chain CDR1 consisting of SEQ ID NO:194, light chain CDR2 consisting of SEQ ID NO:195, and light chain CDR3 consisting of SEQ ID NO:196, as defined by the IMGT numbering system.

[00131] In various embodiments, the anti-her2 antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:27, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:28. In some embodiments, the anti-her2 antibody or antigen-binding fragment thereof comprises the heavy chain variable region amino acid sequence of SEQ ID NO:27 and the light chain variable region amino acid sequence of SEQ ID NO:28, or sequences that are at least 95% identical to the above-mentioned sequences. In some embodiments, the anti-her2 antibody or antigen-binding fragment thereof has a heavy chain variable region amino acid sequence that is at least 96%, at least 97%, at

least 98%, or at least 99% identical to SEQ ID NO:27 and/or a light chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:28.

[00132] In various embodiments, the anti-her2 antibody comprises a human IgG1 heavy chain constant domain and a human Ig kappa light chain constant domain.

[00133] In various embodiments, the anti-her2 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:327 or a sequence that is at least 95% identical to SEQ ID NO:327, and the light chain amino acid sequence of SEQ ID NO:328 or a sequence that is at least 95% identical to SEQ ID NO:328. In particular embodiments, the antibody comprises the heavy chain amino acid sequence of SEQ ID NO:327 and the light chain amino acid sequence of SEQ ID NO:328, or sequences that are at least 95% identical to the above-mentioned sequences. In some embodiments, the anti-her2 antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:327 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:328. In various embodiments, the anti-her2 antibody is trastuzumab, or an antigen-binding fragment thereof.

[00134] In various embodiments, the anti-FRA antibody or antigen-binding fragment thereof comprises the three heavy chain CDRs and three light chain CDRs of MORAb-003 or wherein the CDRs include no more than one, two, three, four, five, or six amino acid additions, deletions or substitutions of HCDR1 (SEQ ID NO:2 according to Kabat, or SEQ ID NO:13 according to IMGT), HCDR2 (SEQ ID NO:3 according to Kabat, or SEQ ID NO:14 according to IMGT), HCDR3 (SEQ ID NO:4 according to Kabat, or SEQ ID NO:15 according to IMGT); LCDR1 (SEQ ID NO:7 according to Kabat, or SEQ ID NO:16 according to IMGT), LCDR2 (SEQ ID NO:8 according to Kabat, or SEQ ID NO:17 according to IMGT), and LCDR3 (SEQ ID NO:9 according to Kabat, or SEQ ID NO:18 according to IMGT).

[00135] In various other embodiments, the anti-her2 antibody or antigen-binding fragment thereof comprises the three heavy chain CDRs and three light chain CDRs of trastuzumab or wherein the CDRs include no more than one, two, three, four, five, or six amino acid additions, deletions or substitutions of HCDR1 (SEQ ID NO:71 according to Kabat, or SEQ ID NO:191 according to IMGT), HCDR2 (SEQ ID NO:72 according to Kabat, or SEQ ID NO:192 according to IMGT), HCDR3 (SEQ ID NO:73

according to Kabat, or SEQ ID NO:193 according to IMGT); LCDR1 (SEQ ID NO:74 according to Kabat, or SEQ ID NO:194 according to IMGT), LCDR2 (SEQ ID NO:75 according to Kabat, or SEQ ID NO:195 according to IMGT), and LCDR3 (SEQ ID NO:76 according to Kabat, or SEQ ID NO:196 according to IMGT).

[00136] In various embodiments, amino acid substitutions are of single residues. Insertions usually will be on the order of from about 1 to about 20 amino acid residues, although considerably larger insertions may be tolerated as long as biological function is retained (e.g., binding to FRA or her2). Deletions usually range from about 1 to about 20 amino acid residues, although in some cases deletions may be much larger. Substitutions, deletions, insertions, or any combination thereof may be used to arrive at a final derivative or variant. Generally these changes are done on a few amino acids to minimize the alteration of the molecule, particularly the immunogenicity and specificity of the antigen binding protein. However, larger changes may be tolerated in certain circumstances. Conservative substitutions are generally made in accordance with the following chart depicted as Table 10.

Table 10

Original Residue	Exemplary Substitutions
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Asn, Gln
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile
Phe	Met, Leu, Tyr
Ser	Thr

Thr	Ser
Trp	Tyr
Tyr	Trp, Phe
Val	Ile, Leu

[00137] Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those shown in Table 10. For example, substitutions may be made which more significantly affect: the structure of the polypeptide backbone in the area of the alteration, for example the alpha-helical or beta-sheet structure; the charge or hydrophobicity of the molecule at the target site; or the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the polypeptide's properties are those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

[00138] In various embodiments where variant antibody sequences are used in an ADC, the variants typically exhibit the same qualitative biological activity and will elicit the same immune response, although variants may also be selected to modify the characteristics of the antigen binding proteins as needed. Alternatively, the variant may be designed such that the biological activity of the antigen binding protein is altered. For example, glycosylation sites may be altered or removed, as discussed herein.

[00139] Various antibodies may be used with the ADCs used herein to target cancer cells. As shown below, the linker-toxins in the ADCs disclosed herein are surprisingly effective with different tumor antigen-targeting antibodies. Suitable antigens expressed on tumor cells but not healthy cells, or expressed on tumor cells at a higher level than on healthy cells, are known in the art, as are antibodies directed against them. These antibodies may be used with the linkers and toxin (e.g., eribulin) disclosed herein. In some embodiments, the antibody moiety targets FRA. In some embodiments, the FRA-targeting antibody moiety is MORAb-003. In some embodiments, while the disclosed linkers and toxin (eribulin) are surprisingly effective with several different tumor-

targeting antibodies, FRA-targeting antibody moieties such as MORAb-003 provided particularly improved drug:antibody ratio, tumor targeting, bystander killing, treatment efficacy, and reduced off-target killing. Improved treatment efficacy can be measured *in vitro* or *in vivo*, and may include reduced tumor growth rate and/or reduced tumor volume.

[00140] In certain embodiments, antibodies to other antigen targets are used and provide at least some of the favorable functional properties of an ADC comprising an FRA-targeting antibody moiety such as MORAb-003 (e.g., improved drug:antibody ratio, improved treatment efficacy, reduced off-target killing, etc.). In some embodiments, some or all of these favorable functional properties are observed when the disclosed linkers and toxin (eribulin) are conjugated to a her2-targeting antibody moiety such as trastuzumab. In some embodiments, the antibody moiety targets her2. In some embodiments, the her2-targeting antibody moiety is trastuzumab. In some embodiments, some or all of these favorable functional properties are observed when the disclosed linkers and toxin (eribulin) are conjugated to a MSLN-targeting antibody moiety such as MORAb-009. In some embodiments, the antibody moiety targets MSLN. In some embodiments, the MSLN-targeting antibody moiety is MORAb-009.

Linkers

[00141] In various embodiments, the linker in an ADC is stable extracellularly in a sufficient manner to be therapeutically effective. In some embodiments, the linker is stable outside a cell, such that the ADC remains intact when present in extracellular conditions (e.g., prior to transport or delivery into a cell). The term "intact," used in the context of an ADC, means that the antibody moiety remains attached to the drug moiety. As used herein, "stable," in the context of a linker or ADC comprising a linker, means that no more than 20%, no more than about 15%, no more than about 10%, no more than about 5%, no more than about 3%, or no more than about 1% of the linkers (or any percentage in between) in a sample of ADC are cleaved (or in the case of an overall ADC are otherwise not intact) when the ADC is present in extracellular conditions.

[00142] Whether a linker is stable extracellularly can be determined, for example, by including an ADC in plasma for a predetermined time period (e.g., 2, 4, 6, 8, 16, or 24 hours) and then quantifying the amount of free drug moiety present in the plasma. Stability may allow the ADC time to localize to target tumor cells and prevent the

premature release of the drug, which could lower the therapeutic index of the ADC by indiscriminately damaging both normal and tumor tissues. In some embodiments, the linker is stable outside of a target cell and releases the drug moiety from the ADC once inside of the cell, such that the drug moiety can bind to its target (e.g., to microtubules). Thus, an effective linker will: (i) maintain the specific binding properties of the antibody moiety; (ii) allow delivery, e.g., intracellular delivery, of the drug moiety via stable attachment to the antibody moiety; (iii) remain stable and intact until the ADC has been transported or delivered to its target site; and (iv) allow for the therapeutic effect, e.g., cytotoxic effect, of the drug moiety after cleavage.

[00143] Linkers may impact the physico-chemical properties of an ADC. As many cytotoxic agents are hydrophobic in nature, linking them to the antibody with an additional hydrophobic moiety may lead to aggregation. ADC aggregates are insoluble and often limit achievable drug loading onto the antibody, which can negatively affect the potency of the ADC. Protein aggregates of biologics, in general, have also been linked to increased immunogenicity. As shown below, linkers disclosed herein result in ADCs with low aggregation levels and desirable levels of drug loading.

[00144] A linker may be "cleavable" or "non-cleavable" (Ducry and Stump, Bioconjugate Chem. (2010) 21:5-13). Cleavable linkers are designed to release the drug when subjected to certain environment factors, e.g., when internalized into the target cell, whereas non-cleavable linkers generally rely on the degradation of the antibody moiety itself.

[00145] In some embodiments, the linker is a non-cleavable linker. In some embodiments, the drug moiety of the ADC is released by degradation of the antibody moiety. Non-cleavable linkers tend to remain covalently associated with at least one amino acid of the antibody and the drug upon internalization by and degradation within the target cell. Non-cleavable linkers commonly include a thioether linkage, which is prepared by the conjugation of a thiol group on the drug or the antibody with a maleimide or haloacetamide group on the antibody or drug, respectively (Goldmacher et. al., In Cancer Drug Discovery and Development: Antibody-Drug Conjugates and Immunotoxins (G. L. Phillips ed., Springer, 2013)). An exemplary non-cleavable linker comprises thioether, cyclohexyl, N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate (SMCC), N-hydroxysuccinimide (NHS), or one or more polyethylene glycol (PEG) moieties, e.g., 1, 2, 3, 4, 5, or 6 PEG moieties. In some embodiments, the

non-cleavable linker comprises $(PEG)_2$. In other embodiments, the non-cleavable linker comprises $(PEG)_4$.

[00146] In some embodiments, the linker is a cleavable linker. A cleavable linker refers to any linker that comprises a cleavable moiety. As used herein, the term "cleavable moiety" refers to any chemical bond that can be cleaved. Suitable cleavable chemical bonds are well known in the art and include, but are not limited to, acid labile bonds, protease/peptidase labile bonds, photolabile bonds, disulfide bonds, and esterase labile bonds. Linkers comprising a cleavable moiety can allow for the release of the drug moiety from the ADC via cleavage at a particular site in the linker. In various embodiments, cleavage of the antibody from the linked toxin activates or increases the activity of the toxin. In some embodiments, an ADC comprising a cleavable linker (e.g., a Val-Cit linker) demonstrates increased on-target cell killing and/or decreased off-target cell killing, as compared to an ADC comprising a non-cleavable linker (e.g., a non-cleavable $(PEG)_2$ or $(PEG)_4$ linker). In some embodiments, an ADC comprising a cleavable linker exhibits improved treatment efficacy relative to an ADC comprising a non-cleavable linker when the cells and/or the cancer treated with the ADC does not express high levels of the target antigen (e.g., FRA or her2). In some embodiments, cleavage of the antibody from the linked toxin is required to achieve improved treatment efficacy of an ADC, as measured *in vitro* and/or *in vivo*.

[00147] In some embodiments, the linker is cleavable under intracellular conditions, such that cleavage of the linker sufficiently releases the drug moiety from the antibody moiety in the intracellular environment to activate the drug and/or render the drug therapeutically effective. In some embodiments, the drug moiety is not cleaved from the antibody moiety until the ADC enters a cell that expresses an antigen specific for the antibody moiety of the ADC, and the drug moiety is cleaved from the antibody moiety upon entering the cell. In some embodiments, the linker comprises a cleavable moiety that is positioned such that no part of the linker or the antibody moiety remains bound to the drug moiety upon cleavage. Exemplary cleavable linkers include acid labile linkers, protease/peptidase-sensitive linkers, photolabile linkers, dimethyl-, disulfide-, or sulfonamide-containing linkers.

[00148] In some embodiments, the linker is a pH-sensitive linker, and is sensitive to hydrolysis at certain pH values. Typically, the pH-sensitive linker is cleavable under acidic conditions. This cleavage strategy generally takes advantage of the lower pH in

the endosomal (pH ~ 5–6) and lysosomal (pH ~ 4.8) intracellular compartments, as compared to the cytosol (pH ~ 7.4), to trigger hydrolysis of an acid labile group in the linker, such as a hydrazone (Jain et al. (2015) Pharm Res 32:3526-40). In some embodiments, the linker is an acid labile and/or hydrolyzable linker. For example, an acid labile linker that is hydrolyzable in the lysosome, and contains an acid labile group (e.g., a hydrazone, a semicarbazone, a thiosemicarbazone, a cis-aconitic amide, an orthoester, an acetal, a ketal, or the like) can be used. *See*, *e.g.*, U.S. Pat. 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. 264:14653-61. 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. Pat. No. 5,622,929.

[00149] In some embodiments, the linker is cleavable under reducing conditions. In some embodiments, the linker is cleavable in the presence of a reducing agent, such as glutathione or dithiothreitol. In some embodiments, the linker is a cleavable disulfide linker or a cleavable sulfonamide linker.

[00150] In some embodiments, the linker is a cleavable disulfide linker. A variety of disulfide linkers are known in the art, including, for example, those that can be formed using SATA (N-succinimidyl-5-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyloxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene), SPDB and SMPT. *See*, *e.g.*, Thorpe et al. (1987) Cancer Res. 47:5924-31; 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. Pat. No. 4,880,935. Disulfide linkers are typically used to exploit the abundance of intracellular thiols, which can facilitate the cleavage of their disulfide bonds. The intracellular concentrations of the most abundance intracellular thiol, reduced glutathione, are generally in the range of 1-10 nM, which is about 1,000-fold higher than that of the most abundant low-molecular thiol in the blood (i.e., cysteine) at about 5 μM (Goldmacher et. al., In Cancer Drug Discovery and Development: Antibody-Drug Conjugates and Immunotoxins (G. L. Phillips ed., Springer, 2013)). The intracellular enzymes of the protein disulfide isomerase family may also contribute to the

intracellular cleavage of a disulfide linker. As used herein, a cleavable disulfide linker refers to any linker that comprises a cleavable disulfide moiety. The term "cleavable disulfide moiety" refers to a disulfide bond that can be cleaved and/or reduced, e.g., by a thiol or enzyme. In some embodiments, the cleavable disulfide moiety is disulfidyl-dimethyl.

[00151] In some embodiments, the linker is a cleavable sulfonamide linker. As used herein, a cleavable sulfonamide linker refers to any linker that comprises a cleavable sulfonamide moiety. The term "cleavable sulfonamide moiety" refers to a sulfonamide group, i.e., sulfonyl group connected to an amine group, wherein the sulfur-nitrogen bond can be cleaved.

[00152] In some embodiments, the linker may be a dendritic type linker for covalent attachment of more than one drug moiety to an antibody moiety through a branching, multifunctional linker moiety. *See*, *e.g.*, Sun et al. (2002) Bioorg. Med. Chem. Lett. 12:2213-5; Sun et al. (2003) Bioorg. Med. Chem. 11:1761-8. Dendritic linkers can increase the molar ratio of drug to antibody, i.e., drug loading, which is related to the potency of the ADC. Thus, where an antibody moiety bears only one reactive cysteine thiol group, for example, a multitude of drug moieties may be attached through a dendritic linker. In some embodiments, the linker moiety or linker-drug moiety may be attached to the antibody via reduced disulfide bridging chemistry or limited lysine utilization technology. *See*, *e.g.*, Intl. Publ. Nos. WO2013173391 and WO2013173393.

[00153] In some embodiments, the linker is cleavable by a cleaving agent, e.g., an enzyme, that is present in the intracellular environment (e.g., within a lysosome or endosome or caveolea). The linker can be, e.g., a peptide linker that is cleaved by an intracellular peptidase or protease enzyme, including, but not limited to, a lysosomal or endosomal protease. In some embodiments, the linker is a cleavable peptide linker. As used herein, a cleavable peptide linker refers to any linker that comprises a cleavable peptide moiety. The term "cleavable peptide moiety" refers to any chemical bond linking amino acids (natural or synthetic amino acid derivatives) that can be cleaved by an agent that is present in the intracellular environment. For instance, a linker may comprise an alanine-alanine-asparagine (Ala-Ala-Asn) sequence or a valine-citrulline (Val-Cit) sequence that is cleavable by a peptidase such as cathepsin, e.g., cathepsin B.

[00154] In some embodiments, the linker is an enzyme-cleavable linker and a cleavable peptide moiety in the linker is cleavable by the enzyme. In some

embodiments, the cleavable peptide moiety is cleavable by a lysosomal enzyme, e.g., cathepsin. In some embodiments, the linker is a cathepsin-cleavable linker. In some embodiments, the cleavable peptide moiety in the linker is cleavable by a lysosomal cysteine cathepsin, such as cathepsin B, C, F, H, K, L, O, S, V, X, or W. In some embodiments, the cleavable peptide moiety is cleavable by cathepsin B. An exemplary dipeptide that may be cleaved by cathepsin B is valine-citrulline (Val-Cit) (Dubowchik et al. (2002) Bioconjugate Chem. 13:855-69). In some embodiments, an ADC that comprises a cleavable peptide moiety demonstrates lower aggregation levels and/or higher drug loading (*p*) relative to an ADC that comprises an alternate cleavable moiety (e.g., a cleavable disulfide moiety or a cleavable sulfonamide moiety).

[00155] In some embodiments, the linker or the cleavable peptide moiety in the linker comprises an amino acid unit. In some embodiments, the amino acid unit allows for cleavage of the linker by a protease, thereby facilitating release of the drug moiety from the ADC upon exposure to one or more intracellular proteases, such as one or more lysosomal enzymes (Doronina et al. (2003) Nat. Biotechnol. 21:778-84; Dubowchik and Walker (1999) Pharm. Therapeutics 83:67-123). Exemplary amino acid units include, but are not limited to, dipeptides, tripeptides, tetrapeptides, and pentapeptides. Exemplary dipeptides include, but are not limited to, valine-citrulline (Val-Cit), alanine-asparagine (Ala-Asn), alanine-phenylalanine (Ala-Phe), phenylalanine-lysine (Phe-Lys), alanine-lysine (Ala-Lys), alanine-valine (Ala-Val), valine-alanine (Val-Ala), valine-lysine (Val-Lys), lysine-lysine (Lys-Lys), phenylalanine-citrulline (Phe-Cit), leucine-citrulline (Leu-Cit), isoleucine-citrulline (Ile-Cit), tryptophan-citrulline (Trp-Cit), and phenylalanine-alanine (Phe-Ala). Exemplary tripeptides include, but are not limited to, alanine-alanine-asparagine (Ala-Ala-Asn), glycine-valine-citrulline (Gly-Val-Cit), glycine-glycine-glycine (Gly-Gly-Gly), phenylalanine-phenylalanine-lysine (Phe-Phe-Lys), and glycine-phenylalanine-lysine (Gly-Phe-Lys). Other exemplary amino acid units include, but are not limited to, Gly-Phe-Leu-Gly, Ala-Leu-Ala-Leu, Phe-N^9-tosyl-Arg, and Phe-N^9-Nitro-Arg, as described in, e.g., U.S. Pat. No. 6,214,345. In some embodiments, the amino acid unit in the linker comprises Val-Cit. In some embodiments, the amino acid unit in the linker comprises Ala-Ala-Asn. In some embodiments, an ADC that comprises Val-Cit demonstrates decreased off-target cell killing, increased on-target cell killing, lower aggregation levels, and/or higher drug loading (*p*) relative to an ADC that comprises an alternate amino acid unit or an

alternate cleavable moiety. An amino acid unit may comprise amino acid residues that occur naturally and/or minor amino acids and/or non-naturally occurring amino acid analogs, such as citrulline. Amino acid units can be designed and optimized for enzymatic cleavage by a particular enzyme, for example, a tumor-associated protease, a lysosomal protease such as cathepsin B, C, D, or S, or a plasmin protease.

[00156] In some embodiments, the linker in any of the ADCs disclosed herein may comprise at least one spacer unit joining the antibody moiety to the drug moiety. In some embodiments, the spacer unit joins a cleavage site (e.g., a cleavable peptide moiety) in the linker to the antibody moiety. In some embodiments, the linker, and/or spacer unit in the linker, is substantially hydrophilic. A hydrophilic linker may be used to reduce the extent to which the drug may be pumped out of resistant cancer cells through multiple drug resistance (MDR) or functionally similar transporters. In some aspects, the linker includes one or more polyethylene glycol (PEG) moieties, e.g., 1, 2, 3, 4, 5, or 6 PEG moieties. In some embodiments, the linker is a shorter PEG linker, and provides improved stability and reduced aggregation over longer PEG linkers.

[00157] In some embodiments, the spacer unit in the linker comprises one or more PEG moieties. In some embodiments, the spacer unit comprises $-(PEG)_m-$, and m is an integer from 1 to 10. In some embodiments, m ranges from 1 to 10; from 2 to 8; from 2 to 6; from 2 to 5; from 2 to 4; or from 2 to 3. In some embodiments, m is 8. In some embodiments, m is 4. In some embodiments, m is 3. In some embodiments, m is 2. In some embodiments, the spacer unit comprises $(PEG)_2$, $(PEG)_4$, $(PEG)_8$, $(PEG)_9$, $(PEG)_3$-triazole-$(PEG)_3$, $(PEG)_4$-triazole-$(PEG)_3$, or dibenzylcyclooctene-triazole-$(PEG)_3$. In some preferred embodiments, the spacer unit comprises $(PEG)_2$. In some embodiments, an ADC that comprises a shorter spacer unit (e.g., $(PEG)_2$) demonstrates lower aggregation levels and/or higher drug loading (p) relative to an ADC that comprises a longer spacer unit (e.g., $(PEG)_8$).

[00158] In some embodiments, the spacer unit in the linker comprises an alkyl moiety. In some embodiments, the spacer unit comprises $-(CH_2)_n-$, and n is an integer from 1 to 10 (i.e., n may be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10). In some embodiments, n is 5. In some embodiments, an ADC that comprises a shorter spacer unit (e.g., $(CH_2)_5$) demonstrates lower aggregation levels and/or higher drug loading (p) relative to an ADC that comprises a longer spacer unit (e.g., $(PEG)_8$).

[00159] A spacer unit may be used, for example, to link the antibody moiety to the drug moiety, either directly or indirectly. In some embodiments, the spacer unit links the antibody moiety to the drug moiety directly. In some embodiments, the antibody moiety and the drug moiety are attached via a spacer unit comprising one or more PEG moieties (e.g., $(PEG)_2$ or $(PEG)_4$). In some embodiments, the spacer unit links the antibody moiety to the drug moiety indirectly. In some embodiments, the spacer unit links the antibody moiety to the drug moiety indirectly through a cleavable moiety (e.g., a cleavable peptide, a cleavable disulfide, or a cleavable sulfonamide) and/or an attachment moiety to join the spacer unit to the antibody moiety, e.g., a maleimide moiety.

[00160] The spacer unit, in various embodiments, attaches to the antibody moiety (i.e., the antibody or antigen-binding fragment) via a maleimide moiety (Mal). In some embodiments, an ADC that comprises a linker attached to the antibody moiety via a maleimide moiety demonstrates higher drug loading (p) relative to an ADC that comprises a linker attached to the antibody moiety via an alternate attachment moiety such as a succinimide moiety.

[00161] A spacer unit that attaches to the antibody or antigen-binding fragment via a Mal is referred to herein as a "Mal-spacer unit." The term "maleimide moiety," as used herein, means a compound that contains a maleimide group and that is reactive with a sulfhydryl group, e.g., a sulfhydryl group of a cysteine residue on the antibody moiety. Other functional groups that are reactive with sulfhydryl groups (thiols) include, but are not limited to, iodoacetamide, bromoacetamide, vinyl pyridine, disulfide, pyridyl disulfide, isocyanate, and isothiocyanate. In some embodiments, the Mal-spacer unit is reactive with a cysteine residue on the antibody or antigen-binding fragment. In some embodiments, the Mal-spacer unit is joined to the antibody or antigen-binding fragment via the cysteine residue. In some embodiments, the Mal-spacer unit comprises a PEG moiety. In some embodiments, the Mal-spacer unit comprises an alkyl moiety.

[00162] In certain embodiments, the linker comprises the Mal-spacer unit and a cleavable peptide moiety. In some embodiments, the cleavable peptide moiety comprises an amino acid unit. In some embodiments, the amino acid unit comprises Val-Cit. In some embodiments, the amino acid unit comprises Ala-Ala-Asn. In some embodiments, the linker comprises the Mal-spacer unit and Val-Cit. In some embodiments, the linker comprises Mal-$(PEG)_2$ and Val-Cit. In some embodiments, the

linker comprises Mal-$(PEG)_m$ and Val-Cit, where *m* is 2 to 8 or 2 to 5, or 2, 3, 4, or 5. In some embodiments, the linker comprises Mal-$(PEG)_8$ and Val-Cit. In certain embodiments, the linker comprises Mal-$(CH_2)_5$ and Val-Cit. In some embodiments, the linker comprises the Mal-spacer unit and Ala-Ala-Asn. In some embodiments, the linker comprises Mal-$(PEG)_2$ and Ala-Ala-Asn.

[00163] In some embodiments, the linker comprises the Mal-spacer unit and a cleavable disulfide moiety. In some embodiments, the cleavable disulfide moiety is disulfidyl-dimethyl. In some embodiments, the linker comprises the Mal-spacer unit and disulfidyl-dimethyl. In some embodiments, the linker comprises Mal-$(PEG)_4$-triazole-$(PEG)_3$ and disulfidyl-dimethyl.

[00164] In some embodiments, the linker comprises the Mal-spacer unit and a cleavable sulfonamide moiety. In some embodiments, the linker comprises Mal-$(PEG)_4$-triazole-$(PEG)_3$ and sulfonamide.

[00165] In various embodiments, the spacer unit attaches to the antibody or antigen-binding fragment via a succinimide moiety (OSu). A spacer unit that attaches to the antibody or antigen-binding fragment via an OSu is referred to herein as an "OSu-spacer unit." The term "succinimide moiety," as used herein, means a compound that contains a succinimide compound that is reactive with an amine group, e.g., an amine group of a lysine residue on the antibody moiety. An exemplary succinimide moiety is N-hydroxysuccinimide (NHS). In some embodiments, the OSu-spacer unit is reactive with a lysine residue on the antibody or antigen-binding fragment. In some embodiments, the OSu-spacer unit is joined to the antibody or antigen-binding fragment via the lysine residue. In some embodiments, the OSu-spacer unit comprises a PEG moiety. In some embodiments, the OSu-spacer unit comprises an alkyl moiety.

[00166] In certain embodiments, the linker comprises the OSu-spacer unit and a cleavable peptide moiety. In some embodiments, the cleavable peptide moiety comprises an amino acid unit. In some embodiments, the amino acid unit comprises Val-Cit. In some embodiments, the amino acid unit comprises Ala-Ala-Asn. In some embodiments, the linker comprises the OSu-spacer unit and Val-Cit. In some embodiments, the linker comprises OSu-$(PEG)_2$ and Val-Cit. In other embodiments, the linker comprises OSu-$(PEG)_9$ and Val-Cit. In other embodiments, the linker comprises OSu-$(CH_2)_5$ and Val-Cit. In certain embodiments, the linker comprises OSu-$(PEG)_3$-triazole-$(PEG)_3$ and Val-Cit. In some embodiments, the linker comprises the OSu-

spacer unit and Ala-Ala-Asn. In some embodiments, the linker comprises OSu-$(PEG)_2$ and Ala-Ala-Asn.

[00167] In some embodiments, the linker comprises the OSu-spacer unit and a cleavable disulfide moiety. In some embodiments, the cleavable disulfide moiety is disulfidyl-dimethyl. In some embodiments, the linker comprises the OSu-spacer unit and disulfidyl-dimethyl. In some embodiments, the linker comprises OSu-$(PEG)_3$-triazole-$(PEG)_3$ and disulfidyl-dimethyl. In other embodiments, the linker comprises OSu-dibenzylcyclooctene-triazole-$(PEG)_3$ and disulfidyl-dimethyl.

[00168] In some embodiments, the linker comprises the OSu-spacer unit and a cleavable sulfonamide moiety. In some embodiments, the linker comprises OSu-$(PEG)_3$-triazole-$(PEG)_3$ and sulfonamide. In other embodiments, the linker comprises OSu-dibenzylcyclooctene-triazole-$(PEG)_3$ and sulfonamide.

[00169] In some embodiments, the Mal-spacer unit or the OSu-spacer unit attaches the antibody moiety (i.e., the antibody or antigen-binding fragment) to the cleavable moiety in the linker. In some embodiments, the Mal-spacer unit or the OSu-spacer unit attaches the antibody or antigen-binding fragment to a cleavable peptide moiety. In some embodiments, the cleavable peptide moiety comprises an amino acid unit. In some embodiments, the linker comprises Mal-spacer unit-amino acid unit or OSu-spacer unit-amino acid unit. In some embodiments, the Mal-spacer unit or the OSu-spacer unit comprises a PEG moiety. In some embodiments, the Mal-spacer-unit or the OSu-spacer unit comprises an alkyl moiety. In some embodiments, the amino acid unit comprises Val-Cit. In other embodiments, the amino acid unit comprises Ala-Ala-Asn.

[00170] In some embodiments, the linker comprises the structure: Mal-spacer unit-Val-Cit. In some embodiments, the linker comprises the structure: Mal-$(PEG)_2$-Val-Cit. In some embodiments, the linker comprises the structure: Mal-$(PEG)_2$-Val-Cit-pAB. In some embodiments, the linker comprises Mal-$(PEG)_8$-Val-Cit. In certain embodiments, the linker comprises Mal-$(CH_2)_5$-Val-Cit. In some embodiments, the linker comprises the Mal-spacer unit-Ala-Ala-Asn. In some embodiments, the linker comprises Mal-$(PEG)_2$-Ala-Ala-Asn.

[00171] In some embodiments, the linker comprises OSu-spacer unit-Val-Cit. In some embodiments, the linker comprises OSu-$(PEG)_2$-Val-Cit. In other embodiments, the linker comprises OSu-$(PEG)_9$-Val-Cit. In other embodiments, the linker comprises OSu-$(CH_2)_5$-Val-Cit. In other embodiments, the linker comprises OSu-$(PEG)_3$-triazole-

$(PEG)_3$-Val-Cit. In some embodiments, the linker comprises the OSu-spacer unit-Ala-Ala-Asn. In some embodiments, the linker comprises OSu-$(PEG)_2$-Ala-Ala-Asn.

[00172] In various embodiments, the Mal-spacer unit or the OSu-spacer unit attaches the antibody or antigen-binding fragment to a cleavable disulfide moiety. In some embodiments, the linker comprises Mal-spacer unit-disulfide or OSu-spacer unit-disulfide. In some embodiments, the disulfide is disulfidyl-dimethyl. In some embodiments, the linker comprises Mal-spacer unit-disulfidyl-dimethyl. In some embodiments, the linker comprises Mal-$(PEG)_4$-triazole-$(PEG)_3$.disulfidyl-dimethyl. In other embodiments, the linker comprises OSu-spacer unit-disulfidyl-dimethyl. In some embodiments, the linker comprises OSu-$(PEG)_3$-triazole-$(PEG)_3$.disulfidyl-dimethyl. In other embodiments, the linker comprises OSu-dibenzylcyclooctene-triazole-$(PEG)_3$-disulfidyl-dimethyl.

[00173] In certain embodiments, the Mal-spacer unit or the OSu-spacer unit attaches the antibody or antigen-binding fragment to a cleavable sulfonamide moiety. In some embodiments, the linker comprises Mal-spacer unit-sulfonamide or OSu-spacer unit-sulfonamide. In some embodiments, the linker comprises Mal-$(PEG)_4$-triazole-$(PEG)_3$-sulfonamide. In some embodiments, the linker comprises OSu-$(PEG)_3$-triazole-$(PEG)_3$-sulfonamide. In other embodiments, the linker comprises OSu-dibenzylcyclooctene-triazole-$(PEG)_3$-sulfonamide.

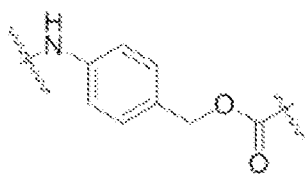
[00174] In various embodiments, the cleavable moiety in the linker is joined directly to the drug moiety. In other embodiments, another spacer unit is used to attach the cleavable moiety in the linker to the drug moiety. In various embodiments, the drug moiety is eribulin. In various embodiments, the eribulin is attached to the cleavable moiety in the linker by a spacer unit. In some embodiments, the eribulin is attached to the cleavable moiety in the linker by a self-immolative spacer unit. In certain embodiments, the eribulin is attached to the cleavable moiety in the linker by a self-immolative spacer unit, the cleavable moiety comprises Val-Cit, and a further spacer unit comprising PEG joins the cleavable moiety to the antibody moiety. In certain embodiments, the eribulin is joined to an anti-FRA antibody via a Mal-spacer unit in the linker joined to a Val-Cit cleavable moiety and a pAB self-immolative spacer unit. In certain other embodiments, the eribulin is joined to an anti-her2 antibody via a Mal-spacer unit in the linker joined to a Val-Cit cleavable moiety and a pAB self-immolative spacer unit.

[00175] A spacer unit may be "self-immolative" or "non-self-immolative." A "non-self-immolative" spacer unit is one in which part or all of the spacer unit remains bound to the drug moiety upon cleavage of the linker. Examples of non-self-immolative spacer units include, but are not limited to, a glycine spacer unit and a glycine-glycine spacer unit. Non-self-immolative spacer units may eventually degrade over time but do not readily release a linked native drug entirely under cellular conditions. A "self-immolative" spacer unit allows for release of the native drug moiety under intracellular conditions. A “native drug” is one where no part of the spacer unit or other chemical modification remains after cleavage/degradation of the spacer unit.

[00176] Self-immolation chemistry is known in the art and could be readily selected for the disclosed ADCs. In various embodiments, the spacer unit attaching the cleavable moiety in the linker to the drug moiety (e.g., eribulin) is self-immolative, and undergoes self-immolation concurrently with or shortly before/after cleavage of the cleavable moiety under intracellular conditions.

[00177] In certain embodiments, the self-immolative spacer unit in the linker comprises a p-aminobenzyl unit. In some embodiments, a p-aminobenzyl alcohol (pABOH) is attached to an amino acid unit or other cleavable moiety in the linker via an amide bond, and a carbamate, methylcarbamate, or carbonate is made between the pABOH and the drug moiety (Hamann et al. (2005) Expert Opin. Ther. Patents 15:1087-103). In some embodiments, the self-immolative spacer unit is or comprises p-aminobenzyloxycarbonyl (pAB). Without being bound by theory, it is thought that the self-immolation of pAB involves a spontaneous 1,6-elimination reaction (Jain et al. (2015) Pharm Res 32:3526-40).

[00178] In various embodiments, the structure of the p-aminobenzyloxycarbonyl (pAB) used in the disclosed ADCs is shown below:



p-amino-benzyloxycarbonyl

[00179] In various embodiments, the self-immolative spacer unit attaches the cleavable moiety in the linker to the C-35 amine on eribulin. In some embodiments, the

self-immolative spacer unit is pAB. In some embodiments, the pAB attaches the cleavable moiety in the linker to the C-35 amine on eribulin. In some embodiments, the pAB undergoes self-immolation upon cleavage of the cleavable moiety, and eribulin is released from the ADC in its native, active form. In some embodiments, an anti-FRA antibody (e.g., MORAb-003) is joined to the C-35 amine of eribulin by a linker comprising Mal-$(PEG)_2$-Val-Cit-pAB. In other embodiments, an anti-her2 antibody (e.g., trastuzumab) is joined to the C-35 amine of eribulin by a linker comprising Mal-$(PEG)_2$-Val-Cit-pAB.

[00180] In some embodiments, the pAB undergoes self-immolation upon cleavage of a cleavable peptide moiety in the linker. In some embodiments, the cleavable peptide moiety comprises an amino acid unit. In some embodiments, the linker comprises amino acid unit-pAB. In some embodiments, the amino acid unit is Val-Cit. In some embodiments, the linker comprises Val-Cit-pAB (VCP). In certain embodiments, the amino acid unit is Ala-Ala-Asn. In some embodiments, the linker comprises Ala-Ala-Asn-pAB.

[00181] In some embodiments, the pAB undergoes self-immolation upon cleavage of a cleavable disulfide moiety in the linker. In some embodiments, the linker comprises disulfide-pAB. In some embodiments, the linker comprises disulfidyl-dimethyl-pAB.

[00182] In some embodiments, the pAB undergoes self-immolation upon cleavage of a cleavable sulfonamide moiety in the linker. In some embodiments, the linker comprises sulfonamide-pAB.

[00183] In various aspects, the antibody moiety of the ADC is conjugated to the drug moiety via a linker, wherein the linker comprises a Mal-spacer unit, a cleavable amino acid unit, and a pAB. In some embodiments, the spacer unit comprises a PEG moiety. In some embodiments, the spacer unit comprises an alkyl moiety. In some embodiments, the linker comprises Mal-$(PEG)_2$-amino acid unit-pAB. In some embodiments, the linker comprises Mal-$(PEG)_2$-Val-Cit-pAB. In other embodiments, the linker comprises Mal-$(PEG)_2$-Ala-Ala-Asn-pAB. In some embodiments, the linker comprises, Mal-$(PEG)_8$-amino acid unit-pAB. In some embodiments, the linker comprises Mal-$(PEG)_8$-Val-Cit-pAB. In some embodiments, the linker comprises Mal-$(CH_2)_5$-amino acid unit-pAB. In some embodiments, the linker comprises Mal-$(CH_2)_5$-Val-Cit-pAB.

[00184] In various embodiments, the antibody moiety of the ADC is conjugated to the drug moiety via a linker, wherein the linker comprises Mal-spacer unit-disulfide-pAB. In some embodiments, the spacer unit comprises a PEG moiety. In some embodiments, the linker comprises Mal-$(PEG)_4$-triazole-$(PEG)_3$-disulfide-pAB. In some embodiments, the linker comprises Mal-$(PEG)_4$-triazole-$(PEG)_3$-disulfidyl-dimethyl-pAB.

[00185] In some embodiments, the antibody moiety of the ADC is conjugated to the drug moiety via a linker, wherein the linker comprises Mal-spacer unit-sulfonamide-pAB. In some embodiments, the spacer unit comprises a PEG moiety. In some embodiments, the linker comprises Mal-$(PEG)_4$-triazole-$(PEG)_3$-sulfonamide-pAB.

[00186] In some aspects, the antibody moiety of the ADC is conjugated to the drug moiety via a linker, wherein the linker comprises OSu-spacer unit-amino acid unit-pAB. In some embodiments, the spacer unit comprises a PEG moiety. In some embodiments, the spacer unit comprises an alkyl moiety. In some embodiments, the linker comprises OSu-$(PEG)_2$-amino acid unit-pAB. In some embodiments, the linker comprises OSu-$(PEG)_2$-Val-Cit-pAB. In other embodiments, the linker comprises OSu-$(PEG)_2$-Ala-Ala-Asn-pAB. In some embodiments, the linker comprises, OSu-$(PEG)_9$-amino acid unit-pAB. In some embodiments, the linker comprises OSu-$(PEG)_9$-Val-Cit-pAB. In some embodiments, the linker comprises OSu-$(CH_2)_5$-amino acid unit-pAB. In some embodiments, the linker comprises OSu-$(CH_2)_5$-Val-Cit-pAB. In some embodiments, the linker comprises OSu-$(PEG)_3$-triazole-$(PEG)_3$-amino acid unit-pAB. In some embodiments, the linker comprises OSu-$(PEG)_3$-triazole-$(PEG)_3$-Val-Cit-pAB.

[00187] In some embodiments, the antibody moiety of the ADC is conjugated to the drug moiety via a linker, wherein the linker comprises OSu-spacer unit-disulfide-pAB. In some embodiments, the spacer unit comprises a PEG moiety. In some embodiments, the linker comprises OSu-$(PEG)_3$-triazole-$(PEG)_3$-disulfide-pAB. In some embodiments, the linker comprises OSu-$(PEG)_3$-triazole-$(PEG)_3$-disulfidyl-dimethyl-pAB. In some embodiments, the linker comprises OSu-dibenzylcyclooctene-triazole-$(PEG)_3$-disulfide-pAB. In some embodiments, the linker comprises OSu-dibenzylcyclooctene-triazole-$(PEG)_3$-disulfidyl-dimethyl-pAB.

[00188] In some embodiments, the antibody moiety of the ADC is conjugated to the drug moiety via a linker, wherein the linker comprises OSu-spacer unit-sulfonamide-pAB. In some embodiments, the spacer unit comprises a PEG moiety. In some

embodiments, the linker comprises OSu-$(PEG)_3$-triazole-$(PEG)_3$-sulfonamide-pAB. In some embodiments, the linker comprises OSu-dibenzylcyclooctene-triazole-$(PEG)_3$-sulfonamide-pAB.

[00189] In various embodiments, the linker is designed to facilitate bystander killing (the killing of neighboring cells) through cleavage after cellular internalization and diffusion of the linker-drug moiety and/or the drug moiety alone to neighboring cells. In some embodiments, the linker promotes cellular internalization. In some embodiments, the linker is designed to minimize cleavage in the extracellular environment and thereby reduce toxicity to off-target tissue (e.g., non-cancerous tissue), while preserving ADC binding to target tissue and bystander killing of cancerous tissue that does not express an antigen targeted by the antibody moiety of an ADC, but surrounds target cancer tissue expressing that antigen. In some embodiments, a linker comprising a maleimide moiety (Mal), a polyethylene glycol (PEG) moiety, valine-citrulline (Val-Cit or "vc"), and a pAB provides these functional features. In some embodiments, a linker comprising Mal-$(PEG)_2$-Val-Cit-pAB is particularly effective in providing these functional features, e.g., when joining an anti-FRA antibody moiety such as MORAb-003 and a drug moiety such as eribulin. In some embodiments, at least some of these functional features may also be observed without an anti-FRA antibody moiety, and/or without MORAb-003. For instance, in some embodiments, a linker comprising Mal-$(PEG)_2$-Val-Cit-pAB is effective in providing some or all of these functional features , e.g., when joining an anti-her2 antibody moiety such as trastuzumab and a drug moiety such as eribulin.

[00190] In some embodiments, the antibody moiety is conjugated to the drug moiety via a linker comprising a maleimide moiety (Mal), a polyethylene glycol (PEG) moiety, valine citrulline (Val-Cit or "vc"), and a pAB. In these embodiments, the maleimide moiety covalently attaches the linker-drug moiety to the antibody moiety, and the pAB acts as a self-immolative spacer unit. Such linker may be referred to as the "m-vc-pAB" linker, the “Mal-VCP” linker, the “Mal-$(PEG)_2$-VCP” linker, or the “Mal-$(PEG)_2$-Val-Cit-pAB” linker. In some embodiments, the drug moiety is eribulin. The structure of Mal-$(PEG)_2$-Val-Cit-pAB-eribulin is provided in Table 46. The pAB of the Mal-$(PEG)_2$-Val-Cit-pAB linker is attached to the C-35 amine on eribulin.

[00191] It has been discovered that ADCs comprising Mal-$(PEG)_2$-Val-Cit-pAB-eribulin demonstrate a particular combination of desirable properties, particularly when

paired with an anti-FRA antibody such as MORAb-003 or an antigen-binding fragment thereof. These properties include, but are not limited to, effective levels of drug loading ($p \sim 4$), low aggregation levels, stability under storage conditions or when in circulation in the body (e.g., serum stability), retained affinity for target-expressing cells comparable to unconjugated antibody, potent cytotoxicity against target-expressing cells, low levels of off-target cell killing, high levels of bystander killing, and/or effective *in vivo* anti-cancer activity, all as compared to ADCs using other linker-toxin and/or antibody moieties. While numerous linker options and combinations of spacers and cleavage sites were known in the art and may provide certain benefits in one or more of these functional categories, the particular combination of a Mal-$(PEG)_2$-Val-Cit-pAB linker joining eribulin to an antibody moiety such as an anti-FRA antibody (e.g., MORAb-003) may provide good or superior properties across the spectrum of desirable functional properties for a therapeutic ADC. In some embodiments, the good or superior functional properties provided by the particular combination of a Mal-$(PEG)_2$-Val-Cit-pAB linker joining eribulin to an antibody moiety may be observed with this linker-toxin conjugated to, e.g., an anti-her 2 antibody such as trastuzumab.

[00192] In some embodiments, the ADC comprises Mal-$(PEG)_2$-Val-Cit-pAB-eribulin and an antibody moiety comprising an internalizing antibody or an antigen-binding fragment thereof that retains the ability to target and internalize in a tumor cell. In some embodiments, the ADC comprises Mal-$(PEG)_2$-Val-Cit-pAB-eribulin and an internalizing antibody or internalizing antigen-binding fragment thereof that targets an FRA-expressing tumor cell. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment thereof that targets an FRA-expressing tumor cell comprises three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:2 (HCDR1), SEQ ID NO:3 (HCDR2), and SEQ ID NO:4 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:7 (LCDR1), SEQ ID NO:8 (LCDR2), and SEQ ID NO:9 (LCDR3), as defined by the Kabat numbering system; or three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:13 (HCDR1), SEQ ID NO:14 (HCDR2), and SEQ ID NO:15 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:16 (LCDR1), SEQ ID NO:17 (LCDR2), and SEQ ID NO:18 (LCDR3), as defined by the

IMGT numbering system. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment thereof that targets an FRA-expressing tumor cell comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:23, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:24. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment thereof that targets an FRA-expressing tumor cell comprises a human IgG1 heavy chain constant domain and an Ig kappa light chain constant domain.

[00193] In some embodiments, the ADC has Formula I:

$$Ab\text{-}(L\text{-}D)_p \qquad (I)$$

wherein:

(i) Ab is an internalizing anti-folate receptor alpha (FRA) antibody or internalizing antigen-binding fragment thereof comprising three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:2 (HCDR1), SEQ ID NO:3 (HCDR2), and SEQ ID NO:4 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:7 (LCDR1), SEQ ID NO:8 (LCDR2), and SEQ ID NO:9 (LCDR3), as defined by the Kabat numbering system; or three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:13 (HCDR1), SEQ ID NO:14 (HCDR2), and SEQ ID NO:15 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:16 (LCDR1), SEQ ID NO:17 (LCDR2), and SEQ ID NO:18 (LCDR3), as defined by the IMGT numbering system;

(ii) D is eribulin;

(iii) L is a cleavable linker comprising Mal-$(PEG)_2$-Val-Cit-pAB; and

(iv) p is an integer from 1 to 20.

[00194] In some embodiments, the internalizing antibody or internalizing antigen-binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:23, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:24. In some embodiments, the internalizing antibody is MORAb-003. In some embodiments, p is from 1 to 8, or 1 to 6. In some embodiments,

p is from 2 to 8, or 2 to 5. In some embodiments, *p* is from 3 to 4. In some embodiments, *p* is 4.

[00195] In other embodiments, the ADC comprises Mal-$(PEG)_2$-Val-Cit-pAB-eribulin and an internalizing antibody or internalizing antigen-binding fragment thereof that targets a her2-expressing tumor cell. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment thereof that targets a her2-expressing tumor cell comprises three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:71 (HCDR1), SEQ ID NO:72 (HCDR2), and SEQ ID NO:73 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:74 (LCDR1), SEQ ID NO:75 (LCDR2), and SEQ ID NO:76 (LCDR3), as defined by the Kabat numbering system; or three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:191 (HCDR1), SEQ ID NO:192 (HCDR2), and SEQ ID NO:193 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:194 (LCDR1), SEQ ID NO:195 (LCDR2), and SEQ ID NO:196 (LCDR3), as defined by the IMGT numbering system. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment thereof that targets a her2-expressing tumor cell comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:27, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:28. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment thereof that targets a her2-expressing tumor cell comprises a human IgG1 heavy chain constant domain and an Ig kappa light chain constant domain.

[00196] In some embodiments, the ADC has Formula I:

$$Ab\text{-}(L\text{-}D)_p \qquad (I)$$

wherein:

(i) Ab is an internalizing anti-human epidermal growth factor receptor 2 (her2) antibody or internalizing antigen-binding fragment thereof comprising three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:71 (HCDR1), SEQ ID NO:72 (HCDR2), and SEQ ID NO:73 (HCDR3); and three light chain complementarity determining regions (LCDRs)

comprising amino acid sequences of SEQ ID NO:74 (LCDR1), SEQ ID NO:75 (LCDR2), and SEQ ID NO:76 (LCDR3), as defined by the Kabat numbering system; or three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:191 (HCDR1), SEQ ID NO:192 (HCDR2), and SEQ ID NO:193 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:194 (LCDR1), SEQ ID NO:195 (LCDR2), and SEQ ID NO:196 (LCDR3), as defined by the IMGT numbering system;

(ii) D is eribulin;

(iii) L is a cleavable linker comprising Mal-$(PEG)_2$-Val-Cit-pAB; and

(iv) *p* is an integer from 1 to 20.

[00197] In some embodiments, the internalizing antibody or internalizing antigen-binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:27, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:28. In some embodiments, the internalizing antibody is trastuzumab. In some embodiments, *p* is from 1 to 8, or 1 to 6. In some embodiments, *p* is from 2 to 8, or 2 to 5. In some embodiments, *p* is from 3 to 4. In some embodiments, *p* is 4.

[00198] In other embodiments, the ADC comprises Mal-$(PEG)_2$-Val-Cit-pAB-eribulin and an internalizing antibody or internalizing antigen-binding fragment thereof that targets a mesothelin (MSLN)-expressing tumor cell. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment thereof that targets a MSLN-expressing tumor cell comprises three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:65 (HCDR1), SEQ ID NO:66 (HCDR2), and SEQ ID NO:67 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:68 (LCDR1), SEQ ID NO:69 (LCDR2), and SEQ ID NO:70 (LCDR3), as defined by the Kabat numbering system; or three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:185 (HCDR1), SEQ ID NO:186 (HCDR2), and SEQ ID NO:187 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:188 (LCDR1), SEQ ID NO:189 (LCDR2), and SEQ ID NO:190 (LCDR3), as defined by the IMGT numbering system. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment thereof that targets a

MSLN-expressing tumor cell comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:25, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:26. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment thereof that targets a MSLN-expressing tumor cell comprises a human IgG1 heavy chain constant domain and an Ig kappa light chain constant domain.

[00199] In some embodiments, the ADC has Formula I:

$$Ab\text{-}(L\text{-}D)_p \qquad (I)$$

wherein:

(i) Ab is an internalizing anti-mesothelin antibody or internalizing antigen-binding fragment thereof comprising three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:65 (HCDR1), SEQ ID NO:66 (HCDR2), and SEQ ID NO:67 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:68 (LCDR1), SEQ ID NO:69 (LCDR2), and SEQ ID NO:70 (LCDR3), as defined by the Kabat numbering system; or three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:185 (HCDR1), SEQ ID NO:186 (HCDR2), and SEQ ID NO:187 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:188 (LCDR1), SEQ ID NO:189 (LCDR2), and SEQ ID NO:190 (LCDR3), as defined by the IMGT numbering system;

(ii) D is eribulin;

(iii) L is a cleavable linker comprising Mal-$(PEG)_2$-Val-Cit-pAB; and

(iv) p is an integer from 1 to 20.

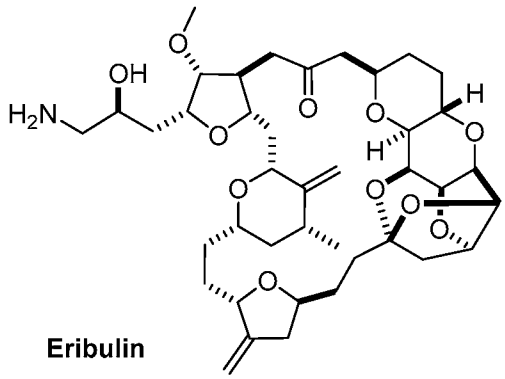
[00200] In some embodiments, the internalizing antibody or internalizing antigen-binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:25, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:26. In some embodiments, the internalizing antibody is MORAb-003, MORAb-009, or trastuzumab. In some embodiments, p is from 1 to 8, or 1 to 6. In some embodiments, p is from 2 to 8, or 2 to 5. In some embodiments, p is from 3 to 4. In some embodiments, p is 4.

Drug Moieties

[00201] The drug moiety (D) of the ADCs described herein can be any chemotherapeutic agent. Useful classes of chemotherapeutic agents include, for example, anti-tubulin agents. In certain embodiments, the drug moiety is an anti-tubulin agent. Examples of anti-tubulin agents include cryptophycin and eribulin. The preferred drug moiety for use in the described ADCs is eribulin.

[00202] In various embodiments, the drug moiety is eribulin. In these embodiments, the linker of the ADC is attached via the C-35 amine on eribulin.

[00203] In various embodiments, the natural form of eribulin used for joining to the linker and antibody moiety is shown below:



Eribulin

[00204] In certain embodiments, an intermediate, which is the precursor of the linker, is reacted with the drug moiety under appropriate conditions. In certain embodiments, reactive groups are used on the drug and/or the intermediate or linker. The product of the reaction between the drug and the intermediate, or the derivatized drug, is subsequently reacted with the antibody or antigen-binding fragment under appropriate conditions. Alternatively, the linker or intermediate may first be reacted with the antibody or a derivatized antibody, and then reacted with the drug or derivatized drug.

[00205] A number of different reactions are available for covalent attachment of drugs and/or linkers to the antibody moiety. This is often accomplished by reaction of one or more amino acid residues of the antibody molecule, including the amine groups of lysine, the free carboxylic acid groups of glutamic acid and aspartic acid, the sulfhydryl groups of cysteine, and the various moieties of the aromatic amino acids. For instance, non-specific covalent attachment may be undertaken using a carbodiimide reaction to link a carboxy (or amino) group on a compound to an amino (or carboxy) group on an antibody moiety. Additionally, bifunctional agents such as dialdehydes or imidoesters

may also be used to link the amino group on a compound to an amino group on an antibody moiety. Also available for attachment of drugs to binding agents is the Schiff base reaction. This method involves the periodate oxidation of a drug that contains glycol or hydroxy groups, thus forming an aldehyde which is then reacted with the binding agent. Attachment occurs via formation of a Schiff base with amino groups of the binding agent. Isothiocyanates may also be used as coupling agents for covalently attaching drugs to binding agents. Other techniques are known to the skilled artisan and within the scope of the present disclosure.

Drug Loading

[00206] Drug loading is represented by *p*, and is also referred to herein as the drug-to-antibody ratio (DAR). Drug loading may range from 1 to 20 drug moieties per antibody moiety. In some embodiments, *p* is an integer from 1 to 20. In some embodiments, *p* is an integer from 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 to 2. In some embodiments, *p* is an integer from 2 to 10, 2 to 9, 2 to 8, 2 to 7, 2 to 6, 2 to 5, 2 to 4, or 2 to 3. In some embodiments, *p* is an integer from 3 to 4. In other embodiments, *p* is 1, 2, 3, 4, 5, or 6, preferably 3 or 4.

[00207] Drug loading may be limited by the number of attachment sites on the antibody moiety. In some embodiments, the linker moiety (L) of the ADC attaches to the antibody moiety through a chemically active group on one or more amino acid residues on the antibody moiety. For example, the linker may be attached to the antibody moiety via a free amino, imino, hydroxyl, thiol, or carboxyl group (e.g., to the N- or C-terminus, to the epsilon amino group of one or more lysine residues, to the free carboxylic acid group of one or more glutamic acid or aspartic acid residues, or to the sulfhydryl group of one or more cysteine residues). The site to which the linker is attached can be a natural residue in the amino acid sequence of the antibody moiety, or it can be introduced into the antibody moiety, e.g., by DNA recombinant technology (e.g., by introducing a cysteine residue into the amino acid sequence) or by protein biochemistry (e.g., by reduction, pH adjustment, or hydrolysis).

[00208] In some embodiments, the number of drug moieties that can be conjugated to an antibody moiety is limited by the number of free cysteine residues. For example, where the attachment is a cysteine thiol group, an antibody may have only one or a few cysteine thiol groups, or may have only one or a few sufficiently reactive thiol groups through which a linker may be attached. Generally, antibodies do not contain many free

and reactive cysteine thiol groups that may be linked to a drug moiety. Indeed, most cysteine thiol residues in antibodies exist as disulfide bridges. Over-attachment of linker-toxin to an antibody may destabilize the antibody by reducing the cysteine residues available to form disulfide bridges. Therefore, an optimal drug:antibody ratio should increase potency of the ADC (by increasing the number of attached drug moieties per antibody) without destabilizing the antibody moiety. In some embodiments, an optimal ratio may be about 3-4.

[00209] In some embodiments, a linker attached to an antibody moiety through a Mal moiety provides a ratio of about 3-4. In some embodiments, a linker attached to an antibody moiety through an alternate moiety (e.g., a OSu moiety) may provide a less optimal ratio (e.g., a lower ratio, such as about 0-3). In some embodiments, a linker comprising a short spacer unit (e.g., a short PEG spacer unit such as $(PEG)_2$ or $(PEG)_4$, or a short alkyl spacer unit such as $(CH_2)_5$) provides a ratio of about 3-4. In some embodiments, a linker that comprises a longer spacer unit (e.g., $(PEG)_8$) may provide a less optimal ratio (e.g., a lower ratio, such as about 0-3). In some embodiments, a linker comprising a peptide cleavable moiety provides a ratio of about 3-4. In some embodiments, a linker that comprises an alternate cleavable moiety (e.g., a cleavable disulfide or a cleavable sulfonamide) may provide a less optimal ratio (e.g., a lower ratio, such as about 0-3). In some embodiments, an ADC comprising Mal-$(PEG)_2$-Val-Cit-pAB-eribulin joined to an antibody such as an anti-FRA antibody (e.g., MORAb-003) has a ratio of about 3-4. In some embodiments, a ratio of about 3-4 is observed with an ADC comprising Mal-$(PEG)_2$-Val-Cit-pAB-eribulin joined to a different antibody, such as an anti-her2 antibody (e.g., trastuzumab). In some embodiments, the optimal ratio observed with ADCs comprising the Mal-$(PEG)_2$-Val-Cit-pAB-eribulin linker-toxin is antibody-independent.

[00210] In some embodiments, an antibody moiety is exposed to reducing conditions prior to conjugation in order to generate one or more free cysteine residues. An antibody, in some embodiments, may be reduced with a reducing agent such as dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine (TCEP), under partial or total reducing conditions, to generate reactive cysteine thiol groups. Unpaired cysteines may be generated through partial reduction with limited molar equivalents of TCEP, which preferentially reduces the interchain disulfide bonds which link the light chain and heavy chain (one pair per H-L pairing) and the two heavy chains in the hinge region

(two pairs per H-H pairing in the case of human IgG1) while leaving the intrachain disulfide bonds intact (Stefano et al. (2013) Methods Mol. Biol. 1045:145-71). In embodiments, disulfide bonds within the antibodies are reduced electrochemically, e.g., by employing a working electrode that applies an alternating reducing and oxidizing voltage. This approach can allow for on-line coupling of disulfide bond reduction to an analytical device (e.g., an electrochemical detection device, an NMR spectrometer, or a mass spectrometer) or a chemical separation device (e.g., a liquid chromatograph (e.g., an HPLC) or an electrophoresis device (see, e.g., U.S. Publ. No. 20140069822)). In certain embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups on amino acid residues, such as lysine or cysteine.

[00211] The drug loading of an ADC may be controlled in different ways, e.g., by: (i) limiting the molar excess of drug-linker intermediate or linker reagent relative to antibody; (ii) limiting the conjugation reaction time or temperature; (iii) partial or limiting reductive conditions for cysteine thiol modification; and/or (iv) engineering by recombinant techniques the amino acid sequence of the antibody such that the number and position of cysteine residues is modified for control of the number and/or position of linker-drug attachments.

[00212] In some embodiments, free cysteine residues are introduced into the amino acid sequence of the antibody moiety. For example, cysteine engineered antibodies can be prepared wherein one or more amino acids of a parent antibody are replaced with a cysteine amino acid. Any form of antibody may be so engineered, i.e. mutated. For example, a parent Fab antibody fragment may be engineered to form a cysteine engineered Fab referred to as a "ThioFab." Similarly, a parent monoclonal antibody may be engineered to form a "ThioMab." A single site mutation yields a single engineered cysteine residue in a ThioFab, whereas a single site mutation yields two engineered cysteine residues in a ThioMab, due to the dimeric nature of the IgG antibody. DNA encoding an amino acid sequence variant of the parent polypeptide can be prepared by a variety of methods known in the art (see, e.g., the methods described in WO2006/034488). These methods include, but are not limited to, preparation by site-directed (or oligonucleotide-mediated) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared DNA encoding the polypeptide. Variants of recombinant antibodies may also be constructed also by restriction fragment manipulation or by overlap extension PCR with synthetic oligonucleotides. ADCs of

Formula I include, but are not limited to, antibodies that have 1, 2, 3, or 4 engineered cysteine amino acids (Lyon et al. (2012) Methods Enzymol. 502:123-38). In some embodiments, one or more free cysteine residues are already present in an antibody moiety, without the use of engineering, in which case the existing free cysteine residues may be used to conjugate the antibody moiety to a drug moiety.

[00213] In some embodiments, higher drug loading (e.g., $p > 5$) may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates. Higher drug loading may also negatively affect the pharmacokinetics (e.g., clearance) of certain ADCs. In some embodiments, lower drug loading (e.g., $p < 3$) may reduce the potency of certain ADCs against target-expressing cells and/or bystander cells. In some embodiments, the drug loading for an ADC of the present disclosure ranges from 1 to about 8; from about 2 to about 6; from about 2 to about 5; from about 3 to about 5; or from about 3 to about 4.

[00214] Where more than one nucleophilic group reacts with a drug-linker intermediate or a linker moiety reagent followed by drug moiety reagent, in a reaction mixture comprising multiple copies of the antibody moiety and linker moiety, then the resulting product can be a mixture of ADC compounds with a distribution of one or more drug moieties attached to each copy of the antibody moiety in the mixture. In some embodiments, the drug loading in a mixture of ADCs resulting from a conjugation reaction ranges from 1 to 20 drug moieties attached per antibody moiety. The average number of drug moieties per antibody moiety (i.e., the average drug loading, or average p) may be calculated by any conventional method known in the art, e.g., by mass spectrometry (e.g., reverse-phase LC-MS), and/or high-performance liquid chromatography (e.g., HIC-HPLC). In some embodiments, the average number of drug moieties per antibody moiety is determined by hydrophobic interaction chromatography-high performance liquid chromatography (HIC-HPLC). In some embodiments, the average number of drug moieties per antibody moiety is determined by reverse-phase liquid chromatography-mass spectrometry (LC-MS). In some embodiments, the average number of drug moieties per antibody moiety is from about 3 to about 4; from about 3.1 to about 3.9; from about 3.2 to about 3.8; from about 3.2 to about 3.7; from about 3.2 to about 3.6; from about 3.3 to about 3.8; or from about 3.3 to about 3.7. In some embodiments, the average number of drug moieties per antibody moiety is from about 3.2 to about 3.8. In some embodiments, the average number of

drug moieties per antibody moiety is about 3.8. In some embodiments, the average number of drug moieties per antibody moiety is from 3 to 4; from 3.1 to 3.9; from 3.2 to 3.8; from 3.2 to 3.7; from 3.2 to 3.6; from 3.3 to 3.8; or from 3.3 to 3.7. In some embodiments, the average number of drug moieties per antibody moiety is from 3.2 to 3.8. In some embodiments, the average number of drug moieties per antibody moiety is 3.8.

[00215] In some embodiments, the average number of drug moieties per antibody moiety is from about 3.5 to about 4.5; from about 3.6 to about 4.4; from about 3.7 to about 4.3; from about 3.7 to about 4.2; or from about 3.8 to about 4.2. In some embodiments, the average number of drug moieties per antibody moiety is from about 3.6 to about 4.4. In some embodiments, the average number of drug moieties per antibody moiety is about 4.0. In some embodiments, the average number of drug moieties per antibody moiety is from 3.5 to 4.5; from 3.6 to 4.4; from 3.7 to 4.3; from 3.7 to 4.2; or from 3.8 to 4.2. In some embodiments, the average number of drug moieties per antibody moiety is from 3.6 to 4.4. In some embodiments, the average number of drug moieties per antibody moiety is 4.0.

[00216] In various embodiments, the term "about" as used with respect to the average number of drug moieties per antibody moiety means +/- 10%.

[00217] Individual ADC compounds, or "species," may be identified in the mixture by mass spectroscopy and separated by UPLC or HPLC, e.g. hydrophobic interaction chromatography (HIC-HPLC). In certain embodiments, a homogeneous or nearly homogenous ADC with a single loading value may be isolated from the conjugation mixture, e.g., by electrophoresis or chromatography.

[00218] In some embodiments, a drug loading and/or an average drug loading of about 4 provides beneficial properties. In some embodiments, a drug loading and/or an average drug loading of less than about 4 may result in an unacceptably high level of unconjugated antibody species, which can compete with the ADC for binding to a target antigen and/or provide for reduced treatment efficacy. In some embodiments, a drug loading and/or average drug loading of more than about 4 may result in an unacceptably high level of product heterogeneity and/or ADC aggregation. A drug loading and/or average drug loading of more than about 4 may also affect stability of the ADC, due to loss of one or more chemical bonds required to stabilize the antibody moiety.

[00219] In some embodiments, an ADC has Formula I:

$$Ab\text{-}(L\text{-}D)_p \qquad (I)$$

wherein:

(i) Ab is an internalizing anti-folate receptor alpha antibody or antigen-binding fragment thereof comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:23, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:24;

(ii) D is eribulin;

(iii) L is a cleavable linker comprising Mal-$(PEG)_2$-Val-Cit-pAB; and

(iv) *p* is an integer from 3 to 4.

[00220] In other embodiments, an ADC has Formula I:

$$Ab\text{-}(L\text{-}D)_p \qquad (I)$$

wherein:

(i) Ab is an internalizing anti-human epidermal growth factor receptor 2 antibody or antigen-binding fragment thereof comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:27, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:28;

(ii) D is eribulin;

(iii) L is a cleavable linker comprising Mal-$(PEG)_2$-Val-Cit-pAB; and

(iv) *p* is an integer from 3 to 4.

[00221] In some embodiments, *p* is 4.

[00222] The present disclosure includes methods of producing the described ADCs. Briefly, the ADCs comprise an antibody or antigen-binding fragment as the antibody moiety, a drug moiety, and a linker that joins the drug moiety and the antibody moiety. In some embodiments, the ADCs can be prepared using a linker having reactive functionalities for covalently attaching to the drug moiety and to the antibody moiety. For example, in some embodiments, a cysteine thiol of an antibody moiety can form a bond with a reactive functional group of a linker or a drug-linker intermediate (e.g., a maleimide moiety) to make an ADC. The generation of the ADCs can be accomplished by any technique known to the skilled artisan.

[00223] In some embodiments, an ADC is produced by contacting an antibody moiety with a linker and a drug moiety in a sequential manner, such that the antibody moiety is covalently linked to the linker first, and then the pre-formed antibody-linker intermediate reacts with the drug moiety. The antibody-linker intermediate may or may not be subjected to a purification step prior to contacting the drug moiety. In other embodiments, an ADC is produced by contacting an antibody moiety with a linker drug compound pre-formed by reacting a linker with a drug moiety. The pre-formed linker-drug compound may or may not be subjected to a purification step prior to contacting the antibody moiety. In other embodiments, the antibody moiety contacts the linker and the drug moiety in one reaction mixture, allowing simultaneous formation of the covalent bonds between the antibody moiety and the linker, and between the linker and the drug moiety. This method of producing ADCs may include a reaction, wherein the antibody moiety contacts the antibody moiety prior to the addition of the linker to the reaction mixture, and vice versa. In certain embodiments, an ADC is produced by reacting an antibody moiety with a linker joined to a drug moiety, such as Mal-$(PEG)_2$-Val-Cit-pAB-eribulin, under conditions that allow conjugation.

[00224] The ADCs prepared according to the methods described above may be subjected to a purification step. The purification step may involve any biochemical methods known in the art for purifying proteins, or any combination of methods thereof. These include, but are not limited to, tangential flow filtration (TFF), affinity chromatography, ion exchange chromatography, any charge or isoelectric point-based chromatography, mixed mode chromatography, e.g., CHT (ceramic hydroxyapatite), hydrophobic interaction chromatography, size exclusion chromatography, dialysis, filtration, selective precipitation, or any combination thereof.

Therapeutic Uses and Compositions

[00225] Disclosed herein are methods of using the disclosed ADCs in treating a subject for a disorder, e.g., an oncologic disorder. ADCs may be administered alone or in combination with a second therapeutic agent, and may be administered in any pharmaceutically acceptable formulation, dosage, and dosing regimen. ADC treatment efficacy may be evaluated for toxicity as well as indicators of efficacy and adjusted accordingly. Efficacy measures include, but are not limited to, a cytostatic and/or cytotoxic effect observed *in vitro* or *in vivo*, reduced tumor volume, tumor growth inhibition, and/or prolonged survival.

[00226] Methods of determining whether an ADC exerts a cytostatic and/or cytotoxic effect on a cell are known. For example, the cytotoxic or cytostatic activity of an ADC can be measured by: exposing mammalian cells expressing a target protein of the ADC in a cell culture medium; culturing the cells for a period from about 6 hours to about 5 days; and measuring cell viability. Cell-based *in vitro* assays may also be used to measure viability (proliferation), cytotoxicity, and induction of apoptosis (caspase activation) of the ADC.

[00227] For determining whether an antibody-drug conjugate exerts a cytostatic effect, a thymidine incorporation assay may be used. For example, cancer cells expressing a target antigen at a density of 5,000 cells/well of a 96-well plated can be cultured for a 72-hour period and exposed to 0.5 μCi of ^{3}H-thymidine during the final 8 hours of the 72-hour period. The incorporation of ^{3}H-thymidine into cells of the culture is measured in the presence and absence of the ADC.

[00228] For determining cytotoxicity, necrosis or apoptosis (programmed cell death) may be measured. Necrosis is typically accompanied by increased permeability of the plasma membrane; swelling of the cell, and rupture of the plasma membrane. Apoptosis is typically characterized by membrane blebbing, condensation of cytoplasm, and the activation of endogenous endonucleases. Determination of any of these effects on cancer cells indicates that an ADC is useful in the treatment of cancers.

[00229] Cell viability may be measured, e.g., by determining in a cell the uptake of a dye such as neutral red, trypan blue, Crystal Violet, or ALAMAR™ blue (see, e.g., Page et al. (1993) Intl. J. Oncology 3:473-6). In such an assay, the cells are incubated in media containing the dye, the cells are washed, and the remaining dye, reflecting cellular uptake of the dye, is measured spectrophotometrically. In certain embodiments,

in vitro potency of prepared ADCs is assessed using a Crystal Violet assay. Crystal Violet is a triarylmethane dye that accumulates in the nucleus of viable cells. In this assay, cells are exposed to the ADCs or control agents for a defined period of time, after which, cells are stained with crystal violet, washed copiously with water, then solubilized with 1% SDS and read spectrophotometrically. The protein-binding dye sulforhodamine B (SRB) can also be used to measure cytoxicity (Skehan et al. (1990) J. Natl. Cancer Inst. 82:1107-12).

[00230] Apoptosis can be quantitated, for example, by measuring DNA fragmentation. Commercial photometric methods for the quantitative *in vitro* determination of DNA fragmentation are available. Examples of such assays, including TUNEL (which detects incorporation of labeled nucleotides in fragmented DNA) and ELISA-based assays, are described in Biochemica (1999) No. 2, pp. 34-37 (Roche Molecular Biochemicals).

[00231] Apoptosis may also be determined by measuring morphological changes in a cell. For example, as with necrosis, loss of plasma membrane integrity can be determined by measuring uptake of certain dyes (e.g., a fluorescent dye such as, for example, acridine orange or ethidium bromide). A method for measuring apoptotic cell number has been described by Duke and Cohen, Current Protocols in Immunology (Coligan et al., eds. (1992) pp. 3.17.1-3.17.16). Cells also can be labeled with a DNA dye (e.g., acridine orange, ethidium bromide, or propidium iodide) and the cells observed for chromatin condensation and margination along the inner nuclear membrane. Other morphological changes that can be measured to determine apoptosis include, e.g., cytoplasmic condensation, increased membrane blebbing, and cellular shrinkage.

[00232] The disclosed ADCs may also be evaluated for bystander killing activity. Bystander killing activity may be determined, e.g., by an assay employing two cell lines, one positive for target antigen and one negative for target antigen. The cell lines are preferably labeled to differentiate them. For example, IGROV1 cells (FRA+) labeled with Nuclight™ Green (NLG) and HL-60 (FRA-) labeled with Nuclight™ Red (NLR) may be co-cultured, treated with an anti-FRA ADC followed by monitoring of cytotoxicity. Killing of the target antigen negative cells when mixed with target antigen positive cells is indicative of bystander killing, whereas killing of the target antigen

negative cells in the absence of the target antigen positive cells is indicative of off-target killing.

[00233] In some aspects, the present disclosure features a method of killing, inhibiting or modulating the growth of, or interfering with the metabolism of, a cancer cell or tissue by disrupting tubulin. The method may be used with any subject where disruption of tubulin provides a therapeutic benefit. Subjects that may benefit from disrupting tubulin include, but are not limited to, those having or at risk of having a gastric cancer, ovarian cancer (e.g., serous ovarian cancer), lung cancer (e.g., non-small cell lung cancer), breast cancer (e.g., triple negative breast cancer), endometrial cancer (e.g., serous endometrial carcinoma), osteosarcoma, Kaposi's sarcoma, testicular germ cell cancer, leukemia, lymphoma (e.g., Hodgkin's disease, non-Hodgkin's lymphoma), myeloma, head and neck cancer, esophageal cancer, pancreatic cancer, prostate cancer, brain cancer (e.g., glioblastoma), thyroid cancer, colorectal cancer, and/or skin cancer (e.g., melanoma), or any metastases thereof (Dumontet and Jordan (2010) Nat. Rev. Drug Discov. 9:790-803). In various embodiments, the disclosed ADCs may be administered in any cell or tissue that expresses FRA, such as an FRA-expressing cancer cell or tissue. An exemplary embodiment includes a method of inhibiting FRA-mediated cell signaling or a method of killing a cell. The method may be used with any cell or tissue that expresses FRA, such as a cancerous cell or a metastatic lesion. Non-limiting examples of FRA-expressing cancers include gastric cancer, serous ovarian cancer, clear cell ovarian cancer, non-small cell lung cancer, colorectal cancer, triple negative breast cancer, endometrial cancer, serous endometrial carcinoma, lung carcinoid, and osteosarcoma. Non-limiting examples of FRA-expressing cells include IGROV1 and OVCAR3 human ovarian carcinoma cells, NCI-H2110 human non-small cell lung carcinoma cells, and cells comprising a recombinant nucleic acid encoding FRA or a portion thereof.

[00234] In various other embodiments, the disclosed ADCs may be administered in any cell or tissue that expresses her2, such as a her2-expressing cancer cell or tissue. An exemplary embodiment includes a method of inhibiting her2-mediated cell signaling or a method of killing a cell. The method may be used with any cell or tissue that expresses her2, such as a cancerous cell or a metastatic lesion. Non-limiting examples of her2-expressing cancers include breast cancer, gastric cancer, bladder cancer, urothelial cell carcinoma, esophageal cancer, lung cancer, cervical cancer, endometrial

cancer, and ovarian cancer (English et al. (2013) Mol. Diagn. Ther. 17:85-99). Non-limiting examples of her2-expressing cells include NCI-N87-luc human gastric carcinoma cells, ZR75 and BT-474 human breast ductal carcinoma cells, and cells comprising a recombinant nucleic acid encoding her2 or a portion thereof.

[00235] In various other embodiments, the disclosed ADCs may be administered in any cell or tissue that expresses mesothelin (MSLN), such as a MSLN-expressing cancer cell or tissue. An exemplary embodiment includes a method of inhibiting MSLN-mediated cell signaling or a method of killing a cell. The method may be used with any cell or tissue that expresses MSLN, such as a cancerous cell or a metastatic lesion. Non-limiting examples of MSLN-expressing cancers include mesothelioma, pancreatic cancer (e.g., pancreatic adenocarcinoma), ovarian cancer, and lung cancer (e.g., lung adenocarcinoma) (Wang et al. (2012) PLoS ONE 7:e33214). Non-limiting examples of MSLN-expressing cells include OVCAR3 human ovarian carcinoma cells, HEC-251 human endometroid cells, H226 human lung squamous cell mesothelioma cells, and cells comprising a recombinant nucleic acid encoding MSLN or a portion thereof.

[00236] Exemplary methods include the steps of contacting the cell with an ADC, as described herein, in an effective amount, i.e., amount sufficient to kill the cell. The method can be used on cells in culture, e.g. *in vitro, in vivo, ex vivo,* or *in situ.* For example, cells that express FRA, her2, and/or MSLN (e.g., cells collected by biopsy of a tumor or metastatic lesion; cells from an established cancer cell line; or recombinant cells), can be cultured *in vitro* in culture medium and the contacting step can be effected by adding the ADC to the culture medium. The method will result in killing of cells expressing FRA, her2, and/or MSLN, including in particular tumor cells expressing FRA, her2, and/or MSLN. Alternatively, the ADC can be administered to a subject by any suitable administration route (e.g., intravenous, subcutaneous, or direct contact with a tumor tissue) to have an effect *in vivo.*

[00237] The *in vivo* effect of a disclosed ADC therapeutic composition can be evaluated in a suitable animal model. For example, xenogenic cancer models can be used, wherein cancer explants or passaged xenograft tissues are introduced into immune compromised animals, such as nude or SCID mice (Klein et al. (1997) Nature Med. 3:402-8). Efficacy may be predicted using assays that measure inhibition of tumor formation, tumor regression or metastasis, and the like.

[00238] *In vivo* assays that evaluate the promotion of apoptosis may also be used. In one embodiment, xenografts from tumor bearing mice treated with the therapeutic composition can be examined for the presence of apoptotic foci and compared to untreated control xenograft-bearing mice. The extent to which apoptotic foci are found in the tumors of the treated mice provides an indication of the therapeutic efficacy of the composition.

[00239] Further provided herein are methods of treating cancer. The ADCs disclosed herein can be administered to a non-human mammal or human subject for therapeutic purposes. The therapeutic methods entail administering to a mammal having a tumor a biologically effective amount of an ADC comprising a selected chemotherapeutic agent (e.g., eribulin) linked to a targeting antibody that binds to an antigen expressed, that is accessible to binding, or is localized on a cancer cell surface. An exemplary embodiment is a method of delivering a chemotherapeutic agent to a cell expressing FRA, comprising conjugating the chemotherapeutic agent to an antibody that immunospecifically binds to an FRA epitope and exposing the cell to the ADC. Exemplary tumor cells that express FRA for which the ADCs of the present disclosure are indicated include cells from a gastric cancer, a serous ovarian cancer, a nonsmall cell lung cancer, a colorectal cancer, a breast cancer (e.g., a triple negative breast cancer), a lung carcinoid, an osteosarcoma, an endometrial cancer, and an endometrial carcinoma with serous histology.

[00240] Another exemplary embodiment is a method of delivering a chemotherapeutic agent to a cell expressing her2, comprising conjugating the chemotherapeutic agent to an antibody that immunospecifically binds to a her2 epitope and exposing the cell to the ADC. Exemplary tumor cells that express her2 for which the ADCs of the present disclosure are indicated include cells from a breast cancer, a gastric cancer, a bladder cancer, an urothelial cell carcinoma, an esophageal cancer, a lung cancer, a cervical cancer, an endometrial cancer, and an ovarian cancer.

[00241] Another exemplary embodiment is a method of delivering a chemotherapeutic agent to a cell expressing MSLN, comprising conjugating the chemotherapeutic agent to an antibody that immunospecifically binds to a MSLN epitope and exposing the cell to the ADC. Exemplary tumor cells that express MSLN for which the ADCs of the present disclosure are indicated include cells from a

mesothelioma, a pancreatic cancer (e.g., an pancreatic adenocarcinoma), an ovarian cancer, and a lung cancer (e.g., lung adenocarcinoma).

[00242] Another exemplary embodiment is a method of treating a patient having or at risk of having a cancer that expresses a target antigen for the antibody moiety of the ADC, such as FRA, her2, or MSLN, comprising administering to the patient a therapeutically effective amount of an ADC of the present disclosure. In some embodiments, the patient is non-responsive or poorly responsive to treatment with an anti-FRA antibody when administered alone, and/or treatment with a drug moiety (e.g., eribulin) when administered alone. In other embodiments, the patient is non-responsive or poorly responsive to treatment with an anti-her2 antibody when administered alone, and/or treatment with a drug moiety (e.g., eribulin) when administered alone. In other embodiments, the patient is non-responsive or poorly responsive to treatment with an anti-MSLN antibody when administered alone, and/or treatment with a drug moiety (e.g., eribulin) when administered alone. In other embodiments, the patient is intolerant to treatment with a drug moiety (e.g., eribulin) when administered alone. For instance, a patient may require doses of eribulin to treat a cancer that lead to systemic toxicity, which are overcome by targeted delivery to a cancer expressing a target antigen for the antibody moiety of the ADC such as FRA, her2, or MSLN, thereby reducing off-target killing.

[00243] Another exemplary embodiment is a method of reducing or inhibiting growth of an target antigen-expressing tumor (e.g., an FRA-expressing tumor, a her2-expressing tumor, or a MSLN-expressing tumor), comprising administering a therapeutically effective amount of an ADC. In some embodiments, the treatment is sufficient to reduce or inhibit the growth of the patient's tumor, reduce the number or size of metastatic lesions, reduce tumor load, reduce primary tumor load, reduce invasiveness, prolong survival time, and/or maintain or improve the quality of life. In some embodiments, the tumor is resistant or refractory to treatment with an anti-FRA antibody when administered alone, and/or treatment with a drug moiety (e.g., eribulin) when administered alone. In other embodiments, the tumor is resistant or refractory to treatment with an anti-her2 antibody when administered alone, and/or treatment with a drug moiety (e.g., eribulin) when administered alone. In some embodiments, the tumor is resistant or refractory to treatment with an anti-MSLN antibody when administered alone, and/or treatment with a drug moiety (e.g., eribulin) when administered alone.

[00244] Moreover, antibodies of the present disclosure may be administered to a non-human mammal expressing an antigen with which the ADC is capable of binding for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of the disclosed ADCs (e.g., testing of dosages and time courses of administration).

[00245] Further provided herein are therapeutic uses of the disclosed ADCs. An exemplary embodiment is the use of an ADC in the treatment of a target antigen-expressing cancer (e.g., an FRA-expressing cancer, a her2-expressing cancer, or a MSLN-expressing cancer). ADCs for use in the treatment of an target antigen-expressing cancer (e.g., an FRA-expressing cancer, a her2-expressing cancer, or a MSLN-expressing cancer) are also disclosed. Methods for identifying subjects having cancers that express FRA, her2, and/or MSLN are known in the art and may be used to identify suitable patients for treatment with a disclosed ADC.

[00246] Another exemplary embodiment is the use of an ADC in a method of manufacturing a medicament for the treatment of a target antigen-expressing cancer (e.g., an FRA-expressing cancer, a her2-expressing cancer, or a MSLN-expressing cancer).

[00247] The therapeutic compositions used in the practice of the foregoing methods may be formulated into pharmaceutical compositions comprising a pharmaceutically acceptable carrier suitable for the desired delivery method. An exemplary embodiment is a pharmaceutical composition comprising an ADC of the present disclosure and a pharmaceutically acceptable carrier. Suitable carriers include any material that, when combined with the therapeutic composition, retains the anti-tumor function of the therapeutic composition and is generally non-reactive with the patient's immune system. Pharmaceutically acceptable carriers include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol, mesylate salt, and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary

substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the ADC.

[00248] Therapeutic formulations may be solubilized and administered via any route capable of delivering the therapeutic composition to the tumor site. Potentially effective routes of administration include, but are not limited to, intravenous, parenteral, intraperitoneal, intramuscular, intratumor, intradermal, intraorgan, orthotopic, and the like. Therapeutic protein preparations can be lyophilized and stored as sterile powders, preferably under vacuum, and then reconstituted in bacteriostatic water (containing for example, benzyl alcohol preservative) or in sterile water prior to injection. Therapeutic formulations may comprise an ADC or a pharmaceutically acceptable salt thereof, e.g., a mesylate salt.

[00249] The ADCs disclosed herein may be administered at a dosage ranging from about 0.2 mg/kg to about 10 mg/kg to a patient in need thereof. In some embodiments, the ADC is administered to the patient daily, bimonthly, or any time period in between. Dosages and administration protocols for the treatment of cancers using the foregoing methods will vary with the method and the target cancer, and will generally depend on a number of other factors appreciated in the art.

[00250] Various delivery systems are known and may be used to administer one or more ADCs of the present disclosure. Methods of administering the ADCs include, but are not limited to, parenteral administration (e.g., intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural administration, intratumoral administration, and mucosal administration (e.g., intranasal and oral routes). In addition, pulmonary administration may be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. *See, e.g.*, the compositions and methods for pulmonary administration described in U.S. Pat. Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publ. Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903. The ADCs may be administered by any convenient route, for example, by infusion or bolus injection, or by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.). Administration can be either systemic or local.

[00251] Therapeutic compositions disclosed herein may be sterile and stable under the conditions of manufacture and storage. In some embodiments, one or more of the

ADCs, or pharmaceutical compositions, is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted (e.g., with water or saline) to the appropriate concentration for administration to a subject. Preferably, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, at least 75 mg, or at least 100 mg, or any amount in between. In some embodiments, the lyophilized ADCs or pharmaceutical compositions is stored at between 2°C and 8°C in the original container. In some embodiments, one or more of the ADCs or pharmaceutical compositions described herein is supplied in liquid form in a hermetically sealed container, e.g., a container indicating the quantity and concentration of the agent. In some embodiments, the liquid form of the administered composition is supplied in a hermetically sealed container of at least 0.25 mg/mL, at least 0.5 mg/mL, at least 1 mg/mL, at least 2.5 mg/mL, at least 5 mg/mL, at least 8 mg/mL, at least 10 mg/mL, at least 15 mg/mL, at least 25 mg/mL, at least 50 mg/mL, at least 75 mg/mL, or at least 100 mg/mL ADC. The liquid form may be stored at between 2°C and 8°C in the original container.

[00252] In some embodiments, the disclosed ADCs can be incorporated into a pharmaceutical composition suitable for parenteral administration. The injectable solution may be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampule, or pre-filled syringe, or other known delivery or storage device.

[00253] The compositions described herein may be in a variety of forms. These include, for example, liquid, semi-solid, and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes, and suppositories. The preferred form depends on the intended mode of administration and therapeutic application.

[00254] In various embodiments, treatment involves single bolus or repeated administration of the ADC preparation via an acceptable route of administration.

[00255] Patients may be evaluated for the levels of target antigen in a given sample (e.g. the levels of target antigen expressing cells) in order to assist in determining the most effective dosing regimen, etc. An exemplary embodiment is a method of determining whether a patient will be responsive to treatment with an ADC of the

present disclosure, comprising providing a biological sample from the patient and contacting the biological sample with the ADC. Exemplary biological samples include tissue or body fluid, such as an inflammatory exudate, blood, serum, bowel fluid, stool sample, or tumor biopsy (e.g., a tumor biopsy derived from a patient having or at risk of a target antigen-expressing cancer, e.g., an FRA-expressing cancer, a her2-expressing cancer, or a MSLN-expressing cancer). In some embodiments, a sample (e.g., a tissue and/or body fluid) can be obtained from a subject, and a suitable immunological method can be used to detect and/or measure protein expression of the target antigen (e.g., FRA, her2, or MSLN). Such evaluations are also used for monitoring purposes throughout therapy, and are useful to gauge therapeutic success in combination with the evaluation of other parameters.

[00256] In some embodiments, the efficacy of an ADC may be evaluated by contacting a tumor sample from a subject with the ADC and evaluating tumor growth rate or volume. In some embodiments, when an ADC has been determined to be effective, it may be administered to the subject.

[00257] The above therapeutic approaches can be combined with any one of a wide variety of additional surgical, chemotherapy, or radiation therapy regimens.

[00258] Also disclosed herein are uses of one or more of the disclosed ADCs in the manufacture of a medicament for treating cancer, e.g., according to the methods described above. In some embodiments, the ADCs disclosed herein are used for treating cancer, e.g., according to the methods described above.

[00259] In various embodiments, kits for use in the laboratory and therapeutic applications described herein are within the scope of the present disclosure. Such kits may comprise a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method disclosed herein, along with a label or insert comprising instructions for use, such as a use described herein. Kits may comprise a container comprising a drug moiety. The present disclosure also provides one or more of the ADCs, or pharmaceutical compositions thereof, packaged in a hermetically sealed container, such as an ampoule or sachette, indicating the quantity of the agent.

[00260] Kits may comprise the container described above and one or more other containers associated therewith that comprise materials desirable from a commercial

and user standpoint, including buffers, diluents, filters, needles, syringes; carrier, package, container, vial and/or tube labels listing contents and/or instructions for use, and package inserts with instructions for use.

[00261] A label may be present on or with the container to indicate that the composition is used for a specific therapy or non-therapeutic application, such as a prognostic, prophylactic, diagnostic, or laboratory application. A label may also indicate directions for either *in vivo* or *in vitro* use, such as those described herein. Directions and or other information may also be included on an insert(s) or label(s), which is included with or on the kit. The label may be on or associated with the container. A label may be on a container when letters, numbers, or other characters forming the label are molded or etched into the container itself. A label may be associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. The label may indicate that the composition is used for diagnosing or treating a condition, such as a cancer a described herein.

[00262] It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the invention described herein are obvious and may be made using suitable equivalents without departing from the scope of the invention or the embodiments disclosed herein. Having now described the invention in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting.

EXAMPLE 1

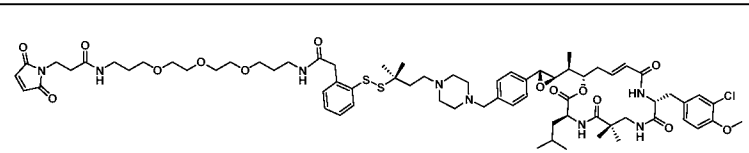
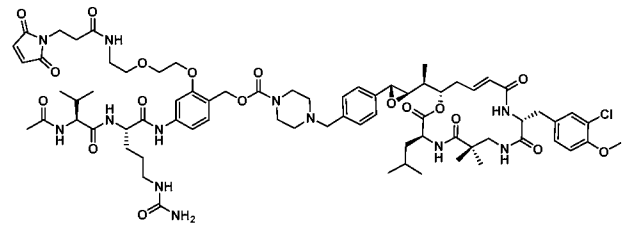
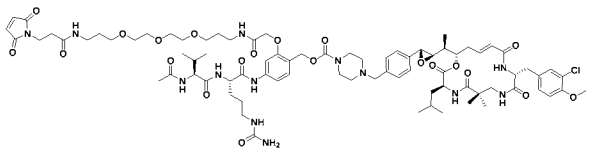
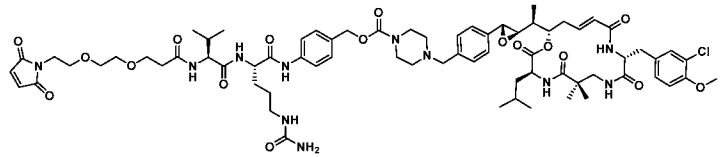
1. Materials and Methods

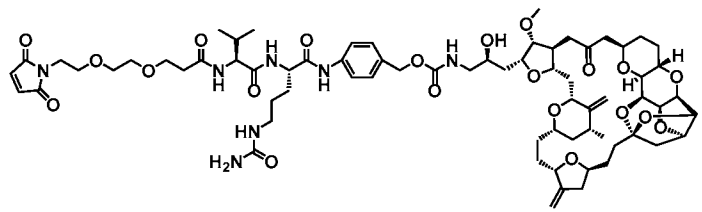
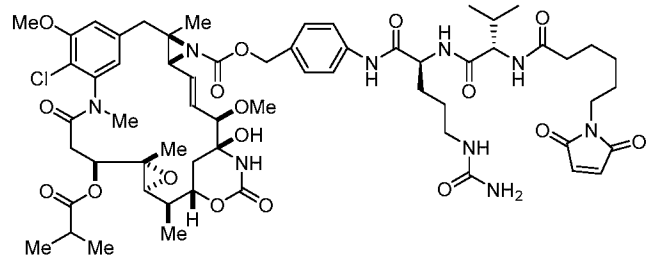
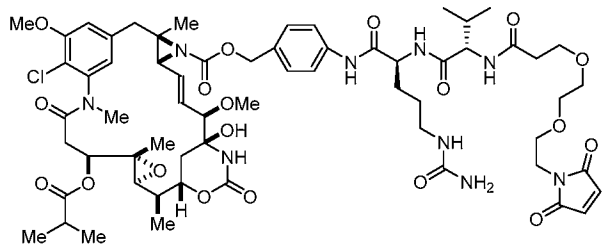
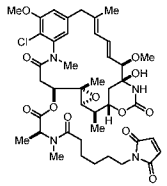
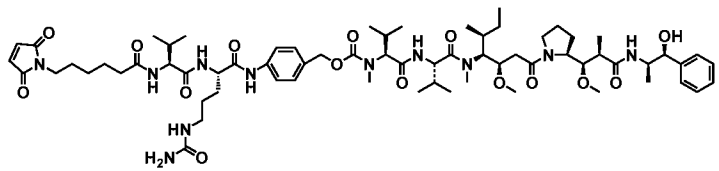
[00263] MORAb-003 used for the preparation of ADCs was from Lot #AA0312.

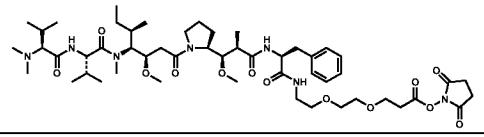
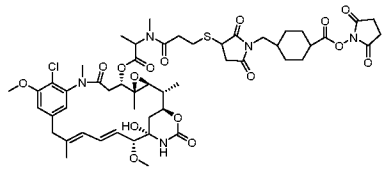
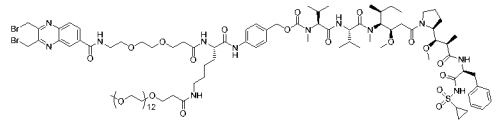
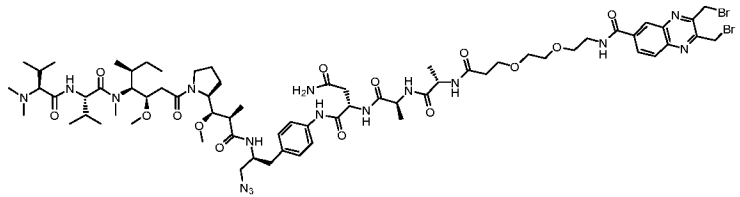
1.1 Cytotoxins

[00264] Structures of conjugatable cytotoxins are shown in Table 11.

Table 11. Conjugatable cytotoxins

Compound name	Linker	Cytotoxin	Cleavability	Structure
PEG3-Bz-disulfidyl-dimethyl-cryptophycin	maleimido-PEG3-Benzyl-disulfidyl-dimethyl	cryptophycin	yes	
LL2-cryptophycin	LL2	cryptophycin	yes	
LL3-cryptophycin	LL3	cryptophycin	yes	
VCP-cryptophycin	maleimido-PEG2-Val-Cit-pAB	cryptophycin	yes	

VCP-eribulin (ER-001159569)	maleimido-PEG2-Val-Cit-pAB	eribulin	yes	
ER-001161318	maleimido-$(CH_2)_5$-Val-Cit-pAB	ER-001150828 (aziridino-maytanzine-P3)	yes	
ER-001161319	maleimido-PEG2-Val-Cit-pAB	ER-001150828 (aziridino-maytanzine-P3)	yes	
ER-001159200	maleimido-$(CH_2)_5$	maytanzine DM1	No	
M-MMAE	maleimido-$(CH_2)_5$-Val-Cit-pAB	monomethyl auristatin E	yes	

NHS-PEG2-AuF	NHS-PEG2	auristatin F	no	
M-DM1	SMCC	maytansine DM1	no	
M-0285	PEG-pAB	duostatin 3	yes	Reduced disulfide bridging chemistry 
M-0115	Asn-Ala	duostatin-5	yes	
M-172	cyclohexyl	duostatin 3	no	Reduced disulfide linking chemistry
M-174	cyclohexyl	duostatin 3	no	Reduced disulfide linking chemistry
M-158	PEG-pAB	duostatin 10	yes	Reduced disulfide linking chemistry
M-0384	PEG-thioether	duostatin 14	no	Reduced disulfide linking chemistry
M-0302	PEG-Asn	duostatin 14	no	Reduced disulfide linking chemistry
M-292	PEG-Asn	duostatin 14	yes	Reduced disulfide linking chemistry
M-0026	PEG	duostatin 14	yes	Reduced disulfide linking chemistry

M-0267	PEG-thioether	duomycin 7	no	Reduced disulfide linking chemistry
M-0272	Asn-Ala	duomycin 7	yes	Reduced disulfide linking chemistry
M-0260	PEG-pAB	duomycin 7	yes	Reduced disulfide linking chemistry
M-0276	Asn-Ala	duomycin 7	yes	Reduced disulfide linking chemistry
M-015-0913	cyclohexyl	duostatin 3	no	Limited lysine utilization
M-030-0132	PEG-pAB	duostatin 6	yes	Limited lysine utilization
M-0161	cyclohexyl	duostatin 10	no	Limited lysine utilization
M-0157	PEG-pAB	duostatin 10	yes	Limited lysine utilization
M-027-0381	thioether	duostatin 14	no	Limited lysine utilization
M-0025	PEG	duostatin 14	no	Limited lysine utilization
M-0301	PEG-Asn	duostatin 14	no	Limited lysine utilization
M-030-0011	PEG-pAB	duostatin 14	yes	Limited lysine utilization
M-030-0291	PEG-Asn	duostatin 14	yes	Limited lysine utilization
M-0114	PEG-pAB	duostatin-5	yes	Reduced disulfide bridging chemistry

Abbreviations: Ala, alanine; Asn, asparagine; Cit, citrulline; NHS, N-hydroxysuccinimide; pAB, p-aminobenzyloxycarbonyl; PEG, polyethylene glycol; SMCC, succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate; Val, valine; VCP, Val-Cit-pAB.

1.2 Antibody-drug conjugation

1.2.1 Partial reduction using TCEP

[00265] Partial reduction conditions for MORAb-003 were established by varying concentration of the non-thiol reducing agent tris(2-carboxyethyl)phosphine (TCEP), antibody concentration, and time of reduction. MORAb-003 was buffer-exchanged into Dulbecco's Phosphate-Buffered Saline (DPBS) containing 1 mM ethylenediaminetetraacetic acid (EDTA), then concentrated to 10 mg/mLusing centrifugal concentration with 10 kD molecular weight cut-off (MWCO) centrifugal filters. Antibodies were diluted to the appropriate concentration and TCEP was added at the indicated final concentration, and gently mixed for 1 hour at room temperature. TCEP was removed by desalting using 5 or 10 mL Zeba™ spin desalting columns with DPBS/1mM EDTA as buffer (Thermo Fisher, 40 kD MWCO), according to the manufacturer's protocol. Samples were analyzed for free thiol content using the Thiol fluorometric quantification kit (Abcam), according to the manufacturer's protocol. SDS-PAGE analysis under non-reducing conditions was performed to determine extent and location of disulfide bond breakage, as described in section 1.3.3. In some cases, desalted MAbs were brought to 1-2 mg/mL by dilution in DPBS and subjected to biotinylation to determine conjugatability and drug-to-antibody (DAR) ratio. 10 mM maleimido-PEG2-biotin (Thermo Fisher) in dimethylsulfoxide (DMSO) was added to antibody (mAb) at a molar ratio of 10:1 and incubated at room temperature for 4 hours with gentle agitation. Following conjugation, unreacted compound was removed by desalting using Zeba™ spin desalting columns (Thermo Fisher). Samples were then analyzed by LC-MS for determination of DAR, as detailed in section 1.3.4.

1.2.2 Cytotoxin conjugation

[00266] Partially-reduced antibody was brought to 2.5 mg/mL in 0.5X DPBS, 0.5 mM EDTA, and mixed thoroughly. Organic co-solvents, if used, were then added and mixed thoroughly. Co-solvents examined were propylene glycol (20% and 50% final concentration), dimethylsulfoxide (DMSO) (10%), *N,N*-dimethylformamide (20%), *N,N*-dimethylacetamide (20%), and *N,N*-dimethylpropionamide (20%). Maleimido-modified cytotoxin (6 mM stock in DMSO) was added to antibodies at a molar ratio of 1:6 (mAb:compound) and mixed thoroughly. Conjugation proceeded at room

temperature for 3.5 hours, with gentle mixing. 50% propylene glycol at 50% was chosen as the final organic modifier and was used in all subsequent conjugation reactions.

1.2.3 Purification

[00267] Conjugated antibody was purified using 26/10 HiTrap® desalting column(s) (GE Healthcare) with chromatography performed on a fast protein liquid chromatography (FPLC) (GE Healthcare), in order to remove unreacted maleimido-cytotoxin and propylene glycol. MORAb-003 ADCs, including MORAb-003-mal-VCP-eribulin (MORAb-202), were formulated in DPBS (formulation buffer was used as running buffer during FPLC chromatography).

1.3 Biophysical characterization

1.3.1 BCA assay

[00268] Prepared bicinchoninic acid (BCA) reagent (200 µL) was added to 25 µL of serially-diluted ADCs or bovine gamma globin (Thermo Fisher) 2 mg/mL standard, and samples were mixed thoroughly. Samples were incubated at 37°C for 20 min. Plates were read at 595 nm on a SpectraMax® M5 plate reader (Molecular Devices). Data was analyzed using SoftMax® Pro (ver 3.2) with a 4-parameter fitting model.

1.3.2 SEC-HPLC analysis

[00269] The antibody aggregation was analyzed by size-exclusion, high-performance liquid chromatography (SEC-HPLC) using an Agilent 1100. The mAb was diluted to 1 mg/mL in DPBS. The antibody (20 µL) was injected onto a TSKgel® SuperSW guard column (4.6 mm x 3.5 cm, 4 µm pore size, Tosoh Bioscience), followed by a TSKgel® SuperSW3000 column (4.6 mm x 30 cm, 4 µm pore size), eluted from the column with 0.1 M sodium phosphate containing 0.15 M NaCl and 0.05% NaN_3, at pH 7.4, at a flow rate of 0.3 mL/min for 20 min. All data were analyzed using Agilent ChemStation software. Percent aggregation was calculated as $[PA_{aggregate}/PA_{total}]*100$, where PA = integrated peak area.

1.3.3 SDS-PAGE analysis

[00270] Protein samples (0.1-10 μg) were brought to 1X with lithium dodecylsulfate (LDS) sample buffer. For non-reduced samples, incubation was performed at room temperature for 10 min prior to electrophoresis. For reduced samples, dithiothreitol (DTT) was added to a final concentration of 20 mM and samples were heated to 95°C for 10 min and placed on ice prior to electrophoresis. Samples were loaded on to 10-, 12-, or 15-well Bis-Tris SDS-PAGE gels (Thermo Fisher) with 1X MOPS or 1X MES as running buffer. Electrophoresis was performed at 185 V (constant voltage) for 1 hour. Gels were stained with InstantBlue staining solution (Expedeon) and destained in water. Documentation was performed on an UltraLum gel documentation system using 600 nm orange filters.

1.3.4 UPLC/ESI-MS analysis of drug-to-antibody ratio (DAR)

[00271] ADCs were deglycosylated using PNGase F (New England BioLabs). G7 buffer (10 μL) and PNGase F (2 μL) were added to the mAb (90 μL, 1 mg/mL in DPBS). The reaction was incubated in a Discover microwave (CEM) for 2 cycles: (1) microwave power 10 W, 37°C, 10 min, followed by a 5-min pause; (2) microwave power 2 W, 37°C, 10 min. A portion of the sample was reduced by adding DTT to a final concentration of 20 mM, followed by incubation at 60°C for 3 min. Samples were then analyzed using a Waters Acquity Ultra Performance Liquid Chromatography (UPLC) and quadrupole time of flight (Q-Tof) Premier mass spectrometer. Samples (0.5-2 μg each) were injected onto a MassPrep™ micro desalting column at 65°C, eluted from the column with a 5 min equilibration in 95% of mobile phase A, a 10 min gradient (5-90% B), and a 10 min re-equilibration in 95% of mobile phase A, at 0.05 mL/min. Mobile phase A was 0.1% formic acid in water. Mobile phase B was 0.1% formic acid in acetonitrile. The Q-Tof mass spectrometer was run in positive ion, V-mode with detection in the range of 500-4000 m/z. The source parameters were as follows: capillary voltage, 2.25 kV (intact antibody)-2.50 kV (reduced antibody); sampling cone voltage, 65.0 V (intact antibody) or 50.0 V (reduced antibody); source temperature, 100°C; desolvation temperature, 250°C; desolvation gas flow, 550 L/hr. The protein peak was deconvoluted using the MassLynx® MaxEnt 1 function. Relative intensities of each unconjugated, singly-conjugated, and multiply-conjugated heavy and light chain masses were combined to calculate the overall DAR using the formula:

$$2\left[\left[I_{LC+1} + 2(I_{LC+2}) + 3(I_{LC+3}) + \ldots n(I_{LC+n})\right]/\Sigma I_{LC}tot\right] + 2\left[\left[I_{HC+1} + 2(I_{HC+2}) + 3(I_{HC+3}) + \ldots n(I_{HC+n})\right]/\Sigma I_{HC}tot\right]$$

where I_{LC+1} is mass intensity of light chain conjugated with one cytotoxin, I_{LC+2} is mass intensity of light chain conjugated with two cytotoxins, etc. I_{HC} are the intensities from the corresponding conjugated heavy chains, and $\Sigma I_{LC}tot$ and $\Sigma I_{HC}tot$ are the combined intensities of all unconjugated and conjugated light chains and heavy chains, respectively.

1.3.5 HIC-HPLC DAR analysis

[00272] In addition to DAR analysis by UPLC/electrospray ionization (ESI)-MS analysis, MORAb-003-vcp-eribulin DAR and MORAb-003-0285 DAR were also analyzed using hydrophobic interaction HPLC (HIC-HPLC). Samples were injected onto a TSKgel® Ether-5 PW, 7.5 mm ID x 7.5cm, 10 μM pore size, and eluted from the column with a 3 min equilibration in 100% of mobile phase A, a 15 min gradient (0-100% B), a 5 min hold in 100% B, a 1 min change to 100% A, and a 5 min re-equilibration in 100% of mobile phase A, at 0.7 mL/min. Mobile phase A was 25 mM sodium phosphate, 1.5 M ammonium sulfate, pH 7.0. Mobile phase B was 25 mM sodium phosphate, 25% isopropanol, pH 7.0. Detection was done at 280 nm (reference 320 nm). DAR was determined by the formula:

$$\left[AUC_{+1} + 2(AUC_{+2}) + 3(AUC_{+3}) + \ldots n(AUC_{+n})\right]/\Sigma AUC_{tot}$$

where AUC_{+1} is the area under the curve for the mAb peak corresponding to ADC conjugated with one cytotoxin, AUC_{+2} is the area under the curve for the mAb peak corresponding to ADC conjugated with two cytotoxins, etc. ΣAUC_{tot} is the combined area under the curve for all peaks.

1.4 Cytotoxicity analyses

1.4.1 Crystal Violet assay

[00273] IGROV1 (FR^{hi}) and SJSA-1 (FR^{neg}) cells were sub-cultured and seeded at 10,000 cells/well in complete growth medium in 96-well tissue culture plates, incubated at 37°C, 5% CO_2 overnight (16 hours). Typically, test reagents were serial diluted 1:4 in 2 mL deep-well dilution plates, starting at 1 μM (10 dilutions total). 100 μL of diluted samples were added to the cell plates (starting concentration of test samples at 500 nM). Plates were incubated at 37°C, 5% CO_2 for an additional 48 hours. Medium was discarded, plates were washed once with 200 μL DPBS, stained with 50 μL of 0.2% Crystal Violet solution at room temperature for 15 min, and then washed extensively with tap water. Plates were air-dried, and Crystal Violet was dissolved with 200 μL of 1% SDS solution. Plates were read at 570 nm. Data was analyzed using GraphPad Prism 6. Assays were performed using a seeding density of 1,000 cells per well and compound exposure was for a total of 5 days. When shorter-term exposure was desired, medium containing cytotoxic agents was removed after 4 hours and replaced with fresh growth medium prior to 5-day incubation. For OVCAR3, CaOV3, and NCI-H2110, cells were seeded at 3,000 cells/well and incubated for 5 days with ADC. For competition experiments, titrated ADCs were pre-incubated with 2 μM (final) unconjugated MORAb-003 prior to incubation with cells.

1.4.2 Bystander killing assay

[00274] The day before study commencement, Nuclight™ Green (NLG) IGROV1 cells were seeded at 5,000 cells/ well into 96-well round bottom plates, followed by centrifugation at 1,000 rpm for 3 min at room temperature to ensure formation of a cell pellet. The plate was placed in the vessel of an Incucyte Zoom® (EssenBio science) and incubated at 37°C/5% CO_2 overnight. The program was set to collect images of cell growth, and to determine total numbers of nuclear green-stained and nuclear red-stained cells as well as phase-confluency of the cells every two hours. The day of the experiment, MORAb-003 ADC or free drug was diluted in complete RPMI medium and serially-diluted, starting at 400 nM. 50 μL of cytotoxin solution was added to the NLG-IGROV1 cells and incubated for 30 min. During the incubation period, Nuclight™ Red (NLR) HL-60 (FR^{neg}) cells were diluted to $2x10^5$, $1x10^5$ or $5x10^4$ cell/mL with fresh

media. 50 µL of the NLR-HL60 cell suspension or medium alone was added to the NLG-IGROV1 wells, followed by centrifugation at 1,000 rpm for 3 min at room temperature to ensure re-formation of the cell pellet. The plate was placed back into the vessel of Incucyte Zoom (EssenBio science) and incubated at 37°C/5% CO_2 for up to 5 days. Relative cell growth of NLG-IGROV1 was determined by comparison to no ADC or free drug alone added samples using green cell counts. Relative cell growth of HL60 was done similarly, except that red cell count was determined. Determination of IC_{50} values for both NLG-IGROV1 and NLR-HL-60 was determined using Prism (GraphPad).

1.4.3 Serum stability assay

[00275] 20 µL of MORAb-003 ADCs were thoroughly mixed with 80 µL of DPBS, normal pooled human serum (Bioreclamation, Lot BRH552911), or normal pooled mouse serum (Bioreclamation, Lot MSE152591), and incubated at 37°C for 0, 4, 24, and 48 hours. Following incubation, samples were frozen and stored at -20°C until evaluation in cytotoxicity and binding assays. For cytotoxicity analyses, samples were evaluated on IGROV1 and SJSA-1 cells, as detailed in section 1.4.1. For binding assessment, samples were evaluated using a solution-based MSD ECL assay. Samples were incubated with biotinylated folate receptor alpha and sulfo-tag anti-MORAb-003 before capture on a streptavidin plate and detected using electrochemiluminescense with a MSD Sector Imager 2400.

2. Results

2.1 Preparation of MORAb-003 ADCs

[00276] In order to select the best combination of linker and cytotoxin to conjugate with MORAb-003, ADCs were prepared using three methodologies. According to the conjugation strategy shown in Figure 1, unpaired cysteines are generated through partial reduction with limited molar equivalents of the non-thiol reducing agent TCEP. This strategy preferentially reduces the interchain disulfide bonds which link the light chain and heavy chain (one pair per H-L pairing) and the two heavy chains in the hinge region (two pairs per H-H pairing in the case of human IgG1), while leaving the intrachain disulfide bonds intact.

[00277] The second conjugation strategy for preparing MORAb-003 ADCs utilized reduced disulfide bridging chemistry. Reduced disulfide bridging chemistry rebridges the free thiols of the cysteine residues released during the partial reduction process, mimicking the role of the disulfide bond and thus retaining the stability and function of the ADC.

[00278] The third conjugation strategy for preparing MORAb-003 ADCs employed limited lysine utilization. Limited lysine utilization results in the conjugation of a very limited number of the estimated 70+ solvent-exposed lysines available on a typical human IgG molecule, and can potentially afford mixtures of ADC product with lower homogeneity relative to strategies involving cysteine modification.

2.1.1 Preparation of VCP-eribulin for MORAb-003 ADCs

[00279] Eribulin (**1**) (10 mg, 14 µmol) (Figure 2) was dissolved in *N,N*-dimethylformamide (DMF) (1mL), and mixed well. *N,N*-diisopropylethylamine (Hunig's Base or iPr$_2$NEt) (3.6 µL, 21 µmol) and Fmoc-Val-Cit-*para*-aminobenzyl-*para*-nitrophenol (Fmoc-VCP-PNP) (**2**) (16 mg, 21 µmol, Concortis Biosystems, cat# VC1003) was added. The reaction mixture was stirred at room temperature for 4-16 hours, monitored using a ninhydrin test kit (Anaspec, cat# 25241) until the reaction was completed. Diethylamine (Et_2NH) (0.014 mL, 0.14 mmol) was then added to the reaction mixture, stirred for 2 hours at 18-25°C to remove the Fmoc protecting group. The reaction was monitored using a ninhydrin test kit. Upon completion, the solvent was evaporated under vacuum to afford crude VCP-eribulin (**3**) (16 mg), purified using a ZOBAX SB-C18 column (5 µm pore size, 9.4 x 150mm) on an Waters Alliance e2695 HPLC system in the mobile phase of H_2O-CH_3CN containing 0.1% formic acid, through a gradient of 15-70%B. VCP-eribulin (**3**) (16 mg) was dissolved in DMF (1 mL). Hunig's Base (7.2 µL, 41 µmol) and maleimido-PEG_2-NHS (**4**) (9.7 mg, 27 µmol) were added. The reaction mixture was stirred at 18-25°C for 3 hours. The reaction mixture was purified by HPLC (H_2O-CH_3CN) containing 0.1% formic acid) through a gradient of 15-70%B. Solvent was removed by lyophilization to yield mal-$(PEG)_2$-Val-Cit-p-aminobenzyloxycarbonyl (pAB)-eribulin (mal-$(PEG)_2$-VCP-eribulin) (**5**).

2.1.2 Optimization of reduction conditions

[00280] MORAb-003 ADCs were prepared by generating unpaired cysteines through partial reduction with limited molar equivalents of the non-thiol reducing agent tris(2-carboxyethyl)phosphine (TCEP). An initial investigation was performed on MORAb-003, whereby antibody concentration, TCEP concentration, and incubation time were varied, with the goal to generate an average of 4 conjugatable sites per antibody molecule. The number of free thiol sites was determined using a fluorometric thiol quantitation assay. The results of this analysis are shown in Table 12. The extent of H-H and H-L bond breakage following a 10 min, 30 min, 60 min, or 120 min incubation was also analyzed by SDS-PAGE (Figure 3). For this analysis, non-reduced and reduced samples were loaded on an SDS-PAGE gel and electrophoresis was performed at 185 V for 1 hour. In Figure 3, lane M corresponds to protein standard. Lane 1 corresponds to untreated, non-reduced MORAb-003. Lane 2 corresponds to MORAb-003 (5.3 mg/mL) reduced in 70.6 μM TCEP. Lane 3 corresponds to MORAb-003 (5.3 mg/mL reduced) in 141.2 μM TCEP. Lane 4 corresponds to MORAb-003 (1.5 mg/mL) reduced in 20 μM TCEP. Lane 5 corresponds to MORAb-003 (1.5 mg/mL) reduced in 40 μM TCEP. The identities of each band are indicated on the lower right gel. "H" indicates heavy chain, whereas "L" indicates light chain.

Table 12. Optimization of reduction conditions of MORAb-003

		10min		30min		60min		120min	
MORAb-003 concentration μM (mg/ml)	TCEP concentration μM	Free thiol μM	Disulfide bonds reduced per MAb	Free thiol μM	Disulfide bonds reduced per MAb	Free thiol μM	Disulfide bonds reduced per MAb	Free thiol μM	Disulfide bonds reduced per MAb
35.3 (5.3)	70.6	215	3.0	247.5	3.5	297.6	4.2	266.8	3.8
35.3 (5.3)	141.2	339	4.8	372.8	5.3	384.2	5.4	479.8	6.8
10 (1.5)	20	13.3	0.7	14.7	0.7	15.2	0.8	14.6	0.7
10 (1.5)	40	21.8	1.1	25.6	1.3	26.9	1.3	27.4	1.4

[00281] Analysis of the SDS-PAGE and thiol content suggested that 60 min incubation of 5.3 mg/mL mAb at 4-fold molar ratio of TCEP to mAb provided a reasonable starting point, as limited reduction of the intramolecular disulfides seemed to be present (as determined by the free thiol content), and very little unreduced mAb was remaining (unreduced mAb would act as a competitive inhibitor in *in vitro* and *in vivo* studies using prepared ADCs). Further studies were conducted with MORAb-003 at

starting concentrations of 5.0 mg/mL to confirm this optimized molar ratio of TCEP to mAb using SDS-PAGE analysis (Figure 4). In Figure 4, lane 1 corresponds to protein standard. Lane 2 corresponds to untreated, non-reduced MORAb-003. Lane 3 corresponds to MORAb-003 treated at a ratio of MORAb-003:TCEP of 1:1. Lane 4 corresponds to MORAb-003 treated at a ratio of MORAb-003:TCEP of 1:2. Lane 5 corresponds to MORAb-003 treated at a ratio of MORAb-003:TCEP of 1:3. Lane 6 corresponds to MORAb-003 treated at a ratio of MORAb-003:TCEP of 1:4. Conjugation using maleimido-PEG2-biotin was also performed subsequent to reduction and TCEP removal, in order to simulate conjugation of cytotoxin for ADC preparation. DAR analysis was performed using LC-MS. The results of these studies are provided in Table 13.

Table 13. Optimization of reduction conditions of MORAb-003 – conjugation levels with maleimido-PEG2-biotin

TCEP		MORAb-003		
TCEP:mAb	TCEP (μM)	LC	HC	DAR
1	33.3	0.29	0.34	1.26
2	66.7	0.48	0.83	2.62
3	100	0.63	1.21	3.68
4	133.2	0.73	1.70	4.86

LC, light chain biotin level; HC, heavy chain biotin level; DAR, biotin per mAb [DAR = 2(LC) + 2(HC)].

[00282] Following biotin conjugation, free thiol analysis indicated that no free thiol was present in MORAb-003-biotin. This indicated that, following reduction of disulfide bonds, conjugation typically occurred at both thiols generated, and that any unconjugated, reduced disulfides underwent re-oxidation to reform disulfide bonds. The final conditions chosen for reduction for ADC generation were antibody concentration of 5.0 mg/mL, TCEP concentration of 110 μM, and incubation time of 60 min. This leads to a mAb with a DAR of 4 following conjugation.

2.1.3 ADC conjugation optimization

[00283] As the first cytotoxin used for ADC preparation was cryptophycin, which is a hydrophobic compound, initial conjugation optimization experiments were performed with a "surrogate" anti-human mesothelin antibody having two unpaired cysteines available for conjugation (one per light chain) at specific locations. This greatly facilitates the analysis of conjugation efficiency by mass spectrometry, as only the light chain needs to be analyzed. Titration of propylene glycol during conjugation of maleimido-LL3-cryptophycin to the surrogate antibody was performed followed by analysis of conjugation efficiency of the light chain by LC-MS (Table 14).

Table 14. Optimization of propylene glycol concentration in conjugation reaction

Propylene glycol (%)	Conjugated Ab LC (%)
0	8 %
20	48 %
50	100 %

LC masses: unconjugated, 23536 Da; conjugated, 24367 Da.

[00284] 50% propylene glycol resulted in full occupation of the available sites, and was chosen as the final concentration to be used. No loss in binding of the mAb was observed following conjugation (data not shown), indicating that the propylene glycol did not have deleterious effects to the antibody. Thus, the final conjugation reaction conditions chosen were 2.5 mg/mL mAb final, 6:1 molar ratio of maleimido-linker-cytotoxin:mAb in 0.5X DPBS (final concentration after propylene glycol addition), 0.5 mM EDTA, 50% propylene glycol, pH 7.2 for 3.5-4 hours at room temperature. In these reactions, propylene glycol is added prior to addition of maleimido-linker-cytotoxin.

2.1.4 Preparation of ADCs and biophysical characterization

[00285] The established reduction and conjugation conditions, described in section 2.1.2, were used to prepare the first 10 MORAb-003 ADCs listed in Table 15. The remaining ADCs were prepared by either reduced disulfide bridging or limited lysine utilization, with the exceptions of M-MMAE and M-DM1. M-MMAE and M-DM1 were prepared by Concortis Biosystems, Inc., and were received in conjugated form.

[00286] Reduced disulfide bridging chemistry bridges across the free thiols produced during the partial reduction process, giving one cytotoxin per disulfide reduced. In

theory, an antibody of DAR = 4 would have both H-L and hinge disulfides re-bridged, providing an ADC with increased stability and homogeneity over traditional conjugation approaches. Limited lysine utilization results in the conjugation of a very limited number of the estimated 70+ solvent-exposed lysines available on a typical human IgG molecule. MORAb-003 conjugates prepared using this method resulted in a DAR of 2.0, suggesting that a single lysine was utilized per H-L pair.

[00287] All ADCs were purified by HiPrep 26/10 desalting chromatography and formulated into DPBS. DAR analysis was performed on all prepared ADCs by LC-MS and aggregation levels were determined by SEC-HPLC. The results of these DAR and aggregation analyses are listed in Table 15 next to the respective ADC.

Table 15. Biophysical analyses of MORAb-003 ADCs

	Compound name	**DAR**	**Aggregation (%)**
1	PEG3-Bz-disulfidyl-dimethyl-cryptophycin	3.7 - 3.9	29
2	LL2-cryptophycin	3.2	18 - 36
3	LL3-cryptophycin	3.2 - 3.7	22 - 36
4	VCP-cryptophycin	3.4	50
5	VCP-eribulin	3.6	0 - 2.6
6	ER-001161318	3.5	3.2
7	ER-001161319	3.5	3.1
8	ER-001159200	2.8	
9	M-MMAE	4.0	2
10	NHS-PEG2-AuF	5.0	
11	M-DM1	3.6	1.8
12	M-0285	4.0	1.2
13	M-0115	4.0	0.4
14	M-172	3.1	3.6
15	M-174	2.8	4.4
16	M-158	4.5	3.8
17	M-0384	4.2	4.2
18	M-0302	4.3	3.3

19	M-292	4.0	4.5
20	M-0026	4.2	3.3
21	M-0267	4.0	2.9
22	M-0272	3.3	1.5
23	M-0260	3.2	1
24	M-0276	4.6	6.2
25	M-015-0913	2.0	<1
26	M-030-0132	2.0	<1
27	M-0161	2.1	2.4
28	M-0157	2.0	<1
29	M-027-0381	2.0	<1
30	M-0025	2.0	1.7
31	M-0301	2.0	1.4
32	M-030-0011	2.0	<1
33	M-030-0291	2.0	<1
34	M-0255	3.6	5.9
35	M-0114	4.0	3.9

[00288] DAR values for all ADCs were in the pre-determined range (DAR between 3 and 4). Aggregate levels for the cryptophycin-based ADCs were significantly higher than desired (>10%), whereas the eribulin-based (VCP-eribulin) and the maytansine-based maleimido-linker-cytotoxins (ER-001161318, ER-001161319, and M-MMAE) all demonstrated acceptable aggregate levels. An investigation into other organic co-solvents was performed on conjugation reactions to MORAb-003 using VCP-cryptophycin. Co-solvents tested were DMSO (10%), *N,N*-dimethylformamide (20%), *N,N*-dimethylacetamide (20%), and *N,N*-dimethylpropionamide (20%). Aggregate levels following conjugation using these co-solvents were all equal to, or higher than, 50% propylene glycol.

[00289] A non-reducing SDS-PAGE analysis was performed on a subset of the ADCs (Figure 5). As DAR for all these ADCs was determined to be 4, it was thought that these ADCs should migrate as intact IgG of ~ 160 kD, as both H-L and both hinge disulfides should be re-bridged. This subset of ADCs included M-MMAE (lane 2), M-

DM1 (lane 3), M-0026 (lane 4), M-0260 (lane 5), M-0267 (lane 6), M-0272 (lane 7), M-0285 (lane 8), M-292 (lane 9), M-027-0381 (lane 10), and M-0384 (lane 11) (Figure 5). In Figure 5, lane 1 corresponds to protein standard.

[00290] It is clear from this analysis that, for the reduced disulfide bridging chemistry ADCs (lanes 4-9, 11), there is significant H-L monovalent species (80 kD), in addition to the intact ADC. This indicates that there is significant intra-chain hinge disulfide bridging, in addition to inter-chain hinge bridging. SEC-HPLC analysis indicates that the ADCs migrate as a single intact IgG, indicating that for those ADCs with intra-chain H-H bridging, the heavy chains are associated non-covalently in the final ADC.

2.2 *In vitro* potency analyses of MORAb-003 ADCs

2.2.1 Cytotoxicity on IGROV1 and SJSA-1 cells

[00291] *In vitro* potency of prepared ADCs was assessed using a Crystal Violet assay as detailed in section 1.4.1.

[00292] Initial screening of all MORAb-003 ADCs was performed on IGROV1 ($FR^{hi(+++)}$) and SJSA-1 ($FR^{neg(-)}$) cells. IGROV1 cells are of human ovarian epithelial carcinoma origin and express high levels of folate receptor alpha (FR), the target antigen of MORAb-003. SJSA-1 cells are a human osteosarcoma tumor cell line that are negative for folate receptor alpha. Screening of selected ADCs was also performed in CaOV3 (human ovarian carcinoma, $FR^{med(++)}$), NCI-H2110 (human non-small cell lung carcinoma, $FR^{med(++)}$), and/or OVCAR3 (human ovarian carcinoma, $FR^{med(++)}$) cells. The results of this screening are provided in Table 16.

Table 16. Cytotoxicity (IC_{50}) screening of MORAb-003 ADCs on various tumor cell lines

Compound name	IGROV1	SJSA-1	CaOV3	NCI-H2110	OVCAR3
PEG3-Bz-disulfidyl-dimethyl-cryptophycin	0.067	0.41			
LL2-cryptophycin	0.023	4.7	0.33		
LL3-cryptophycin	0.086	12.7	0.19		0.094
VCP-cryptophycin	0.03	~100	0.02		
VCP-eribulin	0.054	>100	3.7	0.73	0.16

ER-001161318	0.26	>100	3.1		
ER-001161319	0.49	>100	11.3		
ER-001159200	6.5	>100	9.2		
M-MMAE	0.2	253			
NHS-PEG2-AuF	0.2	>500			
M-DM1	55	132			
M-0285	0.3	>100		14	8.8
M-0115	0.54	>100			
M-172	>500	>500			
M-174	>500	>500			
M-158	>500	>500			
M-0384	2.25	2.45			
M-0302	330	>500			
M-292	1.7	>500			
M-0026	1.38	540			
M-0267	0.029	0.028			
M-0272	0.252	1.02			
M-0260	0.383	0.036			
M-0276	0.43	30			
M-015-0913	>500	>500			
M-030-0132	>500	17.3			
M-0161	>500	>500			
M-0157	>500	>500			
M-027-0381	14.5	28			
M-0025	>500	>500			
M-0301	>500	>500			
M-030-0011	61.6	>500			
M-030-0291	>500	105			
M-0255	0.12	0.46			
M-0114	144	>100			

All values are IC_{50}s in nM, and are mean values of replicate experiments, where performed.

[00293] VCP-eribulin ADC was potent (54 pM) on IGROV1 cells and had little killing on SJSA-1 cells. For these cell lines, the VCP-eribulin ADC demonstrated higher potency and specificity relative to ADCs with equivalent DAR values, such as M-MMAE and M-DM1. VCP-eribulin ADC also demonstrated potent cytotoxicity on additional FR-expressing tumor cell lines of ovarian (CaOV3 and OVCAR3) and non-small cell lung carcinoma (NC-H2110) origin.

[00294] ADCs VCP-eribulin, LL2-cryptophycin, LL3-cryptophycin, VCP-cryptophycin, ER-001161318, ER-001161319, and ER-001159200 displayed specific cytotoxicity (> 2-logs of specificity) in CaOV3 ($FR^{med(++)}$) cells. A number of these ADCs displayed sub-nanomolar potency. Cryptophycin conjugates also demonstrated high levels of potency (23 pM – 86 pM) in IGROV1 cells, but, with the exception of the VCP-cryptophycin, also demonstrated measurable cytotoxicity on SJSA-1 cells. Cleavable maytansine conjugates ER-001161318 and ER-001161319 had intermediate potency on IGROV1 (0.26 nM and 0.49 nM), and little off-target killing of SJSA-1 cells.

[00295] All limited lysine utilization conjugates demonstrated no specificity and were not evaluated further. Cleavable conjugates using reduced disulfide bridging technology of duostatin-3 (M-0285), duostatin-5 (M-0115), and duostatin-14 (M-292 and M-0026) all demonstrated specific cytotoxicity on the IGROV1 cell line, with little cytotoxicity on the SJSA-1 cell line. Duostatin-3 and duostatin-5 conjugates, derivatives of auristatin, were slightly higher in potency then the duostatin-14 conjugates, which is a maytansine derivative. Potencies and specificities were comparable to the control M-MMAE conjugate, which uses a Val-Cit-pAB (VCP) linker attached to monomethyl E. Non-cleavable reduced disulfide chemistry conjugates all either lacked sufficient potency or specificity, and were not analyzed further.

2.2.2 Cytotoxicity on human folate receptor-expressing ovarian cancer cell line CaOV3

[00296] Potency of select MORAb-003 ADCs was also determined on human ovarian tumor cell lines OVCAR3 and CaOV3, as well as the human NSCLC cell line NCI-H2110 (Table 16). On the human ovarian cell line CaOV3, the cryptophycin conjugates

demonstrated measurably higher potency than the VCP-eribulin conjugate, unlike that observed in IGROV1 cells. This may be due to the lower expression level of folate receptor alpha on CaOV3 cells compared with IGROV1, or the higher potency of cryptophycin on these cells, compared with eribulin. The maytansine-based conjugates ER-001161318, ER-001161319, and ER-001159200 all had potencies similar to, or lower than, VCP-eribulin.

2.3 Bystander killing of VCP-eribulin, ER-001161318, and M-0285

[00297] In order to assess bystander killing activity, an assay was set up using two labeled cell lines. In this assay, IGROV1 cells (FR^{hi}) labeled with Nuclight™ Green and HL-60 (FR^{neg}) labeled with Nuclight™ Red were co-cultured in different cell number ratios, and treated with titrations of MORAb-003 ADCs VCP-eribulin, ER-001161318, or M-0285. VCP-eribulin is an eribulin-based ADC comprising a maleimido-PEG_2-Val-Cit-pAB cleavable linker, while ER-001161318 is maytansine-based ADC comprising a maleimido-$(CH_2)_5$-Val-Cit-pAB cleavable linker and M-0285 is a duostatin-based ADC comprising a PEG-pAB cleavable linker. Cytotoxicity was monitored by an Incucyte Zoom® cell imager. The results of this bystander cytotoxicity assay are shown in Table 17 and Figures 6A-C.

Table 17. Bystander killing activity of VCP-eribulin on the co-culture of FR-positive and FR-negative cell lines

EC_{50} (nM)			
IGROV-1	HL-60	HL-60 (co-culture with IGROV-1)	HL-60 (eribulin)
0.0005972	39.74	0.2399	0.1702

[00298] When HL-60 (FR^{neg}) cells were cultured at a 2:1 ratio to IGROV1 (FR^{hi}) cells, treatment with MORAb003-VCP-eribulin resulted in a 2-log increase in killing of the HL-60 cells, compared with HL-60 cells alone (Table 17 and Figure 6A). These data suggest that folate receptor alpha (FR) target-negative cells are killed more effectively by MORAb003-VCP-eribulin when co-cultured with FR target-positive cells, referred to herein as bystander killing. Bystander killing is distinguishable from off-target killing, which is defined as the killing of target-negative cells on their own, in the absence of and independent of co-culturing with target-positive cells. The observed

increase in bystander killing was also almost identical to the increase observed following treatment of HL-60 cells with free eribulin, indicating a potential mechanism for the bystander effect. Without wishing to be bound by any theory, MORAb003-VCP-eribulin may be cleaved in or near FR-positive IGROV1 cells, which also undergo apoptosis and release free eribulin into culture. The released cytotoxin may kill FR-negative HL-60 cells.

[00299] In contrast, only a slight shift was observed for MORAb003-ER-001161318 (Figure 6B), and no shift was observed with MORAb003-0285 (Figure 6C). When the HL-60:IGROV1 ratio was lowered from 2:1 to 1:2, measurable killing of the HL-60 cells was observed, relative to HL-60 cells alone, for MORAb003-ER-001161318, while bystander effect still remained low, albeit detectable, for MORAb003-0285. These data suggest that, in terms of bystander killing, the MORAb-003 ADCs evaluated can be ranked as VCP-eribulin > ER-001161318 > M-0285.

2.4 Serum stability analysis

[00300] Given the long circulating half-life *in vivo* of ADCs and the potential for toxicity if cytotoxins are released in circulation, ADCs should demonstrate stability in serum. MORAb-003 ADCs VCP-eribulin, ER-001161319, and M-0285 were preincubated in human or mouse serum at 37°C for up to 48 hours, then evaluated in a cytotoxicity assay with IGROV1 and SJSA-1 cells. ER-001161319 is maytansine-based ADC comprising the same cleavable linker as VCP-eribulin, maleimido-PEG_2-Val-Cit-pAB. PBS and serum controls were included to correct for any serum effects on assay performance. The results of this study are shown in Table 18.

Table 18. Serum stability of selected MORAb-003 ADCs

		Cell-based cytotoxicity assay, EC_{50}, nM								
		MORAb003-VCP Eribulin			MORAb003-ER001161319			MORAb003-0285		
	Time	PBS	Human Serum	Mouse Serum	PBS	Human Serum	Mouse Serum	PBS	Human Serum	Mouse Serum
IGROV1	0hr-PBS	0.021	0.013	0.02	0.28	0.15	0.2	0.074	0.089	ND
	0hr-Serum	0.022	0.014	0.01	0.15	0.15	0.2	0.063	0.078	0.049
	4hr	0.03	0.018	0.019	0.14	0.17	0.25	0.065	0.075	0.049
	24hr	0.024	0.019	ND	ND	0.27	0.9	0.059	0.074	0.044
	48hr	0.022	0.021	0.03	0.21	0.73	2.56	0.043	0.05	0.051
SJSA-1	0hr-PBS	>10	>10	>10	>10	>10	>10	>10	>10	>10
	0hr-Serum	>10	>10	>10	>10	>10	>10	>10	>10	>10
	4hr	>10	>10	>10	>10	>10	>10	>10	>10	>10
	24hr	>10	>10	>10	>10	>10	>10	>10	>10	>10
	48hr	>10	>10	>10	>10	>10	>10	>10	>10	>10

Shaded boxes indicate significant decrease in potency from T=0 sample.

While VCP-eribulin and M-0285 were stable for at least 48 hours in either serum, ER-001161319 demonstrated a significant drop in potency after 48 hours. This may be due to the aziridino-carbamate linkage to the maytansine, which has not been described in the literature previously. The form of the compound released may not be highly potent, as no increase in cytotoxicity was seen on SJSA-1 cells.

2.5 *In vitro* studies with MORAb003-VCP-eribulin

2.5.1 HIC-HPLC analysis of DAR and product heterogeneity

[00301] MORAb003-VCP-eribulin and MORAb003-0285 were analyzed by HIC-HPLC in order to evaluate DAR by an alternate method and examine product heterogeneity and content of unconjugated antibody (competitor). MORAb003-VCP-eribulin was shown to have DAR species of 0, 2, 4, and 6, which is consistent with the method used for reduction and conjugation (Figure 7A). Very low amounts of DAR = 0 species were observed. Overall DAR, based on AUC calculations, was 3.80, consistent with values determined by LC-MS. MORAb003-0285 migrated as a single peak by HIC-HPLC, indicating a single DAR species (Figure 7B). This was assigned as DAR 4.0.

2.5.2 Specificity by competition assay

[00302] Antigen specificity of MORAb-003-VCP-eribulin cytotoxicity was demonstrated for the VCP-eribulin conjugate using a competition assay format (Figure 8). In this experiment, titrations of the MORAb-003-VCP-eribulin (starting concentration 100 nM) were co-incubated with 2 μM unconjugated MORAb-003. Unconjugated MORAb-003 provided a 2-log shift in potency on IGROV1 cells, similar to results obtained with IMGN853, the anti-human folate receptor alpha-maytansine ADC from Immunogen now in Phase II clinical trials, on KB cells (Moore *et al.*, 2015 American Society of Clinical Oncology (ASCO) Annual Meeting, Abstract 5518).

2.5.3 Cytotoxicity on NCI-H2110 NSCLC cells

[00303] Cytotoxicity for both MORAb003-VCP-eribulin and MORAb003-0285 on the human NSCLC cell line NCI-H2110 was performed using a Crystal Violet assay. The results of this assay are shown in Table 16. MORAb003-VCP-eribulin had an IC_{50} of 0.73 nM, while MORAb003-0285 had an IC_{50} of 14 nM.

2.6 *In vivo* studies

2.6.1 Maximum tolerated dose (MTD) of MORAb-003-VCP-eribulin (MORAb-202) in CD-1 mouse strain

[00304] Naïve CD-1 mice were injected intravenously with 200 μL of MORAb-202 according to the schedule in Table 19. Body weight was measured prior to dose on the

dosing day, 24 hours post dose, and three times a week thereafter. The animals were observed for clinical well-being throughout the study duration. Two weeks after dosing, the terminal body weight was measured and recorded. Euthanized mice at the end of the study (and if any mouse euthanized or found dead during the study) were processed for necropsy. Organs were examined for signs of tissue damage.

Table 19. Study design

Group	# Mice	Treatment	Dose (mg/kg)	Regimen	Route
1	3	Vehicle*	0	single bolus	i.v.
2		MORAb-202	10		
3			20		
4			40		
5			80		

[00305] No significant body weight loss observed in any of the treatment groups compared with PBS-treated control group, or any clinical findings indicating toxicity during the treatment. Body weight of individual mice is shown in Table 20, and the group average and SEM is shown in Table 21. Body weight change kinetics for each group (group average and SEM) are shown in Figure 9. MORAb-202 at doses up to 80 mg/kg via bolus intravenous administration produced no toxicity. Therefore, the MTD is above 80 mg/kg.

Table 20

Days Post Rx	PBS control			MORAb-202 10mg/kg			MORAb-202 20mg/kg			MORAb-202 40mg/kg			MORAb-202 80mg/kg		
X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3	C:Y1	C:Y2	C:Y3	D:Y1	D:Y2	D:Y3	E:Y1	E:Y2	E:Y3
0	22.50	20.60	21.20	22.50	23.50	21.30	27.40	28.20	21.90	23.60	23.30	21.70	21.60	25.60	25.90
1	23.80	21.50	22.00	22.80	21.80	23.30	28.80	27.50	21.40	23.80	22.50	21.80	23.10	27.80	27.80
2	23.30	21.50	22.50	23.30	24.00	22.90	29.30	29.00	22.10	23.60	24.60	21.80	29.30	28.90	28.80
4	23.90	21.90	22.00	23.90	23.90	23.30	30.20	31.00	23.50	24.40	24.10	22.70	23.50	29.50	28.90
7	23.90	23.10	22.70	24.80	25.20	24.50	29.80	32.60	24.00	25.10	25.50	23.40	25.30	30.40	30.10
9	24.50	22.50	22.80	24.90	25.30	25.00	30.50	33.70	23.40	25.50	25.20	23.70	29.60	31.00	30.20
11	24.20	23.40	23.20	24.90	26.10	24.30	31.00	32.40	23.80	25.90	25.00	23.90	23.10	29.10	29.40
14	24.00	23.50	24.40	25.80	26.90	25.70	33.20	32.40	24.10	25.70	25.90	26.30	21.70	28.40	28.90

Table 21

days post injections	PBS			MORAb-202 10 mg/kg			MORAb-202 20mg/kg			MORAb-202 40mg/kg			MORAb-202 80mg/kg		
	mean (g)	sem	n	mean (g)	sem	n	mean (g)	sem	n	mean	mean (g)	n	mean	mean (g)	n
0	21.4	0.6	3	22.6	0.5	3	24.9	2.0	5	22.9	0.6	3	24.7	1.5	3
1	22.2	0.6	3	25.2	0.3	3	25.9	2.3	3	22.9	0.7	3	26.2	1.6	5
2	22.4	0.5	3	23.4	0.3	3	26.8	2.4	3	23.3	0.8	3	26.6	1.7	3
4	22.4	0.5	3	23.5	0.2	3	28.1	2.5	5	23.7	0.5	3	27.2	1.9	3
7	23.2	0.4	3	25.0	0.1	3	28.5	2.4	3	24.7	0.6	3	27.9	2.3	5
9	23.3	0.7	3	25.1	0.1	3	28.6	2.6	3	24.9	0.6	3	28.3	2.3	3
11	23.6	0.3	3	24.6	0.3	3	29.1	2.7	3	24.7	0.5	3	27.2	2.1	3
14	24.0	0.3	3	25.7	0.1	3	29.9	2.9	3	25.8	0.5	3	26.3	2.3	3

2.6.2 Maximum tolerated dose of eribulin in CD-1 mice

[00306] Naïve CD-1 mice were injected intravenously with 200 µL of eribulin according to the schedule in Table 22. Body weight was measured three times a week including prior to dose on each dosing day and 24 hours following each dose. The animals were observed for clinical well-being throughout the study duration (two weeks after the last dose). The terminal body weight was measured and recorded. Euthanized mice at the end of the study (and if any mouse euthanized or found dead during the study) were processed for necropsy. Organs were examined for signs of tissue damage.

Table 22. Study design

Group	# Mice	Treatment	Dose (mg/kg)	Regimen	Route
1	3	PBS	0	q4dx3	i.v.
2		Eribulin	0.4		
3			0.8		
4			1.6		
5			3.2		

[00307] No significant body weight loss or clinical findings indicating toxicity observed in the animals administered eribulin at doses up to 1.6 mg/kg, using q4dx3 dosing regimen (once every four days for 3 doses total). Administration of 3.2 mg/kg with the same schedule induced piloerection in all three mice after the second dose. Severe weight loss (23% loss in one mouse, #552, after the second dose; 17% and 8% in the rest, #551 and #552, after the third dose) was observed, compared with PBS-treated control. No gross changes were observed in the organs of mice during necropsy. The body weight of individual mice is shown in Table 23, and the group average and SEM is shown in Table 24. Body weight change kinetics for each group (group average and SEM) are shown in Figure 10.

[00308] Eribulin at doses up to 1.6 mg/kg, using q4dx3 dosing regimen, produced no toxicity, while 3.2 mg/kg induced severe weight loss. Therefore, the MTD of eribulin, in this study, is 1.6 mg/kg, q4dx3.

Table 23

Day	PBS			eribulin 0.4mg/kg			eribulin 0.8mg/kg			eribulin 1.6mg/kg			eribulin 3.2mg/kg		
X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3	C:Y1	C:Y2	C:Y3	D:Y1	D:Y2	D:Y3	E:Y1	E:Y2	E:Y3
0	26.60	24.40	23.40	22.40	24.60	23.10	23.90	25.50	25.10	21.90	24.40	23.70	24.30	25.50	22.60
1	28.13	25.11	24.35	21.84	24.22	22.43	23.63	23.85	24.46	21.13	24.10	24.13	22.31	23.47	20.57
4	25.80	25.10	24.40	22.70	24.00	24.00	23.80	24.40	24.80	21.70	24.10	23.30	20.90	22.20	17.50
5	26.40	24.80	24.50	22.30	24.10	23.40	24.10	24.00	24.80	21.50	24.50	24.10	20.50	22.70	16.80
8	27.50	25.70	24.50	23.50	23.70	25.20	24.60	24.80	25.90	21.90	24.80	24.20	21.80	23.80	9003.00*
9	27.30	26.50	24.40	22.90	23.40	23.40	24.00	24.70	25.30	21.80	24.60	24.30	20.20	23.60	
11	27.50	25.80	24.40	22.60	23.90	25.60	24.70	25.10	26.00	22.20	25.70	24.70	20.00	23.90	
13	27.40	26.80	25.40	23.70	23.80	26.50	25.30	25.60	26.80	22.80	25.80	25.20	21.40	23.70	
16	27.30	26.90	26.20	24.70	24.10	26.70	25.40	25.90	26.40	23.40	26.70	26.10	23.80	24.50	
18	27.60	27.40	27.30	24.50	24.90	27.90	26.20	26.00	27.90	24.70	27.90	26.20	25.90	25.90	
20	28.10	27.60	28.50	26.10	25.70	28.50	25.40	26.20	28.10	25.40	29.00	28.00	26.80	28.00	

Each column represents an individual animal.
*9003: euthanized for weight loss >20%.

Table 24

days post injections	PBS			eribulin 0.4mg/kg			eribulin 0.8mg/kg			eribulin 1.6mg/kg			eribulin 3.2mg/kg		
	mean (g)	sem	n	mean (g)	sem	n	mean (g)	sem	n	mean	mean (g)	n	mean	mean (g)	n
0	24.8	0.9	3	23.4	0.6	3	24.8	0.5	3	23.3	0.7	3	23.8	0.9	3
1	25.9	1.2	3	22.8	0.7	3	23.7	0.4	3	23.1	1.0	3	22.1	0.8	3
4	25.0	0.3	3	23.6	0.4	3	24.2	0.2	3	23.0	0.7	3	20.2	1.4	3
5	25.2	0.6	3	23.3	0.5	3	24.3	0.3	3	23.4	0.9	3	20.2	1.7	3
8	25.9	0.9	3	24.1	0.6	3	24.9	0.6	3	23.5	0.8	3	22.9	0.9	2
9	25.8	0.9	3	23.9	0.8	3	24.7	0.4	3	23.6	0.9	3	21.6	1.1	2
11	25.8	0.9	3	24.0	0.9	3	25.3	0.4	3	24.2	1.0	3	21.7	1.3	2
13	26.5	0.6	3	24.7	0.9	3	25.8	0.4	3	24.6	0.9	3	22.6	0.9	2
16	26.8	0.3	3	25.2	0.8	3	25.9	0.3	3	25.4	1.0	3	24.0	0.3	2
18	27.2	0.1	3	25.7	1.1	3	27.4	0.6	3	26.3	0.9	3	25.9	0.0	2
20	27.4	0.5	3	26.8	0.9	3	27.2	0.9	3	26.8	1.1	3	26.4	0.3	2

2.6.3 Evaluation of minimum efficacious dose of MORAb003-VCP-eribulin (MORAb-202) in the hNSCLC NCI-H2110 model in CB17-SCID mice

[00309] Human NSCLC, NCI-H2110 cells, passage 47 were implanted subcutaneously in 30 CB17 SCID mice (female, 5 to 6 weeks old, weighing 20 grams). After 14 days post-implantation, mice were randomized into five groups. Average tumor volume in each group on the treatment day (Day 0) ranged between 154-175 mm^3 (Table 27). The enrolled mice were treated with MORAb003-VCP-eribulin (MORAb-202) (Lot# NB2900-87E 10/07/15) at 1, 2.5, or 5 mg/kg, with MORAb-003-0285 (Lot# 042-150-002) as control at 5 mg/kg, or with PBS, according to the study design (Table 25). Each group was removed from the study when tumor volume in any animal in the group was >2000 mm^3. The last group was terminated on Day 61.

Table 25. Study design

Group	# Mice	Treatment	Dose (mg/kg)	Regimen	Route
1	5	PBS	0	single bolus	i.v.
2	5	MORAb-003-VCP-eribulin	1		
3	5	MORAb-003-VCP-eribulin	2.5		
4	4[1]	MORAb-003-VCP-eribulin	5		
5	5	MORAb003-0285	5		

[00310] The tumor volumes in individual mice are shown in Table 26, and the group average and SEM is shown in Table 27. Tumor growth kinetics for each group (group average and standard error of the mean, SEM) are shown in Figure 11, and tumor volumes in individual mice, as well as group average and SEM, are shown in Figure 12. Based on day 17 tumor volumes (when first tumor volume >2000 mm^3 was observed), MORAb-202 caused tumor growth inhibition (TGI) of 47% at 1 mg/kg ($p = 0.002$ vs. saline), TGI of 96% at 2.5 mg/kg ($p < 0.0001$ vs. saline). However, the regressed tumors regrew one to two weeks after end of treatment. No tumor was detected in mice treated with 5 mg/kg of MORAb-202. These mice remained tumor free beyond 60 days

[1] Four mice in this group. One mouse was excluded from this group due to treatment injection error, which was verified by absence of compound in animal sera based on electrochemiluminenscent immunoassay (ECLIA) data.

after a single dose treatment. MORAb-003-0285 caused TGI of 89.7% at 5 mg/kg ($p <$ 0.0001 vs. saline).

[00311] Body weight of individual mice is shown in Table 28, and the group average and SEM is shown in Table 29. Body weight change kinetics for each group (group average and SEM) are shown in Figure 13.

[00312] No significant body weight loss was observed in any of the treatment groups compared with control.

[00313] MORAb-202 showed significant effect on NCI-H2110 tumor growth. Tumor regression was achieved by a bolus treatment at 2.5 mg/kg with TGI of 94% (vs. PBS). Therefore, the minimum efficacious dose of MORAb-202 is 2.5 mg/kg, tested in this model. Complete tumor eradication was achieved by a single dose at 5 mg/kg. No tumor growth was observed for over 60 days.

Table 26. Tumor volumes

days post randomization	PBS					MORAb-202 1mg/kg					MORAb-202 2.5 mg/kg					MORab-202 5mg/kg					003-0285 5mg/kg				
0	164	195	137	300	80	178	218	133	118	150	187	189	92	120	236	110	202	159	146	65	208	241	243	97
3	368	413	279	587	171	178	207	104	106	216	144	97	65	69	148	40	115	68	68	83	259	358	292	164
5	327	481	285	555	190	161	193	83	95	215	75	51	37	35	56	14	52	22	37	54	160	168	239	105
6	467	758	541	894	275	257	258	139	160	348	61	52	58	33	57	7	28	25	20	43	197	235	247	129
7	642	815	621	1055	395	317	306	182	167	476	64	54	53	36	57	8	48	16	20	52	192	255	266	128
10	891	1238	895	1328	662	506	494	230	285	708	24	37	35	15	71	0	0	0	0	39	155	240	181	86
12	993	1274	983	1519	1115	638	655	371	361	865	40	21	51	9	69	0	0	0	0	32	106	206	223	83
14	981	1410	1131	1695	971	848	812	402	418	901	41	30	37	0	89	0	0	0	0	31	115	235	157	79
17	1320	1723	1319	2089	1466	955	980	727	592	946	46	33	64	0	161	0	0	0	0	28	114	346	251	74
19						838	1030	856	602	953	56	37	90	0	282	0	0	0	0	27	144	438	359	94
24											102	37	197	0	702	0	0	0	0	46	391	1244	824	187
26											168	102	319	0	790	0	0	0	0	103	564	1470	1030	287
28											269	54	474	9	990	0	0	0	0	125	703	1898	1112	375
31											362	105	558	13	1187	0	0	0	0	225	1144	2427	1413	657
33											496	124	588	9	1461	0	0	0	0					
35											573	212	669	16	1847	0	0	0	0					
38											764	348	952	20	2367	0	0	0	0					
40																0	0	0	0					
42																0	0	0	0					
45																0	0	0	0					
47																0	0	0	0					
52																0	0	0	0					
54																0	0	0	0					
59																0	0	0	0					
61																0	0	0	0					

Each column represents an individual animal.

Table 27

		PBS			MORAb-202 1mg/kg			MORAb-202 2.5mg/kg			MORAb-202 5mg/kg			MORAb-003-0285 5mg/kg		
days post randomization		MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N
	0	175.2	36.41527	5	159.4	17.68781	5	164.8	25.8917	5	154.25	16.95792	4	170.8	37.46065	5
	3	363.6	69.3831	5	162.2	24.14101	5	104.6	17.7581	5	72.75	13.88661	4	231.2	48.4055	5
	5	367.6	66.21275	5	149.4	26.13343	5	50.8	7.242607	5	31.25	7.500133	4	145.2	31.14683	5
	6	587	108.7468	5	232.4	37.74183	5	52.2	5.005179	5	20	4.140008	4	170.2	37.81015	5
	7	705.6	109.7441	5	289.6	55.74694	5	52.8	4.611415	5	23	7.76666	4	178.6	40.08123	5
	10	1002.8	122.532	5	444.6	85.61518	5	36.4	9.499597	5	0	0	4	140.2	35.30937	5
	12	1176.8	100.25	5	578	95.18355	5	38	10.62087	5	0	0	4	130	36.5513	5
	14	1237.6	138.8994	5	676.2	109.4307	5	39.4	14.30871	5	0	0	4	123.4	34.69758	5
	17	1583.4	146.0629	5	840	76.78507	5	60.8	27.09899	5	0	0	4	162.6	58.96373	5
	19				855.8	72.16584	5	93	49.35207	5	0	0	4	212.4	79.06236	5
	24							207.6	127.8177	5	0	0	4	538.4	219.5123	5
	26							275.8	138.3498	5	0	0	4	690.8	249.2466	5
	28							359.2	177.874	5	0	0	4	842.6	310.8641	5
	31							445	208.4929	5	0	0	4	1173.2	373.2365	5
	33							535.6	255.2269	5	0	0	4			
	35							663.4	318.1881	5	0	0	4			
	38							890.2	402.5237	5	0	0	4			
	40										0	0	4			
	42										0	0	4			
	45										0	0	4			
	47										0	0	4			
	52										0	0	4			
	54										0	0	4			
	59										0	0	4			
	61										0	0	4			

Table 28

days post randomization		PBS					MORAb-202 1mg/kg					MORAb-202 2.5 mg/kg					MORab-202 5mg/kg					MORAb-003-0285 5mg/kg				
	0	19.1	18.2	18.4	18.9	18.8	19.1	18.6	19.3	20.6	18.4	17.8	18.1	18	19.8	16.3		17.6	18.7	16.1	19.7	20.5	17.4	18	17.4	18.8
	3	19.6	18.2	18.9	18.9	19.3	19.3	18.4	20.2	20.9	18.6	18.4	19.1	18.6	19.9	16.4		17.5	18.8	15.9	19.9	20.8	17	18.1	16.3	18.5
	6	19.7	18.4	18.4	19.1	19.1	19	18.3	20.3	21.3	19	18.5	19.4	18.7	20	16.4		17.5	19.3	16.3	19.6	20.8	17.7	18.2	16.8	18.7
	7	19.7	18	18.9	18.8	18.9	18.9	18	20	21.2	18.9	18.7	18.7	18.7	19.7	16.5		17.4	19.4	16.5	19.2	20.6	17.7	18.5	16.8	19
	10	19.7	18	19.2	18.5	19.1	18.4	18	20.1	20.9	19	19.3	19.7	18.8	19.8	16.6		17.6	19.4	16.7	20	20.5	18.2	18.6	17.5	20.3
	12	19.8	17.7	19.1	18.4	19	18.3	17.8	20.5	20.9	19.5	18.9	20	19.7	20.2	17.2		17.9	19.6	16.9	20.2	20.4	18.3	18.8	18.1	20.4
	14	18.8	17.4	18.4	18.2	17.5	17.9	17.7	20.3	21.2	19.9	18.8	19.6	19	19.3	17		17.5	19.3	17	19.2	20	18.2	18.9	18.4	19.7
	17	18.8	17.2	18.3	17.5	17.2	17.4	17.7	20.4	20.7	19.2	18.8	19.8	19.7	19.2	17.3		17.9	20	17.3	19.7	19.8	17.9	18.9	18.6	19.5
	19						16.7	17.2	19.9	20.7	18.9	18.3	19.8	18.7	19.5	16.8		18.1	20	17.1	20.2	19.7	18	19.3	18.4	19.6
	24											18.8	20.2	19.2	19.9	16.9		18.5	20.7	17.5	20.2	20.1	18.5	20	19.1	18.9
	26											18.9	19.6	18.9	19.5	16.5		18.3	20.7	17.6	19.7	20.6	18.4	19.9	18.6	19.1
	28											18.8	19.6	19.5	19.6	16.6		18.6	21.3	17.6	20.1	20.8	18.5	19.9	18.8	19.5
	31											18.9	20.1	19.6	20.7	17		18.6	20.4	17.9	20.9	20.7	18.2	20.9	19.6	19.5
	33											18.9	19.8	19.4	21.2	17.6		18.8	19.6	18.2	21.3					
	35											19.2	19.7	19.5	20.7	17.4		18.7	20.2	18.1	19.6					
	38											19.6	20	19.7	20.6	18		18.8	20.4	19	17.2					
	40																	19.4	20.4	18.7	19.3					
	42																	19.9	20.4	18.8	20.6					
	45																	19.8	21	18.3	21.7					
	47																	19.7	20.7	18.3	21.1					
	52																	20.2	21.1	18.5	21.9					
	54																	20.3	21.4	18.6	22.6					
	59																	20	21.6	18.8	21.7					
	61																	20.5	22.1	19.2	21.3					

Table 29

	PBS			MORAb-202 1mg/kg			MORAb-202 2.5mg/kg			MORAb-202 5mg/kg			MORAb-003-durostatin 5mg/kg		
days post randomization	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N
0	18.68	0.165239	5	19.2	0.385328	5	18	0.554902	5	18.025	0.689078	4	18.42	0.578982	5
3	18.98	0.234959	5	19.48	0.47393	5	18.48	0.579842	5	18.025	0.769253	4	18.14	0.76975	5
6	18.94	0.245739	5	19.58	0.537015	5	18.6	0.609665	5	18.175	0.694839	4	18.44	0.667108	5
7	18.86	0.268971	5	19.4	0.549488	5	18.46	0.525953	5	18.125	0.628577	4	18.52	0.638721	5
10	18.9	0.29444	5	19.28	0.537015	5	18.84	0.585996	5	18.425	0.68618	4	19.02	0.590063	5
12	18.8	0.352933	5	19.4	0.600608	5	19.2	0.545849	5	18.65	0.678513	4	19.2	0.502108	5
14	18.06	0.267112	5	19.4	0.685817	5	18.74	0.454832	5	18.25	0.523801	4	19.04	0.352368	5
17	17.8	0.320373	5	19.08	0.673649	5	18.96	0.451533	5	18.725	0.592675	4	18.94	0.33497	5
19				18.68	0.764423	5	18.62	0.527655	5	18.85	0.670634	4	19	0.338521	5
24							19	0.579498	5	19.225	0.663539	4	19.32	0.313137	5
26							18.68	0.563279	5	19.075	0.621135	4	19.32	0.41086	5
28							18.82	0.573795	5	19.4	0.728103	4	19.5	0.407939	5
31							19.26	0.636533	5	19.45	0.638148	4	19.78	0.484329	5
33							19.38	0.585826	5	19.475	0.60047	4			
35							19.3	0.536644	5	19.15	0.416401	4			
38							19.58	0.430983	5	18.85	0.584918	4			
40										19.45	0.314619	4			
42										19.925	0.359691	4			
45										20.2	0.665164	4			
47										19.96	0.549137	4			
52										20.425	0.651414	4			
54										20.725	0.758819	4			
59										20.525	0.620064	4			
61										20.775	0.552051	4			

2.6.4 Evaluation of minimum efficacious dose of eribulin in the hNSCLC NCI-H2110 model in CB17-SCID mice

[00314] Human NSCLC, H2110 cells, passage 46 were implanted subcutaneously in 30 CB17 SCID mice (female, 5 to 6 weeks old, weighing 20 grams). After 11 days post-implantation, mice were randomized into five groups. The five animals with the tumor volumes deviating the most from the average were excluded. Average tumor volume in each group on the treatment day (Day 0) ranged between 87.6-89.4 mm^3 (Table 32). The enrolled mice were treated with eribulin (Lot# N1201193) at 0.05, 0.2, 0.8, or 1.6 mg/kg, or with PBS, according to the study design (Table 30). Each group was terminated, respectively, when tumor volume >2000 mm^3 was first observed within the group. The study was terminated on Day 38 (30 days after the last dose).

Table 30. Study design

Group	# Mice	Treatment	Dose (mg/kg)	Regimen	Route
1	5	PBS	0	q4dx3	i.v.
2		Eribulin	0.05		
3			0.2		
4	4*		0.8		
5	5		1.6		

[00315] The tumor volumes in individual mice are shown in Table 31, and the group average and SEM is shown in Table 32. Tumor growth kinetics for each group (group average and SEM) are shown in Figure 14, and tumor volumes in individual mice, as well as group average and SEM on Day 24 (when tumor volume >2000mm^3 were observed in PBS treated mice), are shown in Figure 15. Eribulin caused TGI of 50.5% (with no tumor regression observed) at 0.05 mg/kg (p = 0.0026 vs. saline); TGI of ~ 99% at 0.2, 0.8, or 1.6 mg/kg (p values were < 0.0001 for all 3 groups when compared to saline). The minimum efficacious dose that induced tumor regression is 0.2 mg/kg. However, majority of the regressed tumors in these mice (3/5 in 0.2 mg/kg group, 4/5 in 0.8 mg/kg group, and 2/5 in 1.6 mg/kg group) re-grew or remained measurable throughout the study duration (30 days after the last dose).

[00316] Body weight of individual mice is shown in Table 33, and the group average and SEM is shown in Table 34. Body weight change kinetics for each group (group average and SEM) are shown in Figure 16.

[00317] No significant body weight loss in any of the treatment groups compared with saline-treated control group were observed. No clinical findings indicating toxicity during the treatment were observed.

[00318] Eribulin, at 0.2 mg/kg and higher, administered q4dX3 i.v., showed significant effect on H2110 tumor growth. Tumor regression was achieved. When a lower dose was administered (at 0.05 mg/kg), no tumor regression was achieved. Therefore, the minimum efficacious dose tested in this study is 0.2 mg/kg.

Table 31

days post 1st dose	vehicle					eribulin 0.05mg/kg					eribulin 0.2mg/kg					eribulin 0.8mg/kg					eribulin 1.6mg/kg				
0	59	91	118	88	91	105	101	94	61	77	103	68	78	130	62	111	104	81	93	54	70	116	74	91	91
3	62	179	219	236	173	175	149	117	161	64	68	62	52	79	61	54	55	40	51	33	50	44	44	44	47
5	80	255	436	283	257	231	157	228	261	132	60	60	48	90	51	32	29	34	42	25	25	22	32	24	24
7	111	433	440	472	446	357	171	269	247	102	48	47	39	49	47	26	34	17	21	24	19	12	31	15	11
10	230	555	747	622	489	370	200	413	376	226	33	39	28	36	47	17	22	19	5	21	15	0	29	14	0
12	263	677	722	877	620	539	265	448	350	135	19	41	23	13	14	15	23	16	12	24	13	0	14	0	0
17	720	959	960	1158	885	725	514	751	620	531	0	0	0	0	13	17	38	0	0	26	0	0	0	0	0
19	862	1314	940	1097	941	869	437	908	776	837	27	39	29	29	16	0	20	18	0	27	0	0	19	0	0
24	1886	2308	1854	2760	1671	712	718	1489	1225	1040	0	15	19	23	11	14	0	20	0	33	18	0	19	0	0
26											0	24	0	11	14	0	8	14	14	15	8	0	17	0	0
28											0	7	0	14	83	0	16	20	14	17	0	0	16	0	0
31											0	16	0	10	31	0	10	15	26	29	11	0	17	0	0
33											0	27	0	13	22	0	13	8	18	44	8	0	28	0	0
35											0	19	0	16	42	0	13	0	22	50	14	0	17	0	0
38											0	19	0	14	45	0	11	13	13	54	11	0	20	0	0

Table 32

	PBS			eribulin 0.05mg/kg			eribulin 0.2mg/kg			eribulin 0.8mg/kg			eribulin 1.6mg/kg		
days post 1st dose	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N
0	89.4	9.34	5	87.6	8.18	5	88.2	12.56	5	88.6	10.02	5	88.4	8.11	5
3	173.8	30.31	5	133.2	19.74	5	64.4	4.45	5	46.6	4.31	5	45.8	1.20	5
5	262.2	56.43	5	201.8	24.37	5	61.8	7.43	5	32.4	2.83	5	25.4	1.72	5
7	380.4	67.55	5	229.2	43.40	5	46	1.79	5	24.4	2.83	5	17.6	3.62	5
10	528.6	85.83	5	317	43.21	5	36.6	3.17	5	16.8	3.07	5	11.6	5.42	5
12	631.8	101.42	5	347.4	70.14	5	22	5.07	5	18	2.34	5	5.4	3.30	5
17	936.4	70.46	5	628.2	48.40	5	2.6	2.60	5	16.2	7.39	5	0	0.00	5
19	1030.8	80.29	5	765.4	84.75	5	28	3.65	5	13	5.50	5	3.8	3.79	5
24	2095.8	195.76	5	1036.8	149.24	5	13.6	3.94	5	13.4	6.26	5	7.4	4.53	5
26							9.8	4.54	5	10.2	2.83	5	5	3.37	5
28							20.8	15.74	5	13.4	3.48	5	3.2	3.19	5
31							11.4	5.77	5	16	5.29	5	5.6	3.55	5
33							12.4	5.53	5	16.6	7.45	5	7.2	5.42	5
35							15.4	7.72	5	17	9.22	5	6.2	3.82	5
38							15.6	8.25	5	18.2	9.25	5	6.2	4.05	5

Table 33

days post 1st dose	vehicle					eribulin 0.05mg/kg					eribulin 0.2mg/kg					eribulin 0.8mg/kg					eribulin 1.6mg/kg				
0	18.5	16.7	19.1	20.4	19.6	19.1	16.4	18.6	20.1	17.9	18.2	18.5	16.7	19.8	18.9	18.6	17.8	18.4	17.8	18.1	18.2	18.3	19.4	16.3	19.0
3	18.8	16.6	19.6	20.9	20.0	19.4	17.1	18.5	20.4	18.7	18.6	18.5	16.9	19.6	19.8	18.8	18.0	18.4	18.1	19.0	17.8	18.3	20.1	15.8	19.6
5	18.8	16.8	19.3	21.2	20.0	19.4	16.5	18.4	20.4	19.4	18.5	19.1	16.9	20.2	20.1	18.9	18.1	18.5	17.6	18.2	18.1	18.1	19.5	16.0	19.3
7	18.6	16.5	19.3	21.2	19.8	19.4	16.3	18.7	20.3	19.1	18.5	18.5	17.1	19.6	20.7	19.1	18.1	19.0	17.8	19.0	18.1	18.2	19.5	16.3	19.4
8	18.3	16.7	18.8	21.1	19.6	19.3	16.3	18.4	20.4	19.4	18.6	19.1	17.0	19.6	19.9	19.2	18.0	19.0	17.7	18.8	18.0	18.3	20.1	16.2	19.4
9	18.4	16.4	18.5	21.0	19.5	18.8	16.1	18.6	19.9	19.1	18.3	20.0	16.8	19.5	19.5	19.2	17.7	18.5	17.5	18.5	17.4	18.0	19.6	15.8	19.4
10	19.0	17.2	19.2	21.6	20.3	19.7	16.3	19.4	20.5	20.0	18.7	19.4	17.1	20.0	20.1	19.0	17.7	18.9	17.8	19.1	18.1	18.5	21.0	16.1	20.0
12	19.0	15.9	18.5	21.3	19.2	18.8	15.9	18.6	19.6	19.7	18.3	19.3	16.9	19.7	19.9	18.9	17.8	19.0	17.8	19.0	18.0	18.8	20.5	16.3	20.1
17	18.8	15.5	17.8	20.4	18.3	17.5	16.0	18.5	18.0	19.7	19.0	19.3	17.4	20.5	20.0	19.2	18.0	18.8	18.1	19.1	19.0	19.2	21.0	16.4	19.4
19	18.9	15.6	17.2	20.6	18.1	17.8	16.3	18.4	18.0	19.4	19.1	19.0	17.1	21.1	21.0	19.1	17.7	19.4	18.7	19.1	19.1	19.5	21.1	16.3	19.8
24	18.2	15.8	17.4	20.3	18.2	18.8	16.5	18.8	17.4	18.0	19.3	20.2	18.8	21.7	22.0	20.1	18.5	20.3	19.1	20.3	19.4	20.6	21.7	18.1	20.7
26											19.8	20.9	18.6	22.0	20.4	20.3	18.1	19.9	19.3	20.9	19.5	20.7	21.6	18.3	20.6
28											20.1	20.6	18.1	21.5	21.0	20.3	17.8	20.2	19.6	20.6	19.6	20.3	21.2	17.6	21.1
31											18.7	20.1	18.7	22.0	21.2	20.1	18.2	20.3	19.5	20.7	19.8	20.4	21.9	18.1	21.9
33											20.0	20.3	18.4	22.2	21.8	20.2	18.3	21.0	19.2	20.2	20.1	19.9	21.7	17.9	20.7
35											19.5	20.1	18.7	22.1	21.4	20.1	17.8	21.0	19.3	20.3	20.3	18.2	21.8	18.2	20.9
38											19.8	20.8	18.3	21.6	21.6	20.4	18.1	21.4	19.4	21.2	20.0	21.0	21.9	18.4	20.3

Each column represents an individual animal.

Table 34

	PBS			eribulin 0.05mg/kg			eribulin 0.2mg/kg			eribulin 0.8mg/kg			eribulin 1.6mg/kg		
days post 1st dose	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N
0	18.9	0.62	5	18.4	0.62	5	18.4	0.51	5	18.1	0.16	5	18.2	0.53	5
3	19.2	0.73	5	18.8	0.54	5	18.7	0.51	5	18.5	0.19	5	18.3	0.75	5
5	19.2	0.73	5	18.8	0.66	5	19.0	0.60	5	18.3	0.22	5	18.2	0.62	5
7	19.1	0.77	5	18.8	0.67	5	18.9	0.60	5	18.6	0.27	5	18.3	0.58	5
10	18.9	0.72	5	18.8	0.69	5	18.8	0.51	5	18.5	0.29	5	18.4	0.67	5
12	18.8	0.76	5	18.5	0.65	5	18.8	0.59	5	18.3	0.30	5	18.0	0.69	5
17	19.5	0.73	5	19.2	0.74	5	19.1	0.55	5	18.5	0.32	5	18.7	0.84	5
19	18.8	0.86	5	18.5	0.68	5	18.8	0.56	5	18.5	0.28	5	18.7	0.77	5
24	18.2	0.79	5	17.9	0.60	5	19.3	0.53	5	18.6	0.24	5	19.0	0.73	5
26							19.5	0.74	5	18.8	0.30	5	19.2	0.79	5
28							20.4	0.63	5	19.7	0.36	5	20.1	0.62	5
31							20.3	0.56	5	19.7	0.48	5	20.1	0.57	5
33							20.3	0.59	5	19.7	0.50	5	20.0	0.66	5
35							20.1	0.66	5	19.8	0.43	5	20.4	0.71	5
38							20.5	0.68	5	19.8	0.47	5	20.1	0.62	5

EXAMPLE 2

1. Materials and Methods

[00319] MORAb003-VCP-eribulin (MORAb-202) was synthesized by conjugating MORAb-003 (humanized anti-human folate receptor alpha) to the MAL-PEG2-Val-Cit-PAB-eribulin (ER-001159569) compound described in section 1.1 of Example 3. The conjugation method is described in section 1.4.1 of Example 4.

1.1 Tumor models

[00320] Human tumor cell lines used in the additional *in vitro* evaluation of MORAb-202 include IGROV1 (human ovarian carcinoma, $FR^{hi\,(+++)}$), OVCAR3 (human ovarian carcinoma, $FR^{med(++)}$), NCI-H2110 (human non-small cell lung carcinoma, $FR^{med(++)}$), A431-A3 (A431 parental cell line stabily transfected with human mesothelin, $FR^{lo(+/-)}$), SJSA-1 (human osteosarcoma, $FR^{neg(-)}$), and HL-60 (human leukemia, $FR^{neg(-)}$). All of these cell lines were obtained directly from the American Type Culture Collection (ATCC). For *in vivo* studies, non-small cell lung cancer, triple negative breast cancer, and endometrial cancer patient-derived xenograft mouse models were established and maintained at Oncotest GmbH (Freiburg, Germany), Oncodesign (Dijon, France), and EPO Berlin-Buch GmbH (Berlin, Germany), respectively.

1.2 *In vitro* cytotoxicity analyses

1.2.1 Crystal Violet assay

[00321] IGROV1 ($FR^{hi\,(+++)}$), A431-A3 ($FR^{lo(+/-)}$), and SJSA-1 ($FR^{neg(-)}$) cells were sub-cultured and seeded at 10,000 cells/well in complete growth medium in 96-well tissue culture plates, incubated at 37°C, 5% CO_2 overnight (16 hours). Typically, test reagents were serially-diluted 1:4 in 2 mL deep-well dilution plates, starting at 1 μM (10 dilutions total). 100 μL of diluted samples were added to the cell plates (starting concentration of test samples at 100 nM). Plates were incubated at 37°C, 5% CO_2 for an additional 48 hours. Medium was discarded, plates were washed once with 200 μL DPBS, stained with 50 μL of 0.2% Crystal Violet solution at room temperature for 15 min, and then washed extensively with tap water. Plates were air-dried, and Crystal Violet was dissolved with 200 μL of 1% SDS solution. Plates were read at 570 nm.

Data was analyzed using GraphPad Prism 6. For OVCAR3 ($FR^{med(++)}$) and NCI-H2110 ($FR^{med(++)}$), cells were seeded at 3,000 cells/well and incubated for 5 days with MORAb-202.

1.3 *In vivo* studies

1.3.1 NCI-H2110 xenograft model

[00322] *Animal preparation*: CB17 SCID mice (female, 6 weeks old) were housed at 5 mice per ventilated cage. Sterilized food pellets and water bottle were available, ad lib, to the animals. Animals were acclimated for 5-7 days prior to tumor implantation.

[00323] *Cell culture*: Human NCI-H2110 cells were thawed from frozen stock (NB2813-65) and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) in 5% CO_2 at 37°C. After two passages, upon reaching confluence at approximately 70%, the cells were harvested by using cell dissociation solution, washed twice with serum-free medium, and counted.

[00324] *Tumor implantation*: The cell suspension in serum-free medium was mixed with ice-cold matrigel at 1:1 (v:v) to a final concentration of 1.0×10^8 cells/mL. Each mouse was injected subcutaneously with 100 µL of the mixture at 1.0×10^7 cells/mouse. A 27G needle was used for all injections. Mice were monitored for clinical well-being and tumors were measured by digital caliper three times weekly, beginning on Day 3 post-implantation. Tumor volume (mm^3) was calculated using the formula: W (mm) × L (mm) × D (mm) × $\pi/6$. When the tumors reached ~100 mm^3 (in an average of >70 to ~130 mm^3), the animals were randomized to 4-5 per group. The 5 animals with the tumor volumes deviating greatest from the average were excluded.

[00325] *Study design*: The enrolled experimental mice were injected intravenously with 200 µL of vehicle or MORAb-202 at 1.0, 2.5, and 5 mg/kg, according to the study design (Table 35), on the day of randomization. Body weight was measured prior to dose, and two times per week during the study. At the end of the study, terminal body weight was measured and recorded. Animals were euthanized when the individual tumor volume exceeded 2000 mm^3. Early termination criteria prior to reaching the maximum allowed tumor volume included: (1) tumor ulceration greater than 50% of the tumor (v:v); (2) paralysis; (3) body weight loss >20%; and (4) 50% of the animals

within the group had met termination. Any mouse euthanized or found dead during the study was processed following the terminal procedure described above.

Table 35. Study design

Group	# Mice	Treatment	Dose (mg/kg)	Regimen	Route
1	5	Vehicle	0	single bolus	i.v.
2		MORAb-202	1		
3			2.5		
4			5		

1.3.2 Patient-derived xenograft (PDx) models

1.3.2.1 Non-small cell lung cancer (NSCLC) PDx model: LXFA-737 (Oncotest)

[00326] *Tumor implantation*: NSCLC tumor fragments were obtained from LXFA-737 tumor xenografts serially passaged in nude mice. After removal from donor mice, tumors were cut into fragments (3-4 mm edge length) and placed in phosphate-buffered saline (PBS) containing 10% penicillin/streptomycin. Recipient animals were anesthetized by inhalation of isoflurane and received unilateral or bilateral tumor implants subcutaneously in the flank. Tumor xenografts were implanted with one or two tumors per mouse at a take rate < 65%. In the case of a bilateral take, one of these tumors was explanted prior to randomization. Animals and tumor implants were monitored daily until solid tumor growth was detectable in a sufficient number of animals. At randomization, the volume of growing tumors was determined. Animals fulfilling the randomization criteria (i.e. bearing tumors of 50-250 mm^3, preferably 80-200 mm^3) were distributed into experimental groups consisting of 5-6 animals per group, aiming at comparable median and mean group tumor volumes of approximately 100-120 mm^3. Animals not used for experiments were euthanized. The day of randomization was designated as Day 0 of the experiment.

[00327] *Study design*: The enrolled experimental mice were injected intravenously with vehicle, MORAb-003 at 5 mg/kg, or MORAb-202 at 5 mg/kg, according to the study design (Table 36), on the day of randomization. Body weight was measured prior to dose on each dosing day, and two times per week during the study. At the end of the

study, the terminal body weight was measured and recorded. Animals were euthanized when the individual tumor volume exceeded 2000 mm^3.

Table 36. Study design

Group	# Mice	Treatment	Dose (mg/kg)	Regimen	Route
1	6	Vehicle	0	single bolus	i.v.
2	6	MORAb-003	5		
3	6	MORAb-202	5		

1.3.2.2 Triple negative breast cancer (TNBC) PDx model: OD-BRE-0631 (Oncodesign)

[00328] *Tumor implantation*: Nine female SWISS nude mice were injected subcutaneously into the right flank with patient-derived TNBC tumor fragments. Tumor-bearing mice were euthanized when tumor volume reached 500-1000 mm^3, and tumors were surgically excised. Tumor fragments (30-50 mg) were orthotopically implanted into the mammary fat pad region of 34 female SWISS nude mice 24 to 72 hours after a whole-body irradiation with a gamma-source (2 Gy, 60Co, BioMEP, France). When the tumors reached a mean volume of 200-300 mm^3, 24 of the 34 total animals were randomized into two groups (n=12 animals) according to their individual tumor volume using Vivo Manager® software (Biosystemes, Couternon, France). A statistical test (analysis of variance) was performed to evaluate homogeneity between groups. The day of randomization was designated as Day 0 of the experiment.

[00329] *Study design*: On Day 1 (one day after randomization and two days prior to treatment), 3 mice from each of the two untreated groups were terminated. The remaining experimental mice were injected intravenously with vehicle or MORAb-202 at 5 mg/kg, according to the study design (Table 37), on Day 3. On Day 8 (five days after treatment), 3 mice from each of the two treated groups were terminated. Immediately following termination, tumor tissue was collected and fixed in 4% neutral buffered formalin for 24 to 48 hours, and then embedded in paraffin (Histosec®, Merck, Darmstadt, Germany). The paraffin embedded sample was stored at room temperature for subsequent immunohistochemistry analysis.

Table 37. Study design

Group	# Mice	Treatment	Dose (mg/kg)	Regimen	Route
1	3	n/a	n/a	n/a	n/a
	9	Vehicle	0	single bolus	i.v.
2	3	n/a	n/a	n/a	n/a
	9	MORAb-202	5	single bolus	i.v.

[00330] *Immunohistochemistry (IHC) analysis*: IHC staining of formalin-fixed, paraffin-embedded tumor tissues were performed in order to evaluate both MORAb-202 occupation and cancer associated fibroblast expression. Prior to staining, slides were dewaxed and antigen was retreived in a Lab Vision™ PT Module (Thermo Scientific), in citrate buffer (pH 6.0) pre-warmed to 85°C, using the following program: warm to 97°C; incubate at 97°C for 30 min; and cool to 60°C. Slides were then transferred to double distilled water at room temperature for 5 min. Staining was performed in a Lab Vision™ Autostainer 360 (Thermo Scientific). Briefly, slides were washed twice in 1X Tris-buffered saline/Tween-20 (TBST) for 6 min/wash. Tissue sections were then incubated in blocking buffer (300 µL) (10% goat serum (Jackson Immunoresearch Laboratory Inc., Cat No. 005-000-121) diluted in 3% bovine serum albumin (BSA) - phosphate buffered saline (PBS)) for 1 hour, incubated in conjugated antibody (200 µL) (Table 38) for 1 hour, and washed five times in 1X TBST for 6 min/wash. Slides were counterstained with DAPI in mounting media, and coverslipped slides were allowed to harden for 30 min. Slides were processed on a Panoramic MIDI scanner (3DHISTECH), and IHC images were analyzed using Halo software (Indica Labs). The antibodies used in this analysis targeted α-smooth muscle actin (SMA), which is a specific marker for cancer associated fibroblasts, and human IgG,which can detect the presence of MORAb-202.

Table 38. IHC antibodies

Antibody	Conjugated	Vendor	Cat. No.	Lot	Stock Solution	Working Solution
α-smooth muscle actin (SMA)-FITC	FITC	Sigma	F3777	124M4775V	2.0 mg/mL	5.0 µg/mL
mouse IgG1, κ isotype control	AF488	Biolegend	400129	B128493	0.2 mg/mL	1:1000
goat anti-human IgG	AF555	Mol. Probes	A21433	1709318	n/a	1:200

1.3.2.3 Endometrial cancer PDx models: Endo-12961 and Endo-10590 (EPO Berlin)

[00331] *Tumor implantation*: Endometrial cancer tumor fragments were obtained from serially passaged Endo-12961 and Endo-10590 tumor xenografts, and stored as stock in fluid nitrogen. Tumor fragments were implanted subcutaneously into the left flank of 40 NMRI nu/nu female mice, and tumor volume was monitored. Mice with a tumor volume of 100-160 mm^3 were randomized into one of four groups (Groups A-D, Table 39). Satellite mice for randomization were included in a fifth group (Group E, Table 39). Each group consisted of 8 animals. The day of randomization was designated as Day 0 of the experiment.

[00332] *Study design*: The enrolled experimental mice were injected intravenously with PBS, eribulin at 3.2 mg/kg or 0.1 mg/kg, or MORAb-202 at 5 mg/kg, according to the study design (Table 39), on the day of randomization. Tumor growth was evaluated by the measurement of two perpendicular diameters twice weekly, and tumor volume (TV), relative tumor volume (RTV) and treated/control (T/C) values were calculated. Body weight was also evaluated twice weekly as a parameter for toxicity, with the calculation of the body weight per group and body weight changes (BWC) relative to the start of treatment. Animals were sacrificed when the individual tumor volume exceeded 1 cm^3, or at the end of the study.

Table 39. Study design

Group	# Mice	Treatment	Dose (mg/kg)	Regimen	Route
A	8	PBS	0	single bolus	i.v.
B		Eribulin	3.2		
C		Eribulin	0.1		
D		MORAb-202	5		
E		n/a	n/a	n/a	n/a

1.4 Mechanism of action

1.4.1 Three-dimensional (3D) co-culture system in zPredicta

[00333] All mesenchymal stem cell (MSC)-containing 3D co-culture experiments were conducted in zPredicta, using organ-specific 3D extracellular matrix systems such as rStomach™. Bone marrow mesenchymal stem cells (BM-MSCs) in rStomach™ were co-cultured with the Nuc Red Light MKN-74 gastric cancer cell line in quadruplicate in 48-well format for 12 days. MKN-74 cells had been previously shown to express enough folate receptor alpha (FR) for MORAb-202 treatment to induce cellular apoptosis. Prior to culture, BM-MSCs were evaluated for target antigen expression and for markers of MSC differentiation (Table 40) by flow cytometry.

Table 40. Markers of MSC differentiation

Cell population	Markers
Mesenchymal stem cells (MSCs)	Stro-1$^+$/CD105$^+$
Pre-adipocytes	CD34$^+$/CD31$^-$
Adipocytes	Oil red
Cancer associated fibroblasts (CAFs)	Alpha-smooth muscle actin (αSMA), vimentin
Pre-pericytes/pericytes	NG2$^+$, CD13$^+$, CD146$^+$
All	FRA

[00334] rStomach™ cultures were treated with either MORAb-202, unconjugated MORAb-003 antibody, eribulin, or control, as described in Table 41. Controls included

untreated and vehicle-treated (PBS and DMSO) cultures. MSC differentiation was monitored by light microscopy. Once visible differentiation was observed, samples were harvested for staining and flow cytometry analysis.

Table 41. Co-culture treatments

Agent	Working Concentration(s)
MORAb-202	10 nM
MORAb-003 (unconjugated antibody)	10 nM
Eribulin	1.7 nM and 0.2 nM
PBS	
DMSO	0.1%
Untreated control	

1.4.2 Time course analysis of effect of MORAb-202 on cancer associated fibroblasts

[00335] Subcutaneous H2110 xenograft tumor-bearing mice were prepared as described in section 1.3.1. Tumor samples were harvested at Days 0, 3, 5, 7, and 9 following administration of vehicle, or MORAb-202 at 5 mg/kg. Collected tumor samples were processed on slides, and the expression of cancer associated fibroblasts was analyzed by IHC as described in section 1.3.2.2.

2. Results

2.1 *In vitro* cytoxicity analyses

2.1.1 Cytotoxicity of MORAb-202

[00336] *In vitro* potency of MORAb-202 was evaluated using a Crystal Violet assay, as detailed in section 1.2.1. Screening was performed on IGROV1 ($FR^{hi\ (+++)}$), OVCAR3 ($FR^{med(++)}$), NCI-H2110 ($FR^{med(++)}$), A431-A3 ($FR^{lo(+/-)}$), and SJSA-1 ($FR^{neg(-)}$) cells. The results of this screening are provided in Figure 17 and Table 42.

Table 42. Cytotoxicity (EC_{50}) screening of MORAb-202 on various tumor cell lines

EC_{50} (nM)				
IGROV I (FR+++)	OVCAR3 (FR++)	NCI-H2110 (FR++)	A431-A3 (FR+/-)	SJSA-1 (FR-)
0.01	0.16	0.74	23	> 100

[00337] MORAb-202 exhibited folate receptor alpha expression-dependent cytotoxicity against tumor cell lines, and low levels of off-target killing. MORAb-202 demonstrated the highest level of potency (0.01 nM) on IGROV1 cells, with little cytotoxicity (> 100 nM) on folate receptor alpha-negative SJSA-1 cells. Intermediate potency was observed in OVCAR3 and NCI-H2110 cells (0.16 nM and 0.74 nM).

2.2 *In vivo* studies

2.2.1 Efficacy of MORAb-202 in the NC1-H2110 xenograft model

[00338] Subcutaneous H2110 tumor-bearing mice were were injected intravenously with vehicle or MORAb-202 at 1, 2.5, and 5 mg/kg. Significant tumor regression was observed following a single dose of MORAb-202 at 5 mg/kg (Figure 18 and Table 43). Using this xenograft model with high folate receptor alpha expression and single dose administrations, the therapeutic window for MORAb-202 was shown to be 1 mg/kg for tumor growth delay (with stable disease) and ≥ 2.5 mg/kg for tumor regression. In this study, MORAb-202 at a dose of 2.5 mg/kg resulted in a partial response, and MORAb-202 at a dose of 5 mg/kg resulted in a complete response.

Table 43. Anti-tumor activity of MORAb-202 in the NC1-H2110 xenograft model

	Tumor Volume, mm^3 (Tumor Growth Inhibition, %)	
	Day 17	Day 31
Vehicle (n=5)	1583.4 ± 146.1 (100)	n/a
MORAb-202, 1 mg/kg, single dose (n=5)	840.0 ± 76.8 (53.1)	n/a
MORAb-202, 2.5 mg/kg, single dose (n=5)	60.8 ± 27.1 (3.8)	1173.2 ± 373.2
MORAb-202, 5 mg/kg, single dose (n=4)	0.0 (0.0)	0 (0.0)

2.2.2 Efficacy of MORAb-202 in the NSCLC PDx model: LXFA-737

[00339] Subcutaneous NSCLC PDx tumor-bearing mice were injected intravenously with vehicle, MORAb-003 at 5 mg/kg, or MORAb-202 at 5 mg/kg. A single dose of MORAb-202 (5 mg/kg) resulted in significant tumor regression in this model, in contrast to a single dose of unconjugated MORAb-003 antibody (5 mg/kg), which did not demonstrate significant anti-tumor activity (Figure 19A). Five of the six total mice treated with MORAb-202 were considered to be tumor-free at Day 32 of the study (Table 44), and four remained tumor-free through Day 74 (termination of the study). In addition, no significant body weight loss was observed in the treatment group as compared to the vehicle-treated control group, indicating no toxicity during treatment (Figure 19B).

Table 44. Anti-tumor activity of MORAb-202 in the NSCLC PDx model

	Tumor Volume, mm^3 (Tumor Growth Inhibition, %)		
	Day 21	Day 32	Day 74
Vehicle (n=6)	1004.5 (100)	1561.3 (100)	n/a
MORAb-003, 5 mg/kg, single dose (n=6)	860.7 (85.7)	1572.1 (100.7)	n/a
MORAb-202, 5 mg/kg, single dose (n=6)	22.9 (2.3)	4.7 (0.3)	418.3 (4/6 tumor-free)

2.2.3 Relative efficacy of MORAb-202 and eribulin in endometrial cancer PDx models: Endo-12961 and Endo-10590

[00340] Endo-12961 and Endo-10590 xenografts express high levels of folate receptor alpha. Subcutaneous endometrial cancer PDx tumor-bearing mice were injected intravenously with PBS, eribulin at 3.2 mg/kg or 0.1 mg/kg, or MORAb-202 at 5 mg/kg. The maximum tolerated dose (MTD) of eribulin in this model is 3.2 mg/kg, whereas 0.1 mg/kg is equivalent to the dosage of eribulin provided by MORAb-202 administered at 5 mg/kg. Throughout the beginning of the study, significant anti-tumor activity was observed following treatment with MORAb-202 (5 mg/kg) and the MTD dose of eribulin (3.2 mg/kg) in both animal models, while no significant anti-tumor activity was observed following treatment with eribulin at 0.1 mg/kg (Figures 20A and 20C). However, regressed tumors in mice treated with eribulin at 3.2 mg/kg began to

re-grow during the study duration, whereas no significant tumor re-growth was noted in mice treated with MORAb-202. In this study, MORAb-202 was found to be significantly more efficacious than eribulin. Eribulin treatment also temporarily affected body weight in the first week post-treatment (Figures 20B and 20D). In contrast, no body weight loss was observed in animals treated with MORAb-202.

2.3 Mechanism of action of MORAb-202

2.3.1 IHC and efficacy of MORAb-202 in the TNBC PDx model: OD-BRE-0631

[00341] Subcutaneous TNBC PDx tumor-bearing mice were injected intravenously with vehicle or MORAb-202 at 5 mg/kg. Tumor tissue was collected from mice in each group prior to treatment (Day 1) and after treatment (Day 8). IHC analyses of the collected tumor tissues revealed that MORAb-202 occupies folate receptor alpha-expressing tumor cells five days post-treatment (Day 8), following administration on Day 3 as a single dose (5 mg/kg). Cell occupation was evaluated using an anti-human IgG antibody (Figure 21A). MORAb-202 treatment was also shown to diminish the structure of cancer associated fibroblasts, as shown by IHC staining with an anti-α-smooth muscle actin (SMA)-FITC antibody (Figure 21B). In terms of efficacy, MORAb-202 treatment resulted in maximum tumor regression at 11 days post-treatment, measured by a relative tumor volume (RTV) of 0.62 (Figure 21C).

2.3.2 Effect of MORAb-202, MORAb-003, and eribulin on 3D co-culture system

[00342] Bone marrow mesenchymal stem cells (BM-MSCs) in rStomach™ (zPredicta) were co-cultured with the MKN-74 gastric cancer cell line for 12 days. Prior to culture, BM-MSCs were evaluated for folate receptor alpha expression and for markers of MSC differentiation by flow cytometry. rStomach™ cultures were then treated with either MORAb-202, unconjugated MORAb-003 antibody, eribulin, or control. Once visible MSC differentiation was observed by light microscopy, samples were harvested for staining and flow cytometry analysis. The results of these analyses are shown in Figure 22.

[00343] A total treatment duration of 7 days, with treatment replenishment during this period, was sufficient to produce a measureable effect on the differentiation of human BM-MSCs in culture with MKN-74 cells. Relative to vehicle control, treatment with

MORAb-202 (10 nM) resulted in an increase in MSC and adipocyte populations, and a decrease in pericyte populations (Table 45). These data indicate that MORAb-202 may have a significant effect on the tumor microenvironment.

Table 45. Effect of MORAb-202, MORAb-003, and eribulin on 3D co-culture system

	Percentage of live cells		
Treatment	**MSCs**	**Adipocytes**	**Pericytes**
PBS	32.3%	0.72%	14.6%
MORAb-202	43.7%	22.6%	11.4%
MORAb-003	37.1%	0.69%	24.0%
Eribulin	29.9%	2.68%	25.8%

2.3.3 Time course analysis of effect of MORAb-202 on cancer associated fibroblasts

[00344] Tumor samples were harvested from subcutaneous H2110 xenograft tumor-bearing mice at Days 0, 3, 5, 7, and 9 following administration of vehicle, or MORAb-202 at 5 mg/kg. Collected tumor samples were processed on slides, and cancer associated fibroblast (CAF) expression was analyzed by IHC. The CAF network structure, as evaluated and quantified by staining with an anti-α-smooth muscle actin (SMA)-FITC antibody, was prominent on Day 3 and Day 5, following administration of a single dose of MORAb-202 at 5 mg/kg (Figure 23). However, by Day 7, the majority of this structure was significantly diminished.

EXAMPLE 3

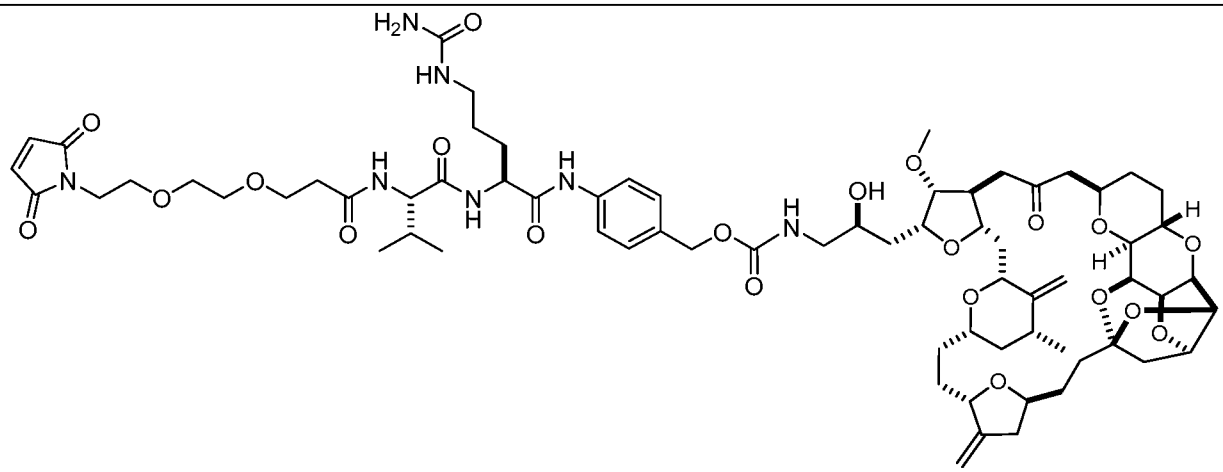
1. Materials and Methods

[00345] Conjugatable eribulin compounds having the structures shown in Table 46 were synthesized according to the following procedures, and used in the preparation of ADCs (Example 4).

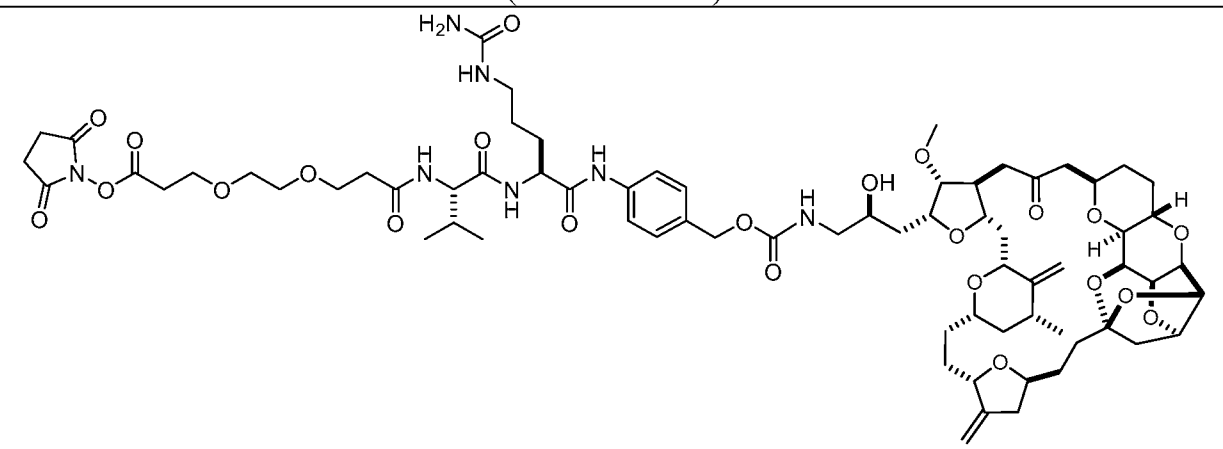
[00346] All solvents used in the synthesis reactions were anhydrous grade (EMD Millipore). All solvents used for workup or purification were high performance liquid chromatography (HPLC) grade (EMD Millipore). Unless indicated otherwise, all chemicals were commercially available. Column chromatography was performed using

a Biotage® SP4. Solvent removal was performed using either a rotary evaporator (Büchi Labortechik AG), or a centrifugal evaporator (Genevac, SP scientific). Preparative liquid chromatography-mass spectrometry (LC/MS) was conducted using a Waters AutoPurification System and an XTerra MS C18 column (5 μm, 19 mm x 100 mm) under acidic mobile phase conditions. Nuclear magnetic resonance (NMR) spectra were taken using deuterated chloroform ($CDCl_3$) unless otherwise stated, and were recorded at 400 or 500 MHz using a Varian instrument (Agilent Technologies). Mass spectra were taken using a Waters Acquity Ultra Performance LC/MS. As used herein, the term "inerted" refers to replacement of the air in a reactor (e.g., a reaction vessel, a flask, a glass reactor) with an essentially moisture-free, inert gas, such as nitrogen or argon. Multiplicities are indicated using the following abbreviations: s=singlet, d=doublet, t=triplet, q=quartet, quint=quintet, sxt=sextet, m=multiplet, dd=doublet of doublets, ddd=doublet of doublets of doublets, dt=doublet of triplets, br s=a broad singlet.

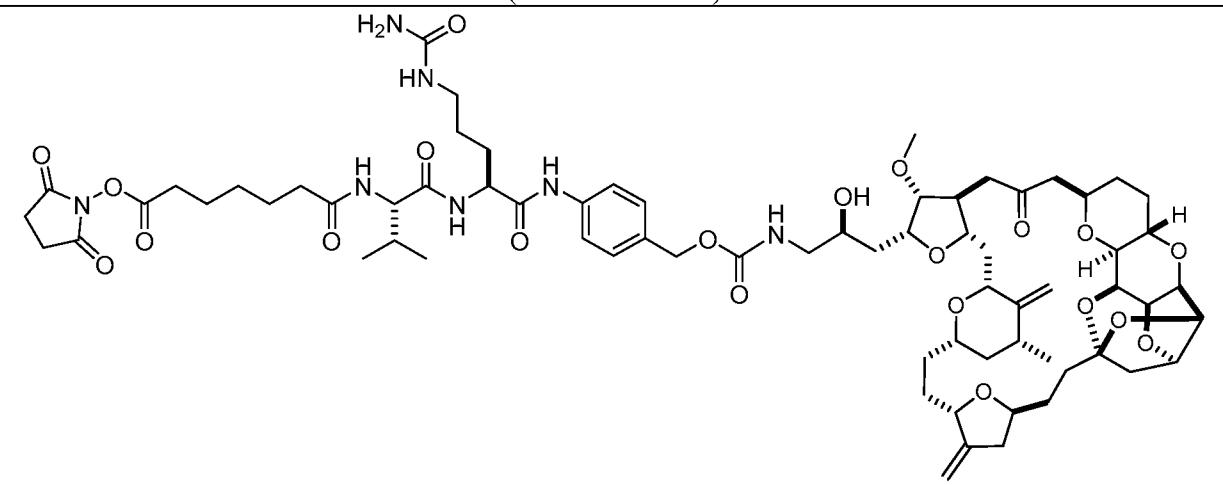
Table 46. Conjugatable eribulin compounds



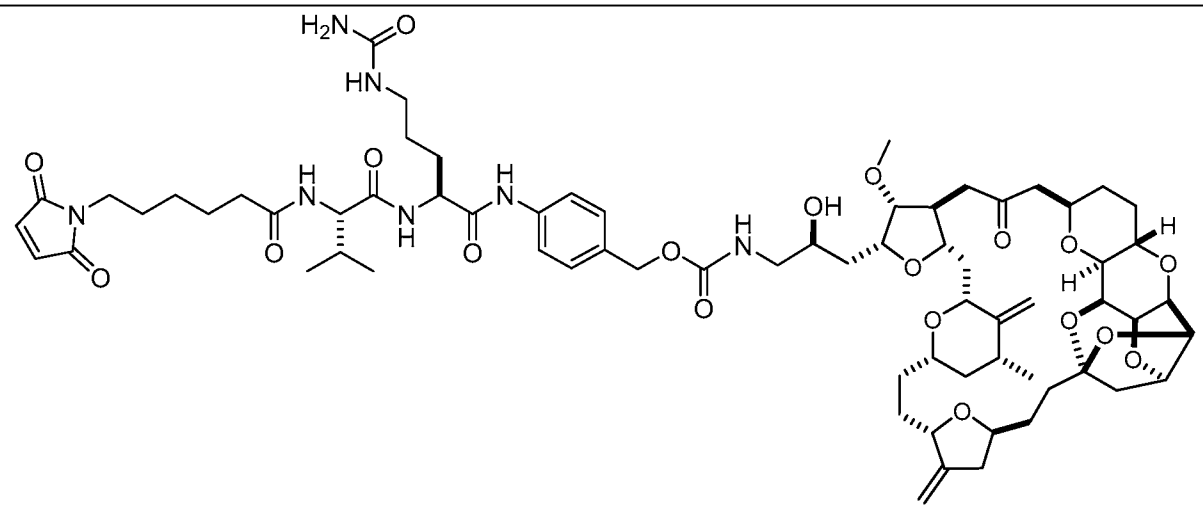
MAL-PEG2-Val-Cit-PAB-eribulin
(ER-001159569)



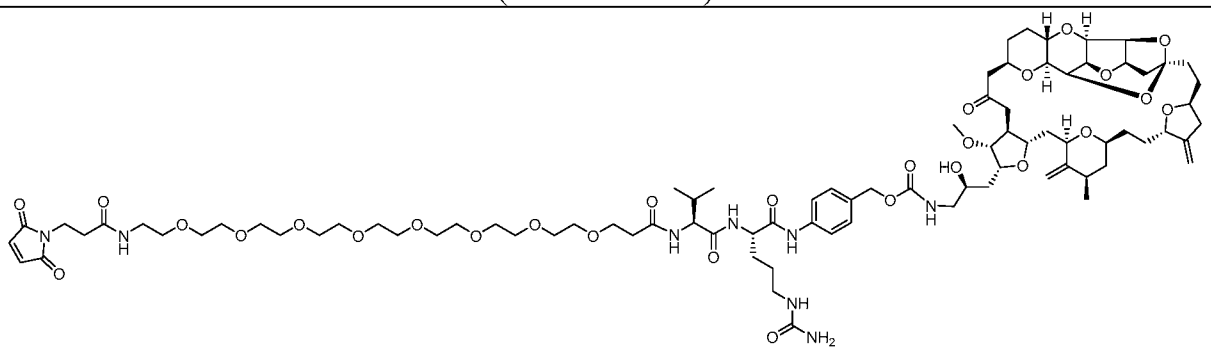
NHS-PEG2-Val-Cit-PAB-eribulin
(ER-001236940)



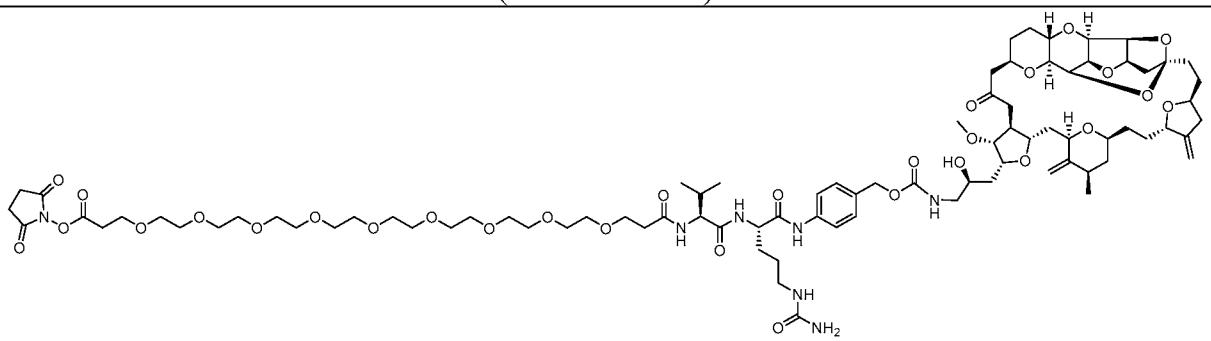
NHS-$(CH_2)_5$-Val-Cit-PAB-eribulin
(ER-001236941)



Mal-$(CH_2)_5$-Val-Cit-PAB-eribulin
(ER-001235638)



Mal-PEG8-Val-Cit-PAB-eribulin
(ER-001242287)



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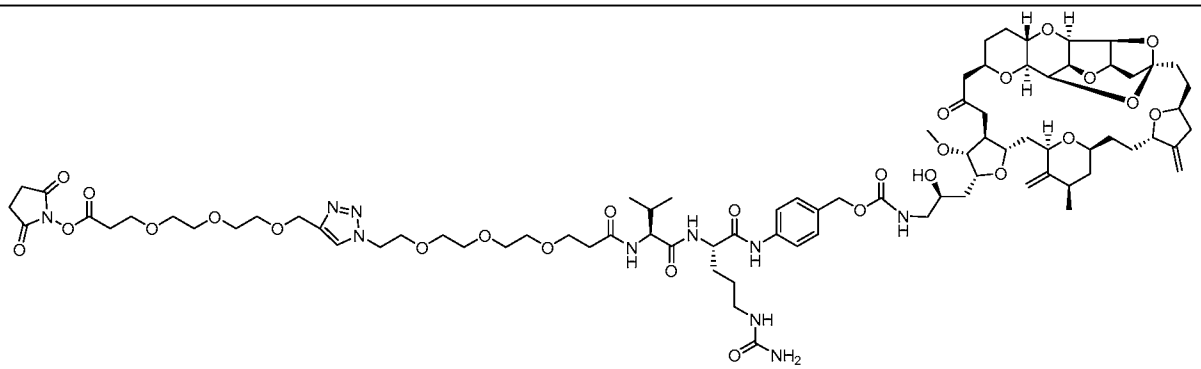
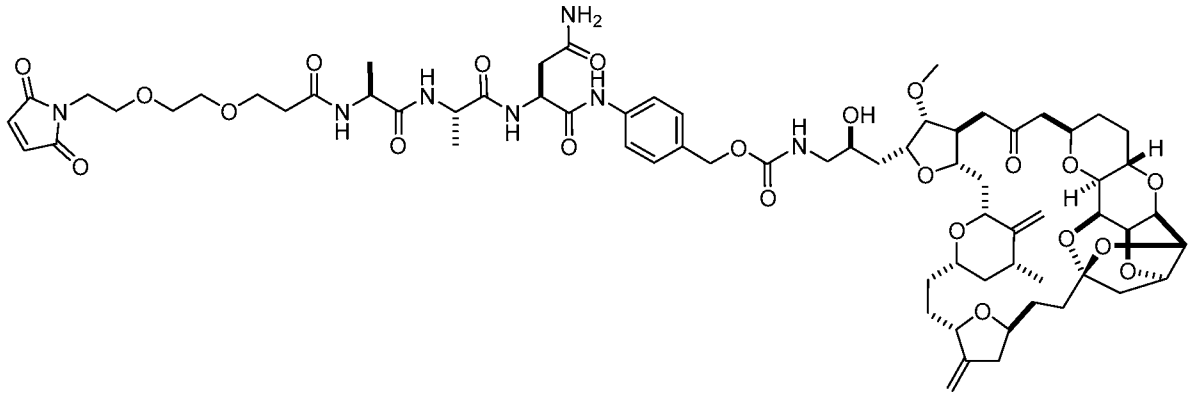
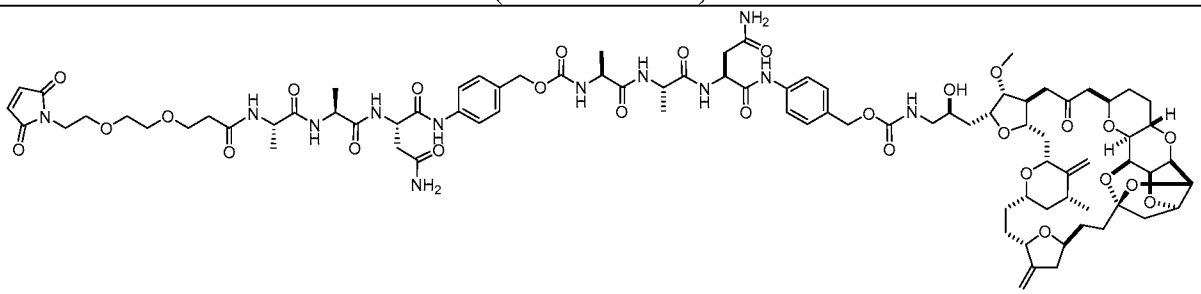
(ER-001242288)



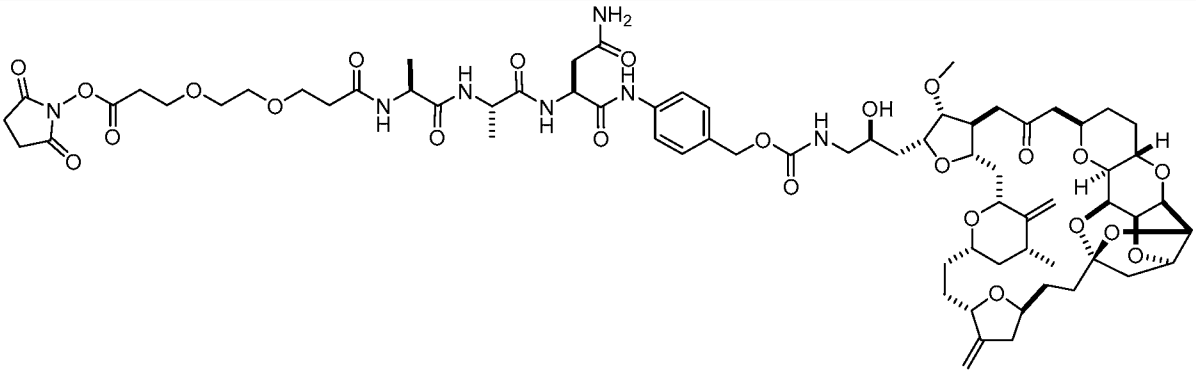
NHS-PEG3-triazole-PEG3-Val-Cit-PAB-eribulin
(ER-001243700)



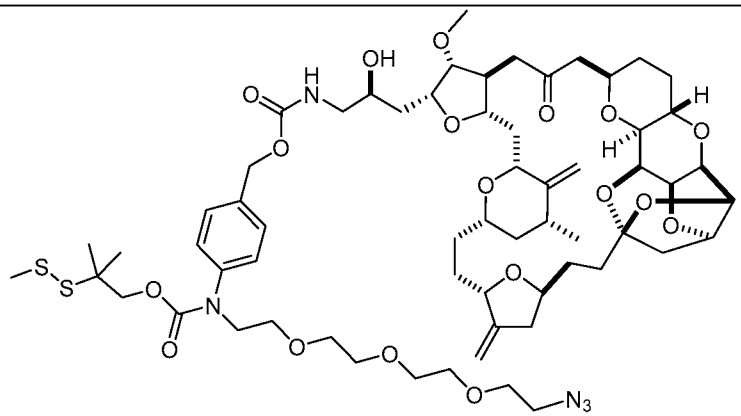
Mal-PEG2-Ala-Ala-Asn-PAB-eribulin
(ER-001231679)



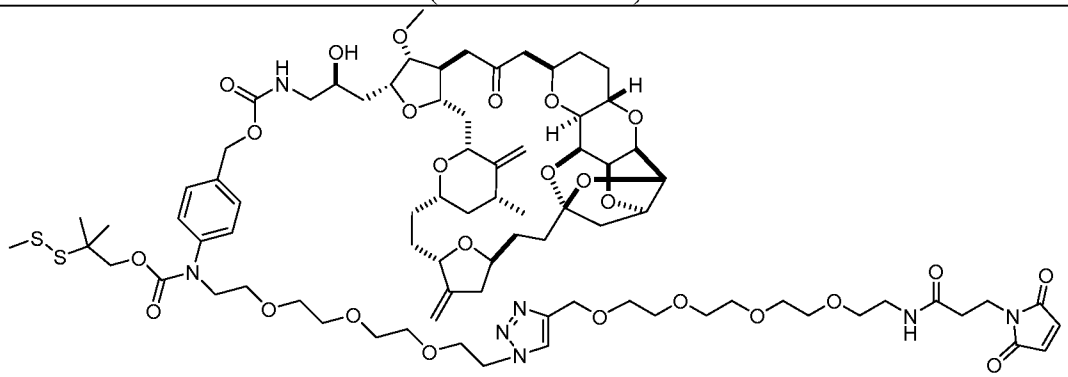
Mal-PEG2-(Ala-Ala-Asn-PAB)2-eribulin
(ER-001231690)



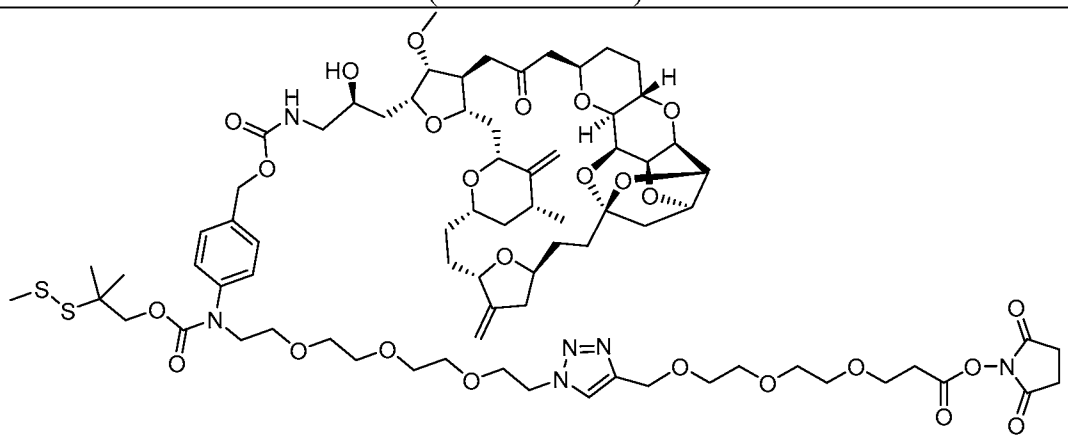
NHS-PEG2-Ala-Ala-Asn-PAB-eribulin
(ER-001231691)



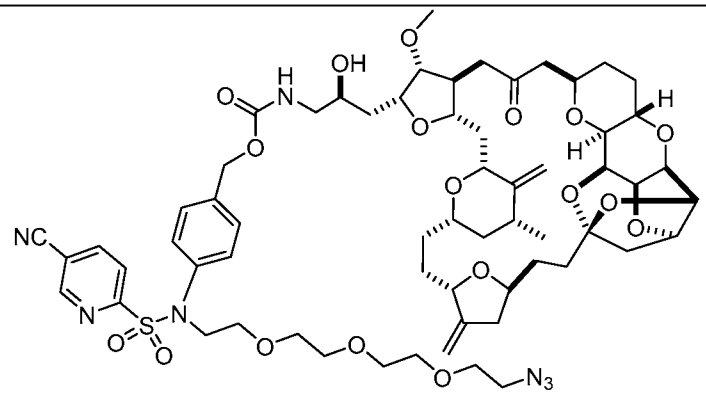
Azide-PEG3-disulfide-PAB-eribulin
(ER-001237508)



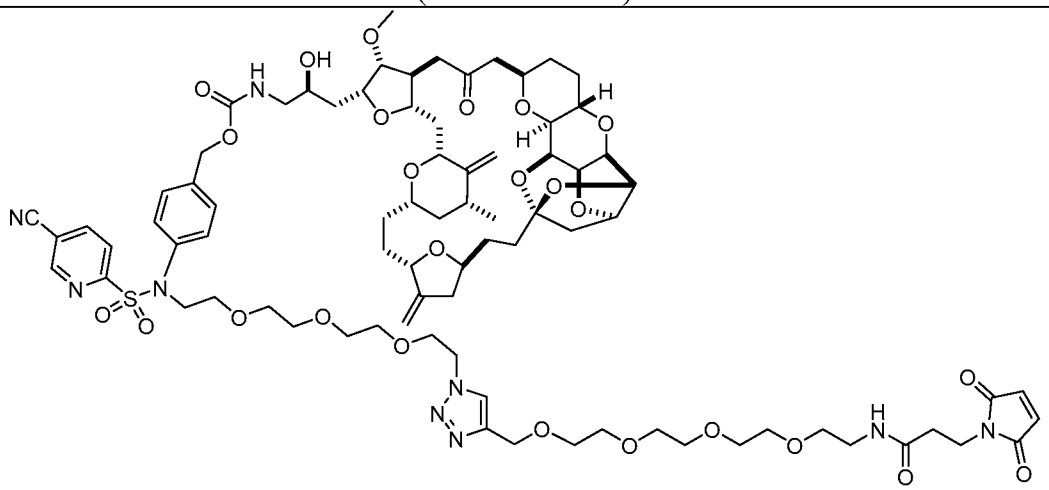
Mal-PEG4-triazole-PEG3-disulfide-PAB-eribulin
(ER-001237504)



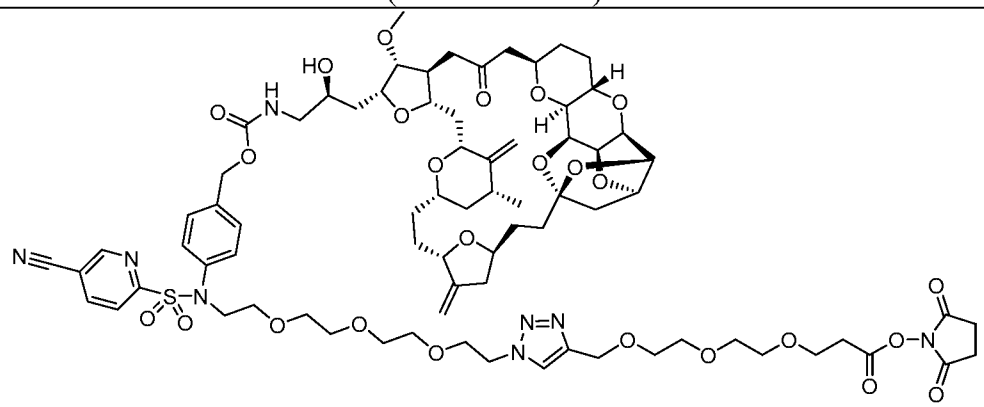
NHS-PEG3-triazole-PEG3-disulfide-PAB-eribulin
(ER-001244129)



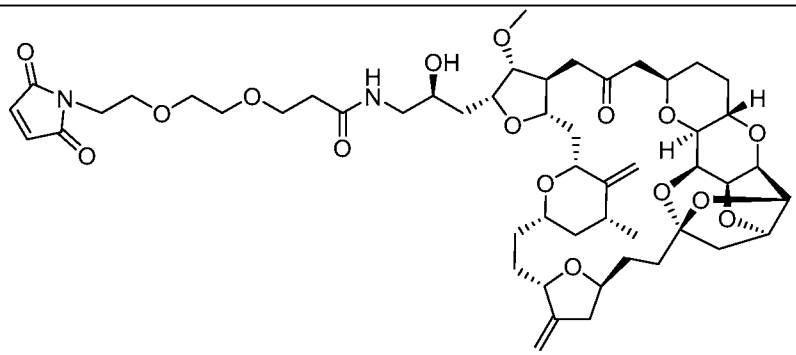
Azide-PEG3-sulfonamide-PAB-eribulin
(ER-001138856)



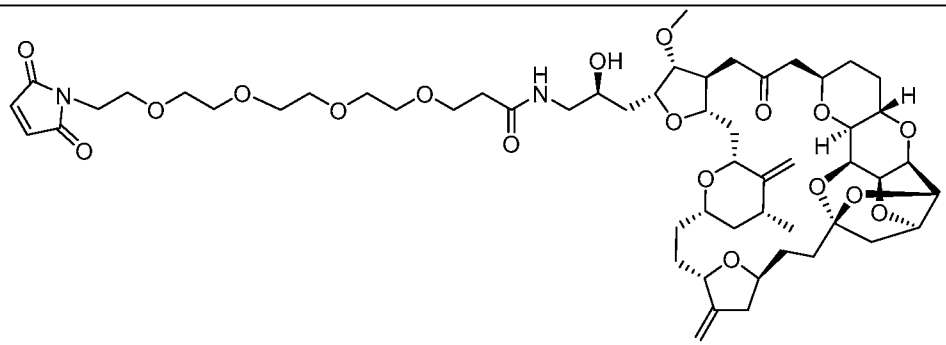
Mal-PEG4-triazole-PEG3-sulfonamide-PAB-eribulin
(ER-001237505)



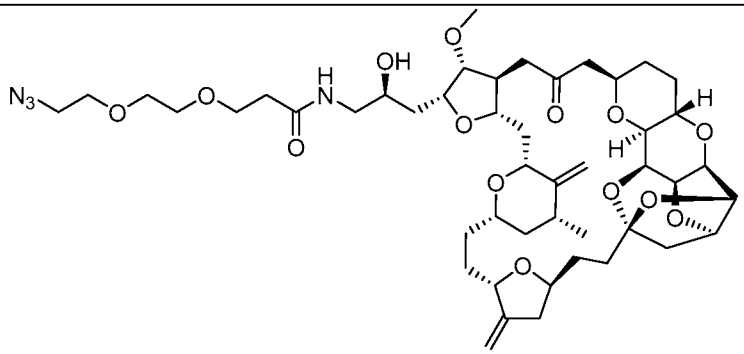
NHS-PEG3-triazole-PEG3-sulfonamide-PAB-eribulin
(ER-001244623)



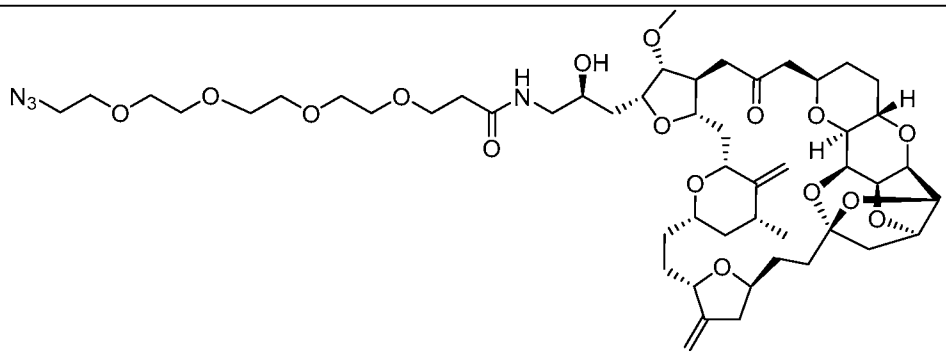
Mal-PEG2-eribulin



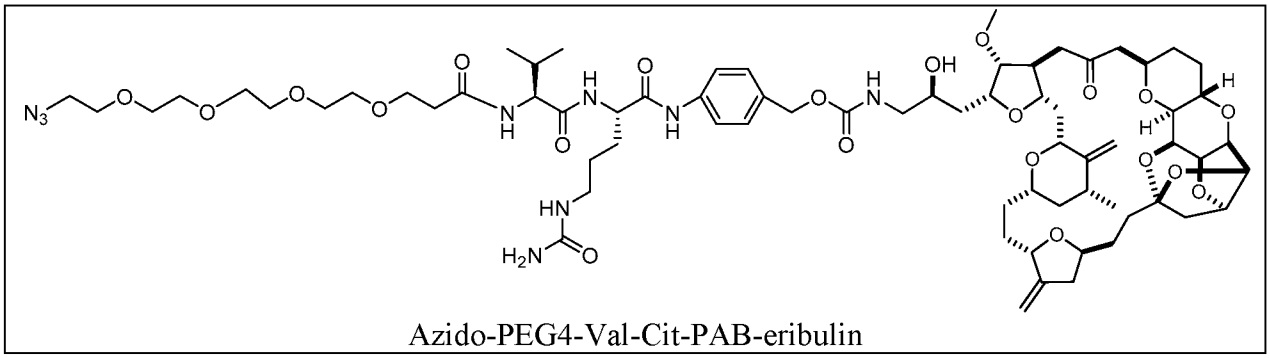
Mal-PEG4-eribulin



Azido-PEG2-eribulin

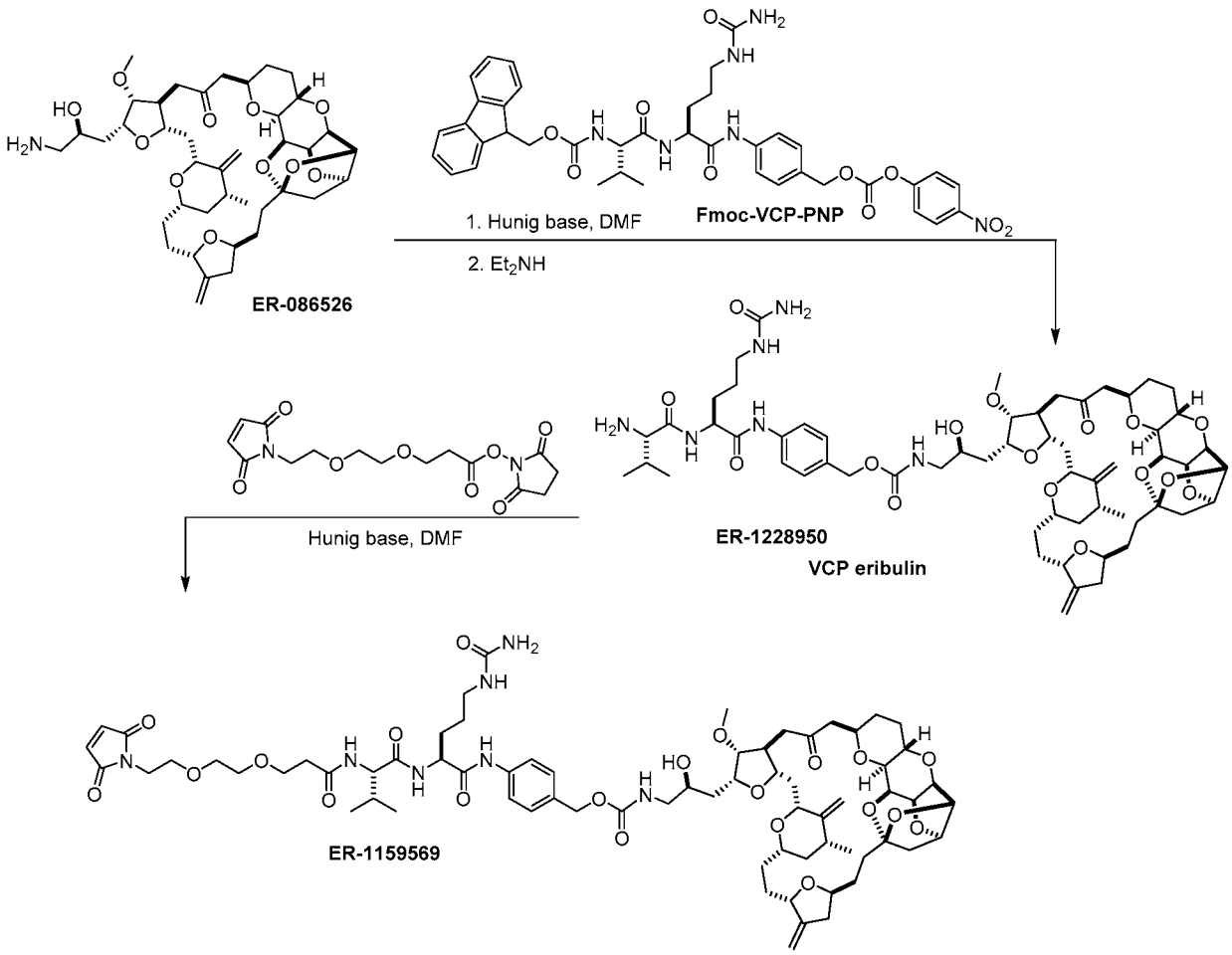


Azido-PEG4-eribulin



Azido-PEG4-Val-Cit-PAB-eribulin

1.1 Preparation of MAL-PEG2-Val-Cit-PAB-eribulin (ER-001159569)

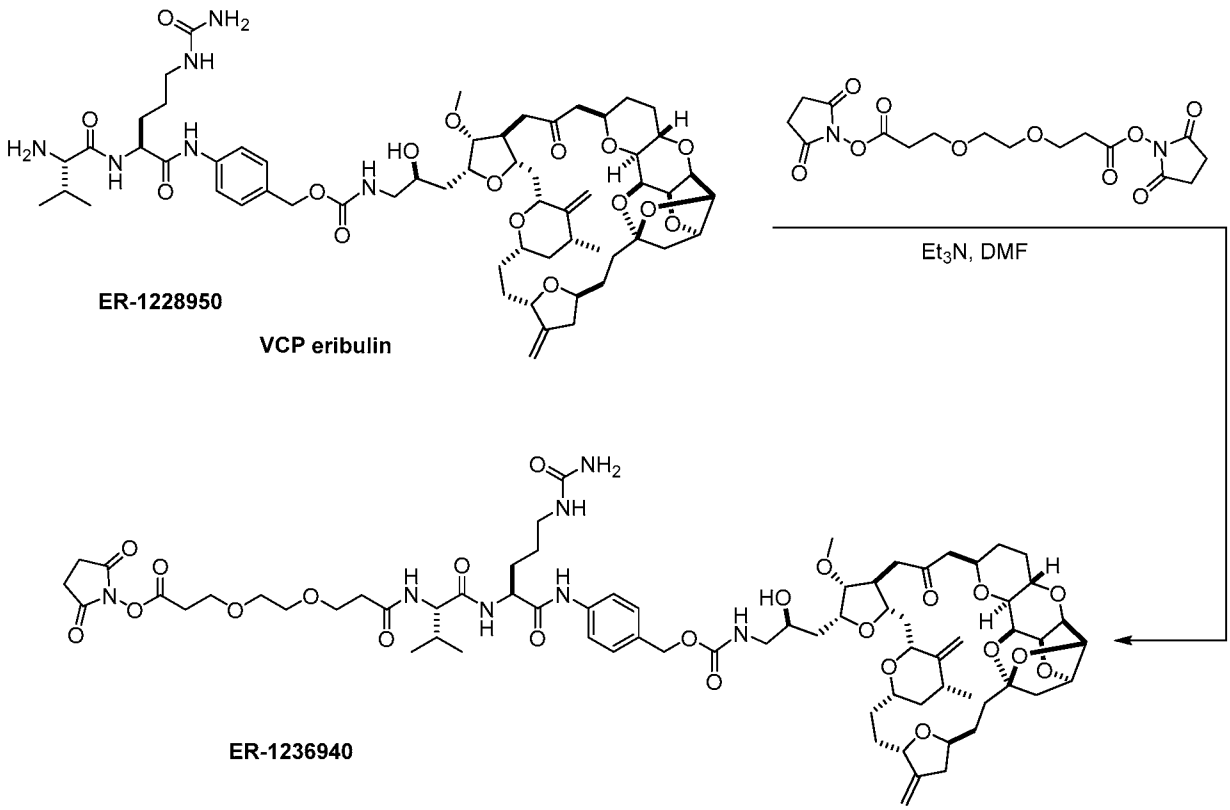


[00347] Eribulin (ER-000086526) (61.5 mg, 0.074 mmol) was dissolved in *N*,*N*-dimethylformamide (DMF) (6.0 mL) and then mixed with Hunig Base (0.027 mL, 0.156 mmol) and Fmoc-Val-Cit-PAB-PNP (86 mg, 0.112 mmol). The reaction was stirred at room temperature for 18 hours until the coupling was complete, as determined by high performance liquid chromatography (HPLC) analysis. Diethylamine (0.078 mL, 0.745

mmol) was added to the mixture, and the mixture was stirred for an additional 2 hours until the reaction was complete. The solvent was removed by evaporation, and the residue was purified by flash chromatography to obtain Val-Cit-PAB-eribulin (ER-001228950) as a white solid (60 mg, 71% yield). ^{1}HNMR (400 MHz, CD_3OD) δ ppm 7.56 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 5.14 (s, 1H), 5.06 (d, *J* = 12.4 Hz, 1H), 5.03 (s, 1H), 5.01 (d, *J* = 12.4 Hz, 1H), 4.87 (s, 1H), 4.83 (s, 1H), 4.71 (t, *J* = 4.4 Hz, 1H), 4.62 (t, *J* = 4.4 Hz, 1H), 4.57 (dd, *J* = 4.8, 8.8 Hz, 1H), 4.47 (d, *J* = 10.8 Hz, 1H), 4.32-4.27 (m, 2H), 4.18 (dd, *J* = 4.8, 6.4 Hz, 1H), 4.13-4.07 (m, 2H), 3.98 (t, *J* = 10.4 Hz, 1H), 3.88-3.82 (m, 3H), 3.76-3.70 (m, 4H), 3.60 (d, *J* = 6.0 Hz, 1H), 3.38 (s, 3H), 3.26-3.10 (m, 3H), 2.93 (dd, *J* = 2.0, 11.2 Hz, 1H), 2.91-2.84 (m, 1H), 2.75-2.64 (m, 2H), 2.44-2.29 (m, 5H), 2.21-1.97 (m, 8H), 1.93-1.83 (m, 3H), 1.79-1.72 (m, 5H), 1.68-1.29 (m, 8H), 1.11 (d, *J* = 6.8 Hz, 3H), 1.07-1.01 (m, 1H), 1.06 (d, *J* = 7.2 Hz, 3H), 1.02 (d, *J* = 7.2 Hz, 3H). LCMS (M+H)=1135.7.

[00348] Val-Cit-PAB-eribulin (ER-001228950) (16 mg, 14 μmol) was dissolved in DMF (1 mL). *N,N*-diisopropylethylamine (7.2 μL, 41 μmol) and Mal-PEG2-NHS (9.7 mg, 27 μmol) were then added to this solution at room temperature, and the reaction mixture was stirred at room temperature for 1 hour. Upon completion of the reaction, the crude mixture was purified by reverse-phase HPLC using an acetonitrile-water mobile phase containing 0.1% formic acid. The collected fractions were concentrated under vacuum at room temperature in a non-heated water bath to yield Mal-PEG2-Val-Cit-PAB-eribulin (ER-001159569) (7.1 mg, 5.2 μmol, 38% yield). ^{1}HNMR (400 MHz, CD_3OD) δ ppm 7.59 (d, *J* = 8.4 Hz, 2H), 7.31 (d, *J* = 8.4 Hz, 2H), 6.81 (s, 2H), 5.13 (s, 1H), 5.06 (d, *J* = 12.4 Hz, 1H), 5.02 (s, 1H), 5.01 (d, *J* = 12.4 Hz, 1H), 4.87 (s, 1H), 4.82 (s, 1H), 4.71 (t, *J* = 4.0 Hz, 1H), 4.61 (t, *J* = 4.4 Hz, 1H), 4.50 (dd, *J* = 5.2, 9.2 Hz, 1H), 4.47 (d, *J* = 10.8 Hz, 1H), 4.32-4.27 (m, 2H), 4.19 (dd, *J* = 6.8, 11.6 Hz, 1H), 4.13-4.07 (m, 2H), 3.98 (t, *J* = 10.4 Hz, 1H), 3.88-3.82 (m, 3H), 3.76-3.64 (m, 6H), 3.62-3.51 (m, 6H), 3.38 (s, 3H), 3.22-3.08 (m, 4H), 2.93 (dd, *J* = 2.4, 9.6 Hz, 1H), 2.92-2.84 (m, 1H), 2.76-2.63 (m, 2H), 2.52 (t, *J* = 6.0 Hz, 2H), 2.44-2.29 (m, 5H), 2.21-1.97 (m, 8H), 1.93-1.83 (m, 3H), 1.80-1.66 (m, 5H), 1.66-1.28 (m, 10H), 1.11 (d, *J* = 6.4 Hz, 3H), 1.07-1.01 (m, 1H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.4 Hz, 3H). LCMS (M+H)=1374.9.

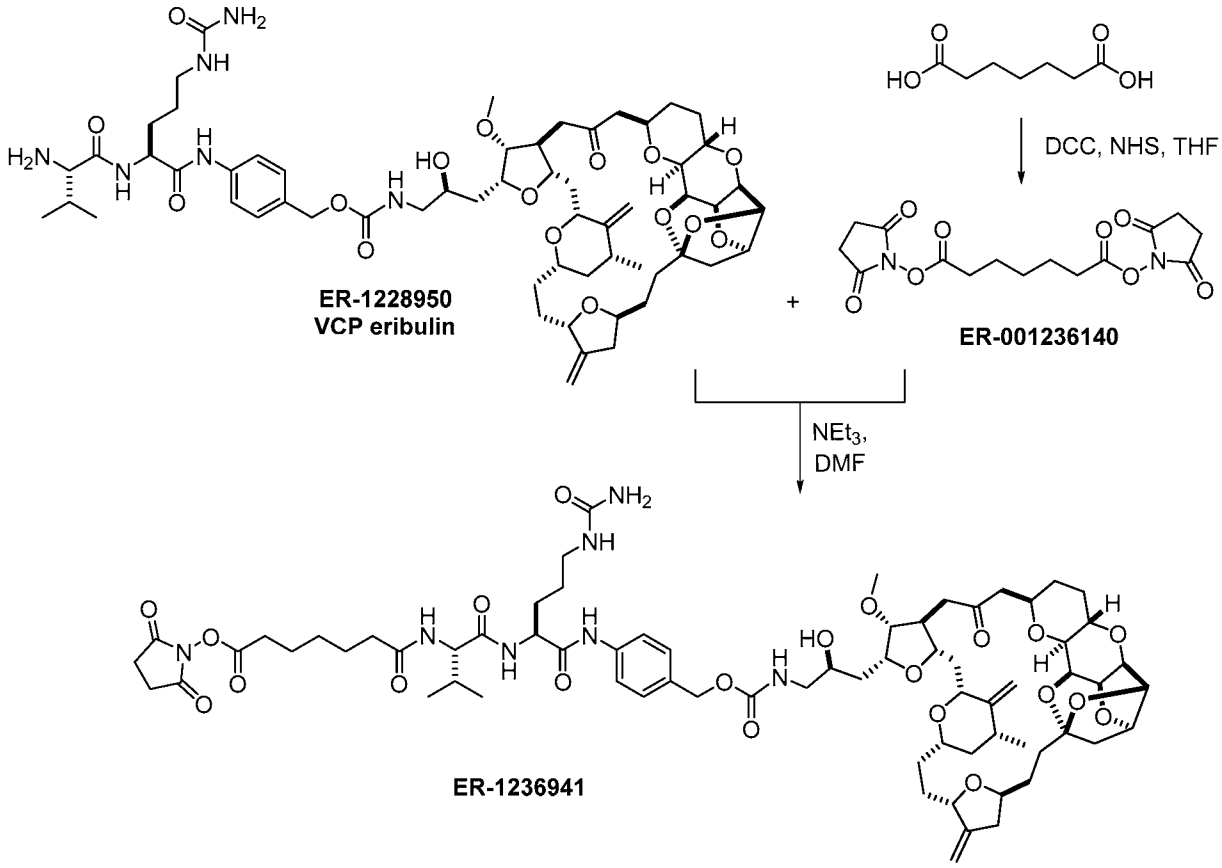
1.2 Preparation of NHS-PEG2-Val-Cit-PAB-eribulin (ER-001236940)



[00349] Val-Cit-PAB-eribulin (ER-001228950) (45 mg, 0.04 mmol) and bis(2,5-dioxopyrrolidin-1-yl) 3,3'-(ethane-1,2-diylbis(oxy))dipropanoate (79 mg, 0.198 mmol) were mixed in DMF (1.5 mL), and Et_3N (44.2 µl, 0.317 mmol) was then added. The mixture was stirred for 18 hours until the reaction was complete, as determined by HPLC analysis. The solvent was evaporated and the residue was purified by flash chromatography to obtain NHS-PEG2-Val-Cit-PAB-eribulin (ER-001236940) as a white solid (38 mg, 68% yield). ^{1}HNMR (400 MHz, CD_3OD) δ ppm 7.58 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8.4 Hz, 2H), 5.14 (s, 1H), 5.05 (d, J = 12.4 Hz, 1H), 5.03 (s, 1H), 5.01 (d, J = 12.4 Hz, 1H), 4.87 (s, 1H), 4.83 (s, 1H), 4.71 (t, J = 4.4 Hz, 1H), 4.62 (t, J = 4.4 Hz, 1H), 4.51 (dd, J = 4.8, 8.8 Hz, 1H), 4.50-4.47 (m, 1H), 4.32-4.27 (m, 2H), 4.21 (dd, J = 4.8, 6.4 Hz, 1H), 4.14-4.08 (m, 2H), 3.99 (t, J = 10.4 Hz, 1H), 3.88-3.82 (m, 3H), 3.78-3.70 (m, 4H), 3.62 (s, 2H), 3.62-3.58 (m, 1H), 3.50-3.46 (m, 2H), 3.39 (s, 4H), 3.36 (s, 3H), 3.22-3.08 (m, 3H), 2.93 (dd, J = 2.0, 11.2 Hz, 1H), 2.91-2.87 (m, 1H), 2.84 (s, 2H), 2.80 (s, 2H), 2.75-2.64 (m, 2H), 2.59-2.52 (m, 2H), 2.44-2.29 (m, 5H), 2.21-1.97 (m, 10H), 1.93-1.83 (m, 3H), 1.79-1.72 (m, 5H), 1.68-1.29 (m, 8H),

1.11 (d, J = 6.8 Hz, 3H), 1.08-0.98 (m, 1H), 1.00 (d, J = 7.2 Hz, 3H), 0.98 (d, J = 7.2 Hz, 3H). LCMS (M+H)=1421.0.

1.3 Preparation of NHS-$(CH_2)_5$-Val-Cit-PAB-eribulin (ER-001236941)

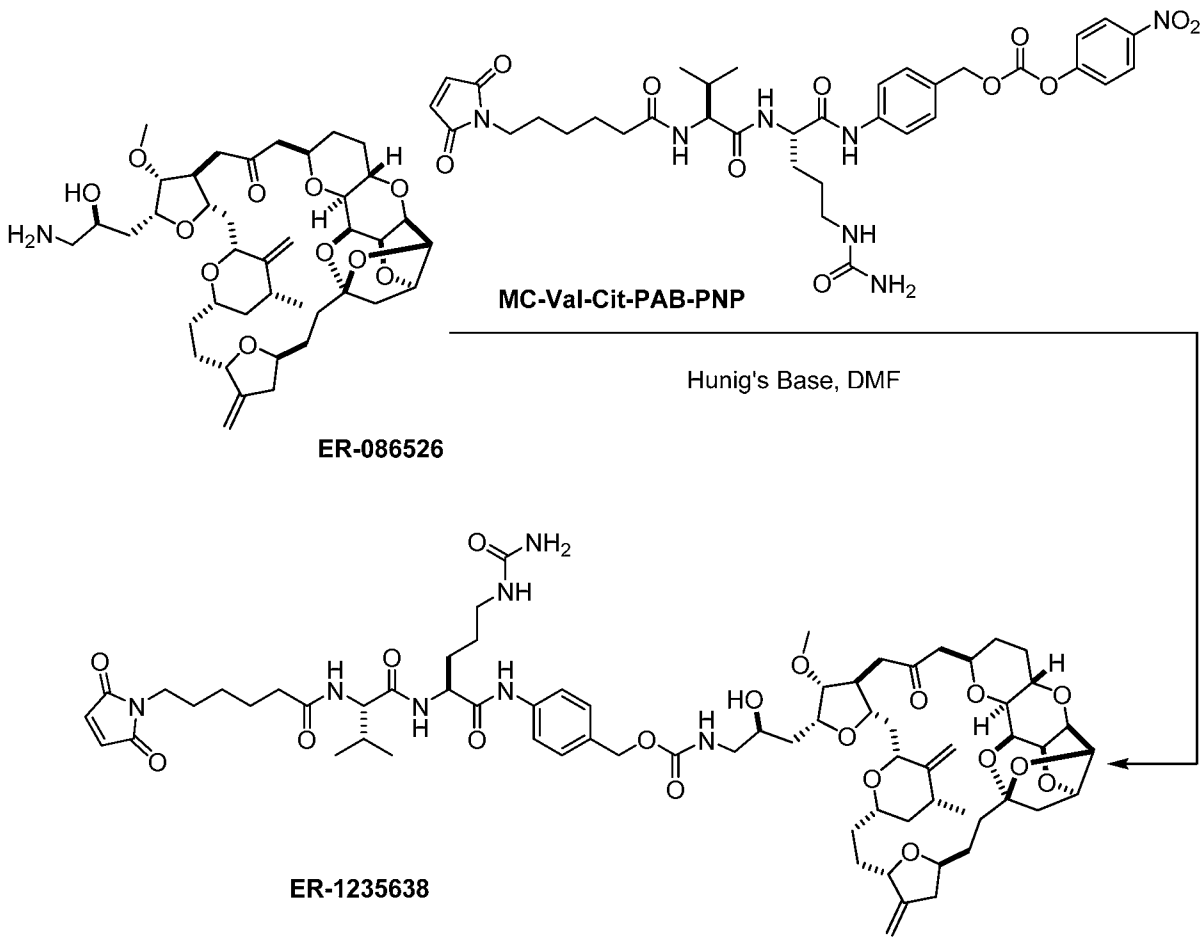


[00350] Heptanedioic acid (1.6 g, 9.99 mmol) was dissolved in tetrahydrofuran (THF) (100 mL), and 1-hydroxypyrrolidine-2,5-dione (2.299 g, 19.98 mmol) was then added, followed by the addition of DCC (4.12 g, 19.98 mmol). The mixture was stirred at room temperature for 18 hours until HPLC analysis indicated the completion of the reaction. The solid was removed by filtration through a celite pad, and washed with THF (3 x 2 mL). The combined filtrate was concentrated and purified by flash chromatography to yield bis(2,5-dioxopyrrolidin-1-yl) heptanedioate (ER-001236140) as a white solid (2.5 g, 71% yield). ^{1}HNMR (400 MHz) δ ppm 2.83 (s, 8H), 2.64 (t, J = 7.6 Hz, 4H), 1.80 (dt, J = 7.6 Hz, 4H), 1.59-1.51 (m, 2H). LCMS (M+H)=355.2.

[00351] NHS-$(CH_2)_5$-Val-Cit-PAB-eribulin (ER-001236941) was prepared (8.5 mg, 47% yield) from VCP-eribulin (ER-001228950) and bis(2,5-dioxopyrrolidin-1-yl)

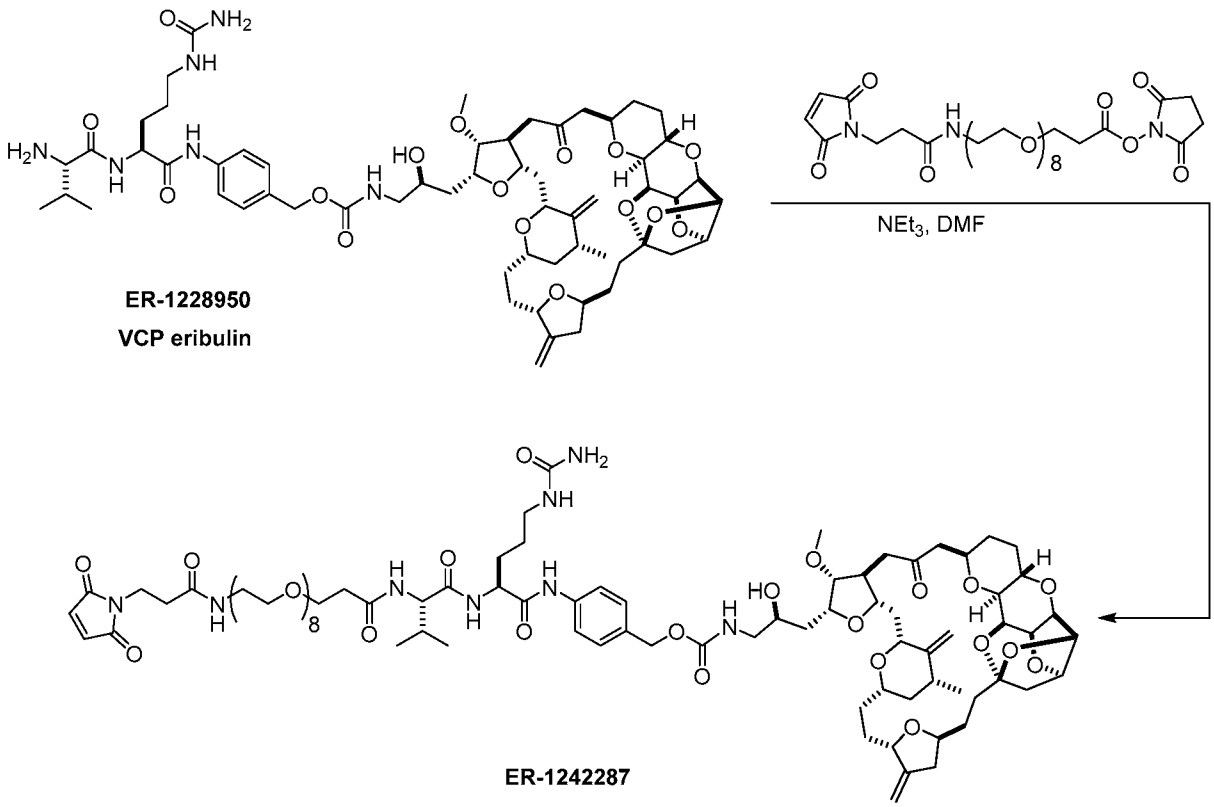
heptanedioate (ER-001236140) using the same procedure as described above for the preparation of NHS-PEG2-Val-Cit-PAB-eribulin (ER-001236940). ^{1}HNMR (400 MHz, CD_3OD) δ ppm 7.56 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 8.4 Hz, 2H), 5.13 (s, 1H), 5.04 (d, *J* = 12.0 Hz, 1H), 5.01 (s, 1H), 5.00 (d, *J* = 12.4 Hz, 1H), 4.86 (s, 1H), 4.82 (s, 1H), 4.70 (t, *J* = 4.4 Hz, 1H), 4.60 (t, *J* = 4.4 Hz, 1H), 4.50 (dd, *J* = 4.8, 8.8 Hz, 1H), 4.46 (d, *J* = 10.8 Hz, 1H), 4.36-4.25 (m, 2H), 4.17 (dd, *J* = 4.8, 6.4 Hz, 1H), 4.13-4.06 (m, 2H), 3.97 (t, *J* = 10.4 Hz, 1H), 3.87-3.80 (m, 3H), 3.74-3.68 (m, 2H), 3.37 (s, 3H), 3.20-3.06 (m, 4H), 2.94 (dd, *J* = 2.0, 11.2 Hz, 1H), 2.90-2.82 (m, 1H), 2.82 (s, 4H), 2.74-2.65 (m, 2H), 2.61 (t, *J* = 8.0 Hz, 2H), 2.46-2.26 (m, 7H), 2.24-1.81 (m, 13H), 1.78-1.28 (m, 19H), 1.10 (d, *J* = 6.8 Hz, 3H), 1.06-0.96 (m, 1H), 0.97 (d, *J* = 7.2 Hz, 3H), 0.95 (d, *J* = 7.2 Hz, 3H). LCMS (M+H)=1375.1.

1.4 Preparation of Mal-$(CH_2)_5$-Val-Cit-PAB-eribulin (ER-001235638)



[00352] Eribulin (ER-000086526) (10 mg, 0.012 mmol) was dissolved in DMF (1 mL), and mixed with MC-Val-Cit-PAB-PNP (9.02 mg, 0.012 mmol) and Hunig's Base (4.44 μL, 0.025 mmol). The mixture was then stirred at room temperature for 12 hours until HPLC analysis indicated the completion of the reaction. The reaction mixture was concentrated and purified by flash chromatography to yield Mal-$(CH_2)_5$-Val-Cit-PAB-eribulin (ER-001235638) as a white solid (11.3 mg, 63% yield). ^{1}HNMR (400 MHz, CD_3OD) δ ppm 7.57 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 6.79 (s, 2H), 5.13 (s, 1H), 5.05 (d, J = 12.4 Hz, 1H), 5.02 (s, 1H), 5.00 (d, J = 12.4 Hz, 1H), 4.87 (s, 1H), 4.83 (s, 1H), 4.71 (t, J = 4.4 Hz, 1H), 4.61 (t, J = 4.4 Hz, 1H), 4.56-4.46 (m, 3H), 4.35-4.27 (m, 2H), 4.20-4.07 (m, 4H), 3.98 (t, J = 10.8 Hz, 1H), 3.87-3.83 (m, 3H), 3.73-3.70 (m, 2H), 3.48 (t, J = 7.6 Hz, 2H), 3.38 (s, 3H), 3.20-3.08 (m, 4H), 2.93 (dd, J = 1.6, 9.6 Hz, 1H), 2.89-2.85 (m, 1H), 2.69 (dt, J = 11.2, 16.8 Hz, 2H), 2.44-2.33 (m, 5H), 2.27-1.83 (m, 13H), 1.78-1.68 (m, 5H), 1.66-1.27 (m, 14H), 1.11 (d, J = 7.2 Hz, 3H), 1.07-0.98 (m, 1H), 0.98 (d, J = 7.2 Hz, 3H), 0.96 (d, J = 7.2 Hz, 3H). LCMS (M+H)=1328.9.

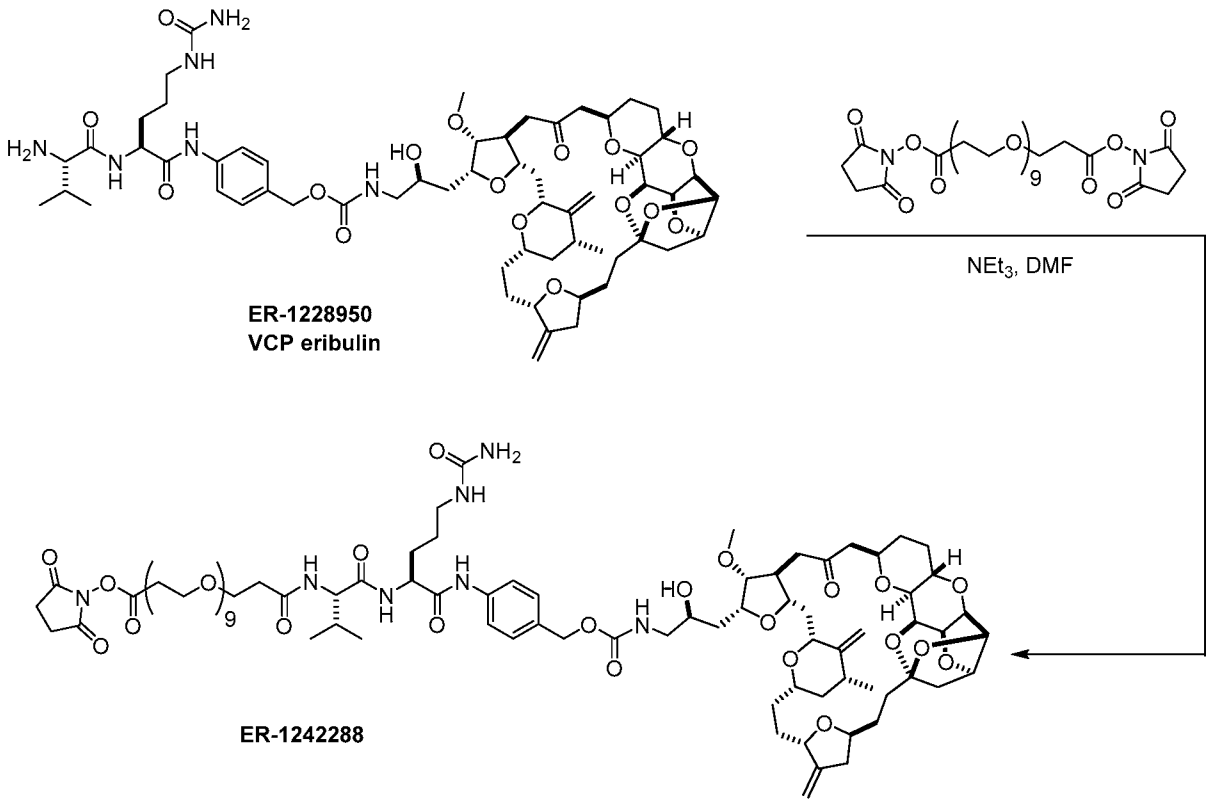
1.5 Preparation of Mal-PEG8-Val-Cit-PAB-eribulin (ER-001242287)



[00353] VCP-eribulin (ER-001228950) (10 mg, 8.808 μmol) and 2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16,19,22,25,28-octaoxa-4-azahentriacontan-31-oate (6.07 mg, 8.808 μmol) were mixed in DMF (1 mL), followed by the addition of Et_3N (9.82 μl, 0.07 mmol). The reaction mixture was stirred at room temperature for 18 hours until HPLC analysis indicated the completion of the reaction. The solvent was removed by evaporation, and the residue was purified by flash chromatography to yield Mal-PEG8-Val-Cit-PAB-eribulin (ER-001242287) as a white solid (3.0 mg, 20% yield). ^{1}HNMR (400 MHz, CD_3OD) δ ppm 7.58 (d, J = 8.4 Hz, 2H), 7.29 (d, J = 8.4 Hz, 2H), 6.80 (s, 2H), 5.12 (s, 1H), 5.04 (d, J = 12.4 Hz, 1H), 5.01 (s, 1H), 4.99 (d, J = 12.4 Hz, 1H), 4.85 (s, 1H), 4.80 (s, 1H), 4.69 (t, J = 4.4 Hz, 1H), 4.59 (t, J = 4.4 Hz, 1H), 4.50-4.42 (m, 2H), 4.32-4.24 (m, 2H), 4.20-4.14 (m, 2H), 4.12-4.04 (m, 3H), 3.96 (t, J = 10.4 Hz, 1H), 3.86-3.80 (m, 3H), 3.76-3.57 (m, 4H), 3.48 (t, J = 6.0 Hz, 1H), 3.36 (s, 3H), 3.20-3.08 (m, 3H), 2.91 (dd, J = 2.0, 11.2 Hz, 1H), 2.90-2.82 (m, 1H), 2.74-2.60 (m, 2H), 2.44-2.29 (m, 5H), 2.21-1.97 (m, 10H), 1.93-

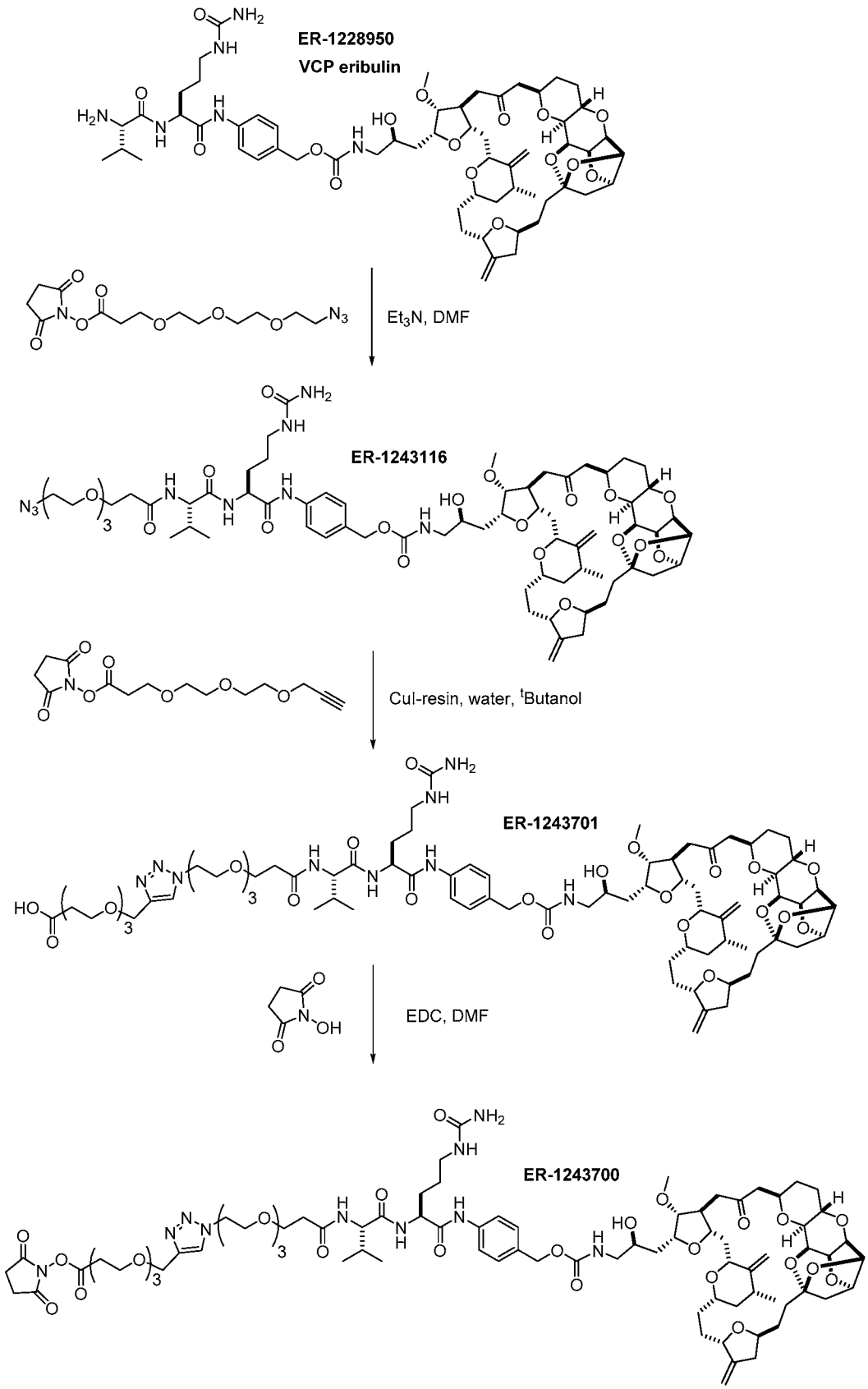
1.83 (m, 3H), 1.79-1.20 (m, 19H), 1.09 (d, *J* = 6.8 Hz, 3H), 1.04-0.98 (m, 1H), 0.99 (d, *J* = 7.2 Hz, 3H), 0.97 (d, *J* = 7.2 Hz, 3H). LCMS (M+H)=1711.6.

1.6 Preparation of NHS-PEG9-Val-Cit-PAB-eribulin (ER-001242288)



[00354] NHS-PEG9-Val-Cit-PAB-eribulin (ER-001242288) was prepared (13 mg, 85% yield) from VCP-eribulin (ER-001228950) and BisNHS-PEG9 using the same procedure as described above for the preparation of NHS-PEG2-Val-Cit-PAB-eribulin (ER-001236940). ^{1}HNMR (400 MHz, CD_3OD) δ ppm 7.61 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 5.16 (s, 1H), 5.06 (d, *J* = 12.4 Hz, 1H), 5.01 (s, 1H), 5.00 (d, *J* = 12.4 Hz, 1H), 4.87 (s, 1H), 4.82 (s, 1H), 4.71 (t, *J* = 4.4 Hz, 1H), 4.61 (t, *J* = 4.4 Hz, 1H), 4.52-4.45 (m, 2H), 4.34-4.26 (m, 2H), 4.20-4.19 (m, 1H), 4.14-4.06 (m, 2H), 3.98 (t, *J* = 10.4 Hz, 1H), 3.88-3.80 (m, 3H), 3.76-3.70 (m, 4H), 3.66-3.58 (m, 37H), 3.38 (s, 3H), 3.24-3.10 (m, 3H), 2.93 (dd, *J* = 2.0, 11.2 Hz, 1H), 2.91-2.84 (m, 1H), 2.84 (s, 4H), 2.76-2.64 (m, 2H), 2.58-2.50 (m, 4H), 2.46-2.28 (m, 5H), 2.22-1.96 (m, 8H), 1.91-1.82 (m, 3H), 1.79-1.68 (m, 5H), 1.64-1.24 (m, 8H), 1.11 (d, *J* = 6.8 Hz, 3H), 1.08-0.96 (m, 1H), 0.99 (d, *J* = 7.2 Hz, 3H), 0.97 (d, *J* = 7.2 Hz, 3H). LCMS (M+H)=1729.7.

1.7 Preparation of NHS-PEG3-triazole-PEG3-Val-Cit-PAB-eribulin (ER-001243700)



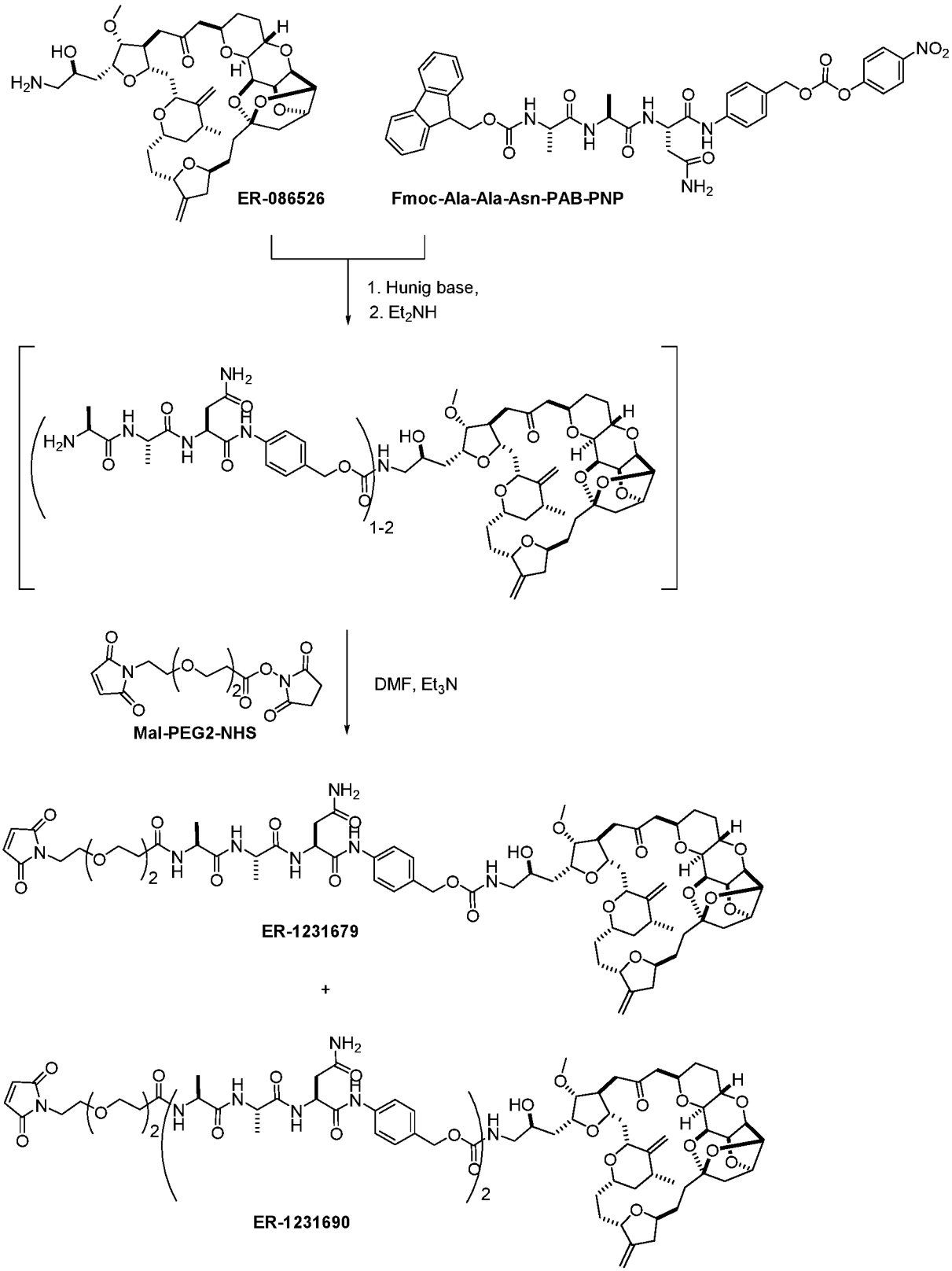
[00355] VCP-eribulin (ER-001228950) (25 mg, 0.022 mmol) was dissolved in DMF (2.5 mL), and then mixed with Et_3N (24.55 µl, 0.176 mmol) and Azide-PEG3-NHS (8.34 mg, 0.024 mmol). The mixture was stirred at room temperature for 18 hours until HPLC analysis indicated the completion of the reaction. The mixture was concentrated under vacuum, and the residue was purified by prep-HPLC (MeCN and water with 0.1% formic acid). The fractions containing azide-PEG3-Val-Cit-PAB-eribulin were extracted with dichloromethane (CH_2Cl_2) (3 x 20 mL), and the CH_2Cl_2 was evaporated to obtain azide-PEG3-Val-Cit-PAB-eribulin (ER-001243116) as a white solid (18.9 mg, 63% yield). ^{1}HNMR (400 MHz, CD_3OD) δ ppm 7.58 (d, J = 8.4 Hz, 2H), 7.30 (d, J = 8.4 Hz, 2H), 5.14 (s, 1H), 5.04 (d, J = 12.4 Hz, 1H), 5.03 (s, 1H), 5.01 (d, J = 12.4 Hz, 1H), 4.85 (s, 1H), 4.81 (s, 1H), 4.70 (t, J = 4.4 Hz, 1H), 4.61 (t, J = 4.4 Hz, 1H), 4.52-4.48 (m, 2H), 4.31-4.25 (m, 2H), 4.20-4.15 (m, 1H), 4.13-4.07 (m, 2H), 3.99 (t, J = 10.4 Hz, 1H), 3.84-3.79 (m, 3H), 3.77-3.65 (m, 4H), 3.64-3.56 (m, 13H), 3.38 (s, 3H), 3.20-3.05 (m, 3H), 2.95-2.80 (m, 2H), 2.75-2.60 (m, 2H), 2.55-2.50 (m, 2H), 2.43-2.25 (m, 5H), 2.21-1.97 (m, 8H), 1.93-1.83 (m, 3H), 1.79-1.72 (m, 5H), 1.68-1.29 (m, 10H), 1.08 (d, J = 6.8 Hz, 3H), 1.05-0.95 (m, 1H), 0.98 (d, J = 7.2 Hz, 3H), 0.95 (d, J = 7.2 Hz, 3H). LCMS (M+H)=1365.1.

[00356] Azide-PEG3-VCP-eribulin (ER-001243116) (9.6 mg, 7.035 µmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)propanoate (6.61 mg, 0.021 mmol) were mixed in water (0.6 mL) and t-Butanol (1.8 mL). The mixture was bubbled with N_2 was for 45 min. Copper iodide on amberlyst-21 (1.23 mmol/g, 10 mg) was added to the mixture and N_2 was bubbled through the mixture for another 30 min. The reaction mixture was then stirred at room temperature for 72 hours until the complete consumption of the starting material. No desired NHS ester product was observed by LCMS analysis, only the hydrolyzed carboxylic acid. The mixture was filtered through a short celite pad to remove CuI resin. The filtrate was concentrated in vacuo, and the resulting residue was purified by preparative thin layer chromatography (prep-TLC) (20% MeOH/CH_2Cl_2) to obtain acid-PEG3-triazole-PEG3-Val-Cit-PAB-eribulin (ER-001243701) as a white solid (3.7 mg, 33% yield). LCMS (ES) (M+H)=1581.2.

[00357] Acid-PEG3-triazole-PEG3-Val-Cit-PAB-eribulin (ER-001243701) (3.0 mg, 1.898 µmol) was dissolved in DMF (200 µL) and 1-hydroxypyrrolidine-2,5-dione

(0.437 mg, 3.796 μmol) was added, followed by the addition of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (0.728 mg, 3.796 μmol). The reaction was approximately 50% complete after stirring at room temperature for 18 hours. EDC (1.46 mg, 7.8 μmol) was added, and the mixture was stirred for another 18 hours until HPLC analysis indicated >95% conversion to NHS-PEG3-triazole-PEG3-Val-Cit-PAB-eribulin. The mixture was concentrated in vacuo, and the residue was purified by prep-TLC (15% MeOH/CH_2Cl_2) to yield NHS-PEG3-triazole-PEG3-Val-Cit-PAB-eribulin (ER-001243700) as a white solid (2.2 mg, 69% yield). ^{1}HNMR (400 MHz, CD_3OD) δ ppm 8.00 (s, 1H), 7.59 (d, J = 8.0 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 5.13 (s, 1H), 5.04 (d, J = 12.4 Hz, 1H), 5.02 (s, 1H), 5.00 (d, J = 12.4 Hz, 1H), 4.87 (s, 1H), 4.83 (s, 1H), 4.71 (t, J = 4.0 Hz, 1H), 4.63 (s, 2H), 4.61 (t, J = 4.4 Hz, 1H), 4.57-4.55 (m, 2H), 4.51-4.45 (m, 1H), 4.32-4.28 (m, 2H), 4.21-4.17 (m, 2H), 4.13-4.10 (m, 2H), 3.98 (t, J = 10.8 Hz, 1H), 3.88-3.80 (m, 5H), 3.75-3.70 (m, 4H), 3.68-3.55 (m, 18H), 3.45-3.40 (m, 2H), 3.38 (s, 3H), 3.20-3.08 (m, 4H), 2.93-2.80 (m, 2H), 2.75-2.50 (m, 2H), 2.68 (s, 4H), 2.48-2.30 (m, 7H), 2.28-1.92 (m, 10H), 1.90-1.68 (m, 8H), 1.65-1.27 (m, 8H), 1.11 (d, J = 6.8 Hz, 3H), 1.05-0.95 (m, 1H), 0.99 (d, J = 7.2 Hz, 3H), 0.97 (d, J = 6.8 Hz, 3H). LCMS (M+H)=1678.3.

1.8 Preparation of Mal-PEG2-Ala-Ala-Asn-PAB-eribulin (ER-001231679) and Mal-PEG2-(Ala-Ala-Asn-PAB)2-eribulin (ER-001231690)



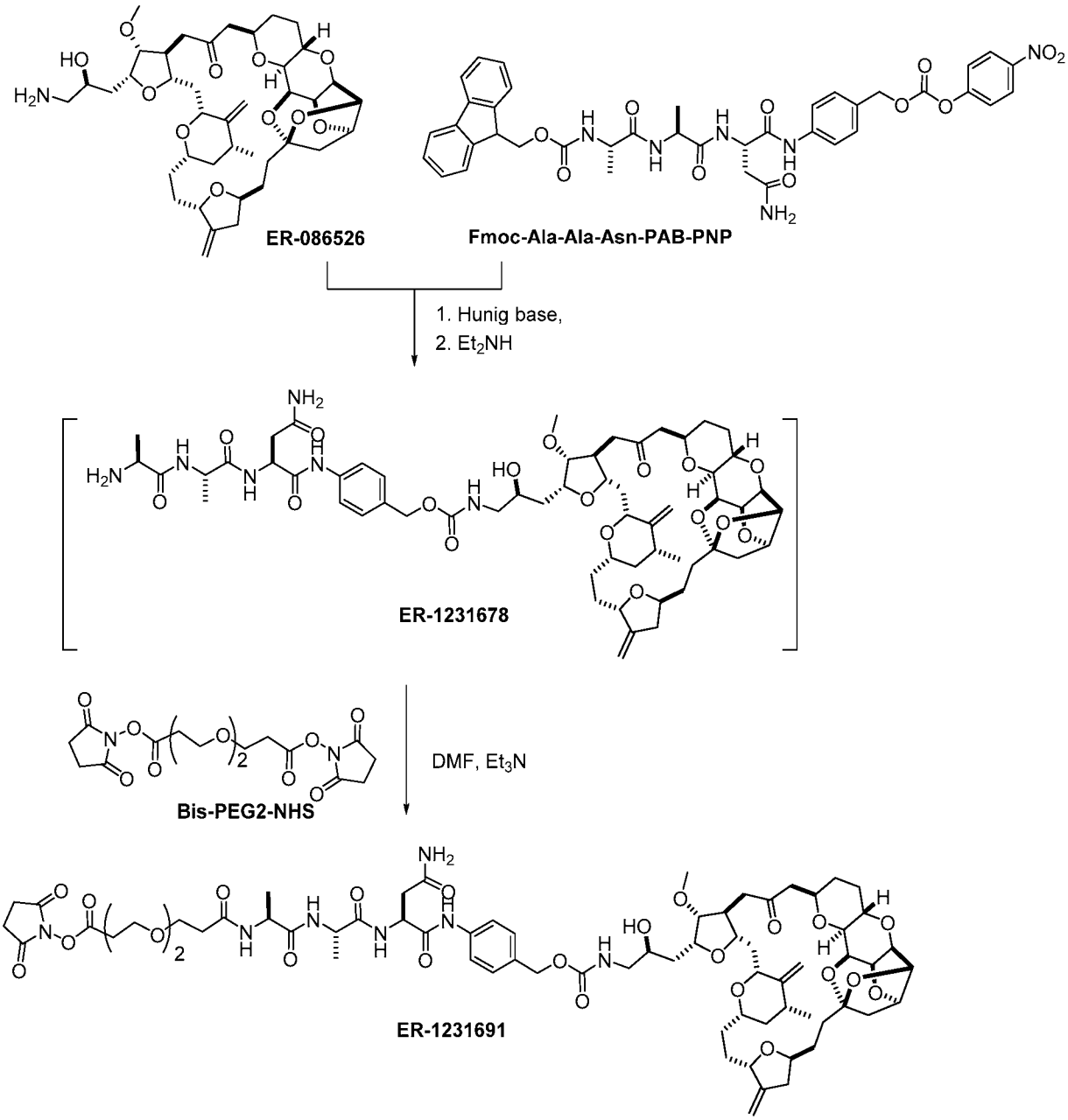
[00358] Eribulin (ER-000086526) (10 mg, 0.014 mmol) was dissolved in DMF (0.5 mL), and mixed with Hunig's Base (3.59 μL, 0.021 mmol). (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-(((S)-4-amino-1-((4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)amino)-1,4-dioxobutan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (15.76 mg, 0.021 mmol) was then added, and the resulting yellow solution was stirred at room temperature for 3 days until HPLC analysis indicated the complete consumption of the starting material. Diethylamine (14.23 μL, 0.137 mmol) was added to the reaction mixture, which was then stirred at room temperature for an additional 2 hours until there was 100% cleavage of Fmoc protection. The reaction mixture was concentrated to remove diethylamine, and the residue was re-dissolved in DMF (1.5 mL). Et_3N (0.015 mL, 0.11 mmol) was added at room temperature, followed by the addition of 2,5-dioxopyrrolidin-1-yl 3-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)propanoate (9.71 mg, 0.027 mmol). The reaction mixture was stirred at room temperature for 16 hours until the reaction was complete, as determined by LCMS analysis. The mixture was concentrated under high vacuum, and purified by flash chromatography to obtain Mal-PEG2-Ala-Ala-Asn-PAB-eribulin (ER-001231679) (9.2 mg, 49% yield) and Mal-PEG2-(Ala-Ala-Asn-PAB)2-eribulin (ER-001231690) (6.0 mg, 18% yield) as colorless oils.

[00359] Mal-PEG2-Ala-Ala-Asn-PAB-eribulin (ER-001231679): ^{1}HNMR (400 MHz) δ ppm 9.23 (s, 1H), 8.00 (d, J = 7.6 Hz, 1H), 7.61 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 6.8 Hz, 1H), 7.24 (d, J = 8.4 Hz, 2H), 7.13 (d, J = 7.2 Hz, 1H), 6.68 (s, 2H), 6.30 (br s, 1H), 6.04-6.00 (m, 1H), 5.77 (br s, 1H), 5.42 (br s, 1H), 5.07 (s, 1H), 5.06-4.98 (m, 2H), 4.93 (s, 1H), 4.88 (s, 1H), 4.90-4.82 (m, 1H), 4.80 (s, 1H), 4.69 (t, J = 4.0 Hz, 1H), 4.60 (t, J = 4.0 Hz, 1H), 4.49-4.42 (m, 1H), 4.38-4.25 (m, 4H), 4.19 (t, J = 4.8 Hz, 1H), 4.15-4.08 (m, 1H), 4.03 (t, J = 4.8 Hz, 1H), 3.97-3.85 (m, 3H), 3.83-3.50 (m, 12H), 3.41 (s, 3H), 3.50-3.10 (m, 3H), 3.02-2.64 (m, 6H), 2.52-2.30 (m, 7H), 2.30-1.65 (m, 14H), 1.65-1.20 (m, 12H), 1.10 (d, J = 6.8 Hz, 3H), 1.13-1.05 (m, 1H). LCMS (M+Na)=1396.6.

[00360] Mal-PEG2-(Ala-Ala-Asn-PAB)2-eribulin (ER-001231690): ^{1}HNMR (400 MHz, CD_3OD) δ ppm 7.65 (d, J = 8.4 Hz, 2H), 7.60 (d, J = 8.4 Hz, 2H), 7.28 (d, J = 8.8 Hz, 2H), 7.23 (d, J = 8.4 Hz, 2H), 6.79 (s, 2H), 5.13 (s, 1H), 5.02 (s, 1H), 5.06-4.98 (m,

4H), 4.87 (s, 1H), 4.82 (s, 1H), 4.85-4.72 (m, 2H), 4.71 (t, J = 4.8 Hz, 1H), 4.61 (t, J = 4.4 Hz, 1H), 4.47 (d, J = 11.2 Hz, 1H), 4.30-4.06 (m, 9H), 3.97 (t, J = 4.8 Hz, 1H), 3.89-3.80 (m, 3H), 3.75-3.48 (m, 12H), 3.38 (s, 3H), 3.17 (d, J = 6.8 Hz, 2H), 2.94-2.62 (m, 8H), 2.50-2.28 (m, 7H), 2.22-1.65 (m, 14H), 1.58-1.30 (m, 18H), 1.10 (d, J = 6.8 Hz, 3H), 1.06-0.97 (m, 1H). LCMS (M+Na)=1802.8.

1.9 Preparation of NHS-PEG2-Ala-Ala-Asn-PAB-eribulin (ER-001231691)

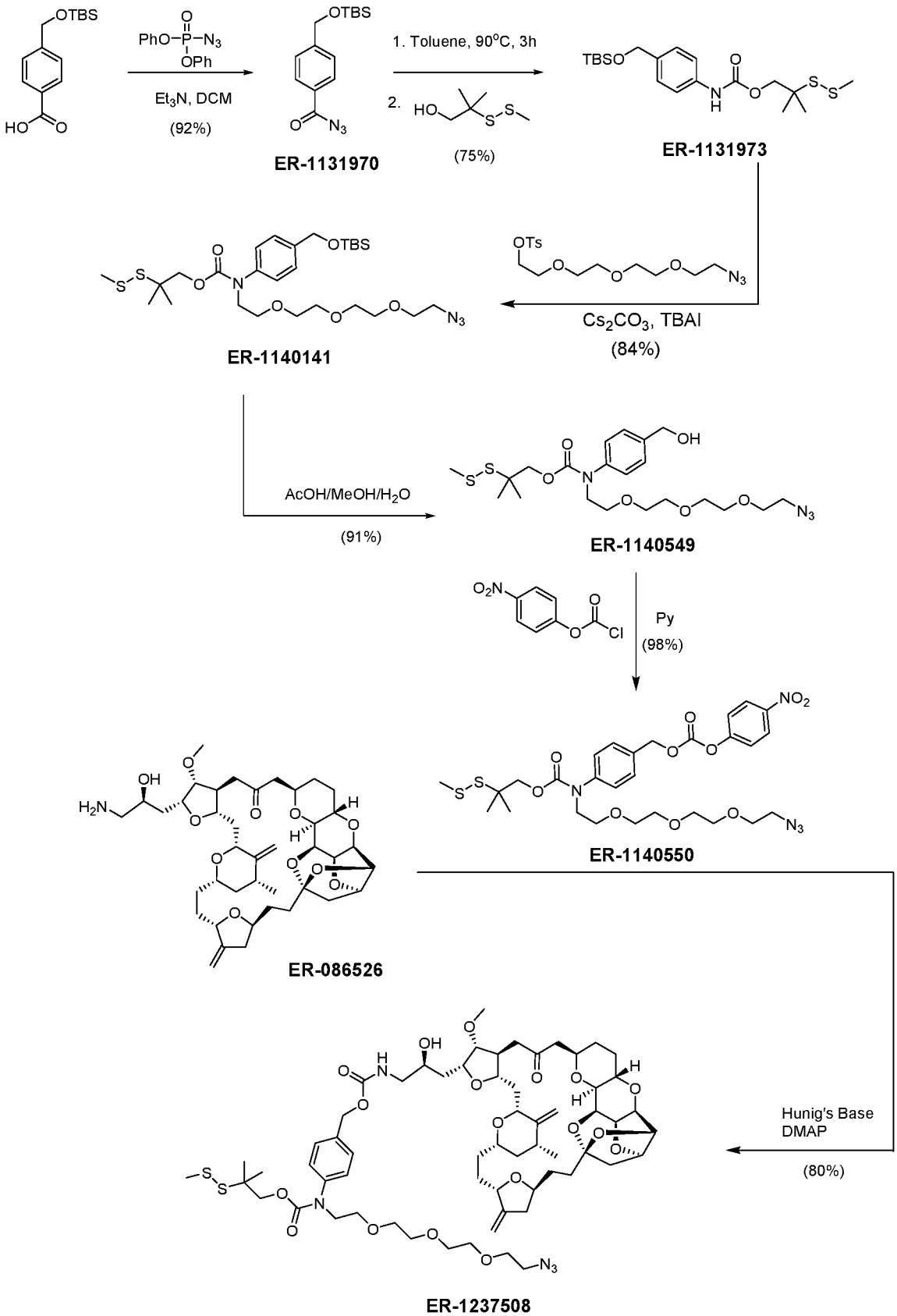


[00361] Ala-Ala-Asn-PAB-eribulin (ER-001231678) was prepared (15 mg, quantitative yield) from eribulin (ER-000086526) and Fmoc-Ala-Ala-Asn-PAB-PNP

using the same procedure as described above for the preparation of Val-Cit-PAB-eribulin (ER-001228950). LCMS (M+H)=1135.5.

[00362] NHS-PEG2-Ala-Ala-Asn-PAB-eribulin (ER-001231691) was prepared (12.4 mg, 64% yield) from Ala-Ala-Asn-PAB-eribulin (ER-001231678) and BisNHS-PEG2 using the same procedure as described above for the preparation of NHS-PEG2-Val-Cit-PAB-eribulin (ER-001236940). ^{1}HNMR (400 MHz) δ ppm 9.21 (s, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.62 (d, J = 8.8 Hz, 2H), 7.58-7.52 (m, 1H), 7.28 (br s, 1H), 7.24 (d, J = 8.4 Hz, 2H), 7.10 (br s, 1H), 6.29 (d, J = 12.4 Hz, 1H), 5.83 (br s, 1H), 5.38 (br s, 1H), 5.07 (s, 1H), 5.05-4.95 (m, 2H), 4.93 (s, 1H), 4.88 (s, 1H), 4.90-4.83 (m, 1H), 4.81 (s, 1H), 4.69 (t, J = 4.4 Hz, 1H), 4.60 (t, J = 4.4 Hz, 1H), 4.46-4.41 (m, 1H), 4.36-4.25 (m, 4H), 4.19 (dd, J = 4.8, 6.0 Hz, 1H), 4.15-4.09 (m, 1H), 4.03 (dd, J = 4.8, 6.0 Hz, 1H), 3.99-3.89 (m, 3H), 3.85-3.50 (m, 10H), 3.41 (s, 3H), 3.40-3.10 (m, 3H), 3.01-2.60 (m, 10H), 2.60-2.35 (m, 7H), 2.35-1.65 (m, 14H), 1.65-1.20 (m, 14H), 1.10 (d, J = 6.8 Hz, 3H), 1.15-1.03 (m, 1H). LCMS (ES) (M+H)=1442.7.

1.10 Preparation of azide-PEG3-disulfide-PAB-eribulin (ER-001237508)



[00363] 4-(((tert-butyldimethylsilyl)oxy)methyl)benzoic acid (1.0 g, 3.754 mmol) was dissolved in dichloromethane (DCM) (25 mL) cooled to 0°C. Triethylamine (0.549 mL, 3.941 mmol) was then added, followed by diphenyl phosphorazidate (1.085 mg, 3.941 mmol). The reaction mixture was slowly warmed to room temperature and stirred for 14 hours. The crude mixture was diluted with ethyl acetate (EtOAc)/Hep (1:1, 100 mL), and passed through a short silica plug eluting with EtOAc/Hep (50%). The solvent was removed under vacuum to yield 1.10 g of 4-(((tert-butyldimethylsilyl)oxy)methyl)benzoyl azide (ER-001131970). ^{1}H NMR (400 MHz) δ ppm 7.98 (d, 2 H, J = 8.0 Hz), 7.40 (d, 2 H, J = 8.0 Hz), 4.79 (s, 2 H), 0.94 (s, 9 H), 0.10 (s, 6 H).

[00364] 4-(((tert-butyldimethylsilyl)oxy)methyl) benzoyl azide (ER-001131970) (1.1 g, 3.775 mmol), dissolved in toluene (20 mL), was heated at 110°C for 3 hours. Although the product did not show as a single spot, thin layer chromatography (TLC) analysis indicated that the starting material was consumed. The reaction mixture was then cooled to room temperature, and transferred to a vial sealed under nitrogen and stored as a solution in toluene (1 mL = 32.6 mg) at -20°C.

[00365] Triethylamine (0.099 mL, 0.709 mmol) was added to a solution of tert-butyl((4-isocyanatobenzyl)oxy)dimethylsilane (165 mg, 0.626 mmol) in toluene (5 mL), followed by alcohol (90.0 mg, 0.591 mmol), and the reaction mixture was stirred for 6 hours at 36°C. Progress of the reaction was monitored by UPLC/MS. A saturated solution of sodium hydrogen carbonate ($NaHCO_3$) (10 mL) was then added, extracted with EtOAc/Hep (1:1, 60 mL), washed with brine, dried over sodium sulfate, and concentrated. The crude material was purified by flash chromatography (EtOAc/Hep 10% to 40%) to obtain 215 mg of 2-methyl-2-(methyldisulfanyl)propyl(4-(((tert-butyldimethylsilyl) oxy)methyl)phenyl)carbamate (ER-001131973). ^{1}H NMR (400 MHz) δ ppm 7.34 (d, 2 H, J = 8.4 Hz), 7.26 (d, 2 H, J = 7.6 Hz), 6.63 (br s, 1 H), 4.69 (s, 2 H), 4.17 (s, 2 H), 2.42 (s, 3 H), 1.35 (s, 6 H), 0.93 (s, 9 H), 0.08 (s, 6 H).

[00366] 2-methyl-2-(methyldisulfanyl)propyl (4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)carbamate (ER-001131973) (198 mg, 0.476 mmol) and 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (325 mg, 0.87 mmol) were dissolved in DMF (6.6 mL). Cesium carbonate (621 mg, 1.905 mmol) was then added, followed by tetrabutylammoniumiodide (45 mg, 0.122 mmol), and the reaction mixture was stirred for 15 hours at 36°C. Progress of the reaction was

monitored by UPLC/MS. A saturated solution of NH_4Cl (30 mL) was then added, extracted with EtOAc/Hep (2:1, 150 mL), washed with brine (10 mL), dried over sodium sulfate, and concentrated under vacuum. The crude material was purified by flash chromatography (EtOAc/Hep 20% to 50%) to obtain 248 mg of 2-methyl-2-(methyldisulfanyl)propyl (2-(2-(2-(2-azidoethoxy)ethoxy) ethoxy)ethyl)(4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)carbamate (ER-001140141). ^{1}H NMR (400 MHz) δ ppm 7.28 (d, 2 H, J = 8.4 Hz), 7.20 (d, 2 H, J = 8.0 Hz), 4.73 (s, 2 H), 4.06 (br s, 2 H), 3.83 (dd, 2 H, J = 6.4, 5.6 Hz), 3.68- 3.56 (m, 12 H), 3.37 (dd, 2 H, J = 5.6, 5.2 Hz), 2.33 (s, 3 H), 1.14 (br s, 6 H), 0.93 (s, 9 H), 0.09 (s, 6 H).

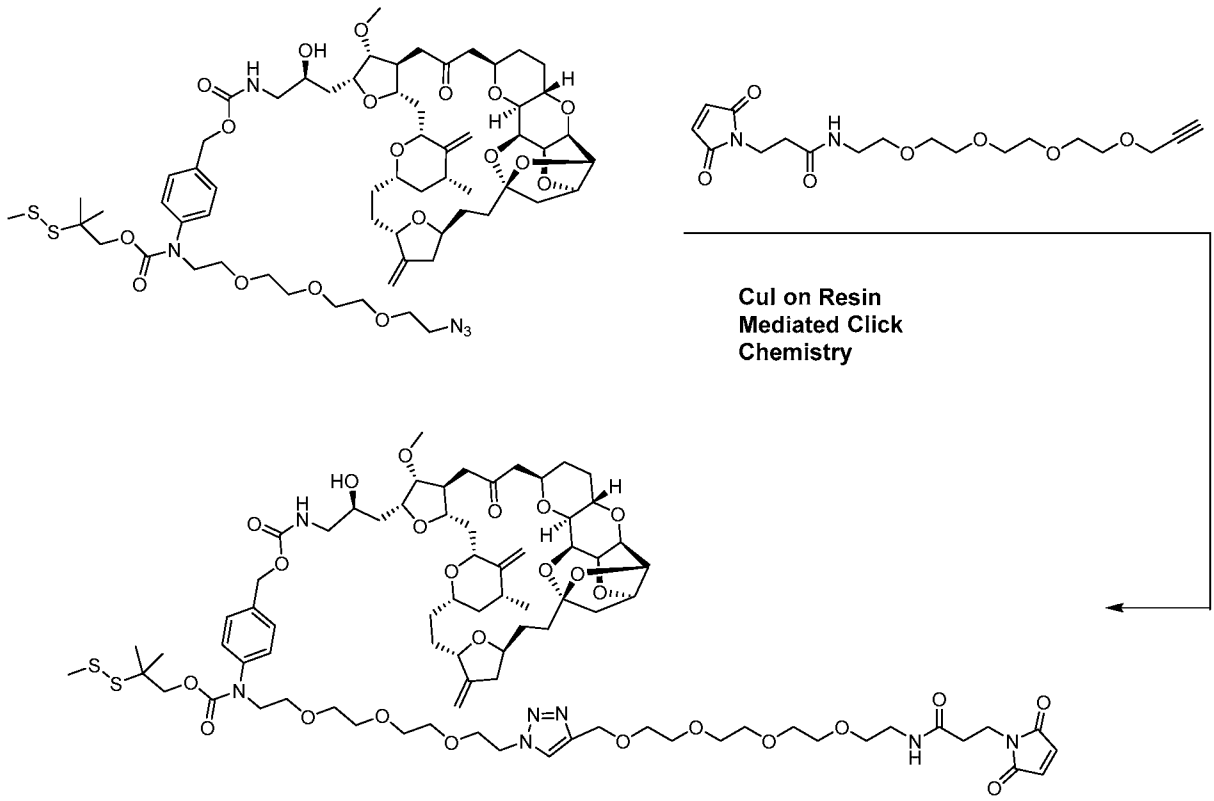
[00367] 2-methyl-2-(methyldisulfanyl)propyl (2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)(4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)carbamate (ER-001140141) (81 mg, 0.131 mmol) was dissolved in a mixture of methanol (5 mL) and water (0.5 mL). Acetic acid (0.5 mL, 8.734 mmol) was then added to the reaction mixture, and stirred for 14 hours at 38°C. The reaction mixture was cooled to room temperature, and the solvent was removed under vacuum. The residue was diluted with EtOAc (30 mL), washed with water (2 X 5 mL), $NaHCO_3$, and brine (3 mL), dried over sodium sulfate, and concentrated under vacuum. The crude material was purified by flash chromatography (EtOAc/Hep 30% to 90%) to obtain 61.0 mg of 2-methyl-2-(methyldisulfanyl)propyl (2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy) ethyl)(4-(hydroxymethyl)phenyl)carbamate (ER-001140549). ^{1}H NMR (400 MHz) δ ppm 7.34 (d, 2 H, J = 8.8 Hz), 7.26 (d, 2 H, J = 8.0 Hz), 4.69 (d, 2 H, J = 4.4 Hz), 4.06 (br s, 2 H), 3.84 (dd, 2 H, J = 6.2, 6.2 Hz), 3.66-3.56 (m, 12 H), 3.37 (dd, 2 H, J = 5.2, 5.2 Hz), 2.33 (s, 3 H), 1.74 (br s, 1 H), 1.14 (br s, 6 H).

[00368] 2-methyl-2-(methyldisulfanyl)propyl (2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)(4-(hydroxymethyl)phenyl)carbamate (ER-001140549) (60 mg, 0.119 mmol) was dissolved in DCM (2 mL) and Py (0.019 mL, 0.239 mmol) cooled to 0°C. 4-nitrophenyl carbonochloridate (38.5 mg, 0.191 mmol) in DCM (2 mL) and dimethylaminopyridine (DMAP) (2.9 mg, 0.024 mmol) were then added, and the reaction mixture was stirred for 30 min at 0°C. The reaction mixture was slowly warmed to room temperature, and stirred until the starting material was consumed (approximately 2.5 hours). The solvent was then removed under vacuum, and the residue was purified by flash chromatography (EtOAc/Hep 10% to 35%) to

obtain 78 mg of 2-methyl-2-(methyldisulfanyl)propyl (2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy) ethyl)(4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)carbamate (ER-001140550). ^{1}H NMR (400 MHz) δ ppm 8.27 (dd, 2 H, *J* = 6.8, 2.4 Hz), 7.41 (d, 2 H, *J* = 8.8 Hz), 7.37 (dd, 2 H, *J* = 7.2, 2.4 Hz), 7.33(d, 2 H, *J* = 8.8 Hz), 5.27 (s, 2 H), 4.08 (br s, 2 H), 3.85 (dd, 2 H, *J* = 5.8, 5.8 Hz), 3.66- 3.57 (m, 12 H), 3.36 (dd, 2 H, *J* = 5.2, 5.2 Hz), 2.33 (br s, 3 H), 1.19 (br s, 6 H).

[00369] 2-methyl-2-(methyldisulfanyl)propyl (2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)(4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)carbamate (ER-001140550) (30 mg, 0.045 mmol) in DCM (3 mL, 46.625 mmol) was placed in a 25-ml flask under nitrogen, and cooled to 0°C. Amine (40.8 mg, 0.049 mmol) in DCM (2 mL) and Hunig's Base (0.024 mL, 0.135 mmol) were added, followed by DMAP (1.4 mg, 0.011 mmol). The reaction mixture was then slowly warmed to room temperature, stirred for 3 hours, concentrated under vacuum, and purified by flash chromatography (EtOAc/Hep 50% to 100%, followed by MeOH/EtOAc 3% to 8%) to obtain 45.0 mg of pure azide-PEG3-disulfide-PAB-eribulin (ER-001237508). ^{1}H NMR (400 MHz) δ ppm 7.32 (d, 2 H, *J* = 8.0 Hz), 7.25 (d, 2 H, *J* = 7.2 Hz), 5.28 (dd, 1 H, *J* = 5.6, 5.6 Hz), 5.11-5.04 (m, 3 H), 4.93 (s, 1 H), 4.88 (s, 1 H), 4.81 (s, 1 H), 4.69 (dd, 1 H, *J* = 4.4, 4.4 Hz), 4.60 (dd, 1 H, *J* = 4.2, 4.2 Hz), 4.36 (br s, 1 H), 4.33(dd, 1 H, *J* = 4.0, 2.0), 4.29 (ddd, 1 H, *J* = 9.6, 4.4, 4.4 Hz), 4.18 (dd, 1 H, *J* = 6.4, 4.4 Hz), 4.14-4.04 (m, 3 H), 4.03 (dd, 1 H, *J* = 6.4, 4.4 Hz), 3.97-3.89 (m, 3 H), 3.84-3.78 (m, 3 H), 3.67-3.56 (m, 14 H), 3.42 (s, 3 H), 3.40-3.35 (m, 1 H), 3.37 (dd, 2 H, *J* = 5.2, 5.2 Hz), 3.27 (d, 1 H, *J* = 3.2 Hz), 3.20 (ddd, 1 H, *J* = 12.8, 6.0, 6.0 Hz), 2.91-2.83 (m, 2 H), 2.70 (dd, 1 H, *J* = 16.0, 10.0 Hz), 2.52-2.40 (m, 3 H), 2.35-2.13 (m, 9 H), 2.10-2.06 (m, 1 H), 2.01-1.89 (m, 4 H), 1.78-1.64 (m, 4 H), 1.60-1.52 (m, 4 H), 1.49-1.28 (m, 5 H), 1.22-1.07 (m, 6 H), 1.09 (d, 3 H, *J* = 6.0 Hz).

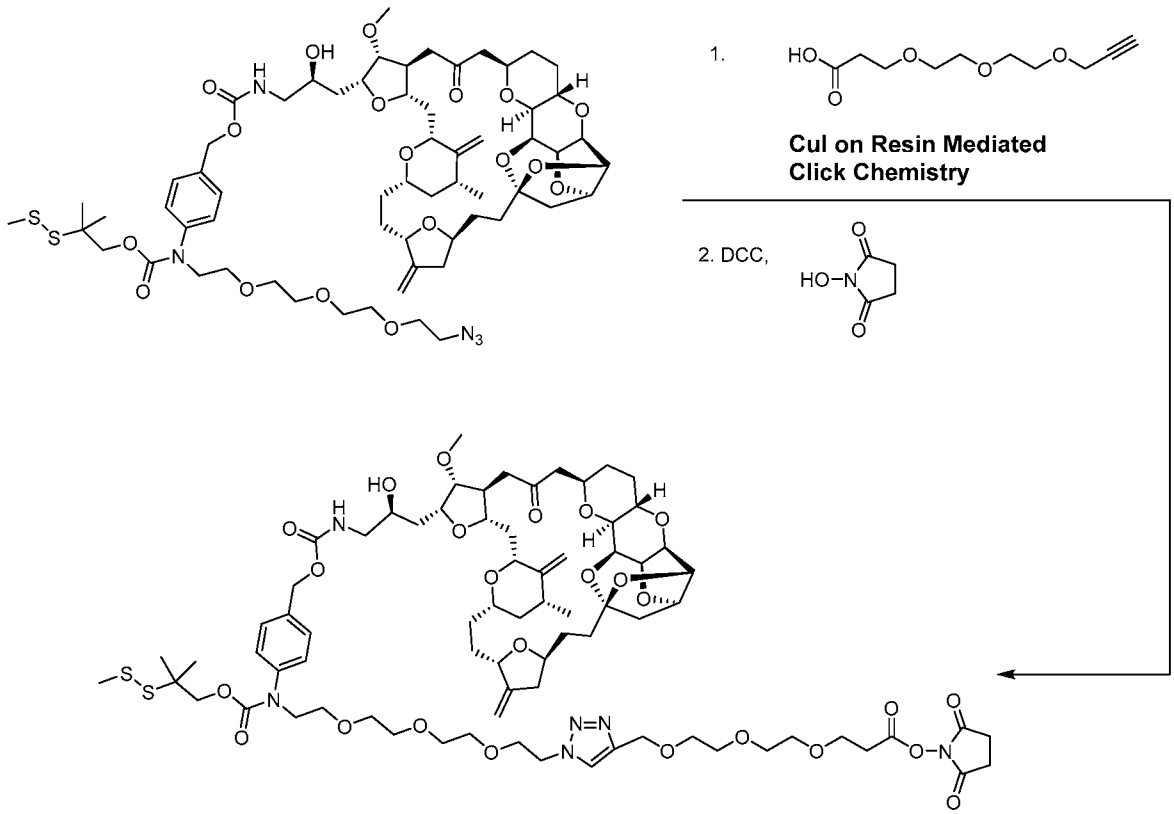
1.11 Preparation of Mal-PEG4-triazole-PEG3-disulfide-PAB-eribulin (ER-001237504)



[00370] A mixture of azide (9.0 mg, 7.151 μmol) and 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-(3,6,9,12-tetraoxapentadec-14-yn-1-yl)propanamide (6.8 mg, 0.018 mmol) in tert-butanol (1.5 mL) and water (0.5 mL) was degassed for 45 min. Copper iodide on amberlyst-21 (1.23 mmol/g, 10 mg) was then added, and degassed for an additional 30 min. The reaction mixture was stirred at room temperature for 18 hours, and monitored by UPLC/MS. The majority of the starting material was consumed, and the desired product showed as a major peak. The mixture was then separated from resin, and purified on HPLC (acetonitril/water with 0.05 % formic acid) to obtain 1.5 mg of Mal-PEG4-triazole-PEG3-disulfide-PAB-eribulin (ER-001237504). ^{1}H NMR (400 MHz) δ ppm 7.74 (s, 1 H), 7.32 (d, 2 H, J = 8.4 Hz), 7.27-7.25 (m, 2 H), 6.69 (br s, 2 H), 5.43 (dd, 1 H, J = 5.6, 5.6 Hz), 5.14-5.06 (m, 3 H), 4.95 (s, 1 H), 4.89 (s, 1 H), 4.82 (s, 1 H), 4.70 (dd, 1 H, J = 4.4, 4.4 Hz), 4.66 (s, 2 H), 4.62 (dd, 1 H, J = 4.4, 4.4 Hz), 4.52(dd, 1 H, J = 5.2, 5.2 Hz), 4.38-4.31 (m, 2 H), 4.30 (ddd, 1 H, J = 10.4, 4.0, 4.0 Hz), 4.20 (dd, 1 H, J = 6.4, 4.4 Hz), 4.16-4.05 (m, 3 H), 4.04 (dd, 1 H, J = 6.4, 4.4 Hz), 3.99-3.91 (m, 3 H), 3.87-3.80 (m, 6 H), 3.70-3.59 (m, 22 H), 3.53 (dd, 2 H, J = 5.2,

5.2 Hz), 3.44 (s, 3 H), 3.43-3.36 (m, 3 H), 3.29 (d, 1 H, *J* = 2.8 Hz), 3.18 (ddd, 1 H, *J* = 12.9, 6.2, 6.2 Hz), 2.92-2.84 (m, 2 H), 2.72 (dd, 1 H, *J* = 16.0, 10.0 Hz), 2.54-2.42 (m, 5 H), 2.37-1.90 (m, 19 H), 178-1.52 (m, 3 H), 1.50-1.14 (m, 16 H), 1.10 (d, 3 H, *J* = 6.0 Hz). LCMS (M+H)=1642.1.

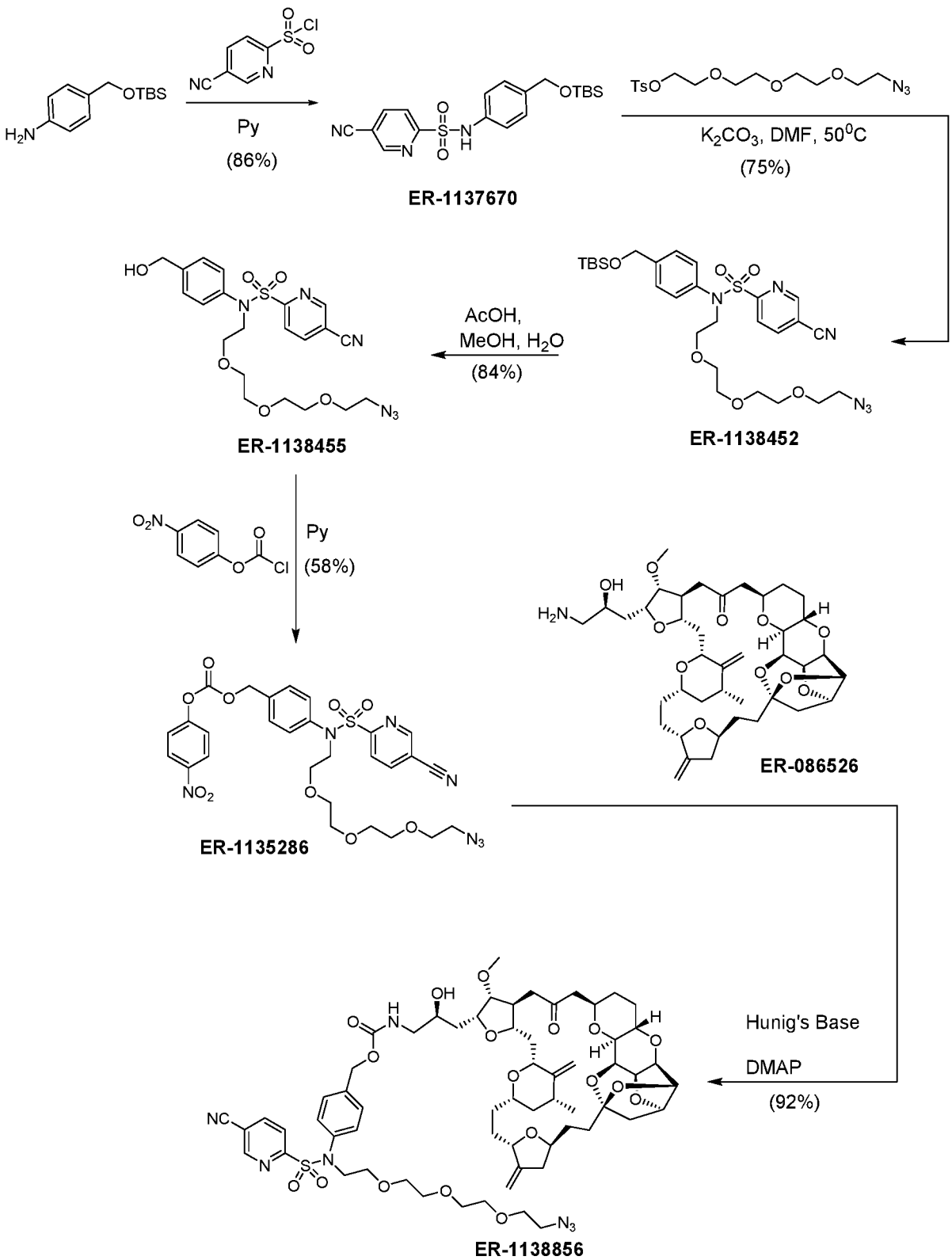
1.12 Preparation of NHS-PEG3-triazole-PEG3-disulfide-PAB-eribulin (ER-001244129)



[00371] A mixture of azide (9 mg, 7.151 µmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)propanoate (4.5 mg, 14.30 µmol) in tert-butanol (1 mL) and water (0.5 mL) was degassed for 45 min. Copper iodide on amberlyst-21 (1.23 mmol/g, 10 mg, 7.151 µmol) was then added, and degassed for an additional 30 min. The reaction mixture was stirred room temperature for 18 hours, and monitored by UPLC/MS. The majority of the starting material was consumed, and the desired product showed as a major peak. The mixture was then separated from resin by filtration, extracted with DCM (15 mL), washed with brine (3 X 3 mL), dried over sodium sulfate, and concentrated under vacuum. The residue (5 mg, 3.39 µmol) was

azeotroped with toluene, dissolved in THF (1 mL), and cooled to 0°C. DCC (4.2 mg, 0.02 mmol) was added, followed by 1-hydroxypyrrolidine-2,5-dione (2.2 mg, 0.019 mmol), and the reaction mixture was stirred at room temperature for 18 hours. The majority of the starting material was consumed, and the desired product showed as a major peak, as determined by UPLC/MS. The reaction mixture was then concentrated and purified on preparative TLC (DCM/*i*-propanol, 8%) to yield 2.5 mg of NHS-PEG3-triazole-PEG3-disulfide-PAB-eribulin (ER-001244129) as a colorless oil. ^{1}H NMR (400 MHz, CD_2Cl_2) δ ppm 7.72 (s, 1 H), 7.32 (d, 2 H, *J* = 8.8 Hz), 7.25 (d, 2 H, *J* = 8.8 Hz), 5.08-5.04 (m, 3 H), 4.93 (s, 1 H), 4.85 (s, 1 H), 4.78 (s, 1 H), 4.64 (dd, 1 H, *J* = 4.4, 4.4 Hz), 4.58 (s, 2 H), 4.55 (dd, 1 H, *J* = 4.4, 4.4 Hz), 4.48 (dd, 2 H, *J* = 5.0, 5.0 Hz), 4.32 (d, 1 H, *J* = 6.6 Hz), 4.27-4.22 (m, 2 H), 4.14 (dd, 1 H, *J* = 6.6, 4.8 Hz), 4.10-4.01 (m, 3 H), 4.00 (dd, 1 H, *J* = 6.8, 4.4 Hz), 3.92-3.78 (m, 9 H), 3.65-3.53 (m, 19 H), 3.44-3.39 (m, 4 H), 3.37 (s, 3 H), 3.26 (d, 1 H, *J* = 3.2 Hz), 3.13 (ddd, 1 H, *J* = 12.4, 6.0, 6.0 Hz), 2.91-2.73 (m, 11 H), 2.70-2.64 (m, 2 H), 2.54-2.41 (m, 3 H), 2.38-1.80 (m, 16 H), 1.74-1.52 (m, 3 H), 1.41-1.13 (m, 10 H), 1.07 (d, 3 H, *J* = 6.4 Hz). LCMS (M+H)=1572.3.

1.13 Preparation of azide-PEG3-sulfonamide-PAB-eribulin (ER-001138856)



[00372] 4-(((tert-butyldimethylsilyl)oxy)methyl)aniline (315 mg, 1.327 mmol) was dissolved in DCM (10 mL) cooled to 0° C. Pyridine (0.268 mL, 3.317 mmol) was then added, followed by 5-cyanopyridine-2-sulfonyl chloride (365 mg, 1.801 mmol) in DCM (10 mL) over 15 min. The reaction mixture was slowly warmed to room temperature over 1 hour, and stirred for 2 hours. The reaction mixture was diluted with EtOAc (50 mL), washed with brine, dried over sodium sulfate, and concentrated under vacuum to obtain 610 mg (103%) of N-(4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)-5-cyanopyridine-2-sulfonamide (ER-001137670). The crude product was reasonably pure, though colored. ^{1}H NMR (400 MHz) δ ppm 8.94 (dd, 1 H, J = 1.8, 0.6 Hz), 8.10 (dd, 1 H, J = 8.4, 2.0 Hz), 7.99 (dd, 1 H, J = 8.0, 0.8 Hz), 7.18 (d, 2 H, J = 8.2 Hz), 7.15 (br s, 1 H), 7.11 (dd, 2 H, J = 6.8, 0.8 Hz), 4.64 (s, 2 H), 0.90 (s, 9 H), 0.05 (s, 6 H).

[00373] N-(4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)-5-cyanopyridine-2-sulfonamide (ER-001137670) (105.0 mg, 0.26 mmol) and 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (143 mg, 0.383 mmol) were dissolved in DMF (4 mL). Potassium carbonate (K_2CO_3) (144 mg, 1.041 mmol) was then added, followed by tetrabutylammonium iodide (19.2 mg, 0.052 mmol), and the reaction mixture was stirred for 36 hours at 50°C. Progress of the reaction was monitored by UPLC/MS. A saturated solution of NH_4Cl (10 mL) was added, extracted with EtOAc/Hep (2:1, 30 mL), washed with brine, dried over sodium sulfate, and concentrated. The crude material was purified by flash chromatography (EtOAc/Hep 25% to 80%) to obtain 118.0 mg of N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-N-(4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)-5-cyanopyridine-2-sulfonamide (ER-001138452) (75%). ^{1}H NMR (400 MHz) δ ppm 8.99 (dd, 1 H, J = 1.8, 0.6 Hz), 8.08 (dd, 1 H, J = 8.2, 2.2 Hz), 7.86 (dd, 1 H, J = 8.0, 0.8 Hz), 7.24 (d, 2 H, J = 10 Hz), 7.09 (d, 2 H, J = 8.8 Hz), 4.69 (s, 2 H), 4.06 (dd, 2 H, J = 6.0, 6.0 Hz), 3.67 (dd, 2 H, J = 5.2, 5.2 Hz), 3.65 – 3.62 (m, 4 H), 3.58 (dd, 2 H, J = 6.2, 6.2 Hz), 3.56 – 3.53 (m, 4 H), 3.38 (dd, 2 H, J = 5.2, 5.2 Hz), 0.93 (s, 9 H), 0.08 (s, 6 H).

[00374] N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-N-(4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)-5-cyanopyridine-2-sulfonamide (ER-001138452) (150 mg, 0.248 mmol) was dissolved in methanol (6 mL). Water (0.60 mL) was then added, followed by acetic acid (AcOH) (0.60 mL, 10.481 mmol). The reaction mixture was slowly warmed to 38°C, and stirred for 14 hours. The majority of the solvent was removed under vacuum. The residue was diluted with EtOAc (30 mL),

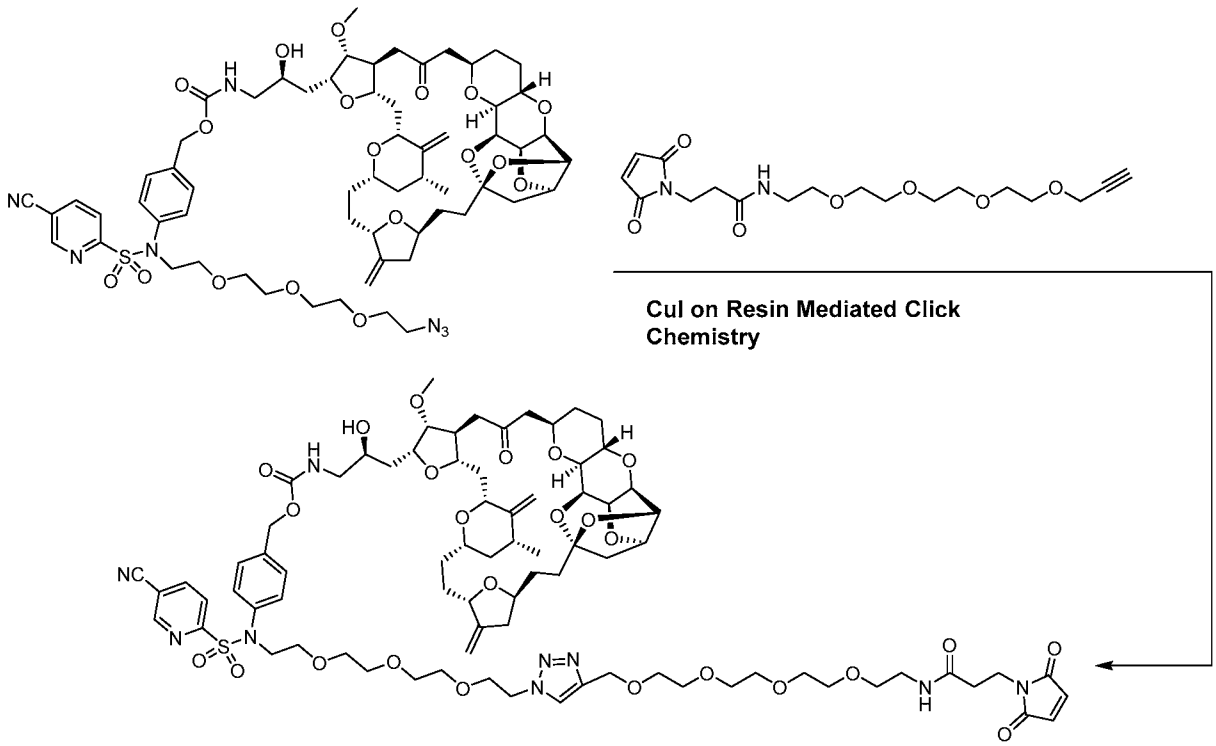
washed with water (2 X 5 mL), $NaHCO_3$, and brine, dried over sodium sulfate, and concentrated under vacuum. The crude material was purified by flash chromatography (EtOAc/Hep 35% to 90%) to obtain 105.0 mg of N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-5-cyano-N-(4-(hydroxymethyl)phenyl)pyridine-2-sulfonamide (ER-001138455) (84%). ^{1}H NMR (400 MHz) δ ppm 8.99 (d, 1 H, J = 1.2 Hz), 8.09 (dd, 1 H, J = 8.4, 2.0 Hz), 7.88 (dd, 1 H, J = 8.4, 0.8 Hz), 7.30 (d, 2 H, J = 8.8 Hz), 7.15 (d, 2 H, J = 8.4 Hz), 4.67 (s, 2 H), 4.06 (dd, 2 H, J = 6.2, 6.2 Hz), 3.66 (dd, 2 H, J = 5.0, 5.0 Hz), 3.65 – 3.58 (m, 6 H), 3.55 – 3.51 (m, 4 H), 3.38 (dd, 2 H, J = 5.2, 5.2 Hz.

[00375] N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-5-cyano-N-(4-(hydroxymethyl)phenyl)pyridine-2-sulfonamide (ER-001138455) (45 mg, 0.092 mmol) was dissolved in DCM (3 mL), and cooled to 0°C following the addition of pyridine (0.015 mL, 0.183 mmol). 4-nitrophenyl carbonochloridate (20.3 mg, 0.101 mmol) in DCM (2 mL) and DMAP (2.3 mg, 0.018 mmol) was then added. The reaction mixture was slowly warmed to room temperature and stirred for 2 hours. UPLC/MS indicated that some starting material remained. The reaction mixture was then concentrated under vacuum, and purified by flash chromatography (EtOAc/Hep 12% to 40%) to obtain 35 mg of 4-((N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-5-cyanopyridine)-2-sulfonamido)benzyl (4-nitrophenyl) carbonate (ER-001235286) (58%), and 20 mg of starting material. ^{1}H NMR (400 MHz) δ ppm 8.99 (d, 1 H, J = 0.8 Hz), 8.27 (dd, 2 H, J = 9.2, 2.0 Hz), 8.12 (dd, 1 H, J = 7.6, 2.0 Hz), 7.92 (d, 1 H, J = 8.4 Hz), 7.38 (d, 4 H, J = 9.6 Hz), 7.26 (d, 2 H, J = 8.8 Hz), 5.45 (s, 2 H), 4.06 (dd, 2 H, J = 5.8, 5.8 Hz), 3.67 – 3.58 (m, 8 H), 3.58 – 3.50 (m, 4 H), 3.38 (dd, 2 H, J = 6.1, 6.1 Hz).

[00376] 4-(N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-5-cyanopyridine-2-sulfonamido)benzyl (4-nitrophenyl) carbonate (ER-001235286) (35.0 mg, 0.053 mmol) was placed in a 25-mL flask under nitrogen, and cooled to 0°C. Amine (48.5 mg, 0.059 mmol) in DCM (3 mL, 46.625 mmol) and Hunig's Base (0.037 mL, 0.214 mmol) was then added, followed by DMAP (2.61 mg, 0.021 mmol). The reaction mixture was stirred for 30 min at 0°C, and then stirred for an additional 6 hours at room temperature. The reaction mixture was concentrated under vacuum, and purified by flash chromatography (EtOAc/Hep 50% to 100%, followed by MeOH/EtOAc 3% to 8%) to obtain 61.0 mg of pure azide-PEG3-sulfonamide-PAB-eribulin (ER-001138856). ^{1}H NMR (400 MHz) δ ppm 8.98 (d, 1 H, J = 1.2 Hz), 8.10 (dd, 1 H, J = 8.2, 1.8 Hz), 7.87

(d, 1 H, J = 8.0 Hz), 7.26 (d, 2 H, J = 6.8 Hz), 7.13 (d, 2 H, J = 8.4 Hz), 5.29 (dd, 1 H, J = 5.6, 5.6 Hz), 5.08-5.00 (m, 3 H), 4.92 (s, 1 H), 4.87 (s, 1 H), 4.80 (s, 1 H), 4.68 (dd, 1 H, J = 4.6, 4.6 Hz), 4.59 (dd, 1 H, J = 4.6, 4.6 Hz), 4.38-4.30 (m, 2 H), 4.28 (ddd, 1 H, J = 10.4, 4.0, 4.0, Hz), 4.17 (dd, 1 H, J = 6.2, 4.6 Hz), 4.13-4.01 (m, 4 H), 3.97-3.88 (m, 3 H), 3.82-3.78 (m, 1 H), 3.67-3.50 (m, 15 H), 3.41 (s, 3 H), 3.40-3.33 (m, 1 H), 3.37 (dd, 2 H, J = 4.8, 4.8 Hz), 3.27 (d, 1 H, J = 3.2 Hz), 3.15 (ddd, 1 H, J = 12.8, 6.4, 6.4 Hz), 2.90-2.82 (m, 2 H), 2.70 (dd, 1 H, J = 16.0, 10.0 Hz), 2.51-2.40 (m, 3 H), 2.34-2.13 (m, 7 H), 2.10-2.05 (m, 1 H), 1.99-1.88 (m, 4 H), 1.78-1.64 (m, 5 H), 1.62-1.52 (m, 2 H), 1.50-1.29 (m, 4 H), 1.08 (d, 3 H, J = 6.8 Hz).

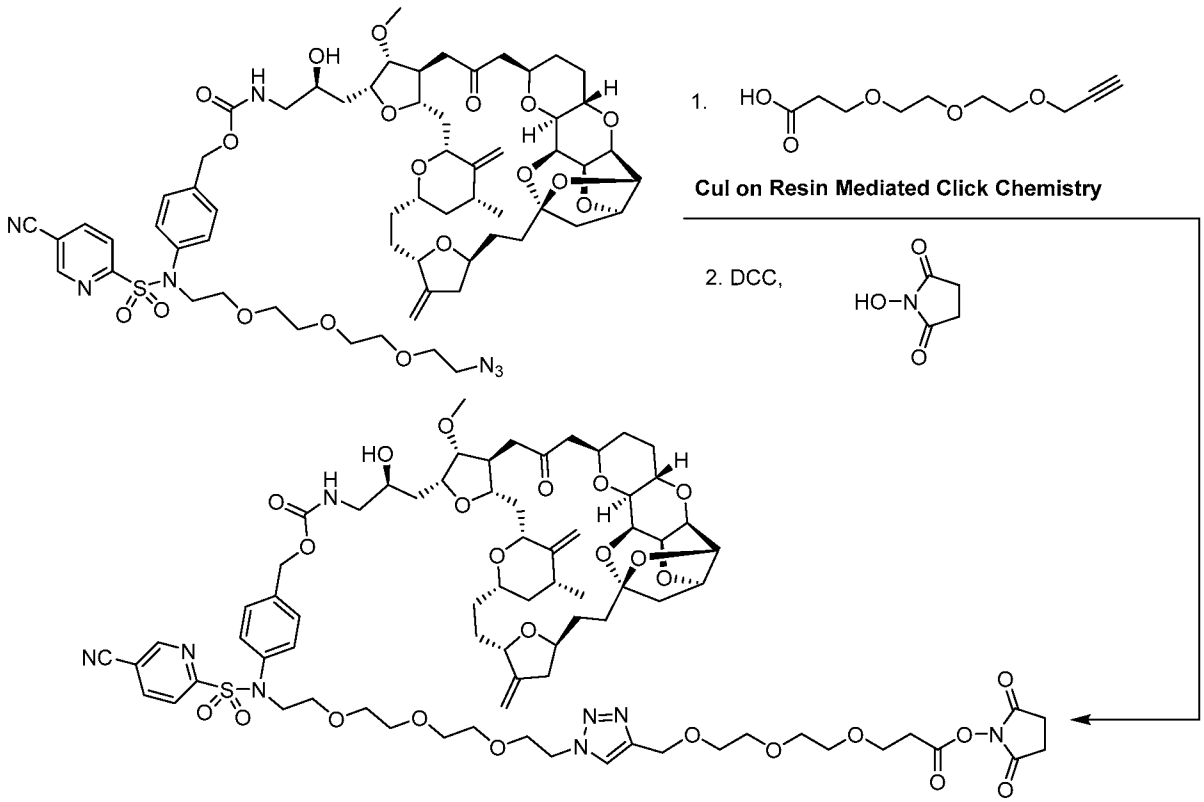
1.14 Preparation of Mal-PEG4-triazole-PEG3-sulfonamide-PAB-eribulin (ER-001237505)



[00377] A mixture of azide (10 mg, 8.023 μmol) and 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-(3,6,9,12-tetraoxapentadec-14-yn-1-yl)propanamide (9.20 mg, 0.024 mmol) in tert-butanol (2.1 mL) and water (0.7 mL) was degassed for 45 min. Copper iodide on amberlyst-21 (1.23 mmol/g, 15 mg) was then added, and degassed for an additional 30 min. The reaction mixture was stirred at room temperature for 18 hours, and was monitored by UPLC/MS. The majority of the starting material was consumed,

and the desired product showed as a major peak. The reaction mixture was then separated from resin, and purified on preparative TLC (DCM/methanol, 7%) to yield 5.5 mg of Mal-PEG4-triazole-PEG3-sulfonamide-PAB-eribulin (ER-001237505). ^{1}H NMR (400 MHz, CD_2Cl_2) δ ppm 9.01 (s, 1 H), 8.15 (dd, 1 H, *J* = 8.0, 1.8 Hz), 7.87 (d, 1 H, *J* = 8.0 Hz), 7.75 (s, 1 H), 7.28 (d, 2 H, *J* = 8.0 Hz), 7.14 (d, 2 H, *J* = 8.4 Hz), 6.68 (s, 2 H), 6.47 (br s, 1 H), 5.44 (br s, 1 H), 5.10-5.02 (m, 3 H), 4.94 (s, 1 H), 4.86 (s, 1 H), 4.80 (s, 1 H), 4.68 (dd, 1 H, *J* = 4.4, 4.4 Hz), 4.59 (s, 2 H), 4.56 (dd, 1 H, *J* = 4.4, 4.4 Hz), 4.51(dd, 2 H, *J* = 5.2, 5.2, Hz), 4.34(d, 1 H, *J* = 7.6, Hz), 4.30-4.23 (m, 2 H), 4.19 – 4.14 (m, 2 H), 4.08 (dd, 1 H, *J* = 4.0, 4.0 Hz), 4.03 – 3.98 (m, 2 H), 3.94 – 3.72 (m, 8 H), 3.68 – 3.46 (m, 28 H), 3.38 (s, 3 H), 3.38 – 3.33 (m, 3 H), 3.27 (d, 1 H, *J* = 3.2 Hz), 3.16 – 3.02 (m, 2 H), 2.90 – 2.81 (m, 2 H), 2.68 (dd, 1 H, *J* = 16.2, 9.8 Hz), 2.54-2.40 (m, 7H), 2.40-1.8 (m, 11 H), 1.80-1.50 (m, 3 H), 1.48-1.25 (m, 3 H), 1.09 (d, 3 H, *J* = 6.4 Hz). LCMS (M+H)=1630.0.

1.15 Preparation of NHS-PEG3-triazole-PEG3-sulfonamide-PAB-eribulin (ER-001244623)



[00378] A mixture of azide (14 mg, 0.011 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)propanoate (8.80 mg, 0.028 mmol) in tert-butanol

(2 mL) and water (1 mL) was degassed for 45 min. Copper iodide on amberlyst-21 (1.23 mmol/g , 20 mg) was then added, and degassed for an additional 30 min. The reaction mixture was stirred at room temperature for 18 hours, and was monitored by UPLC/MS. The majority of the starting material was consumed, and the desired product showed as a major peak. The reaction mixture was then separated from resin by extraction with DCM (2 x 10 mL). The DCM layer was washed with brine (4 x 5 mL), dried over sodium sulfate, and concentrated to the desired product (which was used in the next step without any further purification).

[00379] Crude acid (15.0 mg, 10.255 μmol) was dissolved in THF (1.5 mL), and cooled to 0°C. DCC (15.2 mg, 0.074 mmol) was then added, followed by 1-hydroxypyrrolidine-2,5-dione (8.3 mg, 0.072 mmol). The reaction mixture was stirred at room temperature for 18 hours. UPLC/MS indicated that the majority of the starting material was consumed, and the desired product showed as a major peak. The reaction mixture was concentrated, and purified on preparative TLC (DCM/*i*-propanol, 8%) to yield 2.5 mg of NHS-PEG3-triazole-PEG3-sulfonamide-PAB-eribulin (ER-001244623). ^{1}H NMR (400 MHz, CD_2Cl_2) δ ppm 9.00 (s, 1 H), 8.12 (d, 1 H, J = 8.4 Hz), 8.00 (d, 1 H, J = 8.0 Hz), 7.72 (s, 1 H), 7.26 (d, 2 H, J = 8.0 Hz), 7.12 (d, 2 H, J = 8.0 Hz), 5.37 (br s, 1 H), 5.08-5.02 (m, 3 H), 4.93 (s, 1 H), 4.85 (s, 1 H), 4.78 (s, 1 H), 4.66-4.62 (m, 1 H), 4.58-4.56 (m, 4 H), 4.33 (d, 1 H, J = 10.8 Hz), 4.29-4.21 (m, 2 H), 4.10-3.96 (m, 4 H), 3.93-3.76 (m, 6 H), 3.74-3.44 (m, 27 H), 3.36 (s, 3 H), 3.34-3.24 (m, 2 H), 3.15-3.06 (m, 1 H), 2.97 (br s, 1 H), 2.90-2.78 (m, 8 H), 2.74-2.08 (m, 13 H), 2.05 -1.78 (m, 5 H), 1.73-1.50 (m, 2 H), 1.41-1.25 (m, 4 H), 1.07 (d, 3 H, J = 6.0 Hz). LCMS (M+H)=1560.0.

1.16 Preparation of Mal-PEG2-eribulin

[00380] Eribulin (5 mg, 7 μmol) was dissolved in DMF (0.5 mL), and mixed with maleimido-PEG2-NHS (5 mg, 14 μmol; Broadpharm, Cat No. BP-21680) and Hunig's base (2.4 μL, 14 μmol). The reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was then purified by HPLC (water—acetonitrile gradient 30-70% containing 0.1% formic acid). Eluent was collect by mass, and lyophilized to dryness. Final yield was 3.7 mg (3.8 μmol, 54%). Predicted exact mass was 968.5 Da. Measured mass was 969.6 Da [M+H].

1.17 Preparation of Mal-PEG4-eribulin

[00381] Eribulin (5 mg, 7 μmol) was dissolved in DMF (0.5 mL), and mixed with maleimido-PEG4-NHS (6.2 mg, 14 μmol; Broadpharm, Cat No. BP-20554) and Hunig's base (2.4 μL, 14 μmol). The reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was then purified by HPLC (water—acetonitrile gradient 30-70% containing 0.1% formic acid). Eluent was collect by mass, and lyophilized to dryness. Final yield was 3.7 mg (3.5 μmol, 50%). Predicted exact mass was 1056.5 Da. Measured mass was 1057.7 Da [M+H].

1.18 Preparation of azido-PEG2-eribulin

[00382] Eribulin (5 mg, 7 μmol) was dissolved in DMF (0.5 mL), and mixed with azido-PEG2-NHS (4.2 mg, 14 μmol; Broadpharm, Cat No. BP-20524) and Hunig's base (2.4 μL, 14 μmol). The reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was then purified by HPLC (water—acetonitrile gradient 30-70% containing 0.1% formic acid). Eluent was collect by mass, and lyophilized to dryness. Final yield was 2.2 mg (2.4 μmol, 34%). Predicted exact mass was 914.5 Da. Measured mass was 915.7 Da [M+H].

1.19 Preparation of azido-PEG4-eribulin

[00383] Eribulin (5 mg, 7 μmol) was dissolved in DMF (0.5 mL), and mixed with azido-PEG4-NHS (5.5 mg, 14 μmol; Broadpharm, Cat No. BP-20518) and Hunig's base (2.4 μL, 14 μmol). The reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was then purified by HPLC (water—acetonitrile gradient 30-70% containing 0.1% formic acid). Eluent was collect by mass, and lyophilized to dryness. Final yield was 3.0 mg (3.0 μmol, 43%). Predicted exact mass was 1002.5 Da. Measured mass was 1003.7 Da [M+H].

1.20 Preparation of azido-PEG4-Val-Cit-PAB-eribulin

[00384] Eribulin (15 mg, 21 μmol) was dissolved in DMF (1.5 mL), and mixed well. Hunig's base (5.5 μL, 32 μmol) and Fmoc-VCP-PNP (24 mg, 22 μmol; Levena Biopharma, Cat No. VC1003) were then added. The reaction mixture was stirred at room temperature overnight (16 hours). Upon completion of the reaction, diethylamine (20 μL, 0.21 mmol) was added to the reaction mixture, and stirred for 2 hours at room

temperature to remove the Fmoc protecting group. The deprotection reaction was monitored using a Waters SQD mass spectrometer. Upon completion of the reaction, the reaction mixture was transferred to a pre-weighed 1.5mL microcentrifuge tube. The solvent was evaporated under vacuum using a refrigerated Centrivap concentrator with the temperature set at 30°C. Yield was 16 mg (14 μmol) of crude NH2-Val-Cit-pAB-eribulin (exact mass 1134.6 Da, 67% yield).

[00385] NH2-Val-Cit-pAB-eribulin (16 mg, 14.1 μmol) was dissolved in DMF (1.5 mL). Hunig's Base (7.2 μL, 41 μmol) and azido-PEG4-NHS (11 mg, 28.2 μmol) were then added. The reaction mixture was stirred at room temperature for 3 hours. The reaction mixture was then purified by HPLC (water—acetonitrile gradient 48-72% containing 0.1% formic acid). The eluent was collected at m/z 1409, and lyophilized to afford azido-PEG4-Val-Cit-PAB-eribulin (exact mass 1407.7 Da). 13 mg (9.2 μmol) of azido-PEG4-Val-Cit-PAB-eribulin was obtained (65% step yield, 44% overall).

EXAMPLE 4

1. Materials and Methods

[00386] All reagents used were obtained from commercial suppliers at research-grade or higher, unless otherwise indicated.

1.1 Antibodies

[00387] MORAb-003 (humanized anti-human folate receptor alpha, 25 mg/mL) and MORAb-009 (mouse-human chimeric anti-human mesothelin, 25 mg/mL) used in the following studies were from Lot #NB02962-19 and Lot #030A14, respectively. Trastuzumab was obtained commercially (Clingen), and was from Lot #503345.

[00388] Rabbit-human chimeric and humanized anti-human mesothelin antibodies having an unpaired cysteine at LCcys80 (Table 1) were expressed in 293F cells transiently or as stably-selected pools. Conditioned medium was purified and decysteinylated as described in section 1.4.1.2.1.

1.2 Cytotoxins

[00389] Conjugatable eribulin compounds were synthesized as described in Example 3 (Table 46). Stocks (10 mM) were prepared in DMSO and stored at -20°C until use.

1.3 Tumor cell lines

[00390] Human tumor cell lines used in the analyses of MORAb-003, MORAb-009, and trastuzumab ADCs prepared with maleimido/succinimide (OSu)/azido-linker-eribulin compounds (Table 46) include IGROV1 (human ovarian carcinoma, FR^{hi}, $MSLN^{neg}$), NCI-H2110 (human non-small cell lung carcinoma, FR^{med}, $MSLN^{med}$), A431 (FR^{neg}, $MSLN^{neg}$), NCI-N87-luc (human gastric carcinoma, FR^{lo}, $MSLN^{med}$, $her2^{hi}$), NUGC3 (human gastric adenocarcinoma, FR^{neg}, $MSLN^{neg}$, $her2^{neg}$), ZR75 (human breast ductal carcinoma, FR^{neg}, $MSLN^{neg}$, $her2^{med}$), and BT-474 (human breast ductal carcinoma, FR^{neg}, $MSLN^{neg}$, $her2^{hi}$). Human tumor cell lines used in the analyses of rabbit-human chimeric and humanized anti-human mesothelin LCcys80 antibodies conjugated with MAL-PEG2-Val-Cit-PAB-eribulin (ER-001159569) were A3 (A431 stably transfected with human mesothelin, $MSLN^{hi}$), OVCAR3 (human ovarian carcinoma, $MSLN^{hi}$), HEC-251 (human endometroid, $MSLN^{med}$), H226 (human lung squamous cell mesothelioma, $MSLN^{lo}$), and A431 parental ($MSLN^{neg}$). All cell lines used were obtained directly from the American Type Culture Collection (ATCC), with the exceptions of IGROV1 (obtained from the National Cancer Institute, with permission) and A3 (generated at Morphotek from parental A431).

1.4 Antibody-drug conjugation

1.4.1 Cysteine-based conjugation using maleimides

1.4.1.1 Conjugation to interchain disulfides

1.4.1.1.1 Partial reduction

[00391] MORAb-003 and MORAb-009 were buffer-exchanged into Dulbecco's phosphate-buffered saline (DPBS), and then concentrated to 20 mg/mL using centrifugal concentration. An equal volume of 270 μM tris(2-carboxyethyl)phosphine (TCEP) in 1X DPBS with 2 mM EDTA was added, and the reduction was carried out by gentle mixing for 80 min at room temperature. Trastuzumab was partially-reduced in a similar manner, except the reduction was carried out by gentle mixing for 40 min at room temperature.

1.4.1.1.2 Conjugation

[00392] Maleimido-linker-eribulin compound (in DMSO) was conjugated to the partially reduced antibodies at a molar ratio of 1:6 (mAb:compound). The compound was added to 50% propylene glycol in DPBS and mixed well. An equal volume of partially-reduced antibody was then added, and mixed gently (final propylene glycol concentration of 25%). Conjugation proceeded for 3.5 to 4 hours at room temperature.

1.4.1.2 Conjugation to LCcys80

1.4.1.2.1 Decysteinylation

[00393] Using an ÄKTA Explorer (GE Healthcare), a protein A column (GE Healthcare) was equilibrated with 10 column volumes (CV) of 20 mM sodium phosphate, 10 mM EDTA, pH 7.2 (equilibration buffer). Conditioned medium was then loaded, followed by the washing of unbound material with 10 CV of equilibration buffer. The column was washed with 16 CV of 20 mM sodium phosphate, 10 mM EDTA, 5 mM cysteine, pH 7.2 at 0.5 mL/min for 16 hours to remove the capping group. The column was then washed with 60 CV of 20 mM Tris, pH 7.5 at 0.5 mL/min for 60 hours. The decysteinylated antibody was eluted using 5 CV of 0.1 M glycine, pH 2.9 and immediately neutralized using 5% volume of 2 M Tris, pH 9.0. The fractions containing the antibodies were pooled and dialyzed in DPBS using a MWCO 20K Slide-A-Lyzer (Thermo Fisher).

1.4.1.2.2 Conjugation

[00394] Decysteinylated antibody was brought to 5.0 mg/mL in DPBS, 1 mM EDTA, and 50% propylene glycol was prepared in DPBS, 1mM EDTA. MAL-PEG2-Val-Cit-PAB-eribulin (ER-001159569) (12 mM in DMSO) was added to the 50% propylene glycol and mixed thoroughly. An equal volume of decysteinylated antibody was then added at a molar ratio of 1:4 (mAb:compound), and mixed gently. Conjugation proceeded for 3.5 to 4 hours at room temperature.

1.4.2 Amine-based conjugation using succinimides

1.4.2.1 Conjugation

[00395] Antibody (MORAb-003 or MORAb-009, non-reduced) was brought to 10.0 mg/mL in 0.1 M sodium bicarbonate, pH 8.3. 50% propylene glycol was prepared in

0.1 M sodium bicarbonate, pH 8.3. Succinimide (OSu)-linker-eribulin (in DMSO) was added to the 50% propylene glycol and mixed thoroughly. An equal volume of antibody was then added at a molar ratio of 1:4 (mAb:compound), and mixed thoroughly. Conjugation proceeded for 1 hour at room temperature. The conjugation reaction was quenched with the addition of 1:20 volume of 1 M Tris, pH 8.0, and the ADC was purified as described in section 1.4.4.

1.4.3 Two-step amine-based conjugation using strain-promoted alkyne-azide chemistry (SPAAC)

1.4.3.1 Dybenzylcyclooctyne (DBCO) derivatization

[00396] Antibody (MORAb-003 or MORAb-009, non-reduced) was brought to 10.0 mg/mL in 0.1 M sodium bicarbonate, pH 8.3. 50% propylene glycol was prepared in 0.1 M sodium bicarbonate, pH 8.3. NHS-PEG_4-DBCO (Click Chemistry Tools, 50 mM in DMSO) was added to the 50% propylene glycol and mixed thoroughly. An equal volume of antibody was then added at a molar ratio of 1:4 (mAb:compound), and mixed thoroughly. Conjugation proceeded for 1 hour at room temperature. Unreacted NHS-PEG_4-DBCO was removed, as described in section 1.4.4.

1.4.3.2 Conjugation

[00397] 50% propylene glycol was prepared in DPBS. Azido-linker-eribulin compounds were added to the 50% propylene glycol and mixed thoroughly. An equal volume of the DBCO-modified MORAb-003 or MORAb-009 was then added to the mixture at a molar ratio of 1:4 (mAb:compound), and mixed thoroughly. SPAAC conjugation was allowed to proceed overnight at room temperature. Unreacted NHS-PEG_4-DBCO was removed, as described in section 1.4.4.

1.4.4 Purification

[00398] Conjugated antibody was purified using HiTrap desalting column(s) (GE Healthcare). Chromatography was performed on a fast protein liquid chromatogaphy (FPLC) (GE Healthcare), using 1X DPBS as running buffer, in order to remove maleimido/OSu/azido-linker-eribulin and propylene glycol. Final protein content was determined by BCA assay, as described in section 1.3.1 of Example 1.

1.5 Biophysical characterization

1.5.1 SEC-HPLC analysis

[00399] The aggregation of ADCs was analyzed by size-exclusion, high-performance liquid chromatography (SEC-HPLC) using an Agilent 1260 HPLC. ADC was diluted to 1 mg/mL in DPBS. ADC (10 µL) was then injected onto an Advanced SEC 300A guard column (4.6 mm x 3.5 cm, 2.7 µm pore size, Agilent), followed by a AdvancedBio 300A column (4.6 mm x 30 cm, 2.7 µm pore size). ADC was eluted from the column with 0.1 M sodium phosphate containing 0.15 M NaCl and 5% IPA, pH 7.4 at a flow rate of 0.25 mL/min for 28 min. All data were analyzed using Agilent ChemStation software. Percent aggregation was calculated as $[PA_{aggregate}/PA_{total}]*100$, where PA = integrated peak area.

1.5.2 HIC-HPLC analysis of drug-to-antibody ratio (DAR)

[00400] DAR was analyzed using hydrophobic interaction HPLC (HIC-HPLC). Samples were injected onto a TSKgel® Butyl-NP5, 4.6 mm ID x 3.5 cm, 2.5 µM nonporous size column (Tosoh Bioscience), and eluted with a 3 min equilibration in 100% of mobile phase A, a 15 min gradient (0-100% B), a 5 min hold in 100% B, a 1 min change to 100% A, and a 5 min re-equilibration in 100% of mobile phase A, at 0.7 mL/min. Mobile phase A was 25 mM sodium phosphate, 1.5 M ammonium sulfate, pH 7.0. Mobile phase B was 25 mM sodium phosphate, 25% isopropanol, pH 7.0. Detection was performed at 280 nm (reference 320 nm). DAR was determined by the formula:

$[AUC_{+1} + 2(AUC_{+2}) + 3(AUC_{+3}) + \ldots n(AUC_{+n})]/\Sigma AUC_{tot}]$

where AUC_{+1} is the area under the curve for the antibody peak corresponding to ADC conjugated with one cytotoxin, AUC_{+2} is the area under the curve for the antibody peak corresponding to ADC conjugated with two cytotoxins, etc. ΣAUC_{tot} is the combined area under the curve for all peaks.

1.5.3 LC-MS DAR analysis

[00401] DAR was also analyzed using an LC-MS method with a Waters Alliance HPLC with SQD/PDA detection. Samples were injected onto a Proteomix RP-1000 column (5 µM, 1000A, 4.6 mm x 15 cm, Sepax) at 65°C, and eluted with a 3 min

equilibration in 25%B, a 27 min linear gradient from 25%-55%B, a 5 min hold at 55%B, a 1 min change to 90%B, a 5 min hold at 90%B, a 1 min change back to 25%B, and a 5 min reequilibration at 25%B. Mobile phase A was 0.1% TFA in water, and mobile phase B was 0.1% TFA in acetonitrile. The elute was then split (10:1) into PDA and SQD detectors. The SQD detector was set up as ES positive, capillary voltage at 3.2 kV, cone voltage at 40 V, extractor at 3 V, and RF lens at 0.2 V, source temperature at 150°C, and desolvation temperature at 250°C. Mass data was acquired at 200-2000m/z for 40 min, continuum mode, scan time 1 second. Data was analyzed and deconvoluted offline using MassLynx and MaxEnt1. DAR was calculated using the formula:

$2[[AUC_{LC+1} + 2(AUC_{LC+2}) + 3(AUC_{LC+3}) + \ldots n(AUC_{LC+n})]/\Sigma I_{LC}tot]+$

$2[[AUC_{HC+1} + 2(AUC_{HC+2}) + 3(AUC_{HC+3}) + \ldots n(AUC_{HC+n})]/\Sigma AUC_{HC}tot]$

where AUC_{LC+1} is the area under the curve of the light chain peak conjugated with one cytotoxin, AUC_{LC+2} is the area under the curve of the light chain peak conjugated with two cytotoxins, etc. AUC_{HC} is the area under the curve of the corresponding heavy chains, and $\Sigma AUC_{LC}tot$ and $\Sigma AUC_{HC}tot$ are the combined area under the curve of all unconjugated and conjugated light chains and heavy chains, respectively.

1.5.4 UPLC/ESI-MS DAR analysis of LCcys80 ADCs

[00402] ADC (1 mg/mL) was reduced by adding DTT to a final concentration of 20 mM, followed by incubation at 60°C for 3 min. Samples were then analyzed using a Waters Acquity Ultra Performance Liquid Chromatography and Q-Tof Premier mass spectrometer. Samples (0.5-2 μg each) were injected onto a MassPrep micro desalting column at 65°C, eluted from the column with a 5 min equilibration in 95% of mobile phase A, a 10 min gradient (5-90% B), and a 10 min re-equilibration in 95% of mobile phase A, at 0.05 mL/min. Mobile phase A was 0.1% formic acid in water. Mobile phase B was 0.1% formic acid in acetonitrile. The Q-Tof mass spectrometer was run in positive ion, V-mode with detection in the range of 500-4000 m/z. The source parameters were as follows: capillary voltage, 2.25 kV (intact antibody)-2.50 kV (reduced antibody); sampling cone voltage, 65.0 V (intact antibody) or 50.0 V (reduced antibody); source temperature, 105°C; desolvation temperature, 250°C; desolvation gas flow, 550 L/hr. The light chain protein peak was deconvoluted using the MassLynx

MaxEnt 1 function. Relative intensities of unconjugated and singly-conjugated light chain masses were used to calculate the overall DAR using the formula:
$2[LC_{+1}/\Sigma LC_{tot}]$

where LC_{+1} is mass intensity of light chain conjugated with one cytotoxin, and ΣLC_{tot} is the combined intensities of unconjugated and conjugated light chain.

1.6 Binding characterization

1.6.1 BIAcore

[00403] Antibody concentrations were adjusted to 2 μg/mL in HBS-P+ buffer (GE Healthcare). Unmodified antibodies, or ADCs, were injected over an anti-human IgG sensor on a BIAcore T100 (GE Healthcare) for 1 min at a flow rate of 10 μL/min. To record the antigen association to the captured antibody, a series of increasing concentrations of antigen was injected for 300 sec at a flow rate of 30 μL/min. For anti-mesothelin antibodies, the range of concentrations was 10 nM – 0.041 nM. For MORAb-003 and MORAb-009 ADCs, the range of concentrations was 100 nM – 0.41 nM. The dissociation of antigen was monitored for 30 min at the same flow rate. The sensor surface was regenerated by injecting 3 M $MgCl_2$ for 2 x 30 sec at a flow rate of 30 μL/min. Sensograms were analyzed with Biacore T100 Evaluation Software using a 1:1 Langmuir binding model.

1.6.2 ELISA - Folate receptor alpha

[00404] Recombinant human folate receptor alpha was diluted to 115 ng/mL in coating buffer (50 mM carbonate-bicarbonate buffer, pH 9.6), and coated onto 96-well Maxisorp black plates (Thermo, Cat No. 43711, 100 μL/well) at 4°C, overnight. Coating solution was discarded and the plates were washed three times using 1X PBS with 0.05% Tween-20 (PBST) buffer. Plates were blocked in 300 μL blocking buffer (1% BSA in PBST) at room temperature for 2 hours on an orbital shaker. MORAb-003 and MORAb-003 ADCs were diluted to 1000 ng/mL in blocking buffer, then serially-diluted 2-fold to obtain a range from 1000 ng/mL to 0.98 ng/mL. Blocking buffer was discarded and 100 μL/well of diluted antibody was added to the plates. Plates were incubated at room temperature for 2 hours on an orbital shaker. Antibody solution was discarded and plates were washed three times using PBST. 100 μL/well of goat-anti-

human IgG (H+L)-HRP (1:10,000 dilution in blocking buffer) solution was added to the plates, and plates were incubated at room temperature for 1 hour on an orbital shaker. Secondary antibody solution was discarded and plates were washed three times using PBST. 100 µL/well of QuantaBlu fluorogenic peroxidase substrate working solution (Thermo, Cat No. 15169) was added to the plates, and plates were incubated at room temperature for 30 min. Fluorescence was read at excitation 325 nm/emission 420 nm using a SpectraMax M5 (Molecular Devices). Data was analyzed using SoftMaxPro 5.4.2 software with 4-parameter fitting.

1.6.3 ELISA - Mesothelin

[00405] Recombinant human mesothelin was diluted to 1 µg/mL in coating buffer (50 mM carbonate-bicarbonate buffer, pH 9.6), and coated onto 96-well Maxisorp black plates (Thermo, Cat No. 43711, 100 µL/well) at 4 °C, overnight. Coating solution was discarded and the plates were washed three times using 1X PBS with 0.05% Tween-20 (PBST) buffer. Plates were blocked in 300 µL blocking buffer (1% BSA in PBST) at room temperature for 2 hours on an orbital shaker. MORAb009 and MORAb-009 ADCs were diluted to 1000 ng/mL in blocking buffer, then serially-diluted 2.5-fold to obtain a range from 1000 ng/mL to 0.105 ng/mL. Blocking buffer was discarded and 100µL/well of diluted antibody was added to the plates. Plates were incubated at room temperature for 2 hours on an orbital shaker. Antibody solution was discarded and plates were washed three times using PBST. 100 µL/well of goat-anti-human IgG (H+L)-HRP (1:10,000 dilution in blocking buffer) solution was added to the plates, and plates were incubated at room temperature for 1 hour on an orbital shaker. Secondary antibody solution was discarded and plates were washed three times using PBST. 100 µL/well of QuantaBlu fluorogenic peroxidase substrate working solution (Thermo, Cat No. 15169) was added to the plates, and plates were incubated at room temperature for 30 min. Fluorescence was read at excitation 325 nm/emission 420 nm using a SpectraMax M5 (Molecular Devices). Data was analyzed using SoftMaxPro 5.4.2 software with 4-parameter fitting.

1.7 Cytotoxicity analyses

1.7.1 Crystal Violet assay

[00406] IGROV1 (FR^{hi}, $MSLN^{neg}$), NCI-H2110 (FR^{med}, $MSLN^{med}$), and A431 (FR^{neg}, $MSLN^{neg}$) cells were sub-cultured and seeded at 5,000 cells/well in complete growth medium in 96 well tissue culture plates, incubated at 37°C, 5% CO_2 overnight (16 hours). Test reagents were serial diluted 1:3 in 2 mL deep-well dilution plates, starting at 200 nM (10 dilutions total). Diluted samples (100 µL) were added to the cell plates (starting concentration of test samples at 100 nM). Plates were incubated at 37°C, 5% CO_2 for an additional 5 days. Medium was then discarded. The plates were washed once with 200 µL DPBS, stained with 50 µL of 0.2% Crystal Violet solution at room temperature for 15 min, and then washed extensively with tap water. Plates were air-dried, and Crystal Violet was dissolved with 200 µL of 1% SDS solution. Plates were read at 570 nm. Data was analyzed using GraphPad Prism 6.

2. Results

2.1 Biophysical characterization of MORAb-003, MORAb-009, and trastuzumab ADCs

[00407] MORAb-003 (humanized anti-human folate receptor alpha) , MORAb-009 (mouse-human chimeric anti-human mesothelin), and trastuzumab (humanized anti-human her2) ADCs were prepared using the conjugatable eribulin compounds listed in Table 46 according to one of three conjugation methods, including: (1) partial reduction of antibody interchain disulfides using the non-thiol reductant TCEP, followed by conjugation using thiol-reactive maleimido-spacer-linker-eribulin constructs; (2) direct conjugation to antibody lysine residues using succinimide (OSu)-spacer-linker-eribulin constructs; and (3) conjugation to antibody lysine residues using a two-step approach, whereby OSu-PEG4-dibenzylcyclooctyne was first conjugated to lysine residues, then orthogonal conjugation of azido-spacer-linker-eribulin constructs was performed using SPAAC.

[00408] Following purification, aggregation levels for all MORAb-003, MORAb-009, and trastuzumab ADCs were determined by SEC-HPLC and the drug-to-antibody ratio (DAR) was analyzed using reverse-phase LC-MS and/or HIC-HPLC. The DAR for all maleimide-based ADCs was analyzed using both reverse-phase LC-MS and HIC-

HPLC. A difference in DAR values of less than 0.3 was typically observed between the two methods. In contrast, the DAR for all ADCs prepared via conjugation through lysine residues was analyzed only by LC-MS, since the high degree of heterogeneity of these ADCs prevents the resolution of individual DAR species by HIC-HPLC. Binding to target antigen was also analyzed using ELISA, for MORAb-003 and MORAb-009 ADCs. The results of the DAR and aggregation analyses are shown in Table 47 next to the respective ADC.

Table 47.
Biophysical analyses of MORAb-003, MORAb-009, and trastuzumab ADCs

					DAR Analysis		SEC-HPLC Analysis			Antigen Binding	
ADCs	antibody	conjugation chemistry	spacer	cleavage chemistry	DAR (LC-MS)	DAR (HIC-HPLC)	% Aggr.	% Monomer	% Frag.	ELISA, EC_{50}, ng/mL	ELISA, EC_{50}, nM
MORAb003		N/A	N/A	N/A			3.62	96.38	0	6.29	0.04
MORAb009		N/A	N/A	N/A			0	100	0	42.60	0.28
trastuzumab		N/A	N/A	N/A			3.52	96.48	0	N/A	N/A
MORAb003-ER1159569 (Lot NB3073-88L)	MORAb-003	maleimide	PEG2	val-cit-pAB	3.58	3.91	3.12	96.88	0	22.60	0.15
MORAb009-ER1159569 (Lot NB3073-88F)	MORAb-009	maleimide	PEG2	val-cit-pAB	3.63	3.93	3.23	96.77	0	43.70	0.29
MORAb003-ER1159569 (Lot NB3142-62A)	MORAb-003	maleimide	PEG2	val-cit-pAB	4.80	4.88	3.21	96.79	0	18.20	0.12
MORAb009-ER1159569 (Lot NB3142-62D)	MORAb-009	maleimide	PEG2	val-cit-pAB	4.68	4.57	0.90	99.10	0	33.10	0.22
trastuzumab-ER1159569	trastuzumab	maleimide	PEG2	val-cit-pAB	3.10	3.11	1.26	98.74	0	N/A	N/A
MORAb003-ER1242287	MORAb-003	maleimide	PEG8	val-cit-pAB	2.31	2.35	18.63	81.37	0	21.50	0.14
MORAb009-ER1242287	MORAb-009	maleimide	PEG8	val-cit-pAB	1.13	2.00	11.24	88.76	0	58.60	0.39
MORAb003-ER1235638	MORAb-003	maleimide	pentyl	val-cit-pAB	3.65	3.89	3.95	96.05	0	15.30	0.10
MORAb009-ER1235638	MORAb-009	maleimide	pentyl	val-cit-pAB	3.99	4.10	4.5	95.5	0	65.60	0.44
MORAb003-ER1231679	MORAb-003	maleimide	PEG2	ala-ala-asn-pAB	3.60	3.83	3.09	96.91	0	18.30	0.12
MORAb009-	MORAb-009	maleimide	PEG2	ala-ala-asn-	3.27	3.94	4.39	95.61	0	41.40	0.28

Table 47.
Biophysical analyses of MORAb-003, MORAb-009, and trastuzumab ADCs

					DAR Analysis		SEC-HPLC Analysis			Antigen Binding	
ADCs	antibody	conjugation chemistry	spacer	cleavage chemistry	DAR (LC-MS)	DAR (HIC-HPLC)	% Aggr.	% Monomer	% Frag.	ELISA, EC_{50}, ng/mL	ELISA, EC_{50}, nM
ER1231679				pAB							
MORAb003-ER1231690	MORAb-003	maleimide	PEG2	ala-ala-asn-pAB-ala-ala-asn-pAB	3.02	3.23	4.44	95.56	0	8.92	0.06
MORAb009-ER1231690	MORAb-009	maleimide	PEG2	ala-ala-asn-pAB-ala-ala-asn-pAB	2.36	3.17	6.22	93.78	0	58.70	0.39
MORAb003-ER1237504	MORAb-003	maleimide	PEG4-triazole-PEG3	disylfidyl-dimethyl-pAB	0.52	1.61	13.73	86.27	0	29.80	0.20
MORAb009-ER1237504	MORAb-009	maleimide	PEG4-triazole-PEG3	disylfidyl-dimethyl-pAB	0.72	1.03	9.78	90.22	0	55.90	0.37
MORAb003-ER1237505	MORAb-003	maleimide	PEG4-triazole-PEG3	sulfonamide	1.85	3.88	5.72	94.28	0	18.30	0.12
MORAb009-ER1237505	MORAb-009	maleimide	PEG4-triazole-PEG3	sulfonamide	2.33	3.91	5.44	94.56	0	61.00	0.41
MORAb003-PEG2-eribulin	MORAb-003	maleimide	PEG2	non-cleavable	4.15	4.49	3.97	96.03	0	6.96	0.05
MORAb009-PEG2-eribulin	MORAb-009	maleimide	PEG2	non-cleavable	4.55	4.30	1.15	97.11	1.74	8.84	0.06
MORAb003-PEG4-eribulin	MORAb-003	maleimide	PEG4	non-cleavable	4.70	4.79	9.84	89.76	0	9.31	0.06
MORAb009-PEG4-eribulin	MORAb-009	maleimide	PEG4	non-cleavable	4.48	4.57	1.03	97.13	1.84	11.60	0.08
MORAb003-ER1236940	MORAb-003	succinimide	PEG2	val-cit-pAB	0.72		3.65	96.35	0	17.00	0.11
MORAb009-ER1236940	MORAb-009	succinimide	PEG2	val-cit-pAB	0.89		2.75	97.25	0	66.30	0.44
MORAb003-ER1242288	MORAb-003	succinimide	PEG9	val-cit-pAB	0.00		2.85	97.15	0	14.40	0.10

Table 47.
Biophysical analyses of MORAb-003, MORAb-009, and trastuzumab ADCs

					DAR Analysis		SEC-HPLC Analysis			Antigen Binding	
ADCs	antibody	conjugation chemistry	spacer	cleavage chemistry	DAR (LC-MS)	DAR (HIC-HPLC)	% Aggr.	% Monomer	% Frag.	ELISA, EC_{50}, ng/mL	ELISA, EC_{50}, nM
MORAb009-ER1242288	MORAb-009	succinimide	PEG9	val-cit-pAB	0.21		1.69	98.31	0	15.30	0.10
MORAb003-ER1236941	MORAb-003	succinimide	pentyl	val-cit-pAB	0.77		3.13	96.87	0	13.00	0.09
MORAb009-ER1236941	MORAb-009	succinimide	pentyl	val-cit-pAB	0.93		3.04	96.96	0	44.60	0.30
MORAb003-ER1243700	MORAb-003	succinimide	PEG3-triazole-PEG3	val-cit-pAB	0.00		3.92	96.08	0	6.22	0.04
MORAb009-ER1243700	MORAb-009	succinimide	PEG3-triazole-PEG3	val-cit-pAB	0.06		1.97	98.03	0	46.70	0.31
MORAb003-ER1231691	MORAb-003	succinimide	PEG2	ala-ala-asn-pAB	0.37		3.46	96.54	0	11.50	0.08
MORAb009-ER1231691	MORAb-009	succinimide	PEG2	ala-ala-asn-pAB	0.29		2.45	97.55	0	43.30	0.29
MORAb003-ER1244129	MORAb-003	succinimide	PEG3-triazole-PEG3	disylfidyl-dimethyl-pAB	0.24		10.87	89.13	0	14.30	0.10
MORAb009-ER1244129	MORAb-009	succinimide	PEG3-triazole-PEG3	disylfidyl-dimethyl-pAB	0.47		12.79	87.21	0	57.70	0.38
MORAb003-ER1244623	MORAb-003	succinimide	PEG3-triazole-PEG3	sulfonamide	0.55		5.21	94.79	0	4.54	0.03
MORAb009-ER1244623	MORAb-009	succinimide	PEG3-triazole-PEG3	sulfonamide	1.14		0	100	0	39.00	0.26
MORAb003-DBCO-ER1237508	MORAb-003	succinimide/ click	dibenzylcyclooctene-triazole-PEG3	disylfidyl-dimethyl-pAB	2.19		4.1	95.9	0	24.10	0.16
MORAb009-DBCO-ER1237508	MORAb-009	succinimide/ click	dibenzylcyclooctene-triazole-PEG3	disylfidyl-dimethyl-pAB	2.33		0	100	0	53.80	0.36

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Table 47.
Biophysical analyses of MORAb-003, MORAb-009, and trastuzumab ADCs

					DAR Analysis		SEC-HPLC Analysis			Antigen Binding	
ADCs	antibody	conjugation chemistry	spacer	cleavage chemistry	DAR (LC-MS)	DAR (HIC-HPLC)	% Aggr.	% Monomer	% Frag.	ELISA, EC_{50}, ng/mL	ELISA, EC_{50}, nM
MORAb003-DBCO-ER1138856	MORAb-003	succinimide/ click	dibenzylcyclooctene-triazole-PEG3	sulfonamide	1.82		3.49	96.51	0	15.00	0.10
MORAb009-DBCO-ER1138856	MORAb-009	succinimide/ click	dibenzylcyclooctene-triazole-PEG3	sulfonamide	1.59		0	100	0	44.70	0.30
MORAb003-DBCO-PEG4 VCP eribulin	MORAb-003	succinimide/ click	dibenzylcyclooctene-triazole-PEG4	val-cit-pAB	3.09		2.87	97.13	0	16.00	0.11
MORAb009-DBCO-PEG4 VCP eribulin	MORAb-009	succinimide/ click	dibenzylcyclooctene-triazole-PEG4	val-cit-pAB	2.91		0.22	99.78	0	33.70	0.22
MORAb003-DBCO-PEG2 eribulin	MORAb-003	succinimide/ click	dibenzylcyclooctene-triazole-PEG2	non-cleavable	3.43		3.88	96.12	0	19.10	0.13
MORAb009-DBCO-PEG2 eribulin	MORAb-009	succinimide/ click	dibenzylcyclooctene-triazole-PEG2	non-cleavable	3.07		1.15	98.85	0	23.30	0.16
MORAb003-DBCO-PEG4 eribulin	MORAb-003	succinimide/ click	dibenzylcyclooctene-triazole-PEG4	non-cleavable	2.96		3.64	96.36	0	13.30	0.09
MORAb009-DBCO-PEG4 eribulin	MORAb-009	succinimide/ click	dibenzylcyclooctene-triazole-PEG4	non-cleavable	2.8		1.12	98.88	0	45.20	0.30

Abbreviations: % Aggr., % aggregation; % Frag, % fragmentation.

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2.1.1 MORAb-003, MORAb-009, and trastuzumab ADCs

[00409] No significant differences between MORAb-003, MORAb-009, and trastuzumab were observed, in terms of both conjugation efficiency and biophysical parameters. All ADCs demonstrated similar DAR values and levels of aggregrate formation.

2.1.2 Maleimide-based ADCs

[00410] For maleimide-based ADCs, both pentyl and PEG_2 spacers paired with a val-cit-pAB cleavage site, and a PEG_2 spacer paired with an ala-ala-asn-pAB cleavage site, provided DAR values between 3.5 and 4.0 by reverse-phase LC-MS and HIC-HPLC, in addition to low (<5%) aggregate levels. However, when the spacer was lengthened to PEG_8 (paired with a val-cit-pAB cleavage site), aggregate levels increased (11-18%) and conjugation efficiency decreased, resulting in DAR values between 1.1 and 2.3. *See, e.g.*, percent aggregation and DAR values of MORAb003/MORAb009-ER-001159569 (short PEG linker) and MORAb003/MORAb009-1242287 (long PEG linker) in Table 47.

[00411] For ADCs prepared with a disulfidyl-pAB cleavage site, low DAR values were observed (1.0-1.6), together with relatively high aggregate levels (10-14%). Significantly lower DAR values were observed when these ADCs were analyzed by LC-MS than by HIC-HPLC (see, e.g., LC-MS/HIC-HPLC DAR values for MORAb003/MORAb009-ER1237504 and MORAb003/MORAb009-ER1237505 in Table 47). This result suggests the linker cleavage site exhibits pH instability, as the mobile phase of LC-MS analysis is approximately 3.0, whereas the mobile phase of HIC-HPLC analysis is neutral.

[00412] For ADCs prepared with a sulfonamide cleavage site, low (< 5%) aggregate levels were observed. Similar to the disulfidyl-pAB ADCs, lower DAR values were observed when analyzed by LC-MS (1.8-2.3) than by HIC-HPLC (3.9), which again indicates that the linker cleavage site exhibits pH instability.

[00413] For the PEG_2 and PEG_4 non-cleavable linkers, efficient conjugation was observed, resulting in DAR values between 4.0 and 4.7. MORAb-009 ADCs with these non-cleavable linkers also demonstrated low aggregation levels (< 2%), while slightly higher aggregation levels were observed for the corresponding MORAb-003 ADCs (4% and 10% for PEG_2 and PEG_4, respectively).

2.1.3 Succinimide-based ADCs

[00414] All ADCs prepared using succinimide coupled with spacer-linker-eribulin resulted in DAR values < 1.0. To confirm that this lower conjugation efficiency (relative to maleimides) was not a consequence of the conjugation procedure itself, these ADCs were remade using a higher compound:antibody ratio and reanalyzed using the same DAR analysis methods. Similar results were obtained, which suggests, without being bound by theory, that lower DAR values are an inherent property of the combination of succinimide and eribulin, and that maleimides may be conjugated more efficiently. Efficiency of succinimide conjugation was increased through use of a two-step method, whereby DBCO was first added to the antibody using NHS-DBCO, followed by the addition of the azido compounds. This approach results in higher DAR values, as measured by reverse-phase HPLC analysis, as compared to conjugation directly to antibody lysine residues. For succinimide-based ADCs having sulfonamide (cleavable), val-cit-PAB (cleavable), or PEG_2/PEG_4 (non-cleavable) linkers, DAR values resulting from the two-step conjugation were similar to those determined for maleimide-based ADCs having a sulfonamide cleavage site. Without being bound by theory, this result again suggests that lower DAR values for succinimide-spacer-linker-eribulin conjugation reactions are an inherent property of the combination of succinimide and eribulin.

2.2 Binding Characterization of MORAb-003 and MORAb-009 ADCs

[00415] For MORAb-003 ADCs, no significant differences were observed between non-cleavable maleimide-based linker-eribulin ADCs and parental MORAb-003 in terms of target antigen binding. For other maleimide-based linker-eribulin MORAb-003 ADCs, a 2- to 3-fold loss in target antigen binding relative to parental MORAb-003 was typically observed by ELISA analysis. However, there was no apparent correlation between either linker length or linker composition and lower EC_{50} values. Similarly, for succinimide-based linker-eribulin MORAb-003 ADCs, a 0- to 3-fold loss in target antigen binding relative to unconjugated MORAb-003 was generally observed. Again, no correlation between either linker length or linker composition and lower EC_{50} values was apparent. For MORAb-009 ADCs, all ADCs had less than a 2-fold decrease in EC_{50} values, relative to parental MORAb-009.

2.3 *In vitro* cytoxicity analyses of MORAb-003, MORAb-009, and trastuzumab ADCs

[00416] *In vitro* potency of prepared MORAb-003, MORAb-009, and trastuzumab ADCs was evaluated using a Crystal Violet cell-based cytotoxicity assay. The cell lines selected for screening MORAb-003 and MORAb-009 ADCs were IGROV1, NCI-H2110, and A431. IGROV1 cells are of human ovarian epithelial carcinoma origin and express high levels of folate receptor alpha, but no mesothelin (i.e., MORAb-003-reactive). NCI-H2110 cells are of human non-small cell lung carcinoma origin and express moderate levels of both folate receptor alpha and mesothelin (i.e., MORAb-003- and MORAb-009-reactive). A431 control cells are of human epidermal carcinoma origin and do not express either target antigen. The results of this screening are shown in Table 48. MORAb-003, MORAb-009, and trastuzumab ADCs comprising the linker-toxin maleimido-PEG2-val-cit-pAB-eribulin (VCP-eribulin) were also evaluated in additional gastric and breast cancer cell lines, including NCI-N87 (FR^{lo}, $MSLN^{med}$, $her2^{hi}$), BT-474 (FR^{neg}, $MSLN^{neg}$, $her2^{hi}$), ZR-75 (FR^{neg}, $MSLN^{neg}$, $her2^{med}$), and NUGC3 (FR^{neg}, $MSLN^{neg}$, $her2^{neg}$). The results of this screening are shown in Table 49.

Table 48. Cytotoxicity (IC_{50}) screening of MORAb-003 and MORAb-009 ADCs on IGROV1, NCI-H2110, and A431 cells

					Cytotoxicity Analysis					
					IGROV1 (FR^{hi}, $MSLN^{neg}$)		NCI-H2110 (FR^{med}, $MSLN^{med}$)		A431 (FR^{neg}, $MSLN^{neg}$)	
ADCs	antibody	conjugation chemistry	spacer	cleavage chemistry	IC_{50} (nM)	SD	IC_{50} (nM)	SD	IC_{50} (nM)	SD
MORAb003		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MORAb009		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
trastuzumab		N/A	N/A	N/A						
eribulin	N/A	N/A	N/A	N/A	0.320	0.212	0.199	0.034	0.653	0.159
MORAb003-ER1159569 (Lot NB3073-88L)	MORAb-003	maleimide	PEG2	val-cit-pAB	0.155	0.064	3.685	0.417	>100	
MORAb009-ER1159569 (Lot NB3073-88F)	MORAb-009	maleimide	PEG2	val-cit-pAB	9.450	2.093	14.945	1.747	>100	
MORAb003-ER1159569 (Lot NB3142-62A)	MORAb-003	maleimide	PEG2	val-cit-pAB	0.020		1.550		>100	
MORAb009-ER1159569 (Lot NB3142-62D)	MORAb-009	maleimide	PEG2	val-cit-pAB	5.687		6.784		>100	
trastuzumab-ER1159569	trastuzumab	maleimide	PEG2	val-cit-pAB						
MORAb003-ER1242287	MORAb-003	maleimide	PEG8	val-cit-pAB	0.115	0.035	7.065	0.417	85.960	
MORAb009-ER1242287	MORAb-009	maleimide	PEG8	val-cit-pAB	25.765	8.478	34.455	3.033	>100	
MORAb003-ER1235638	MORAb-003	maleimide	pentyl	val-cit-pAB	0.105	0.092	3.920	1.032	>100	
MORAb009-ER1235638	MORAb-009	maleimide	pentyl	val-cit-pAB	6.830	0.962	13.965	6.611	>100	

Table 48. Cytotoxicity (IC_{50}) screening of MORAb-003 and MORAb-009 ADCs on IGROV1, NCI-H2110, and A431 cells

					Cytotoxicity Analysis					
					IGROV1 (FR^{hi}, $MSLN^{neg}$)		NCI-H2110 (FR^{med}, $MSLN^{med}$)		A431 (FR^{neg}, $MSLN^{neg}$)	
ADCs	antibody	conjugation chemistry	spacer	cleavage chemistry	IC_{50} (nM)	SD	IC_{50} (nM)	SD	IC_{50} (nM)	SD
MORAb003-ER1231679	MORAb-003	maleimide	PEG2	ala-ala-asn-pAB	0.080	0.028	3.800	0.566	31.630	1.202
MORAb009-ER1231679	MORAb-009	maleimide	PEG2	ala-ala-asn-pAB	8.890	0.976	7.080	1.867	34.390	3.536
MORAb003-ER1231690	MORAb-003	maleimide	PEG2	ala-ala-asn-pAB-ala-ala-asn-pAB	0.125	0.021	4.745	2.114	38.555	0.403
MORAb009-ER1231690	MORAb-009	maleimide	PEG2	ala-ala-asn-pAB-ala-ala-asn-pAB	16.980	5.176	12.310	3.422	54.960	5.360
MORAb003-ER1237504	MORAb-003	maleimide	PEG4-triazole-PEG3	disylfidyl-dimethyl-pAB	0.265	0.092	0.845	0.177	7.005	0.290
MORAb009-ER1237504	MORAb-009	maleimide	PEG4-triazole-PEG3	disylfidyl-dimethyl-pAB	6.375	2.751	1.220	0.325	8.130	0.608
MORAb003-ER1237505	MORAb-003	maleimide	PEG4-triazole-PEG3	sulfonamide	0.370	0.269	0.690	0.283	6.800	0.834
MORAb009-ER1237505	MORAb-009	maleimide	PEG4-triazole-PEG3	sulfonamide	6.370	3.012	0.990	0.453	9.030	1.527
MORAb003-PEG2-eribulin	MORAb-003	maleimide	PEG2	non-cleavable	0.330		38.300		>100	
MORAb009-PEG2-eribulin	MORAb-009	maleimide	PEG2	non-cleavable	42.770		50.040		>100	
MORAb003-PEG4-eribulin	MORAb-003	maleimide	PEG4	non-cleavable	0.277		21.630		>100	
MORAb009-PEG4-eribulin	MORAb-009	maleimide	PEG4	non-cleavable	76.320		31.600		>100	
MORAb003-ER1236940	MORAb-003	succinimide	PEG2	val-cit-pAB	0.325	0.106	30.545	3.132	>100	

Table 48. Cytotoxicity (IC_{50}) screening of MORAb-003 and MORAb-009 ADCs on IGROV1, NCI-H2110, and A431 cells

					Cytotoxicity Analysis					
					IGROV1 (FR^{hi}, $MSLN^{neg}$)		NCI-H2110 (FR^{med}, $MSLN^{med}$)		A431 (FR^{neg}, $MSLN^{neg}$)	
ADCs	antibody	conjugation chemistry	spacer	cleavage chemistry	IC_{50} (nM)	SD	IC_{50} (nM)	SD	IC_{50} (nM)	SD
MORAb009-ER1236940	MORAb-009	succinimide	PEG2	val-cit-pAB	31.915	2.510	36.500	11.031	90.060	
MORAb003-ER1242288	MORAb-003	succinimide	PEG9	val-cit-pAB	38.105	45.601	64.010	8.075	>100	
MORAb009-ER1242288	MORAb-009	succinimide	PEG9	val-cit-pAB	>100		>100		>100	
MORAb003-ER1236941	MORAb-003	succinimide	pentyl	val-cit-pAB	0.330	0.071	42.105	12.594	>100	
MORAb009-ER1236941	MORAb-009	succinimide	pentyl	val-cit-pAB	>100		49.485	13.569	>100	
MORAb003-ER1243700	MORAb-003	succinimide	PEG3-triazole-PEG3	val-cit-pAB	1.150		>100		>100	
MORAb009-ER1243700	MORAb-009	succinimide	PEG3-triazole-PEG3	val-cit-pAB	>100		>100		>100	
MORAb003-ER1231691	MORAb-003	succinimide	PEG2	ala-ala-asn-pAB	12.320		31.795	4.448	>100	
MORAb009-ER1231691	MORAb-009	succinimide	PEG2	ala-ala-asn-pAB	>100		20.000	5.954	>100	
MORAb003-ER1244129	MORAb-003	succinimide	PEG3-triazole-PEG3	disylfidyl-dimethyl-pAB	0.370	0.184	0.750	0.071	12.005	1.534
MORAb009-ER1244129	MORAb-009	succinimide	PEG3-triazole-PEG3	disylfidyl-dimethyl-pAB	6.595	4.052	0.840	0.057	9.230	0.014
MORAb003-ER1244623	MORAb-003	succinimide	PEG3-triazole-PEG3	sulfonamide	0.980	0.396	1.820	0.410	37.235	15.733
MORAb009-ER1244623	MORAb-009	succinimide	PEG3-triazole-PEG3	sulfonamide	24.505	4.702	2.235	0.629	36.665	14.206
MORAb003-DBCO-ER1237508	MORAb-003	succinimide/ click	dibenzylcyclooctene-triazole-PEG3	disylfidyl-dimethyl-pAB	0.545	0.389	0.900	0.071	9.670	0.382

Table 48. Cytotoxicity (IC_{50}) screening of MORAb-003 and MORAb-009 ADCs on IGROV1, NCI-H2110, and A431 cells

					Cytotoxicity Analysis					
					IGROV1 (FR^{hi}, $MSLN^{neg}$)		NCI-H2110 (FR^{med}, $MSLN^{med}$)		A431 (FR^{neg}, $MSLN^{neg}$)	
ADCs	antibody	conjugation chemistry	spacer	cleavage chemistry	IC_{50} (nM)	SD	IC_{50} (nM)	SD	IC_{50} (nM)	SD
MORAb009-DBCO-ER1237508	MORAb-009	succinimide/ click	dibenzylcyclooctene-triazole-PEG3	disylfidyl-dimethyl-pAB	10.245	3.486	1.040	0.297	11.280	2.277
MORAb003-DBCO-ER1138856	MORAb-003	succinimide/ click	dibenzylcyclooctene-triazole-PEG3	sulfonamide	1.775	1.421	1.655	0.007	24.990	2.022
MORAb009-DBCO-ER1138856	MORAb-009	succinimide/ click	dibenzylcyclooctene-triazole-PEG3	sulfonamide	19.155	5.438	1.960	0.113	28.070	0.636
MORAb003-DBCO-PEG4 VCP eribulin	MORAb-003	succinimide/ click	dibenzylcyclooctene-triazole-PEG4	val-cit-pAB	0.038		4.281		>100	
MORAb009-DBCO-PEG4 VCP eribulin	MORAb-009	succinimide/ click	dibenzylcyclooctene-triazole-PEG4	val-cit-pAB	12.960		31.400		>100	
MORAb003-DBCO-PEG2 eribulin	MORAb-003	succinimide/ click	dibenzylcyclooctene-triazole-PEG2	non-cleavable	4.250		38.070		>100	
MORAb009-DBCO-PEG2 eribulin	MORAb-009	succinimide/ click	dibenzylcyclooctene-triazole-PEG2	non-cleavable	75.680		85.680		>100	
MORAb003-DBCO-PEG4 eribulin	MORAb-003	succinimide/ click	dibenzylcyclooctene-triazole-PEG4	non-cleavable	1.323		46.280		>100	
MORAb009-DBCO-PEG4 eribulin	MORAb-009	succinimide/ click	dibenzylcyclooctene-triazole-PEG4	non-cleavable	61.490		39.330		>100	

All IC_{50} values are in nM, and represent mean values of replicate experiments. SD – standard deviation.

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Table 49. Cytotoxicity (IC_{50}) screening of MORAb-003, MORAb-009, and trastuzumab ADCs on NCI-N87, BT-474, ZR-75, and NUGC3 cells

					Cytotoxicity Analysis			
					NCI-N87-Luc (FR^{lo}, $MSLN^{med}$, $her2^{hi}$)	BT-474 (FR^{neg}, $MSLN^{neg}$, $her2^{hi}$)	ZR-75-1 (FR^{neg}, $MSLN^{neg}$, $her2^{med}$)	NUGC3-Luc (FR^{neg}, $MSLN^{neg}$, $her2^{neg}$)
ADCs	antibody	conjugation chemistry	spacer	cleavage chemistry	IC_{50} (nM)	IC_{50} (nM)	IC_{50} (nM)	IC_{50} (nM)
MORAb003		N/A	N/A	N/A				
MORAb009		N/A	N/A	N/A				
trastuzumab		N/A	N/A	N/A	0.78	0.641	>100	>100
eribulin	N/A	N/A	N/A	N/A	0.257	0.151	0.236	0.445
MORAb003-ER1159569 (Lot NB3073-88L)	MORAb-003	maleimide	PEG2	val-cit-pAB				
MORAb009-ER1159569 (Lot NB3073-88F)	MORAb-009	maleimide	PEG2	val-cit-pAB				
MORAb003-ER1159569 (Lot NB3142-62A)	MORAb-003	maleimide	PEG2	val-cit-pAB	4.528	11.46	14.74	20.45
MORAb009-ER1159569 (Lot NB3142-62D)	MORAb-009	maleimide	PEG2	val-cit-pAB	0.013	10.21	12.8	29.93
trastuzumab-ER1159569	trastuzumab	maleimide	PEG2	val-cit-pAB	0.006	0.003	0.023	20.06

All IC_{50} values are in nM, and represent mean values of replicate experiments. SD – standard deviation.

2.3.1 Cytotoxicity of maleimide-based ADCs

[00417] All maleimide-based MORAb-003 and MORAb-009 ADCs displayed specific cytotoxicity on IGROV1 cells, with a 2-3 orders of magnitude difference in potency observed between antibodies. The val-cit-pAB-eribulin MORAb-003 ADCs demonstrated higher potency on the IGROV1 cell line than either the PEG_2 or PEG_4 non-cleavable MORAb-003 ADCs, but fold-specificity was unchanged. Similar trends were observed for MORAb-009 ADCs, with the non-cleavable MORAb-009 ADCs demonstrating lower cytotoxicity on IGROV1 cells than val-cit-pAB-eribulin MORAb-009 ADCs.

[00418] Maleimide-based MORAb-009 ADCs with disulfidyl- and sulfonamide-based linkers demonstrated higher potency on the NCI-H2110 cell line than the IGROV1 cell line. This may be due to the potential instability of the linkers in culture, as described below. Potent cytotoxicity was also observed with the corresponding MORAb-003 ADCs. In contrast, maleimide-based MORAb-003 and MORAb-009 ADCs with non-cleavable linkers demonstrated relatively low potency on NCI-H2110 cells. Without being bound by theory, this result suggests that with lower target expression, efficient cleavage and release of the payload may improve cytotoxicity.

[00419] ADCs with a val-cit-pAB enzyme-cleavable linker or a non-cleavable linker demonstrated low levels of off-target killing on A431 control cells (IC_{50} > 100 nM), whereas ADCs with an ala-ala-asn-pAB enzyme-cleavable linker displayed weak but detectable killing of these control cells. This indicates that val-cit-pAB enzyme-cleavable linkers may be more stable in culture ala-ala-asn-pAB enzyme-cleavable linkers. In addition, MORAb-009 ADCs with a shorter PEG_2 spacer demonstrated higher cytoxicity in IGROV1 cells than corresponding ADCs with a longer PEG_8 spacer. This same trend was observed in NCI-H2110 cells for both MORAb-003 and MORAb-009 ADCs, with shorter spacer lengths resulting in higher cytotoxicity.

[00420] ADCs with sulfonamide-based linkers generally demonstrated higher DAR values and lower aggregate levels than the corresponding ADCs with disulfidyl-based linkers. However, nM-level killing of A431 control cells was observed in both of these categories of ADCs, suggesting that the disulfidyl- and sulfonamide-based linkers were less stable in culture than the enzyme-cleavable linkers under the assay conditions examined.

[00421] The specific linker-toxin maleimido-PEG_2-val-cit-pAB-eribulin (VCP-eribulin) was further examined for specificity and potency on different gastric and breast cancer cell lines. VCP-eribulin was conjugated to MORAb-003 and MORAb-009, in addition to the anti-human her2 antibody trastuzumab. MORAb-003-VCP-eribulin demonstrated weak but specific killing on NCI-N87 cells, which express low levels of folate receptor alpha (FR), and little killing on the remaining three FR-negative cell lines. MORAb-009-VCP-eribulin also demonstrated potent cytotoxicity on NCI-N87 cells, which express moderate levels of mesothelin. Trastuzumab-VCP-eribulin was very potent (3 – 6 pM, IC_{50}) on NCI-N87 and BT-474 cells, the two cell lines that express high levels of her2, and also potent on ZR-75 breast cancer cells, which only moderately express her2. MORAb-003, MORAb-009, and trastuzumab VCP-eribulin ADCs all demonstrated low cytotoxicity on NUGC3 cells, with do not express FR, mesothelin, or her2, the respective target antigens.

2.3.2 Cytoxicity of succinimide-based ADCs

[00422] Trends in cytotoxicity of the succinimide-based ADCs were similar to the maleimide-based ADCs for IGROV1 cells, with PEG_8 spacer ADCs demonstrating low cytotoxicity in addition to low DAR values. Lower cytotoxicity on both IGROV1 and NCI-H2110 cells was generally observed for succinimide-based ADCs with enzyme-cleavable linkers compared with the corresponding maleimide-based ADCs, which was most likely due to their lower DAR values. Off-target killing of A431 cells was also observed with the disulfidyl- and sulfonamide-based linkers, similar to the corresponding maleimide-based ADCs. This points to increased instability potentially arising from the cleavage site, rather than the conjugation chemistry.

[00423] When a two-step conjugation was performed, higher DAR values were observed relative to those obtained with the direct succinimide conjugation approach. These higher DAR values correlated with higher potency. For the VCP-eribulin MORAb-003 ADC, potent cytotoxicity on both IGROV1 and NCI-H2110 cells was observed. While non-cleavable MORAb-003 ADCs demonstrated potency on IGROV1 cells (1- 4 nM), they were still less potent than the VCP-eribulin MORAb-003 ADC prepared with this method (38 pM), even though DAR values were comparable. In addition, non-cleavable MORAb-003 ADCs prepared using the two-step method were slightly less potent than the corresponding maleimide-based ADCs on the IGROV1 cell

line, which may be due to their lower DAR values. Similar to their maleimide-based counterparts, non-cleavable ADCs prepared using the two-step method also lost nearly all cytotoxicity on NCI-2110 cells.

2.4 Biophysical characterization of anti-human mesothelin (LCcys80) ADCs

[00424] MAL-PEG2-Val-Cit-PAB-eribulin (ER-001159569) was conjugated to eight different anti-human mesothelin antibodies (Table 1). Binding affinities of the parental antibodies were determined by BIAcore analysis, as described above in section 1.6.1. Aggregation levels for all anti-human mesothelin ADCs were determined by SEC-HPLC and the DAR was analyzed using HIC-HPLC. *In vitro* potency was evaluated using a Crystal Violet cell-based cytotoxicity assay in A3 (A431 stably transfected with human mesothelin (MSLN), $MSLN^{hi}$), OVCAR3 (human ovarian, $MSLN^{hi}$), HEC-251 (human endometroid, $MSLN^{med}$), H226 (human lung squamous cell mesothelioma, $MSLN^{lo}$), and A431 parental ($MSLN^{neg}$) cells. The results of the DAR, aggregation, and cytotoxicity analyses are shown in Table 50.

Table 50. Biophysical characterization of anti-human mesothelin (LCcys80) ADCs

		Parental MAb			ADC								
		Affinity			Payload	HIC	SEC-HPLC		Cell based Cytotoxicity assay, EC50, nM				
		k_a (10^5 $M^{-1}sec^{-1}$)	k_d (10^{-4} sec^{-1})	K_d (10^{-9} M)	Drug-linker	DAR	% aggregate	% monomer	A431	OVCAR3	HEC-251	H226	A3
33O11	xi				ER-001159569-000	1.92	8.97	91.03	40.67	0.008	3.950	>100	0.14
	zu	2.2	0.65	3.4	ER-001159569-000	1.69	1.42	98.58	~100	0.064	26.500	>100	0.28
111B10	xi	6.5	3.9	6.3	ER-001159569-000	1.90	4.25	95.75	38.10	0.004	13.960	~100	0.05
	zu	5.1	3	6.5	ER-001159569-000	1.81	3.64	96.36	68.92	0.014	27.42	>100	0.12
201C15	xi	2.4	0.28	1.1	ER-001159569-000	1.85	1.62	98.38	48.50	0.004	14.82	~100	0.27
	zu	3.1	1.1	4.2	ER-001159569-000	1.80	5.84	94.16	68.88	0.250	20.42	>100	0.41
346C6	xi	3.8	0.49	1.4	ER-001159569-000	1.56	5.28	94.72	34.49	0.087	5.73	~100	0.11
	zu	133	93	8.9	ER-001159569-000	1.63	4.48	95.52	72.86	1.180	32.54	>100	0.55

Abbreviations: xi – chimeric; zu – humanized.

[00425] All anti-human mesothelin ADCs retained low aggregation levels (< 10% aggregate) and demonstrated high potency on target cell lines. High potency was observed on A3 and OVCAR3, whereas HEC-251 and H226 cells were relatively resistant to ADC cytotoxicity.

[00426] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

[00427] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Selected sequences:

SEQ ID NO: 1 (MORAb-003 Heavy chain (HC))

```
   1   EVQLVESGGG VVQPGRSLRL SCSASGFTFS GYGLSWVRQA PGKGLEWVAM
  51   ISSGGSYTYY ADSVKGRFAI SRDNAKNTLF LQMDSLRPED TGVYFCARHG
 101   DDPAWFAYWG QGTPVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
 151   YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY
 201   ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP SVFLFPPKPK
 251   DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
 301   TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
 351   YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL
 401   DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK
```

SEQ ID NO: 2 (MORAb-003 HC CDR1; Kabat): GYGLS

SEQ ID NO: 3 (MORAb-003 HC CDR2; Kabat): MISSGGSYTYYADSVKG

SEQ ID NO: 4 (MORAb-003 HC CDR3; Kabat): HGDDPAWFAY

SEQ ID NO: 5 (MORAb-003 Heavy Chain full length pre-protein amino acid sequence; leader sequence underlined)

```
   1   MGWSCIILFL VATATGVHSE VQLVESGGGV VQPGRSLRLS CSASGFTFSG
  51   YGLSWVRQAP GKGLEWVAMI SSGGSYTYYA DSVKGRFAIS RDNAKNTLFL
 101   QMDSLRPEDT GVYFCARHGD DPAWFAYWGQ GTPVTVSSAS TKGPSVFPLA
 151   PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL
 201   YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKKVEPKS CDKTHTCPPC
 251   PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
 301   DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
 351   APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV
 401   EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
 451   EALHNHYTQK SLSLSPGK
```

SEQ ID NO: 6 (MORAb-003 Light chain (LC))

```
   1   DIQLTQSPSS LSASVGDRVT ITCSVSSSIS SNNLHWYQQK PGKAPKPWIY
  51   GTSNLASGVP SRFSGSGSGT DYTFTISSLQ PEDIATYYCQ QWSSYPYMYT
 101   FGQGTKVEIK RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ
 151   WKVDNALQSG NSQESVTEQD SKDSTYSLSS TLTLSKADYE KHKVYACEVT
 201   HQGLSSPVTK SFNRGEC
```

SEQ ID NO: 7 (MORAb-003 LC CDR1; Kabat): SVSSSISSNNLH

SEQ ID NO: 8 (MORAb-003 LC CDR2: Kabat): GTSNLAS

SEQ ID NO: 9 (MORAb-003 LC CDR3; Kabat): QQWSSYPYMYT

SEQ ID NO: 10 MORAb-003 Light Chain full length pre-protein amino acid sequence (leader sequence underlined)

```
   1   MGWSCIILFL VATATGVHSD IQLTQSPSSL SASVGDRVTI TCSVSSSISS
  51   NNLHWYQQKP GKAPKPWIYG TSNLASGVPS RFSGSGSGTD YTFTISSLQP
 101   EDIATYYCQQ WSSYPYMYTF GQGTKVEIKR TVAAPSVFIF PPSDEQLKSG
 151   TASVVCLLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYSLSST
```

```
 201   LTLSKADYEK HKVYACEVTH QGLSSPVTKS FNRGEC
```

SEQ ID NO: 11 (MORAb-003 HC nt)

```
   1   ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT
  51   CCACTCCGAG GTCCAACTGG TGGAGAGCGG TGGAGGTGTT GTGCAACCTG
 101   GCCGGTCCCT GCGCCTGTCC TGCTCCGCAT CTGGCTTCAC CTTCAGCGGC
 151   TATGGGTTGT CTTGGGTGAG ACAGGCACCT GGAAAAGGTC TTGAGTGGGT
 201   TGCAATGATT AGTAGTGGTG GTAGTTATAC CTACTATGCA GACAGTGTGA
 251   AGGGTAGATT TGCAATATCG CGAGACAACG CCAAGAACAC ATTGTTCCTG
 301   CAAATGGACA GCCTGAGACC CGAAGACACC GGGGTCTATT TTTGTGCAAG
 351   ACATGGGGAC GATCCCGCCT GGTTCGCTTA TTGGGGCCAA GGGACCCCGG
 401   TCACCGTCTC CTCAGCCTCC ACCAAGGGCC CATCGGTCTT CCCCCTGGCA
 451   CCCTCCTCCA AGAGCACCTC TGGGGGCACA GCGGCCCTGG GCTGCCTGGT
 501   CAAGGACTAC TTCCCCGAAC CGGTGACGGT GTCGTGGAAC TCAGGCGCCC
 551   TGACCAGCGG CGTGCACACC TTCCCGGCTG TCCTACAGTC CTCAGGACTC
 601   TACTCCCTCA GCAGCGTGGT GACCGTGCCC TCCAGCAGCT TGGGCACCCA
 651   GACCTACATC TGCAACGTGA ATCACAAGCC CAGCAACACC AAGGTGGACA
 701   AGAAAGTTGA GCCCAAATCT TGTGACAAAA CTCACACATG CCCACCGTGC
 751   CCAGCACCTG AACTCCTGGG GGGACCGTCA GTCTTCCTCT TCCCCCCAAA
 801   ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG
 851   TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
 901   GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA
 951   CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
1001   GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
1051   GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC
1101   ACAGGTGTAC ACCCTGCCCC CATCCCGGGA TGAGCTGACC AAGAACCAGG
1151   TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
1201   GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC
1251   CGTGCTGGAC TCCGACGGCT CCTTCTTCTT ATATTCAAAG CTCACCGTGG
1301   ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
1351   GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCCGG
1401   GAAATGA
```

SEQ ID NO: 12 (MORAb-003 LC nt)

```
   1   ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT
  51   CCACTCCGAC ATCCAGCTGA CCCAGAGCCC AAGCAGCCTG AGCGCCAGCG
 101   TGGGTGACAG AGTGACCATC ACCTGTAGTG TCAGCTCAAG TATAAGTTCC
 151   AACAACTTGC ACTGGTACCA GCAGAAGCCA GGTAAGGCTC CAAAGCCATG
 201   GATCTACGGC ACATCCAACC TGGCTTCTGG TGTGCCAAGC AGATTCAGCG
 251   GTAGCGGTAG CGGTACCGAC TACACCTTCA CCATCAGCAG CCTCCAGCCA
 301   GAGGACATCG CCACCTACTA CTGCCAACAG TGGAGTAGTT ACCCGTACAT
 351   GTACACGTTC GGCCAAGGGA CCAAGGTGGA AATCAAACGA ACTGTGGCTG
 401   CACCATCTGT CTTCATCTTC CCGCCATCTG ATGAGCAGTT GAAATCTGGA
 451   ACTGCCTCTG TTGTGTGCCT GCTGAATAAC TTCTATCCCA GAGAGGCCAA
 501   AGTACAGTGG AAGGTGGATA ACGCCCTCCA ATCGGGTAAC TCCCAGGAGA
 551   GTGTCACAGA GCAGGACAGC AAGGACAGCA CCTACAGCCT CAGCAGCACC
 601   CTGACGCTGA GCAAAGCAGA CTACGAGAAA CACAAAGTCT ACGCCTGCGA
 651   AGTCACCCAT CAGGGCCTGA GCTCGCCCGT CACAAAGAGC TTCAACAGGG
 701   GAGAGTGTTA A
```

SEQ ID NO: 13 (MORAb-003 HC CDR1; IMGT): GFTFSGYG

SEQ ID NO: 14 (MORAb-003 HC CDR2; IMGT): ISSGGSYT

SEQ ID NO: 15 (MORAb-003 HC CDR3; IMGT): ARHGDDPAWFAY

SEQ ID NO: 16 (MORAb-003 LC CDR1; IMGT): SSISSNN

SEQ ID NO: 17 (MORAb-003 LC CDR2; IMGT): GTS

SEQ ID NO: 18 (MORAb-003 LC CDR3; IMGT): QQWSSYPYMYT

SEQ ID NO: 19 (human FRA)

```
  1 maqrmttqll lllvwvavvg eaqtriawar tellnvcmna khhkekpgpe dklheqcrpw
 61 rknaccstnt sqeahkdvsy lyrfnwnhcg emapackrhf iqdtclyecs pnlgpwiqqv
121 dqswrkervl nvplckedce qwwedcrtsy tcksnwhkgw nwtsgfnkca vgaacqpfhf
181 yfptptvlcn eiwthsykvs nysrgsgrci qmwfdpaqgn pneevarfya aamsgagpwa
241 awpfllslal mllwlls
```

SEQ ID NO: 20 (human FRA nucleotide)

```
  1 cattccttgg tgccactgac cacagctctt tcttcaggga cagacatggc tcagcggatg
 61 acaacacagc tgctgctcct tctagtgtgg gtggctgtag taggggaggc tcagacaagg
121 attgcatggg ccaggactga gcttctcaat gtctgcatga acgccaagca ccacaaggaa
181 aagccaggcc ccgaggacaa gttgcatgag cagtgtcgac cctggaggaa gaatgcctgc
241 tgttctacca acaccagcca ggaagcccat aaggatgttt cctacctata tagattcaac
301 tggaaccact gtggagagat ggcacctgcc tgcaaacggc atttcatcca ggacacctgc
361 ctctacgagt gctcccccaa cttggggccc tggatccagc aggtggatca gagctggcgc
421 aaagagcggg tactgaacgt gcccctgtgc aaagaggact gtgagcaatg gtgggaagat
481 tgtcgcacct cctacacctg caagagcaac tggcacaagg gctggaactg gacttcaggg
541 tttaacaagt gcgcagtggg agctgcctgc caacctttcc atttctactt ccccacaccc
601 actgttctgt gcaatgaaat ctggactcac tcctacaagg tcagcaacta cagccgaggg
661 agtggccgct gcatccagat gtggttcgac ccagcccagg gcaaccccaa tgaggaggtg
721 gcgaggttct atgctgcagc catgagtggg gctgggccct gggcagcctg gcctttcctg
781 cttagcctgg ccctaatgct gctgtggctg ctcagctgac ctccttttac cttctgatac
841 ctggaaatcc ctgccctgtt cagccccaca gctcccaact atttggttcc tgctccatgg
901 tcgggcctct gacagccact ttgaataaac cagacaccgc acatgtgtct tgagaattat
961 ttggaaaaaa aaaaaaaaaa aa
```

SEQ ID NO: 21 (human her2)

```
1     melaalcrwg lllallppga astqvctgtd mklrlpaspe thldmlrhly
qgcqvvqgnl
61    eltylptnas lsflqdiqev qgyvliahnq vrqvplqrlr ivrgtqlfed
nyalavldng
121   dplnnttpvt gaspgglrel qlrslteilk ggvliqrnpq lcyqdtilwk
difhknnqla
181   ltlidtnrsr achpcspmck gsrcwgesse dcqsltrtvc aggcarckgp
lptdccheqc
241   aagctgpkhs dclaclhfnh sgicelhcpa lvtyntdtfe smpnpegryt
fgascvtacp
301   ynylstdvgs ctlvcplhnq evtaedgtqr cekcskpcar vcyglgmehl
revravtsan
```

361 iqefagckki fgslaflpes fdgdpasnta plqpeqlqvf etleeitgyl
yisawpdslp
421 dlsvfqnlqv irgrilhnga ysltlqglgi swlglrslre lgsglalihh
nthlcfvhtv
481 pwdqlfrnph qallhtanrp edecvgegla chqlcarghc wgpgptqcvn
csqflrgqec
541 veecrvlqgl preyvnarhc lpchpecqpq ngsvtcfgpe adqcvacahy
kdppfcvarc
601 psgvkpdlsy mpiwkfpdee gacqpcpinc thscvdlddk gcpaeqrasp
ltsiisavvg
661 illvvvlgvv fgilikrrqq kirkytmrrl lqetelvepl tpsgampnqa
qmrilketel
721 rkvkvlgsga fgtvykgiwi pdgenvkipv aikvlrents pkankeilde
ayvmagvgsp
781 yvsrllgicl tstvqlvtql mpygclldhv renrgrlgsq dllnwcmqia
kgmsyledvr
841 lvhrdlaarn vlvkspnhvk itdfglarll dideteyhad ggkvpikwma
lesilrrrft
901 hqsdvwsygv tvwelmtfga kpydgipare ipdllekger lpqppictid
vymimvkcwm
961 idsecrprfr elvsefsrma rdpqrfvviq nedlgpaspl dstfyrslle
dddmgdlvda
1021 eeylvpqqgf fcpdpapgag gmvhhrhrss strsgggdlt lglepseeea
prsplapseg
1081 agsdvfdgdl gmgaakglqs lpthdpsplq rysedptvpl psetdgyvap
ltcspqpeyv
1141 nqpdvrpqpp spregplpaa rpagatlerp ktlspgkngv vkdvfafgga
venpeyltpq
1201 ggaapqphpp pafspafdnl yywdqdpper gappstfkgt ptaenpeylg ldvpv

SEQ ID NO: 22 (human her2 nucleotide)

1 ATGGAGCTGG CGGCCTTGTG CCGCTGGGGG CTCCTCCTCG CCCTCTTGCC
CCCCGGAGCC
61 GCGAGCACCC AAGTGTGCAC CGGCACAGAC ATGAAGCTGC GGCTCCCTGC
CAGTCCCGAG
121 ACCCACCTGG ACATGCTCCG CCACCTCTAC CAGGGCTGCC AGGTGGTGCA
GGGAAACCTG
181 GAACTCACCT ACCTGCCCAC CAATGCCAGC CTGTCCTTCC TGCAGGATAT
CCAGGAGGTG

241 CAGGGCTACG TGCTCATCGC TCACAACCAA GTGAGGCAGG TCCCACTGCA
GAGGCTGCGG
301 ATTGTGCGAG GCACCCAGCT CTTTGAGGAC AACTATGCCC TGGCCGTGCT
AGACAATGGA
361 GACCCGCTGA ACAATACCAC CCCTGTCACA GGGGCCTCCC CAGGAGGCCT
GCGGGAGCTG
421 CAGCTTCGAA GCCTCACAGA GATCTTGAAA GGAGGGGTCT TGATCCAGCG
GAACCCCCAG
481 CTCTGCTACC AGGACACGAT TTTGTGGAAG GACATCTTCC ACAAGAACAA
CCAGCTGGCT
541 CTCACACTGA TAGACACCAA CCGCTCTCGG GCCTGCCACC CCTGTTCTCC
GATGTGTAAG
601 GGCTCCCGCT GCTGGGGAGA GAGTTCTGAG GATTGTCAGA GCCTGACGCG
CACTGTCTGT
661 GCCGGTGGCT GTGCCCGCTG CAAGGGGCCA CTGCCCACTG ACTGCTGCCA
TGAGCAGTGT
721 GCTGCCGGCT GCACGGGCCC CAAGCACTCT GACTGCCTGG CCTGCCTCCA
CTTCAACCAC
781 AGTGGCATCT GTGAGCTGCA CTGCCCAGCC CTGGTCACCT ACAACACAGA
CACGTTTGAG
841 TCCATGCCCA ATCCCGAGGG CCGGTATACA TTCGGCGCCA GCTGTGTGAC
TGCCTGTCCC
901 TACAACTACC TTTCTACGGA CGTGGGATCC TGCACCCTCG TCTGCCCCCT
GCACAACCAA
961 GAGGTGACAG CAGAGGATGG AACACAGCGG TGTGAGAAGT GCAGCAAGCC
CTGTGCCCGA
1021 GTGTGCTATG GTCTGGGCAT GGAGCACTTG CGAGAGGTGA GGGCAGTTAC
CAGTGCCAAT
1081 ATCCAGGAGT TTGCTGGCTG CAAGAAGATC TTTGGGAGCC TGGCATTTCT
GCCGGAGAGC
1141 TTTGATGGGG ACCCAGCCTC CAACACTGCC CCGCTCCAGC CAGAGCAGCT
CCAAGTGTTT
1201 GAGACTCTGG AAGAGATCAC AGGTTACCTA TACATCTCAG CATGGCCGGA
CAGCCTGCCT
1261 GACCTCAGCG TCTTCCAGAA CCTGCAAGTA ATCCGGGGAC GAATTCTGCA
CAATGGCGCC
1321 TACTCGCTGA CCCTGCAAGG GCTGGGCATC AGCTGGCTGG GGCTGCGCTC
ACTGAGGGAA
1381 CTGGGCAGTG GACTGGCCCT CATCCACCAT AACACCCACC TCTGCTTCGT
GCACACGGTG

1441 CCCTGGGACC AGCTCTTTCG GAACCCGCAC CAAGCTCTGC TCCACACTGC
CAACCGGCCA
1501 GAGGACGAGT GTGTGGGCGA GGGCCTGGCC TGCCACCAGC TGTGCGCCCG
AGGGCACTGC
1561 TGGGGTCCAG GGCCCACCCA GTGTGTCAAC TGCAGCCAGT TCCTTCGGGG
CCAGGAGTGC
1621 GTGGAGGAAT GCCGAGTACT GCAGGGGCTC CCCAGGGAGT ATGTGAATGC
CAGGCACTGT
1681 TTGCCGTGCC ACCCTGAGTG TCAGCCCCAG AATGGCTCAG TGACCTGTTT
TGGACCGGAG
1741 GCTGACCAGT GTGTGGCCTG TGCCCACTAT AAGGACCCTC CCTTCTGCGT
GGCCCGCTGC
1801 CCCAGCGGTG TGAAACCTGA CCTCTCCTAC ATGCCCATCT GGAAGTTTCC
AGATGAGGAG
1861 GGCGCATGCC AGCCTTGCCC CATCAACTGC ACCCACTCCT GTGTGGACCT
GGATGACAAG
1921 GGCTGCCCCG CCGAGCAGAG AGCCAGCCCT CTGACGTCCA TCATCTCTGC
GGTGGTTGGC
1981 ATTCTGCTGG TCGTGGTCTT GGGGGTGGTC TTTGGGATCC TCATCAAGCG
ACGGCAGCAG
2041 AAGATCCGGA AGTACACGAT GCGGAGACTG CTGCAGGAAA CGGAGCTGGT
GGAGCCGCTG
2101 ACACCTAGCG GAGCGATGCC CAACCAGGCG CAGATGCGGA TCCTGAAAGA
GACGGAGCTG
2161 AGGAAGGTGA AGGTGCTTGG ATCTGGCGCT TTTGGCACAG TCTACAAGGG
CATCTGGATC
2221 CCTGATGGGG AGAATGTGAA AATTCCAGTG GCCATCAAAG TGTTGAGGGA
AAACACATCC
2281 CCCAAAGCCA ACAAAGAAAT CTTAGACGAA GCATACGTGA TGGCTGGTGT
GGGCTCCCCA
2341 TATGTCTCCC GCCTTCTGGG CATCTGCCTG ACATCCACGG TGCAGCTGGT
GACACAGCTT
2401 ATGCCCTATG GCTGCCTCTT AGACCATGTC CGGGAAAACC GCGGACGCCT
GGGCTCCCAG
2461 GACCTGCTGA ACTGGTGTAT GCAGATTGCC AAGGGGATGA GCTACCTGGA
GGATGTGCGG
2521 CTCGTACACA GGGACTTGGC CGCTCGGAAC GTGCTGGTCA AGAGTCCCAA
CCATGTCAAA
2581 ATTACAGACT TCGGGCTGGC TCGGCTGCTG GACATTGACG AGACAGAGTA
CCATGCAGAT

2641 GGGGGCAAGG TGCCCATCAA GTGGATGGCG CTGGAGTCCA TTCTCCGCCG
GCGGTTCACC
2701 CACCAGAGTG ATGTGTGGAG TTATGGTGTG ACTGTGTGGG AGCTGATGAC
TTTTGGGGCC
2761 AAACCTTACG ATGGGATCCC AGCCCGGGAG ATCCCTGACC TGCTGGAAAA
GGGGGAGCGG
2821 CTGCCCCAGC CCCCCATCTG CACCATTGAT GTCTACATGA TCATGGTCAA
ATGTTGGATG
2881 ATTGACTCTG AATGTCGGCC AAGATTCCGG GAGTTGGTGT CTGAATTCTC
CCGCATGGCC
2941 AGGGACCCCC AGCGCTTTGT GGTCATCCAG AATGAGGACT TGGGCCCAGC
CAGTCCCTTG
3001 GACAGCACCT TCTACCGCTC ACTGCTGGAG GACGATGACA TGGGGGACCT
GGTGGATGCT
3061 GAGGAGTATC TGGTACCCCA GCAGGGCTTC TTCTGTCCAG ACCCTGCCCC
GGGCGCTGGG
3121 GGCATGGTCC ACCACAGGCA CCGCAGCTCA TCTACCAGGA GTGGCGGTGG
GGACCTGACA
3181 CTAGGGCTGG AGCCCTCTGA AGAGGAGGCC CCCAGGTCTC CACTGGCACC
CTCCGAAGGG
3241 GCTGGCTCCG ATGTATTTGA TGGTGACCTG GGAATGGGGG CAGCCAAGGG
GCTGCAAAGC
3301 CTCCCCACAC ATGACCCCAG CCCTCTACAG CGGTACAGTG AGGACCCCAC
AGTACCCCTG
3361 CCCTCTGAGA CTGATGGCTA CGTTGCCCCC CTGACCTGCA GCCCCCAGCC
TGAATATGTG
3421 AACCAGCCAG ATGTTCGGCC CCAGCCCCCT TCGCCCCGAG AGGGCCCTCT
GCCTGCTGCC
3481 CGACCTGCTG GTGCCACTCT GGAAAGGCCC AAGACTCTCT CCCCAGGGAA
GAATGGGGTC
3541 GTCAAAGACG TTTTTGCCTT TGGGGGTGCC GTGGAGAACC CCGAGTACTT
GACACCCCAG
3601 GGAGGAGCTG CCCCTCAGCC CCACCCTCCT CCTGCCTTCA GCCCAGCCTT
CGACAACCTC
3661 TATTACTGGG ACCAGGACCC ACCAGAGCGG GGGGCTCCAC CCAGCACCTT
CAAAGGGACA
3721 CCTACGGCAG AGAACCCAGA GTACCTGGGT CTGGACGTGC CAGTGTGA

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An antibody-drug conjugate of Formula (I):

$$Ab\text{-}(L\text{-}D)_p \qquad (I)$$

wherein Ab is an internalizing anti-folate receptor alpha antibody or internalizing antigen-binding fragment thereof comprising three heavy chain complementarity determining regions (HCDRs) consisting of amino acid sequences of SEQ ID NO:2 (HCDR1), SEQ ID NO:3 (HCDR2), and SEQ ID NO:4 (HCDR3); and three light chain complementarity determining regions (LCDRs) consisting of amino acid sequences of SEQ ID NO:7 (LCDR1), SEQ ID NO:8 (LCDR2), and SEQ ID NO:9 (LCDR3), as defined by the Kabat numbering system; or three heavy chain complementarity determining regions (HCDRs) consisting of amino acid sequences of SEQ ID NO:13 (HCDR1), SEQ ID NO:14 (HCDR2), and SEQ ID NO:15 (HCDR3); and three light chain complementarity determining regions (LCDRs) consisting of amino acid sequences of SEQ ID NO:16 (LCDR1), SEQ ID NO:17 (LCDR2), and SEQ ID NO:18 (LCDR3), as defined by the IMGT numbering system;

D is eribulin;

L is a cleavable linker comprising valine-citrulline-p-aminobenzyloxycarbonyl (Val-Cit-pAB); and

p is an integer from 1 to 8.

2. The antibody-drug conjugate of claim 1, wherein *p* is from 3 to 4.

3. The antibody-drug conjugate of claim 1 or claim 2, wherein the cleavable linker is positioned such that no part of the linker or the antibody or antigen-binding fragment remains bound to eribulin upon cleavage.

4. The antibody-drug conjugate of any one of claims 1 to 3, wherein the cleavable linker comprises at least one spacer unit.

5. The antibody-drug conjugate of claim 4, wherein the spacer unit comprises a polyethylene glycol (PEG) moiety.

6. The antibody-drug conjugate of claim 5, wherein the PEG moiety comprises -$(PEG)_m$- and *m* is an integer from 1 to 10.

7. The antibody-drug conjugate of claim 6, wherein *m* is 2.

8. The antibody-drug conjugate of claim 4, wherein the spacer unit or cleavable linker comprises an alkyl moiety.

9. The antibody-drug conjugate of claim 8, wherein the alkyl moiety comprises -$(CH_2)_n$- and *n* is an integer from 1 to 10.

10. The antibody-drug conjugate of claim 9, wherein *n* is 5.

11. The antibody-drug conjugate of any one of claims 4 to 10, wherein the spacer unit attaches to the antibody or antigen-binding fragment via a maleimide moiety ("Mal-spacer unit").

12. The antibody-drug conjugate of claim 11, wherein the Mal-spacer unit is reactive with a cysteine residue on the antibody or antigen-binding fragment.

13. The antibody-drug conjugate of claim 11 or claim 12, wherein the Mal-spacer unit is joined to the antibody or antigen-binding fragment via a cysteine residue on the antibody or antigen-binding fragment.

14. The antibody-drug conjugate of any one of claims 11 to 13, wherein the Mal-spacer unit attaches the antibody or antigen-binding fragment to the Val-Cit-pAB.

15. The antibody-drug conjugate of any one of claims 4 to 10, wherein the spacer unit attaches to the antibody or antigen-binding fragment via a succinimide moiety ("OSu-spacer unit").

16. The antibody-drug conjugate of claim 15, wherein the OSu-spacer unit is reactive with a lysine residue on the antibody or antigen-binding fragment.

17. The antibody-drug conjugate of claim 15 or claim 16, wherein the OSu-spacer unit is joined to the antibody or antigen-binding fragment via a lysine residue.

18. The antibody-drug conjugate of any one of claims 15 to 17, wherein the OSu-spacer unit attaches the antibody or antigen-binding fragment to the Val-Cit-pAB.

19. The antibody-drug conjugate of any one of claims 1 to 18, wherein the pAB covalently attaches to eribulin via a C-35 amine.

20. The antibody-drug conjugate of any one of claims 1 to 19, wherein the antibody or antigen-binding fragment comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:23, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:24.

21. The antibody-drug conjugate of any one of claims 1 to 20, wherein the cleavable linker comprises Mal-$(PEG)_2$-Val-Cit-pAB.

22. An antibody-drug conjugate of Formula (I):

$$Ab\text{-}(L\text{-}D)_p \qquad (I)$$

wherein

Ab is an internalizing anti-folate receptor alpha antibody or internalizing antigen-binding fragment thereof comprising three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:2 (HCDR1), SEQ ID NO:3 (HCDR2), and SEQ ID NO:4 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:7 (LCDR1), SEQ ID NO:8 (LCDR2), and SEQ ID NO:9 (LCDR3), as defined by the Kabat numbering system; or three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:13 (HCDR1), SEQ ID NO:14 (HCDR2), and SEQ ID NO:15 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:16 (LCDR1), SEQ ID NO:17 (LCDR2), and SEQ ID NO:18 (LCDR3), as defined by the IMGT numbering system;

D is eribulin;

L is a cleavable linker comprising Mal-$(PEG)_2$-Val-Cit-pAB; and

p is an integer from 1 to 8.

23. The antibody-drug conjugate of claim 22, wherein the antibody or antigen-binding fragment comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:23, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:24.

24. The antibody-drug conjugate of claim 22 or claim 23, wherein *p* is from 3 to 4.

25. An antibody-drug conjugate of Formula (I):

$$Ab\text{-}(L\text{-}D)_p \qquad (I)$$

wherein

Ab is an internalizing anti-folate receptor alpha antibody or antigen-binding fragment thereof comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:23, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:24;

D is eribulin;

L is a cleavable linker comprising Mal-$(PEG)_2$-Val-Cit-pAB; and

p is an integer from 3 to 4.

26. The antibody-drug conjugate of any one of claims 1 to 25, wherein the antibody comprises a human IgG1 heavy chain constant domain and a human Ig kappa light chain constant domain.

27. A composition comprising multiple copies of the antibody-drug conjugate of any one of claims 1 to 26, wherein the average *p* of the antibody-drug conjugates in the composition is from about 3.2 to about 4.4.

28. A method of treating a patient having or at risk of having a cancer that expresses a target antigen, comprising administering to the patient a therapeutically effective amount of the antibody-drug conjugate of any one of claims 1 to 26 or the composition of claim 27, wherein the target antigen is folate receptor alpha.

29. The method of claim 28, wherein the folate receptor alpha-expressing cancer is a gastric cancer, a serous ovarian cancer, a clear cell ovarian cancer, a non-small cell lung cancer, a colorectal cancer, a triple negative breast cancer, an endometrial cancer, a serous endometrial carcinoma, a lung carcinoid, or an osteosarcoma.

30. A method of reducing or inhibiting growth of a target antigen-expressing tumor, comprising administering a therapeutically effective amount of the antibody-drug conjugate of any one of claims 1 to 26 or the composition of claim 27, wherein the target antigen is folate receptor alpha.

31. The method of claim 30, wherein the tumor is a folate receptor alpha-expressing gastric cancer, serous ovarian cancer, clear cell ovarian cancer, non-small cell lung cancer, colorectal cancer, triple negative breast cancer, endometrial cancer, serous endometrial carcinoma, lung carcinoid, or osteosarcoma.

32. Use of an antibody-drug conjugate of any one of claims 1 to 26 or the composition of claim 27 in the treatment of a target antigen-expressing cancer, wherein the target antigen is folate receptor alpha.

33. The use of claim 32, wherein the folate receptor alpha-expressing cancer is a gastric cancer, a serous ovarian cancer, a clear cell ovarian cancer, a non-small cell lung cancer, a colorectal cancer, a triple negative breast cancer, an endometrial cancer, a serous endometrial carcinoma, a lung carcinoid, or an osteosarcoma.

34. Use of an antibody-drug conjugate of any one of claims 1 to 26 or the composition of claim 27 in the manufacture of a medicament for the treatment of a target antigen-expressing cancer, wherein the target antigen is folate receptor alpha.

35. The use of claim 34, wherein the folate receptor alpha-expressing cancer is a gastric cancer, a serous ovarian cancer, a clear cell ovarian cancer, a non-small cell lung cancer, a colorectal cancer, a triple negative breast cancer, an endometrial cancer, a serous endometrial carcinoma, a lung carcinoid, or an osteosarcoma.

36. A pharmaceutical composition comprising the antibody-drug conjugate of any one of claims 1 to 26 or the composition of claim 27, and a pharmaceutically acceptable carrier.

37. A method of producing the antibody-drug conjugate of any one of claims 1 to 26 or the composition of claim 27, comprising reacting an antibody or antigen-binding fragment with a cleavable linker joined to eribulin under conditions that allow conjugation.

38. A method of determining whether a patient will be responsive to treatment with the antibody-drug conjugate of any one of claims 1 to 26 or the composition of claim 27, comprising providing a biological sample from the patient and contacting the biological sample with the antibody-drug conjugate of any one of claims 1 to 26 or the composition of claim 27.

39. The method of claim 38, wherein the biological sample is a tumor biopsy derived from a patient having or at risk of having a folate receptor alpha-expressing cancer, wherein the cancer is a gastric cancer, a serous ovarian cancer, a clear cell ovarian cancer, a non-small cell lung cancer, a colorectal cancer, a triple negative breast cancer, an endometrial cancer, a serous endometrial carcinoma, a lung carcinoid, or an osteosarcoma.

40. A composition comprising multiple copies of an antibody-drug conjugate of Formula (I):

$$Ab\text{-}(L\text{-}D)_p \qquad (I)$$

wherein

Ab is an internalizing anti-folate receptor alpha antibody or internalizing antigen-binding fragment thereof comprising three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:2 (HCDR1), SEQ ID NO:3 (HCDR2), and SEQ ID NO:4 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:7 (LCDR1), SEQ ID NO:8 (LCDR2), and SEQ ID NO:9 (LCDR3), as defined by the Kabat numbering system; or three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:13 (HCDR1), SEQ ID NO:14 (HCDR2), and SEQ ID NO:15 (HCDR3); and three light chain complementarity determining regions

(LCDRs) comprising amino acid sequences of SEQ ID NO:16 (LCDR1), SEQ ID NO:17 (LCDR2), and SEQ ID NO:18 (LCDR3), as defined by the IMGT numbering system;

D is eribulin;

L is a cleavable linker comprising Mal-$(PEG)_2$-Val-Cit-pAB; and

p is the average number of -L-D moieties per Ab, wherein the average *p* of the antibody-drug conjugates in the composition is from about 3.6 to about 4.4; and the average *p* is determined by hydrophobic interaction chromatography-high performance liquid chromatography (HIC-HPLC).

41. The composition of claim 40, wherein the antibody or antigen-binding fragment comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:23, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:24.

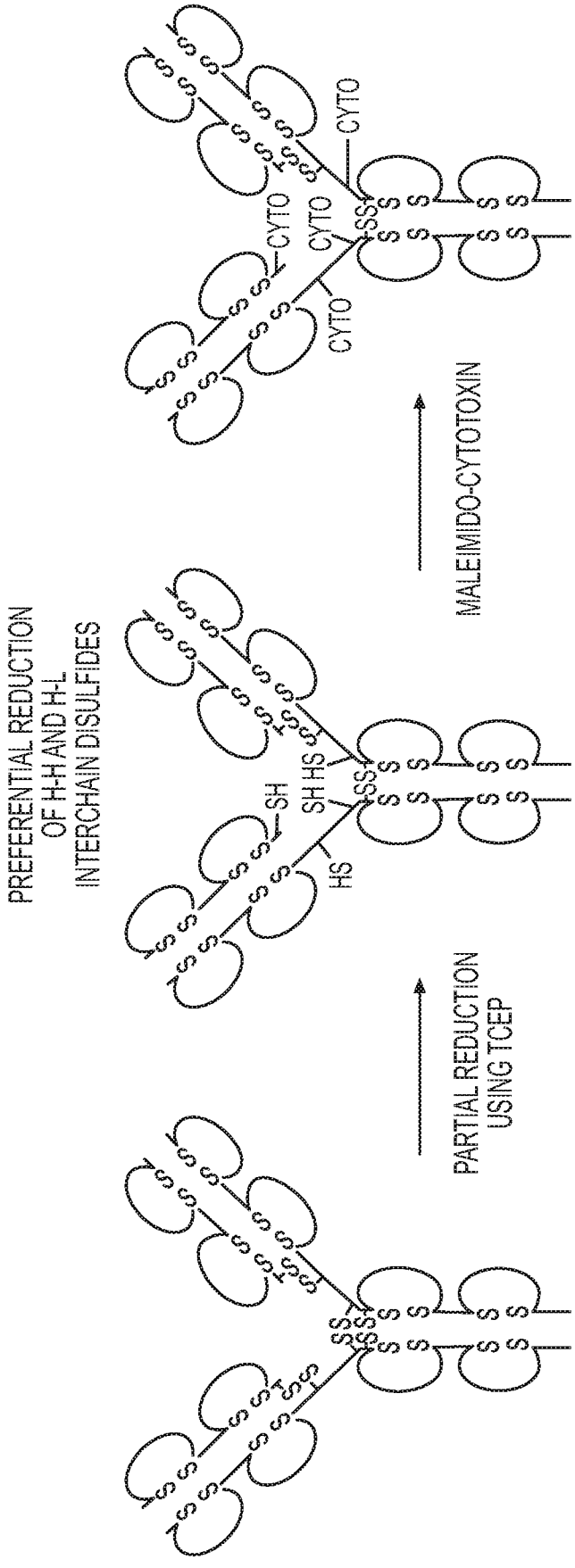
PREFERENTIAL REDUCTION
OF H-H AND H-L
INTERCHAIN DISULFIDES
PARTIAL REDUCTION
USING TCEP
MALEIMIDO-CYTOTOXIN
SH
SH HS
HS
CYTO
CYTO
CYTO
CYTO
CYTO

FIG. 1

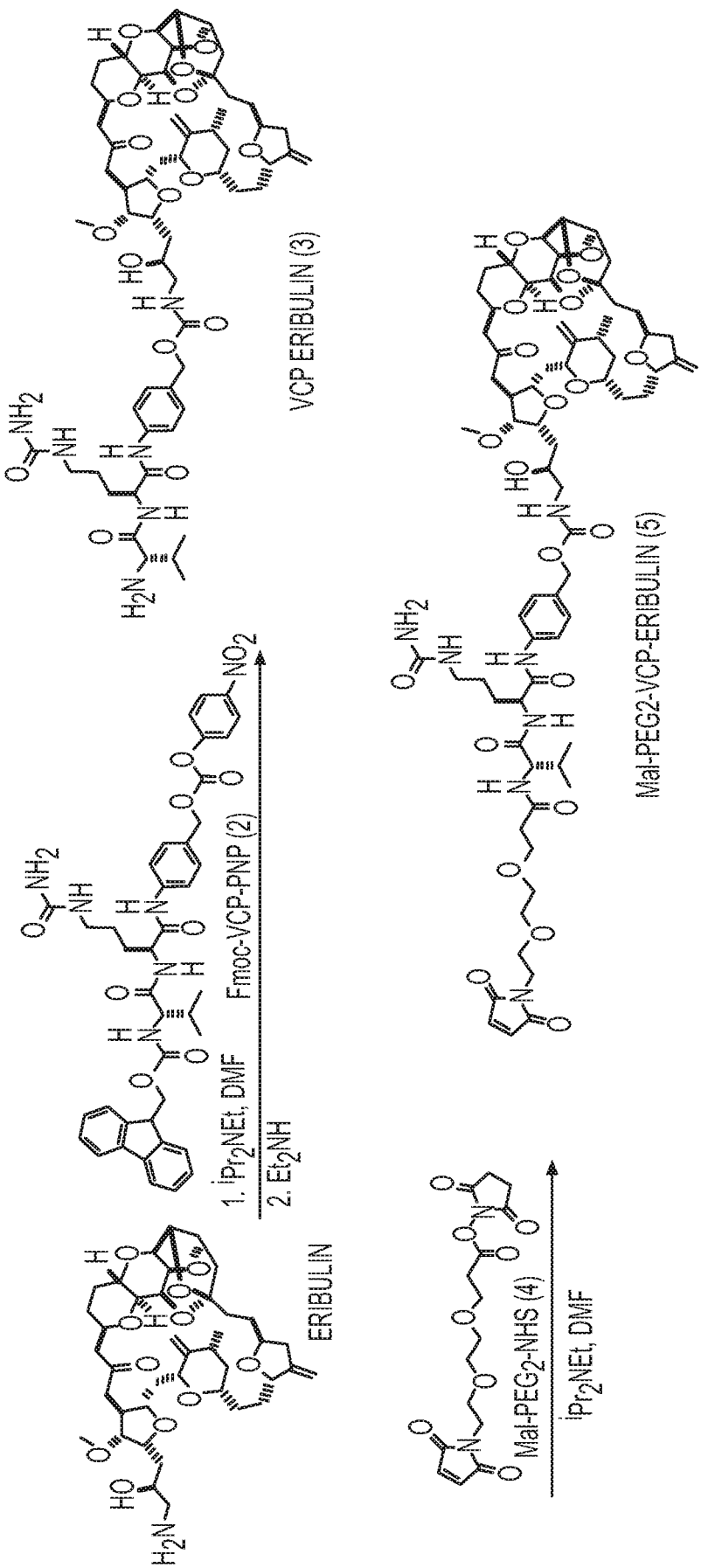
ERIBULIN
1. iPr2NEt, DMF
2. Et2NH
Fmoc-VCP-PNP (2)
VCP ERIBULIN (3)
Mal-PEG2-NHS (4)
iPr2NEt, DMF
Mal-PEG2-VCP-ERIBULIN (5)

FIG. 2

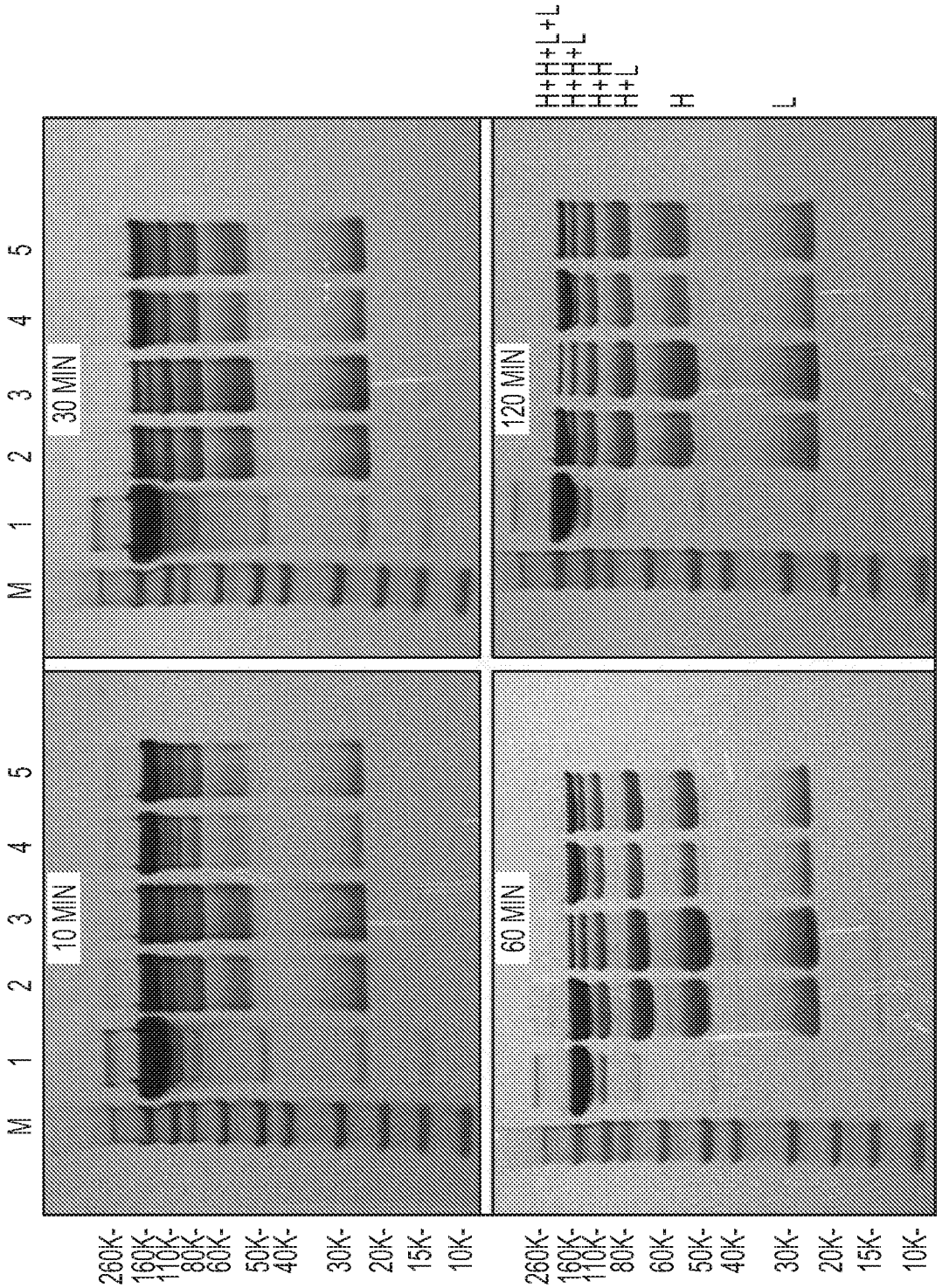
M 1 2 3 4 5
10 MIN
30 MIN
60 MIN
120 MIN
H+H+L+L
H+H+L
H+H
H+L
H
L
260K-
160K-
110K-
80K-
60K-
50K-
40K-
30K-
20K-
15K-
10K-

FIG. 3

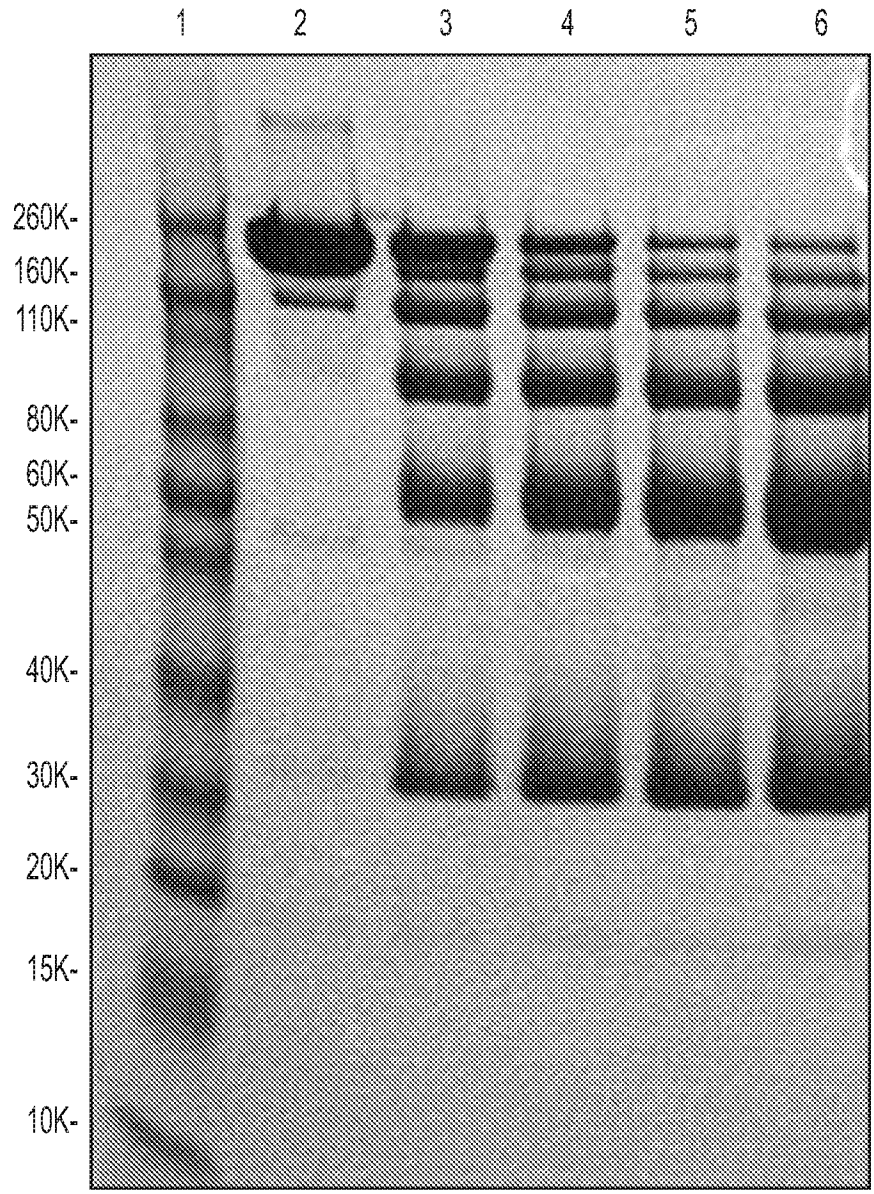

FIG. 4

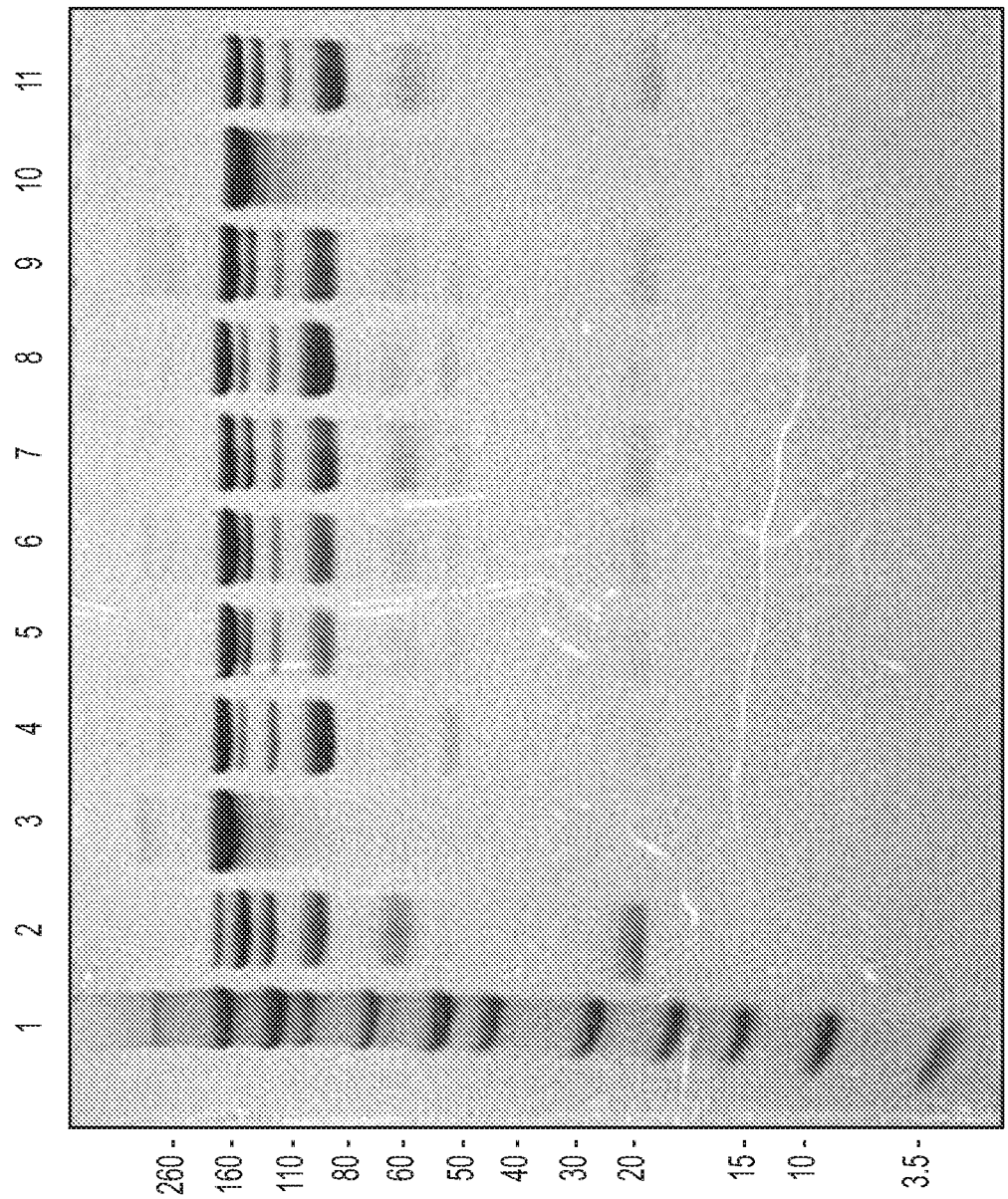

FIG. 5

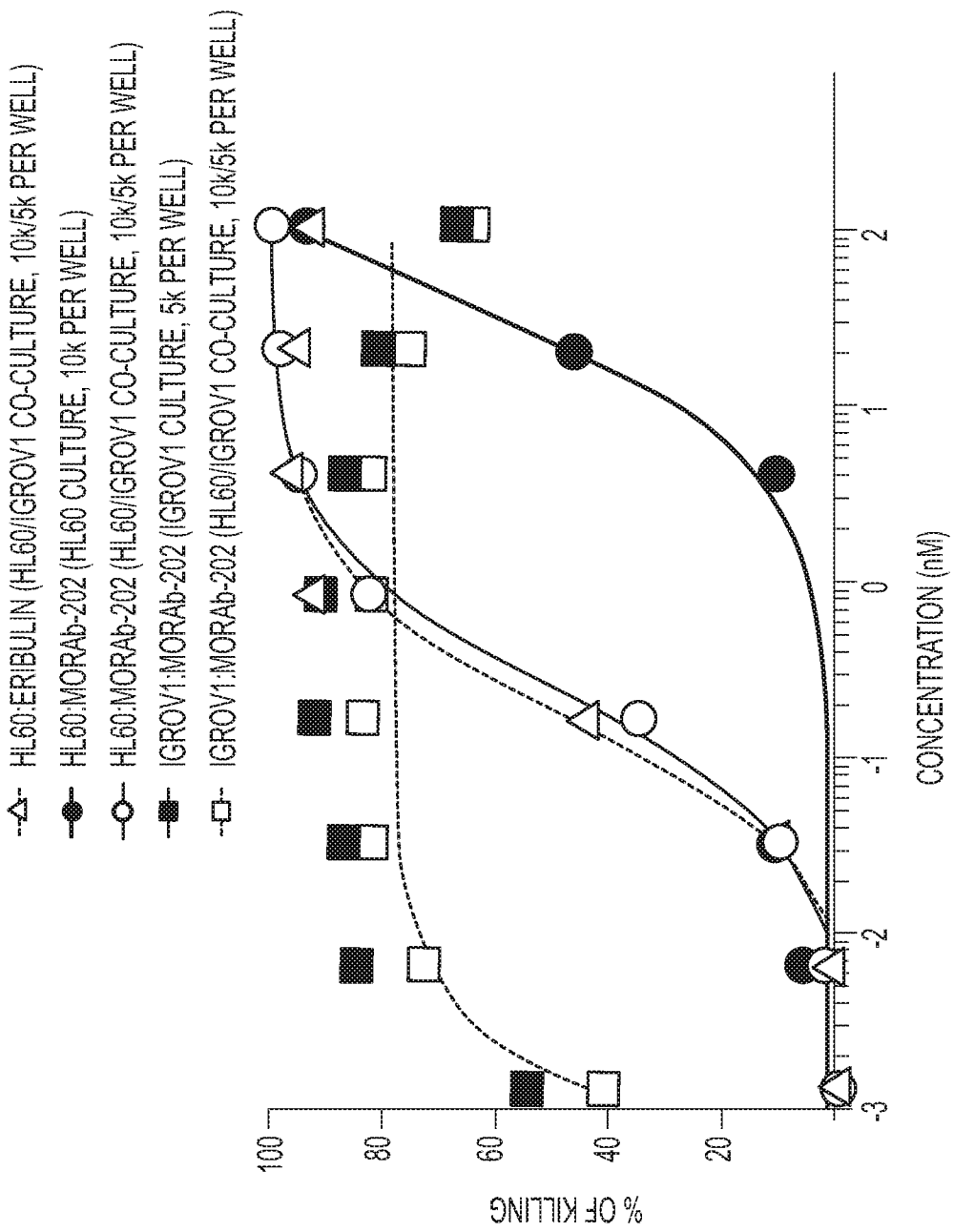
HL60:ERIBULIN (HL60/IGROV1 CO-CULTURE, 10k/5k PER WELL)
HL60:MORAb-202 (HL60 CULTURE, 10k PER WELL)
HL60:MORAb-202 (HL60/IGROV1 CO-CULTURE, 10k/5k PER WELL)
IGROV1:MORAb-202 (IGROV1 CULTURE, 5k PER WELL)
IGROV1:MORAb-202 (HL60/IGROV1 CO-CULTURE, 10k/5k PER WELL)
% OF KILLING
100
80
60
40
20
-3
-2
-1
0
1
2
CONCENTRATION (nM)

FIG. 6A

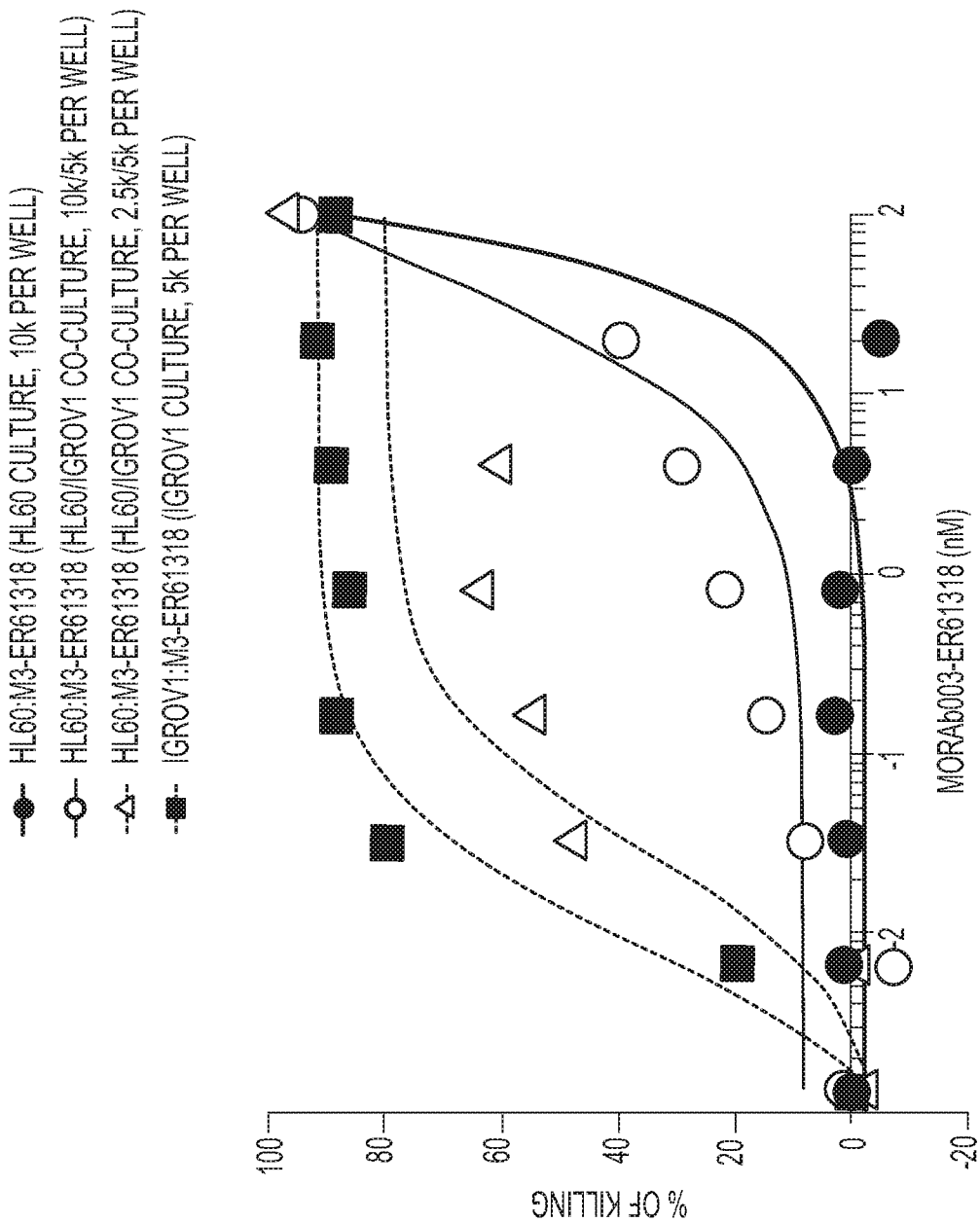
HL60:M3-ER61318 (HL60 CULTURE, 10k PER WELL)
HL60:M3-ER61318 (HL60/IGROV1 CO-CULTURE, 10k/5k PER WELL)
HL60:M3-ER61318 (HL60/IGROV1 CO-CULTURE, 2.5k/5k PER WELL)
IGROV1:M3-ER61318 (IGROV1 CULTURE, 5k PER WELL)
% OF KILLING
100
80
60
40
20
0
-20
-2
-1
0
1
2
MORAb003-ER61318 (nM)

FIG. 6B

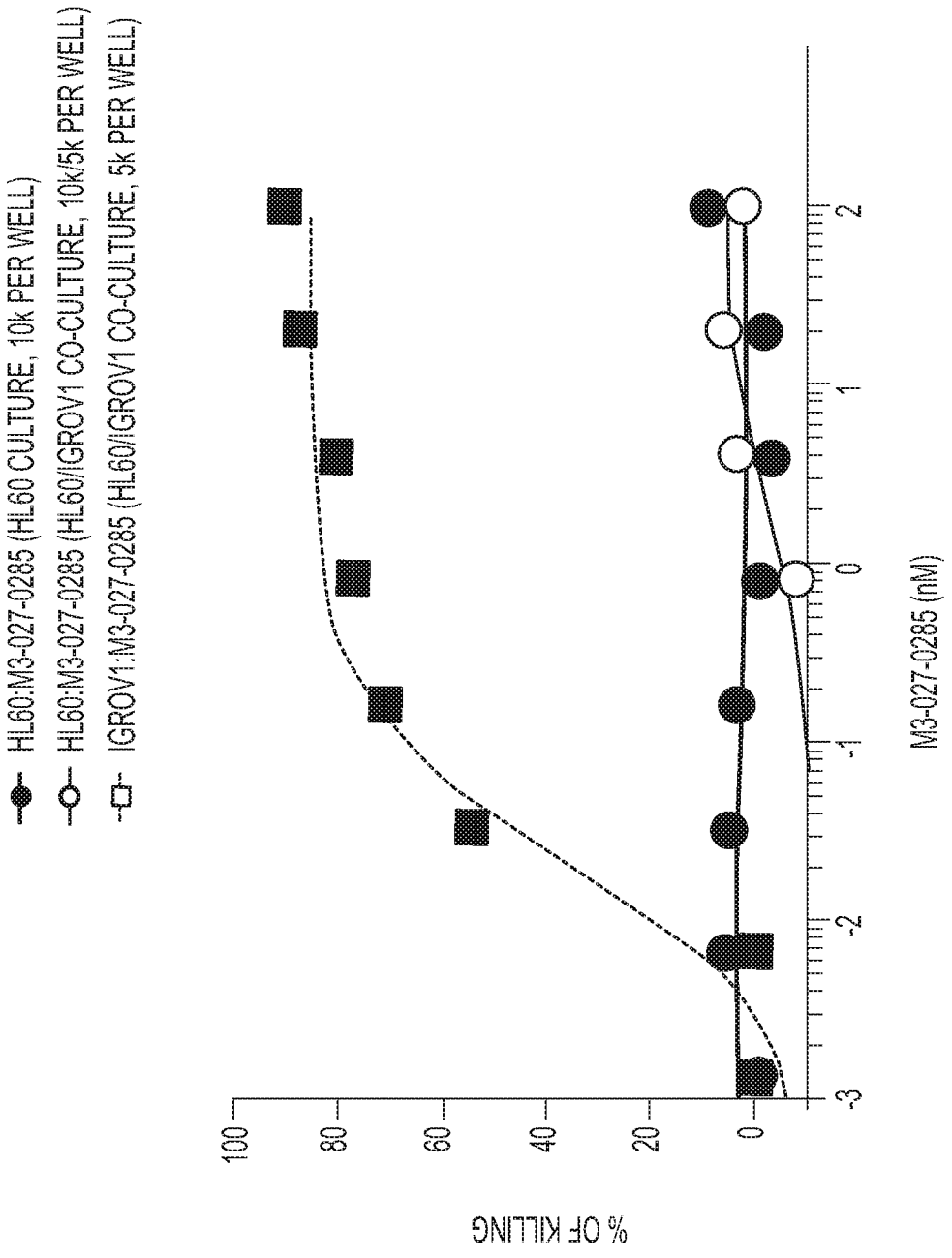
HL60:M3-027-0285 (HL60 CULTURE, 10k PER WELL)
HL60:M3-027-0285 (HL60/IGROV1 CO-CULTURE, 10k/5k PER WELL)
IGROV1:M3-027-0285 (HL60/IGROV1 CO-CULTURE, 5k PER WELL)
% OF KILLING
100
80
60
40
20
0
-3
-2
-1
0
1
2
M3-027-0285 (nM)

FIG. 6C

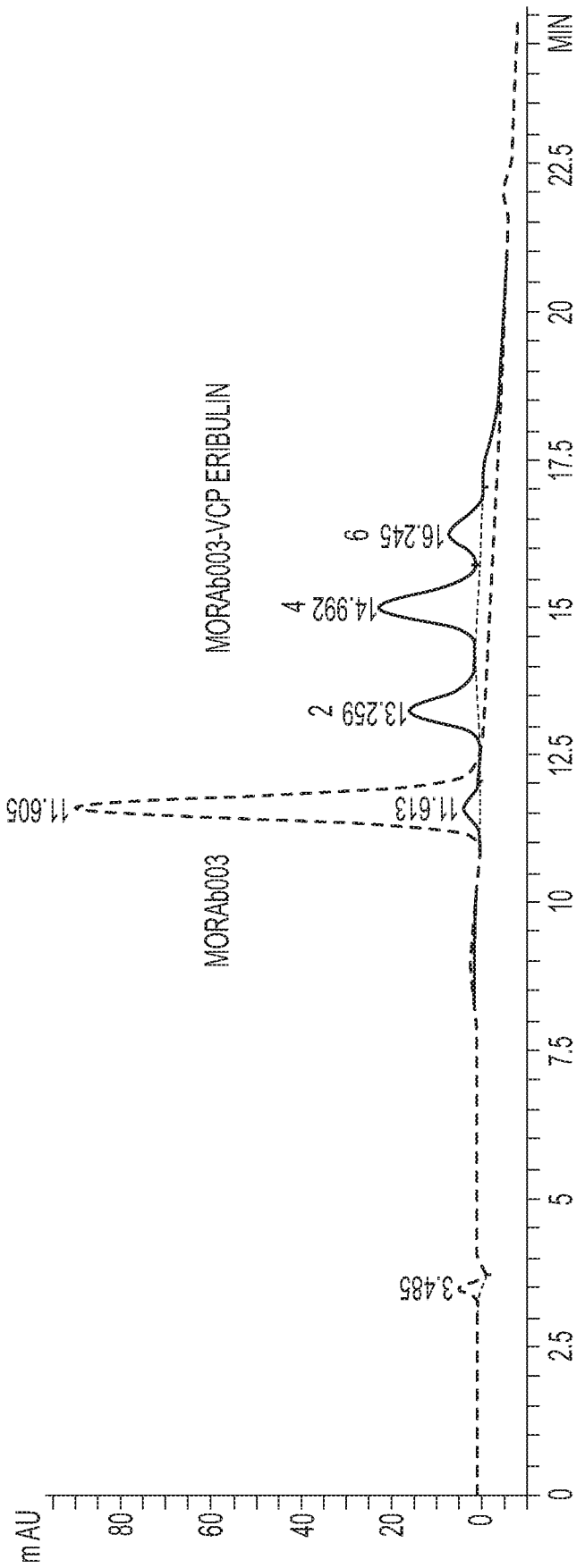
MORAb003-VCP ERIBULIN
MORAb003
11.605
11.613
13.259
14.992
16.245
3.485
mAU
MIN
0
20
40
60
80
2.5
5
7.5
10
12.5
15
17.5
20
22.5

FIG. 7A

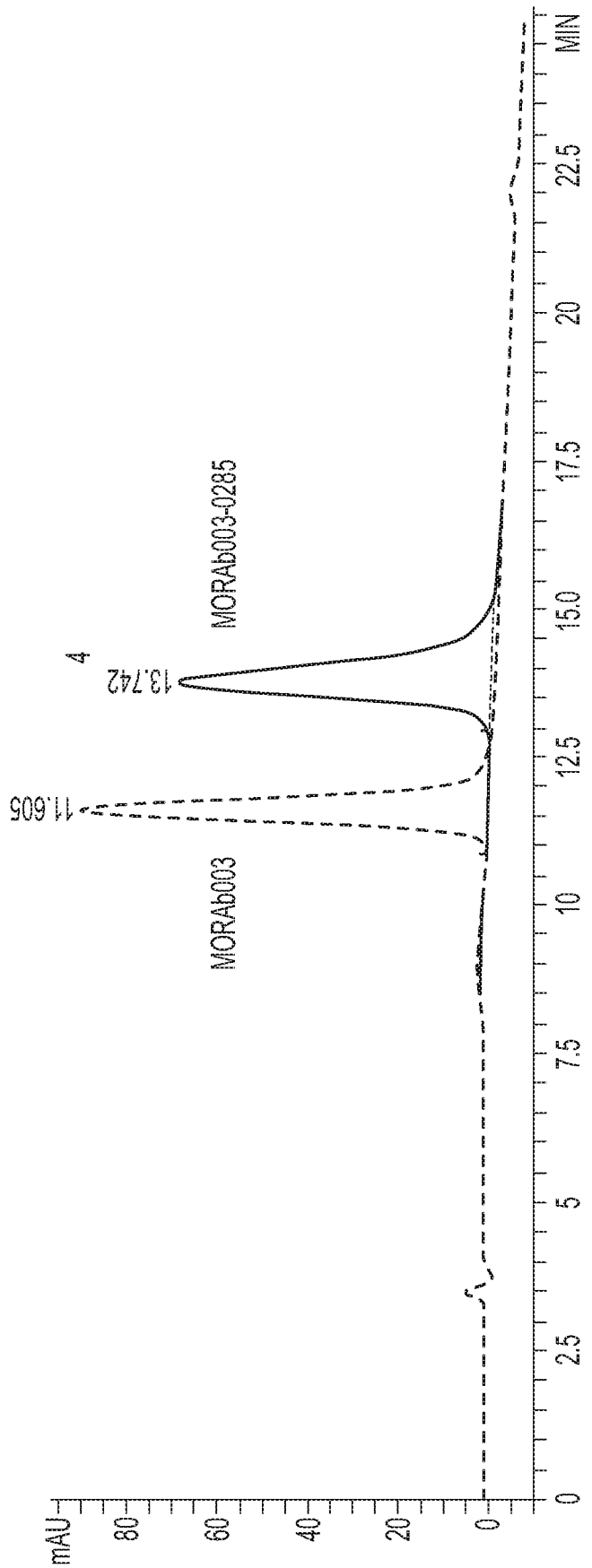
mAU
80
60
40
20
0
0
2.5
5
7.5
10
12.5
15.0
17.5
20
22.5
MIN
MORAb003
11.605
4
13.742
MORAb003-0285

FIG. 7B

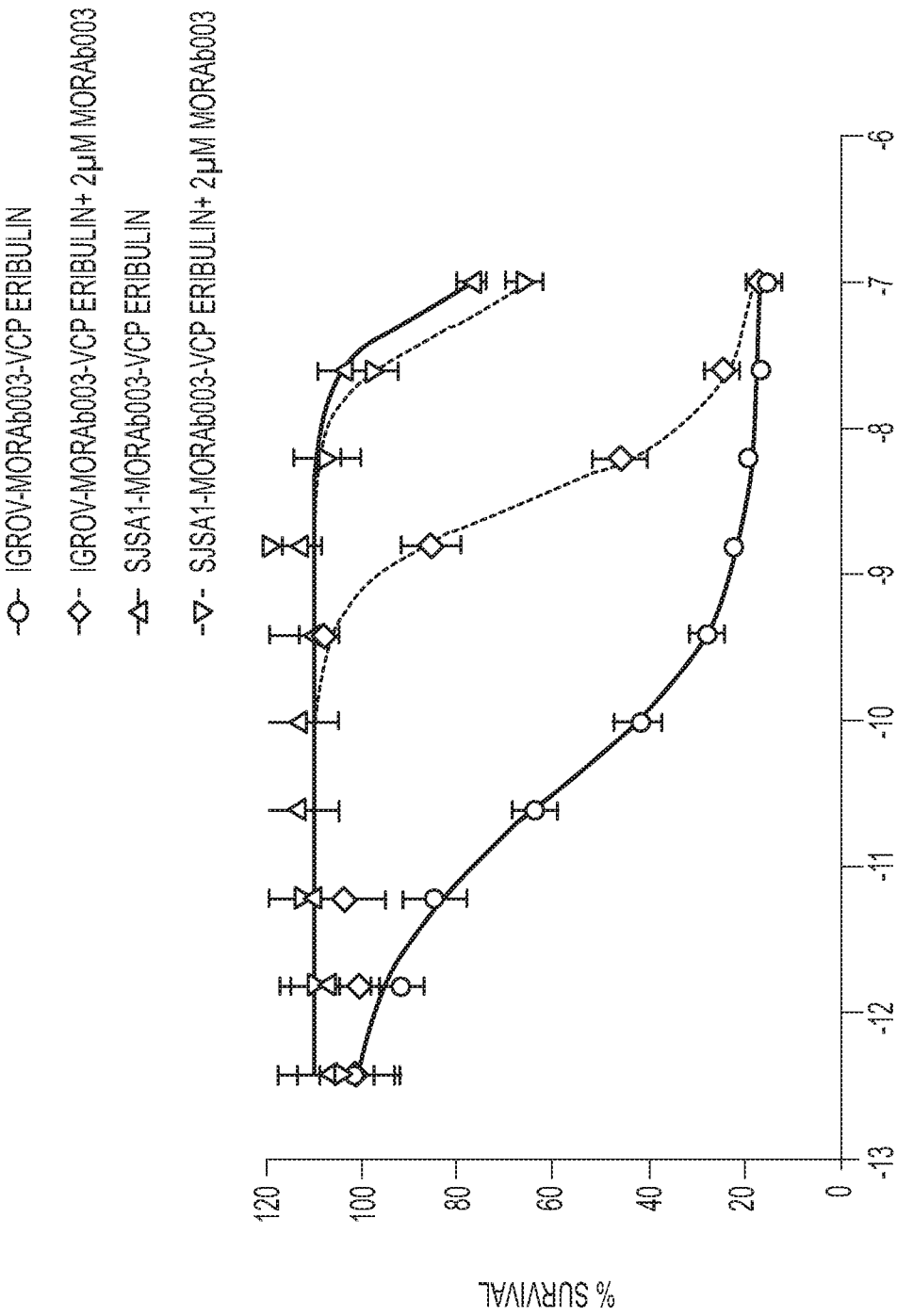
IGROV-MORAb003-VCP ERIBULIN
IGROV-MORAb003-VCP ERIBULIN+ 2μM MORAb003
SJSA1-MORAb003-VCP ERIBULIN
SJSA1-MORAb003-VCP ERIBULIN+ 2μM MORAb003
% SURVIVAL
120
100
80
60
40
20
0
-6
-7
-8
-9
-10
-11
-12
-13
CONJUGATE CONCENTRATION, LOG (M)

FIG. 8

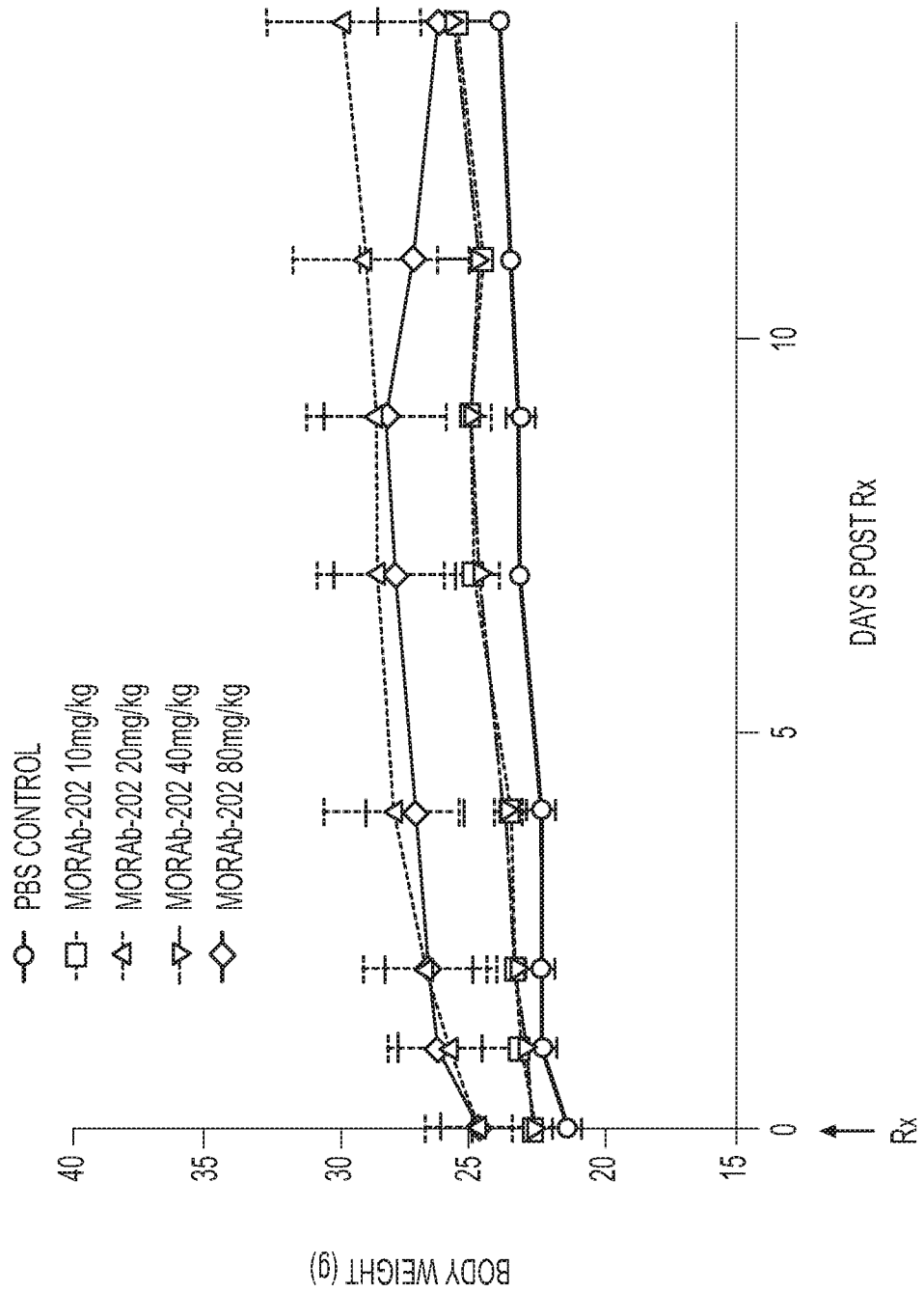
PBS CONTROL
MORAb-202 10mg/kg
MORAb-202 20mg/kg
MORAb-202 40mg/kg
MORAb-202 80mg/kg
BODY WEIGHT (g)
40
35
30
25
20
15
0
5
10
Rx
DAYS POST Rx

FIG. 9

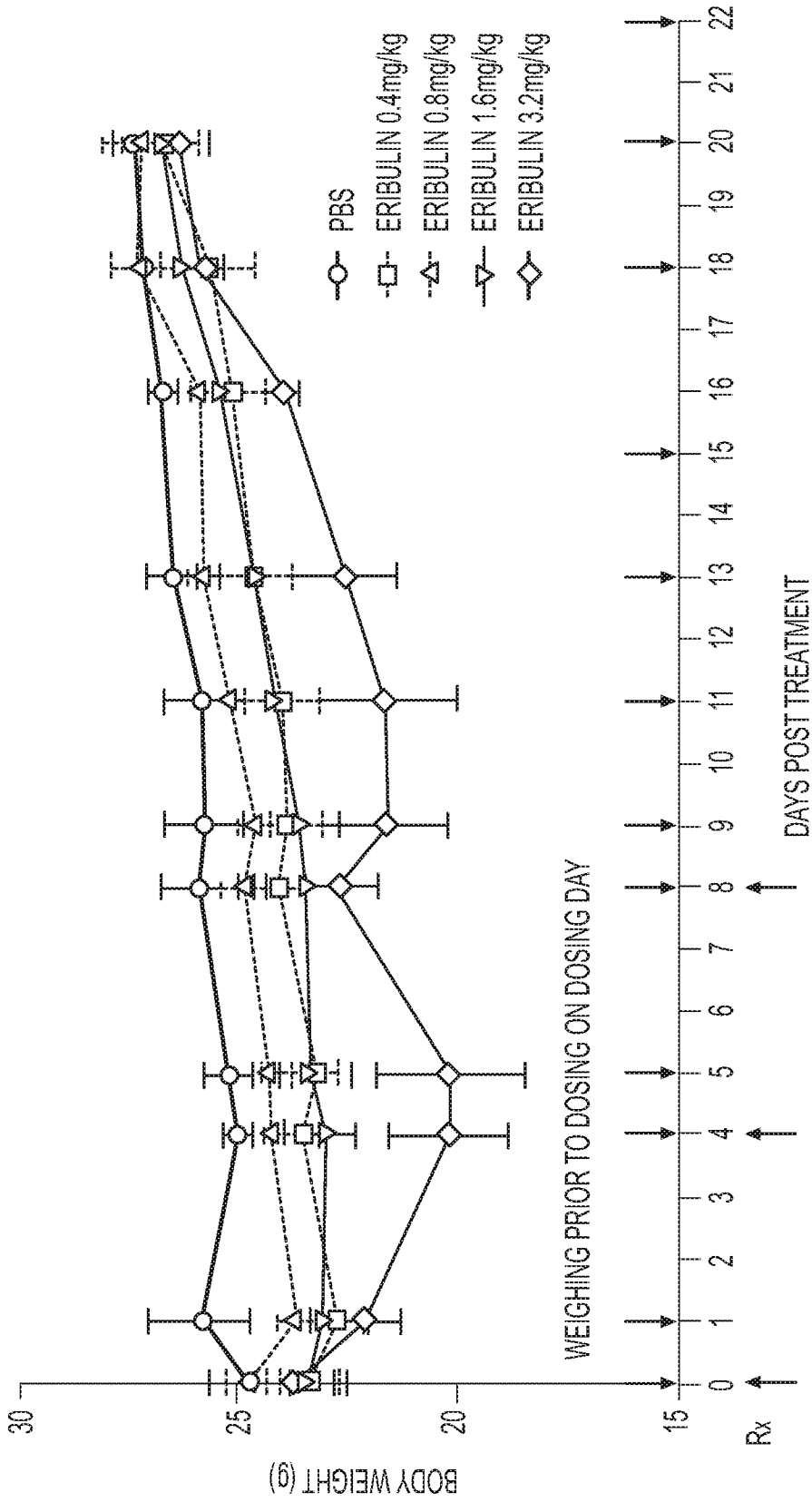
PBS
ERIBULIN 0.4mg/kg
ERIBULIN 0.8mg/kg
ERIBULIN 1.6mg/kg
ERIBULIN 3.2mg/kg
BODY WEIGHT (g)
30
25
20
15
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Rx
WEIGHING PRIOR TO DOSING ON DOSING DAY
DAYS POST TREATMENT

FIG. 10

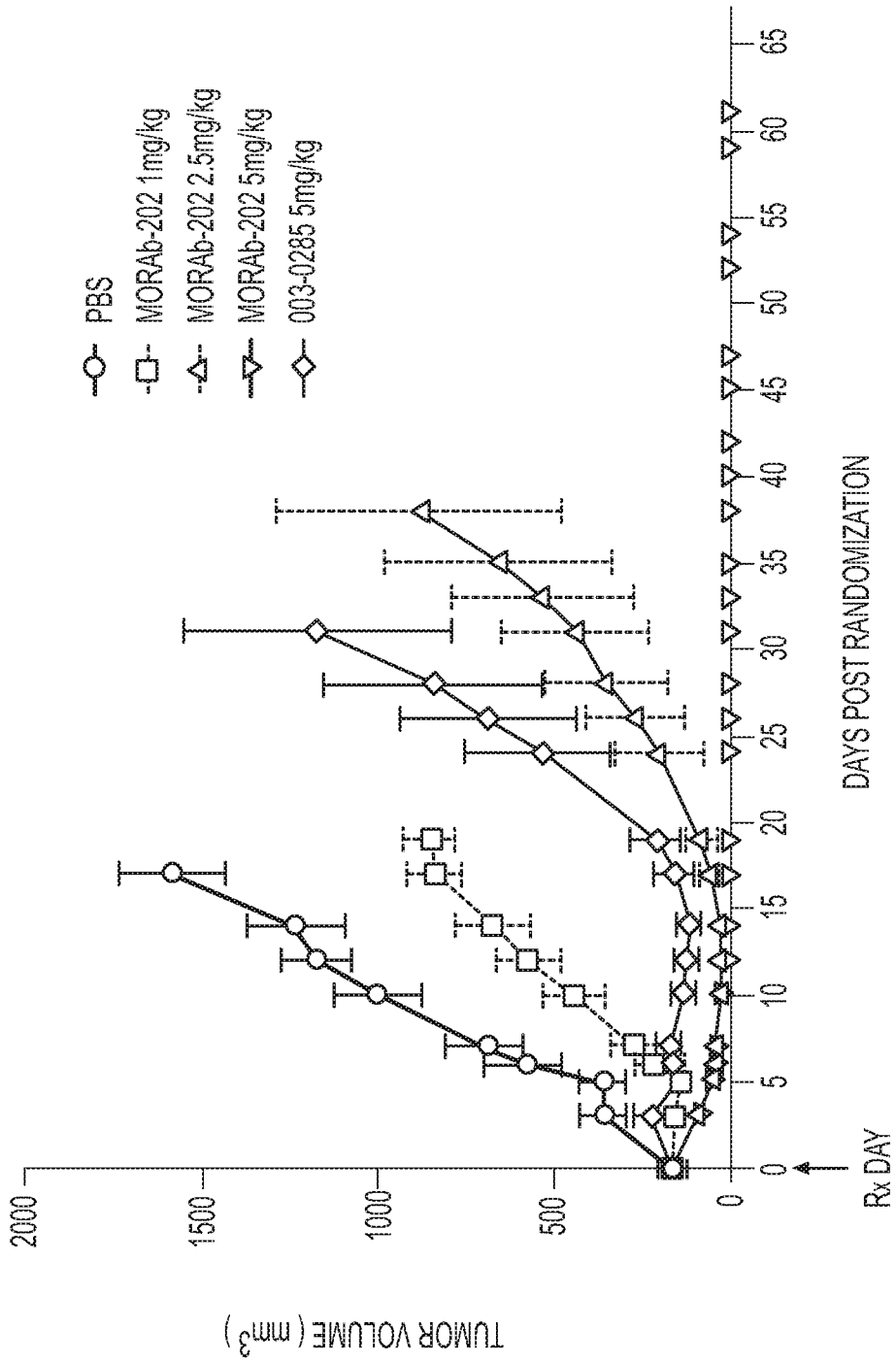
PBS
MORAb-202 1mg/kg
MORAb-202 2.5mg/kg
MORAb-202 5mg/kg
003-0285 5mg/kg
TUMOR VOLUME (mm3)
2000
1500
1000
500
0
Rx DAY
0
5
10
15
20
25
30
35
40
45
50
55
60
65
DAYS POST RANDOMIZATION

FIG. 11

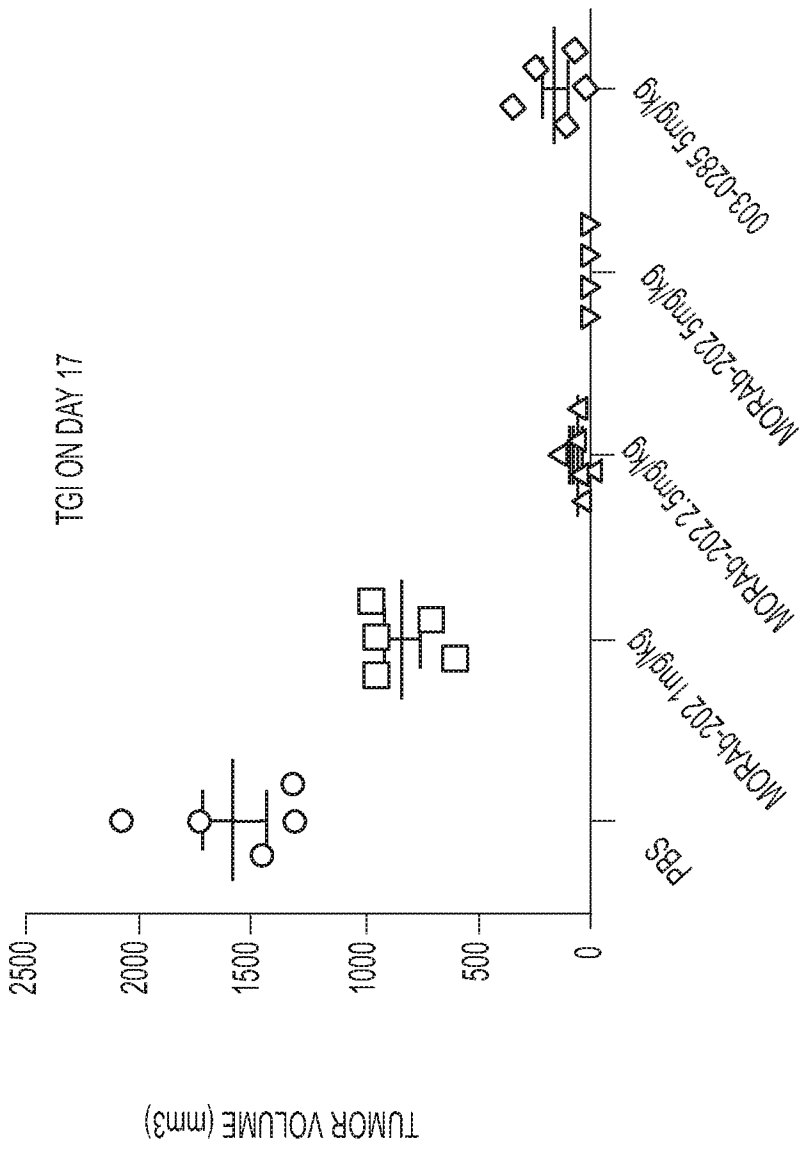
TGI ON DAY 17
TUMOR VOLUME (mm3)
2500
2000
1500
1000
500
0
PBS
MORAb-202 1mg/kg
MORAb-202 2.5mg/kg
MORAb-202 5mg/kg
003-0285 5mg/kg

FIG. 12

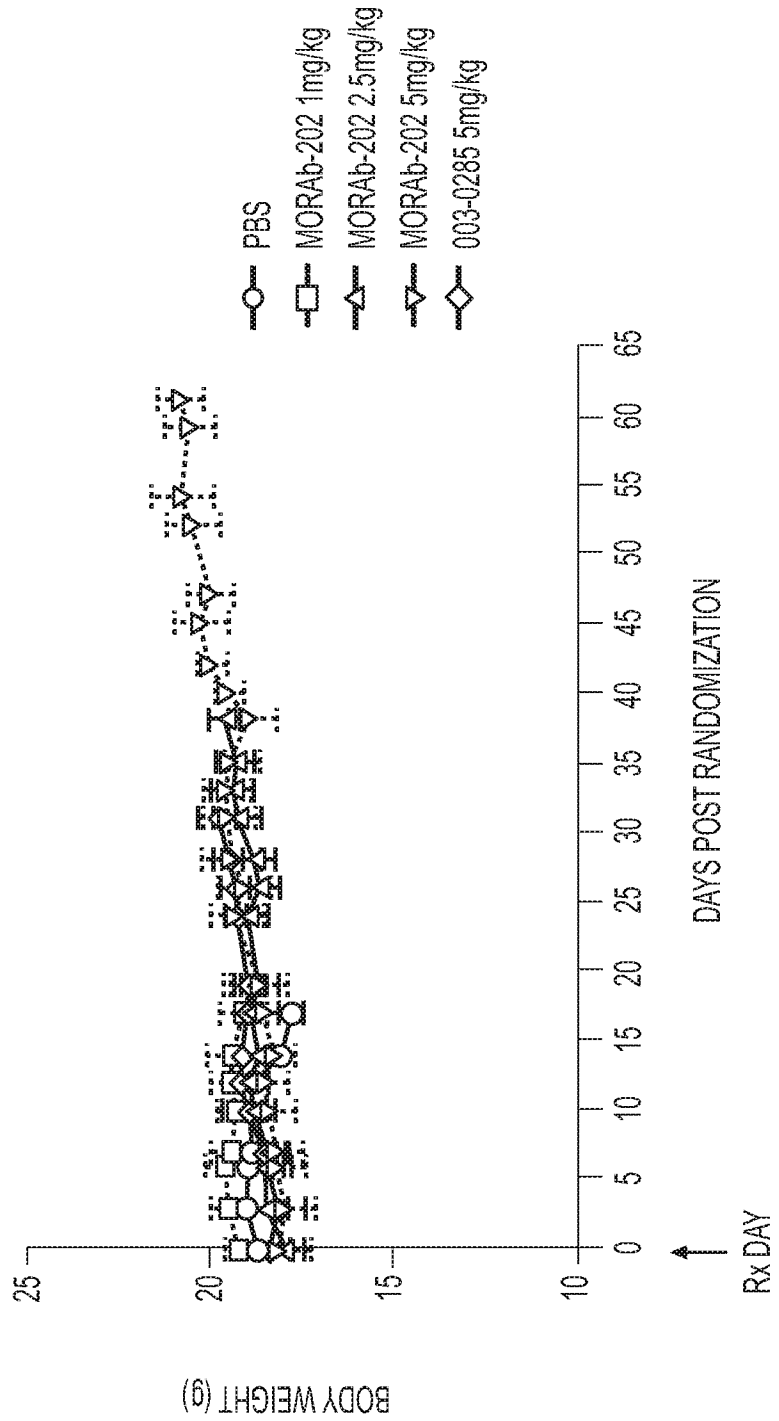
PBS
MORAb-202 1mg/kg
MORAb-202 2.5mg/kg
MORAb-202 5mg/kg
003-0285 5mg/kg
BODY WEIGHT (g)
25
20
15
10
0
5
10
15
20
25
30
35
40
45
50
55
60
65
DAYS POST RANDOMIZATION
Rx DAY

FIG. 13

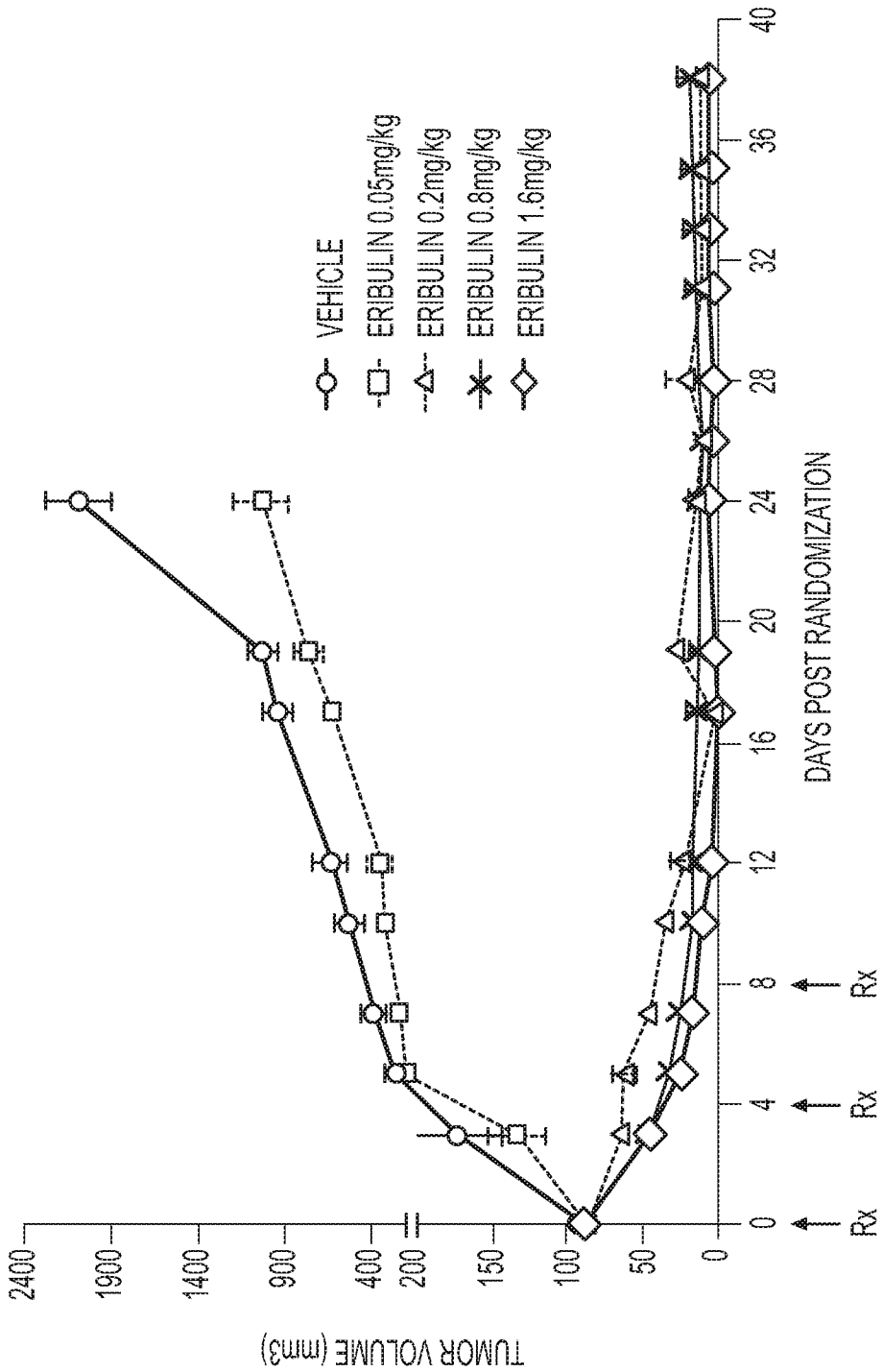
VEHICLE
ERIBULIN 0.05mg/kg
ERIBULIN 0.2mg/kg
ERIBULIN 0.8mg/kg
ERIBULIN 1.6mg/kg
TUMOR VOLUME (mm3)
2400
1900
1400
900
400
200
150
100
50
0
DAYS POST RANDOMIZATION
0
4
8
12
16
20
24
28
32
36
40
Rx
Rx
Rx

FIG. 14

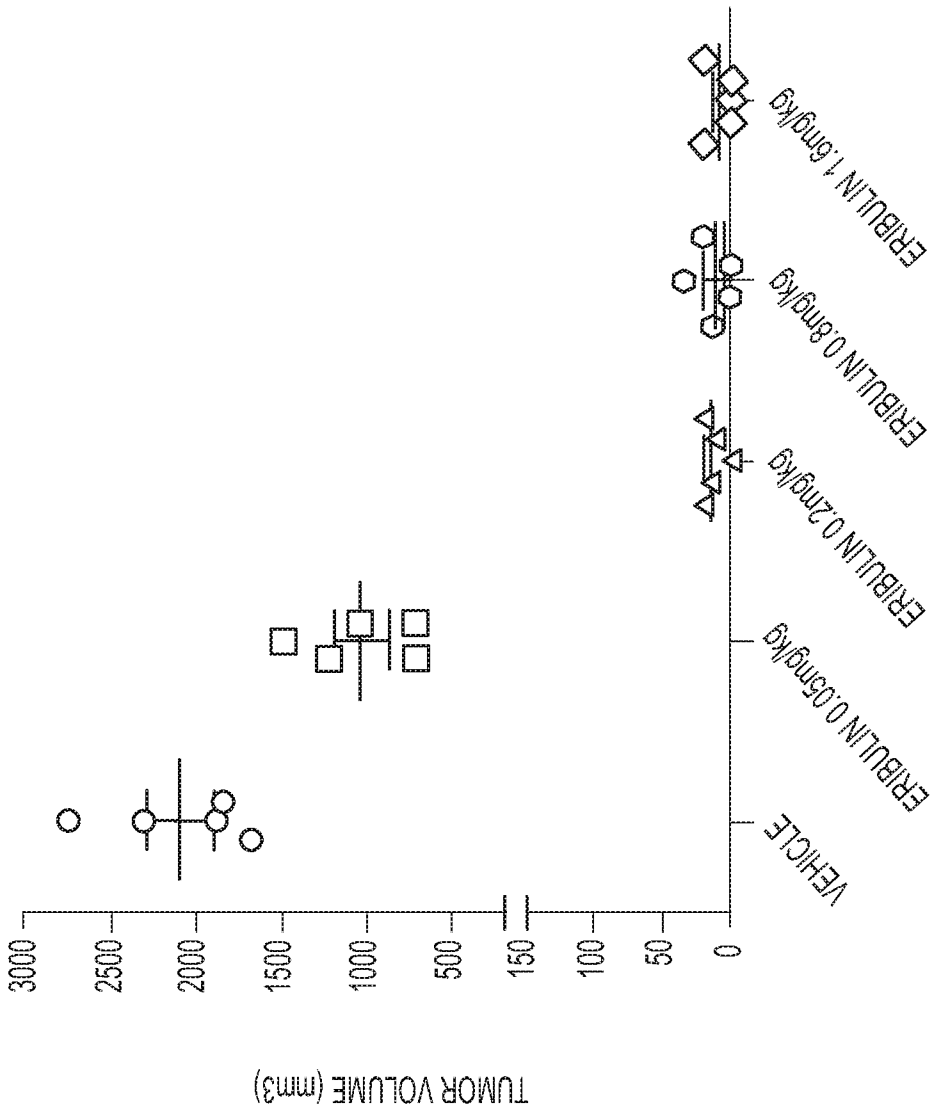
TUMOR VOLUME (mm3)
3000
2500
2000
1500
1000
500
150
100
50
0
VEHICLE
ERIBULIN 0.05mg/kg
ERIBULIN 0.2mg/kg
ERIBULIN 0.8mg/kg
ERIBULIN 1.6mg/kg

FIG. 15

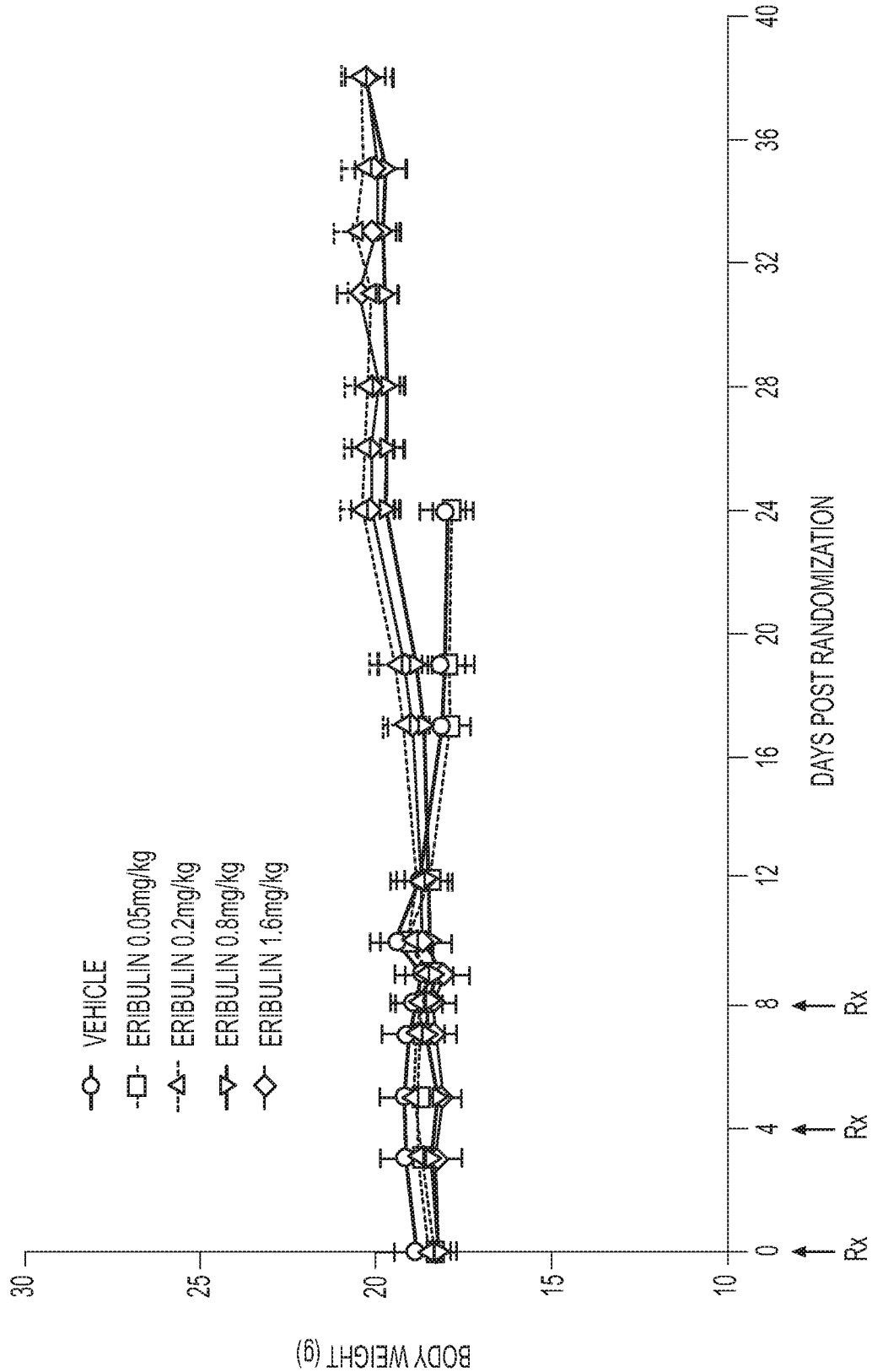
VEHICLE
ERIBULIN 0.05mg/kg
ERIBULIN 0.2mg/kg
ERIBULIN 0.8mg/kg
ERIBULIN 1.6mg/kg
BODY WEIGHT (g)
30
25
20
15
10
0
4
8
12
16
20
24
28
32
36
40
DAYS POST RANDOMIZATION
Rx
Rx
Rx

FIG. 16

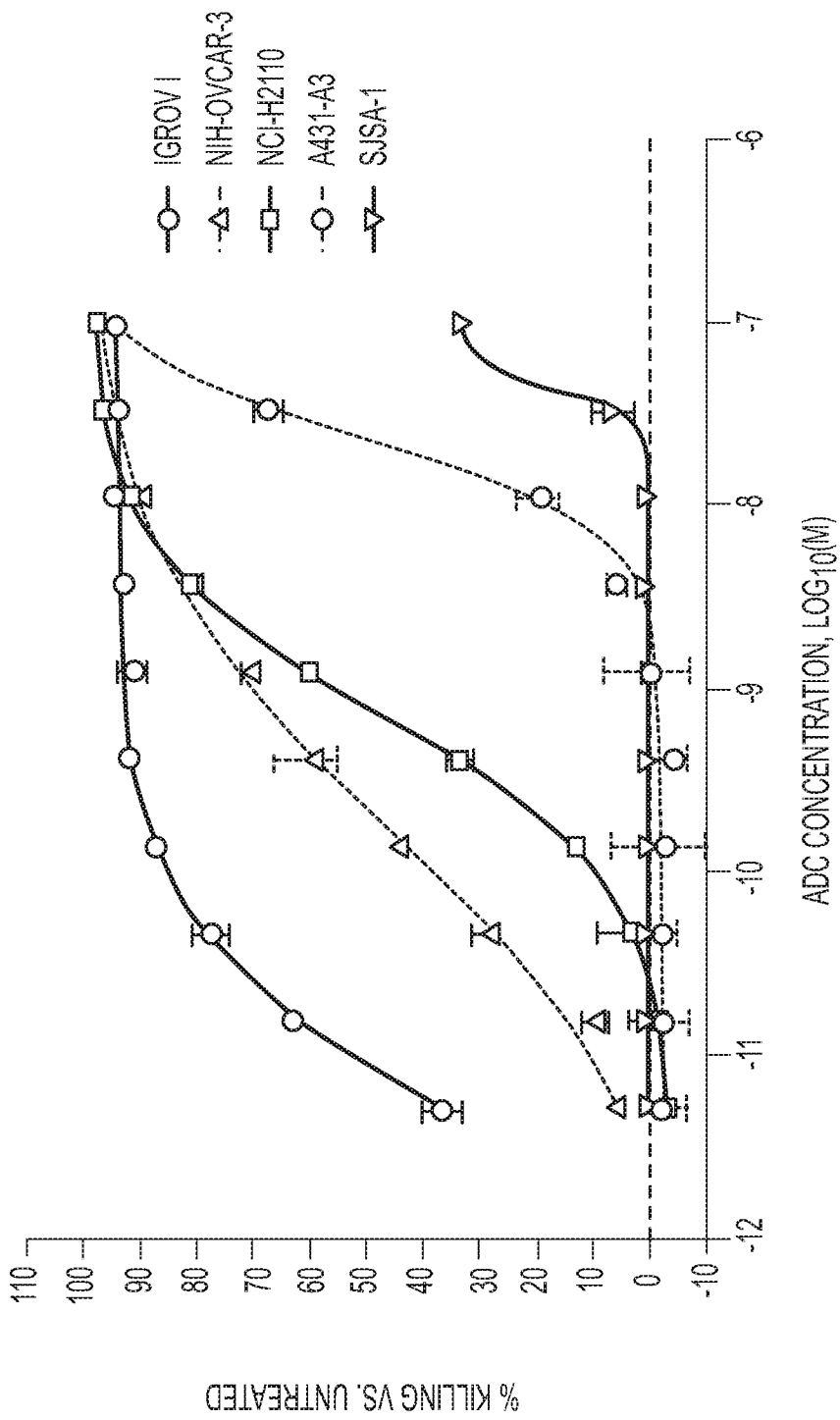
IGROV I
NIH-OVCAR-3
NCI-H2110
A431-A3
SJSA-1
% KILLING VS. UNTREATED
110
100
90
80
70
60
50
40
30
20
10
0
-10
-12
-11
-10
-9
-8
-7
-6
ADC CONCENTRATION, LOG10(M)

FIG. 17

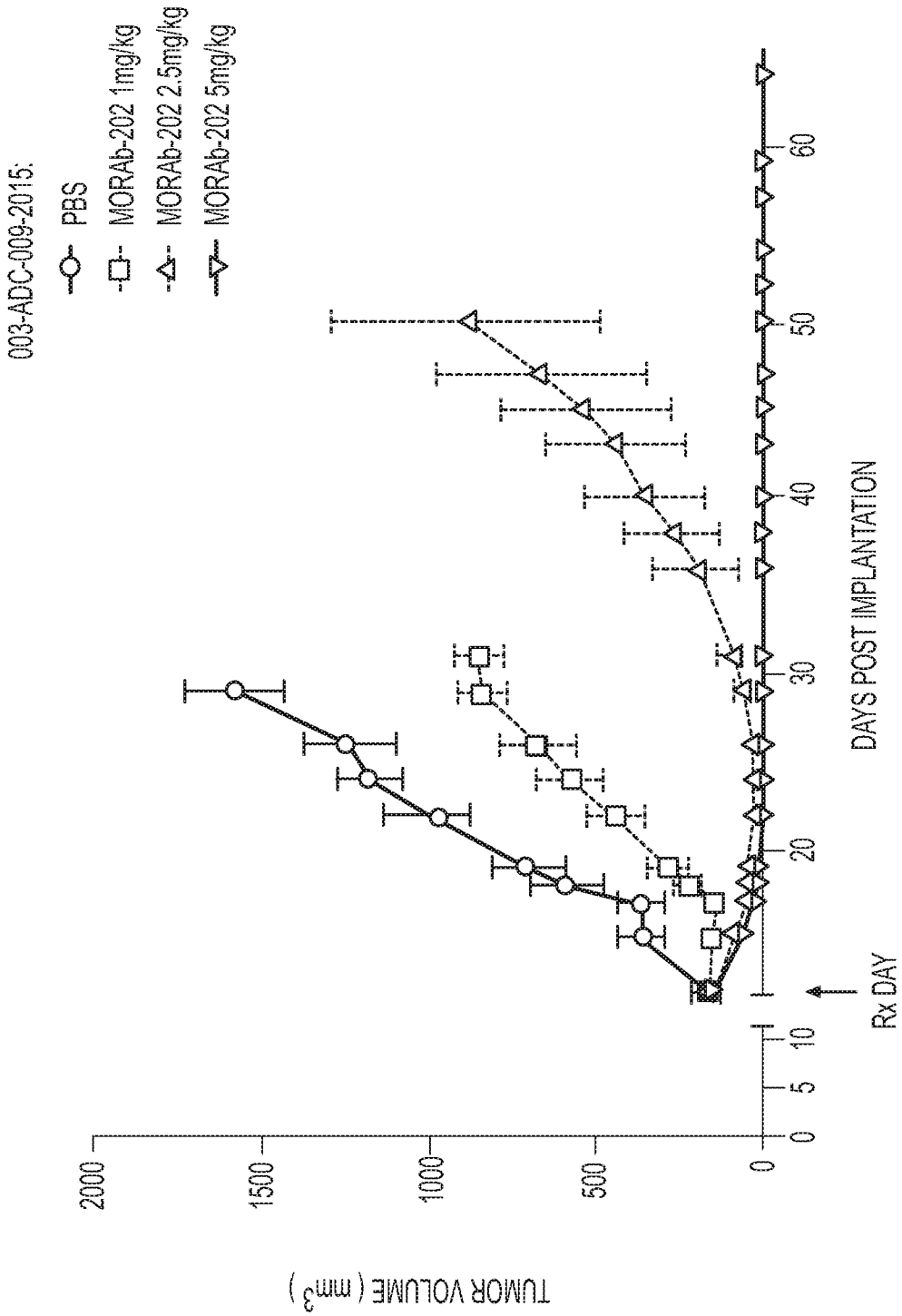
003-ADC-009-2015:
PBS
MORAb-202 1mg/kg
MORAb-202 2.5mg/kg
MORAb-202 5mg/kg
TUMOR VOLUME (mm3)
2000
1500
1000
500
0
0
5
10
20
30
40
50
60
Rx DAY
DAYS POST IMPLANTATION

FIG. 18

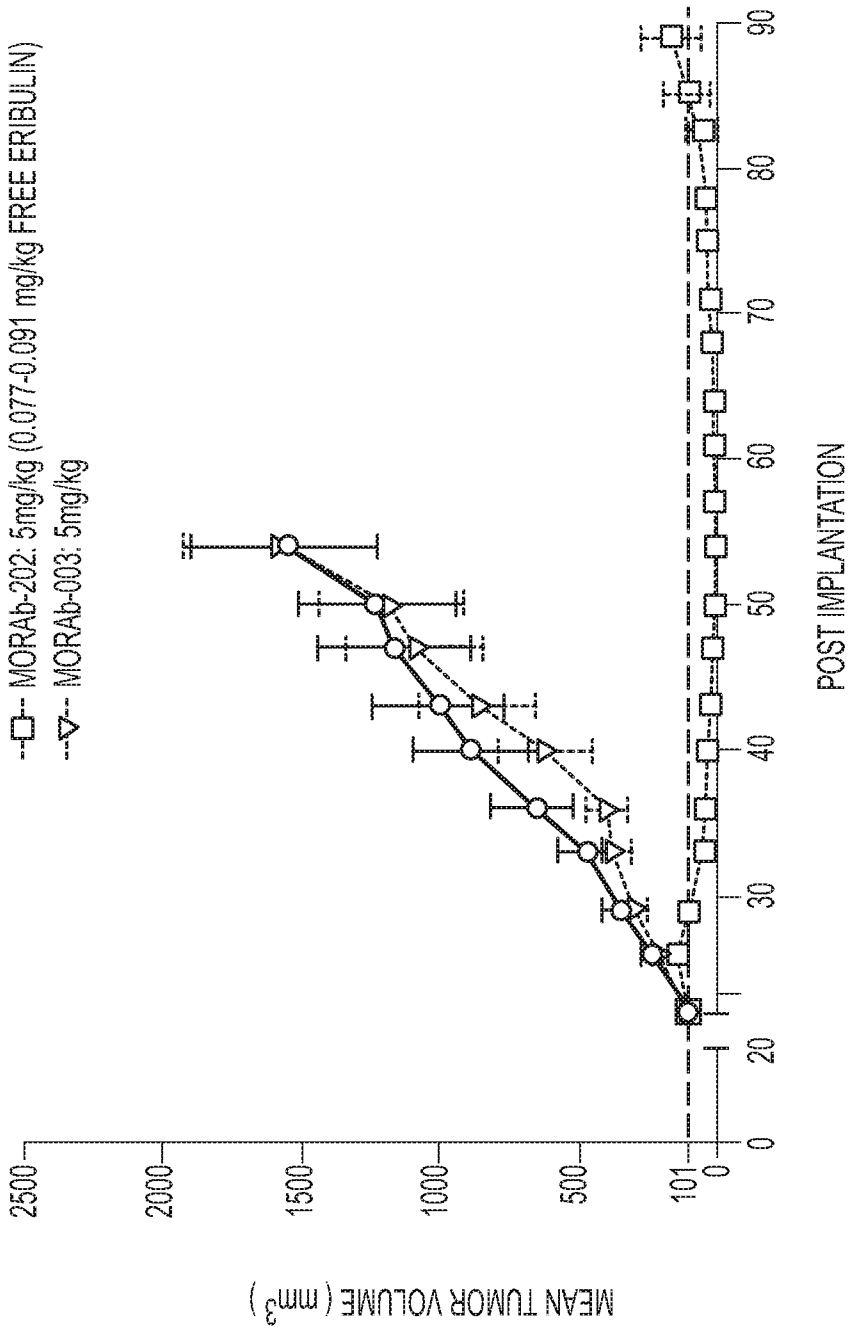
MORAb-202: 5mg/kg (0.077-0.091 mg/kg FREE ERIBULIN)
MORAb-003: 5mg/kg
MEAN TUMOR VOLUME (mm^3)
2500
2000
1500
1000
500
101
0
0
20
30
40
50
60
70
80
90
POST IMPLANTATION

FIG. 19A

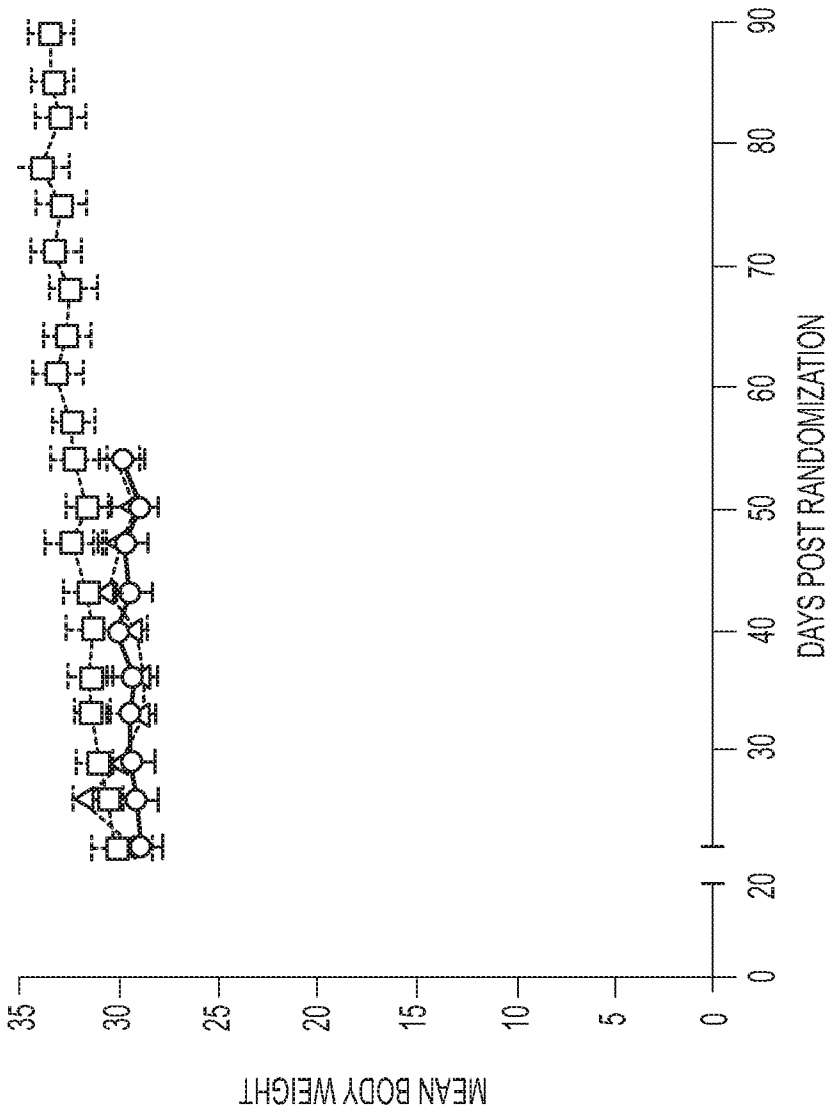
MEAN BODY WEIGHT
35
30
25
20
15
10
5
0
0
20
30
40
50
60
70
80
90
DAYS POST RANDOMIZATION

FIG. 19B

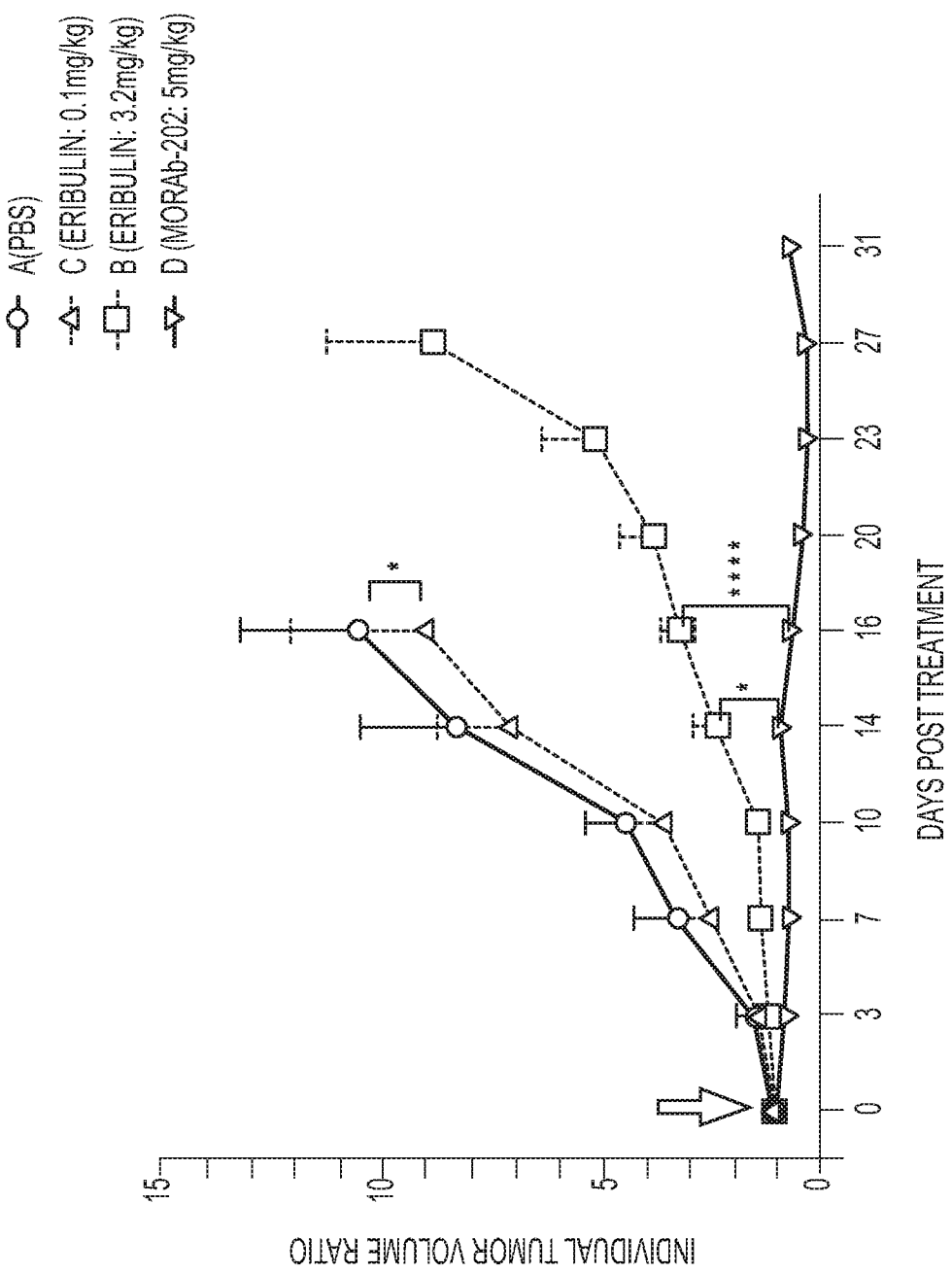
A(PBS)
C (ERIBULIN: 0.1mg/kg)
B (ERIBULIN: 3.2mg/kg)
D (MORAb-202: 5mg/kg)
INDIVIDUAL TUMOR VOLUME RATIO
0
5
10
15
DAYS POST TREATMENT
0
3
7
10
14
16
20
23
27
31
*
*
****

FIG. 20A

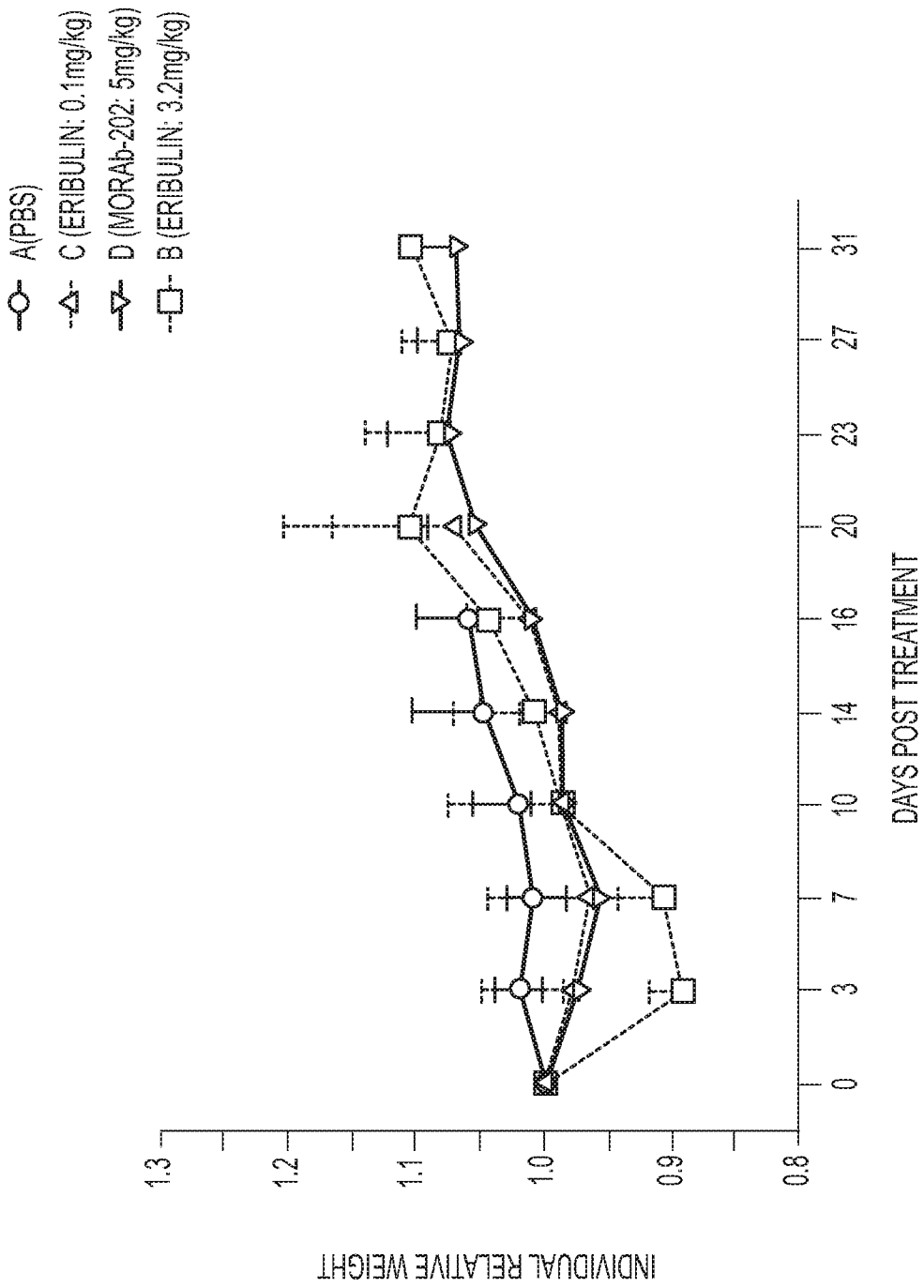
A(PBS)
C (ERIBULIN: 0.1mg/kg)
D (MORAb-202: 5mg/kg)
B (ERIBULIN: 3.2mg/kg)
INDIVIDUAL RELATIVE WEIGHT
1.3
1.2
1.1
1.0
0.9
0.8
0
3
7
10
14
16
20
23
27
31
DAYS POST TREATMENT

FIG. 20B

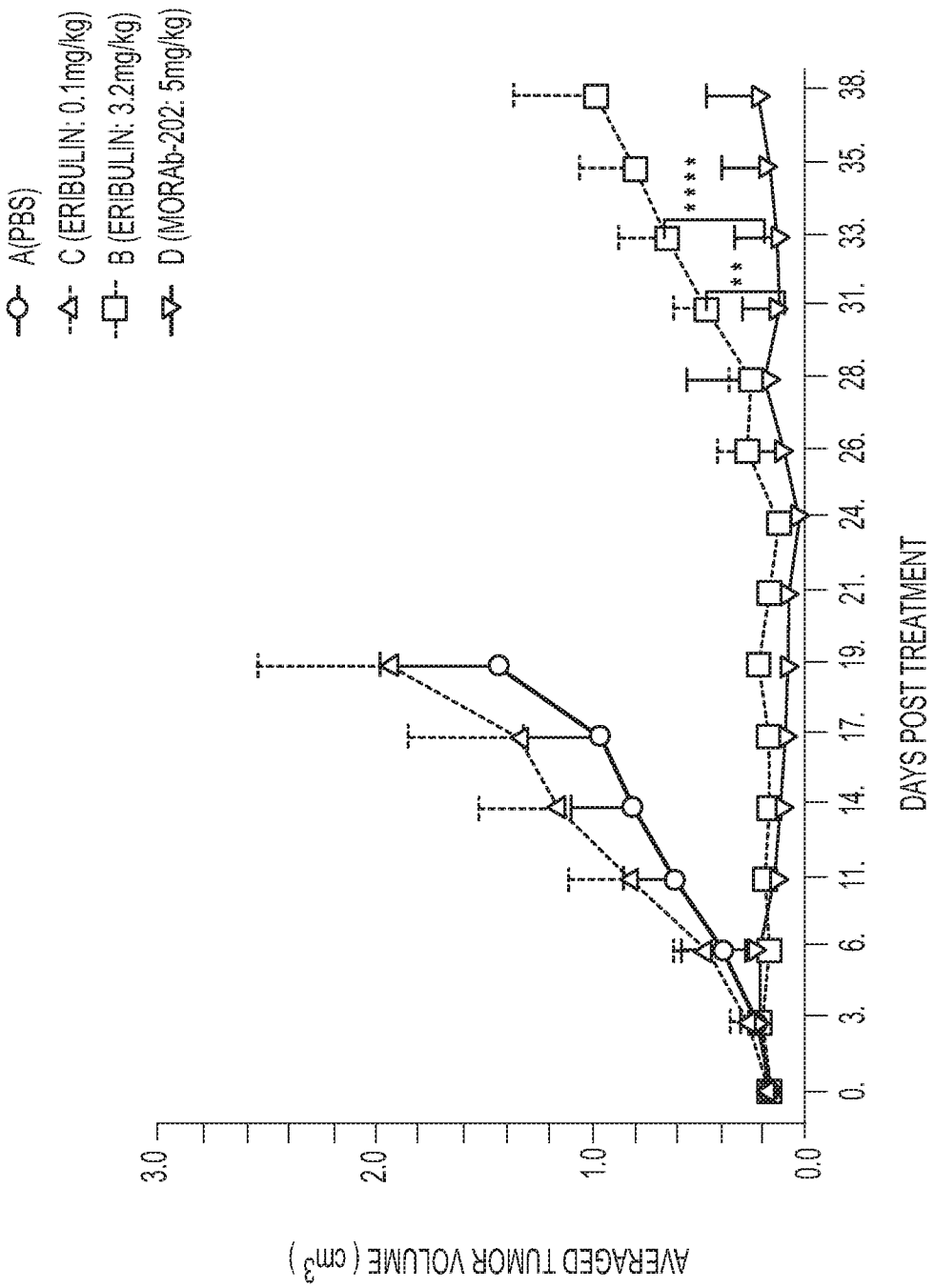
A(PBS)
C (ERIBULIN: 0.1mg/kg)
B (ERIBULIN: 3.2mg/kg)
D (MORAb-202: 5mg/kg)
AVERAGED TUMOR VOLUME (cm3)
3.0
2.0
1.0
0.0
0.
3.
6.
11.
14.
17.
19.
21.
24.
26.
28.
31.
33.
35.
38.
DAYS POST TREATMENT
****
**

FIG. 20C

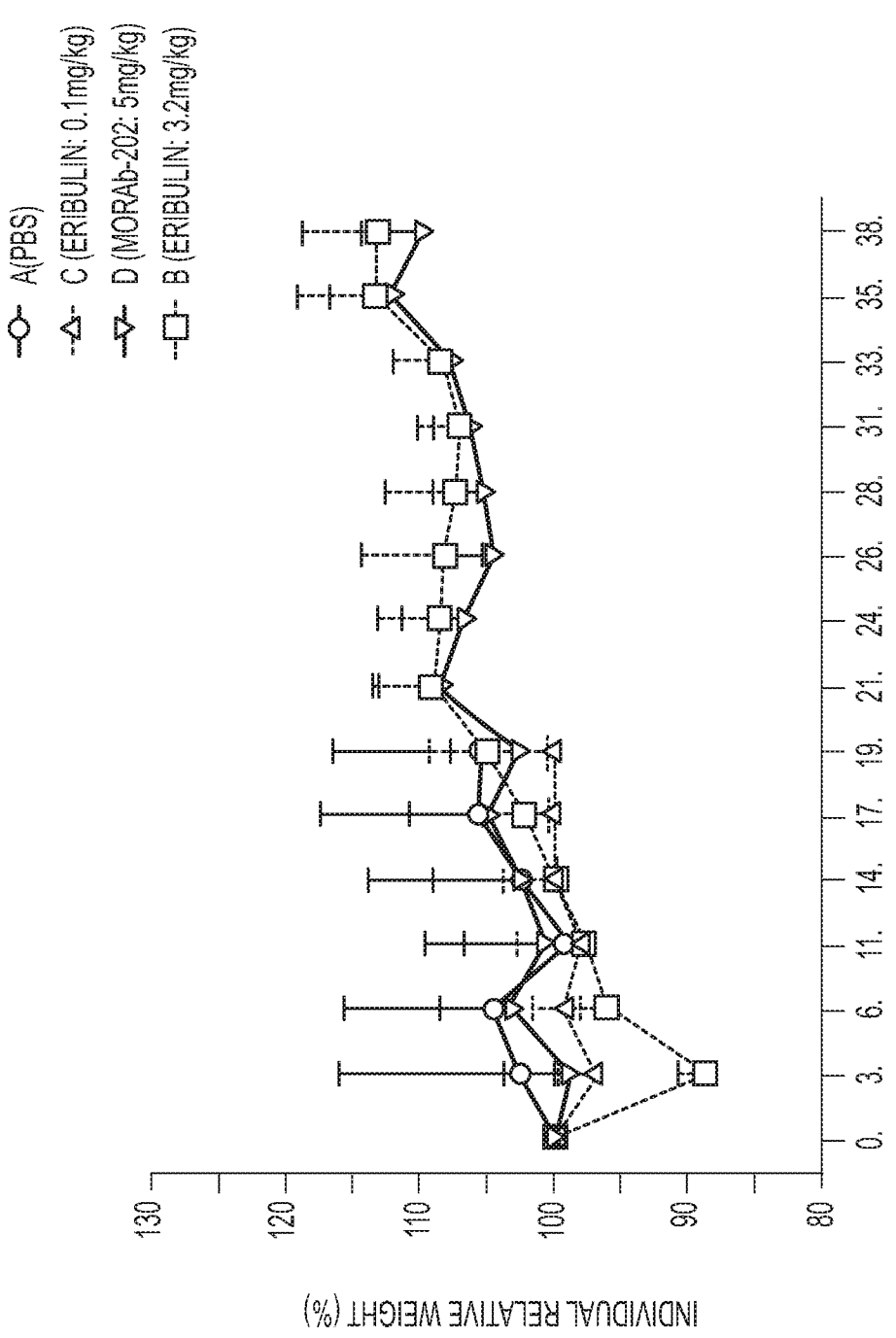
A(PBS)
C (ERIBULIN: 0.1mg/kg)
D (MORAb-202: 5mg/kg)
B (ERIBULIN: 3.2mg/kg)
INDIVIDUAL RELATIVE WEIGHT (%)
130
120
110
100
90
80
0.
3.
6.
11.
14.
17.
19.
21.
24.
26.
28.
31.
33.
35.
38.
DAYS POST TREATMENT

FIG. 20D

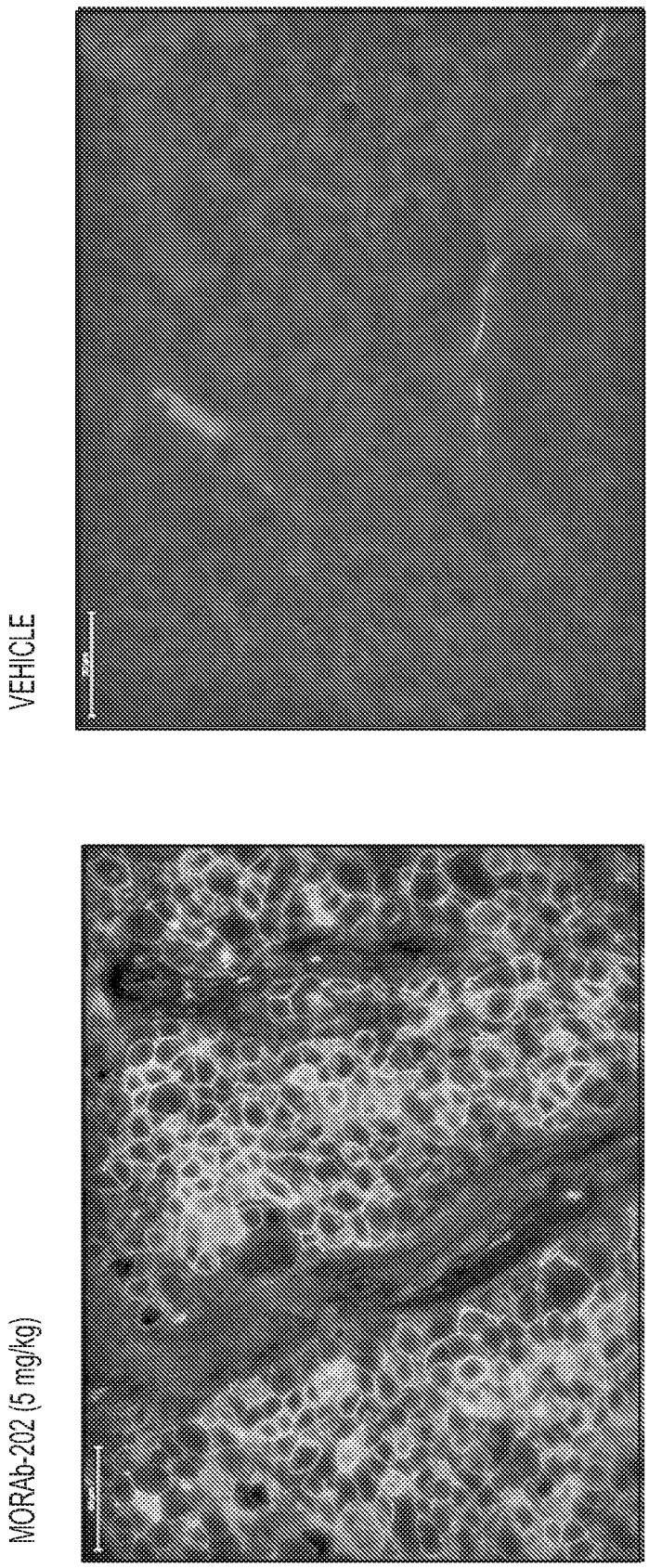

FIG. 21A

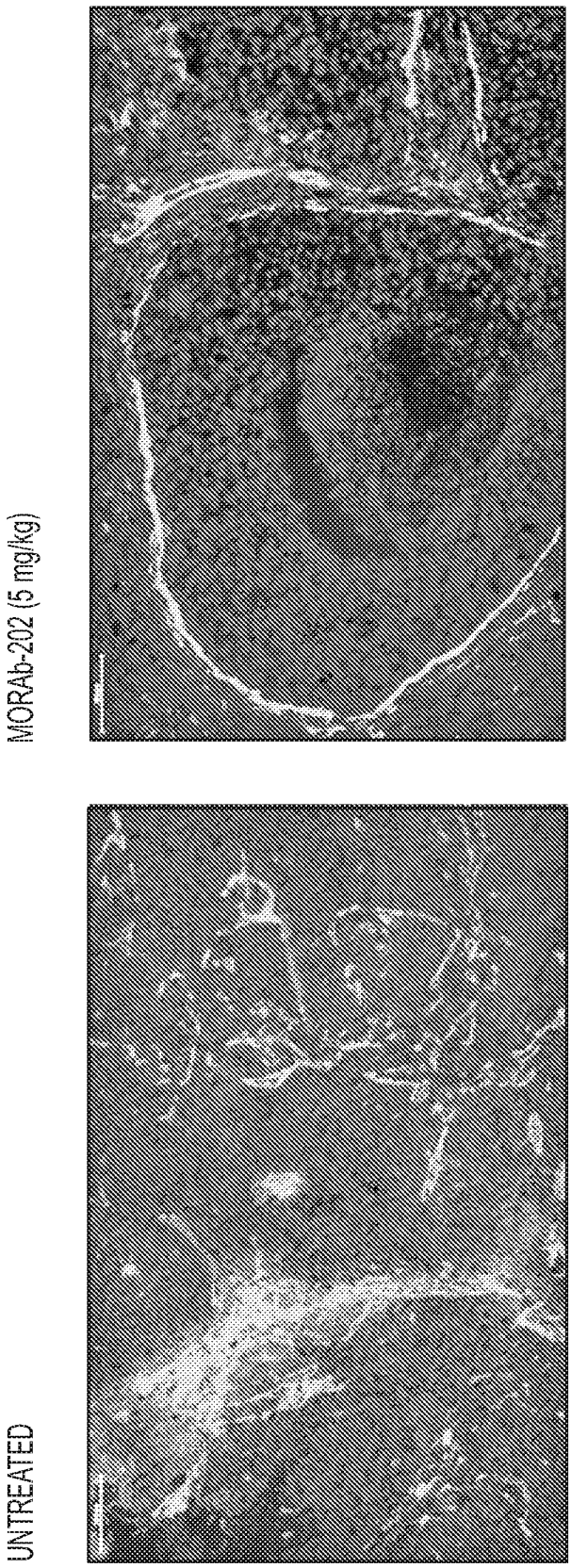

FIG. 21B

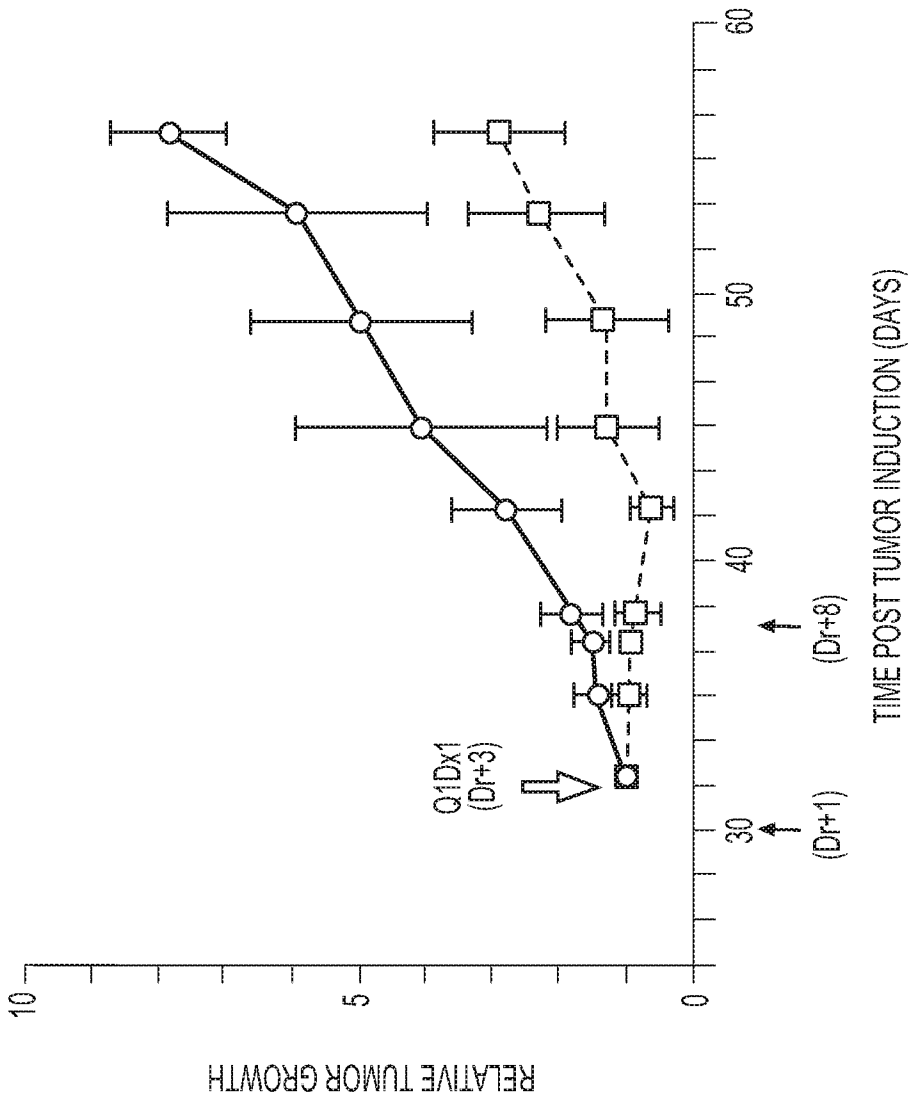
RELATIVE TUMOR GROWTH
10
5
0
60
50
40
30
TIME POST TUMOR INDUCTION (DAYS)
(Dr+8)
(Dr+1)
Q1Dx1
(Dr+3)

FIG. 21C

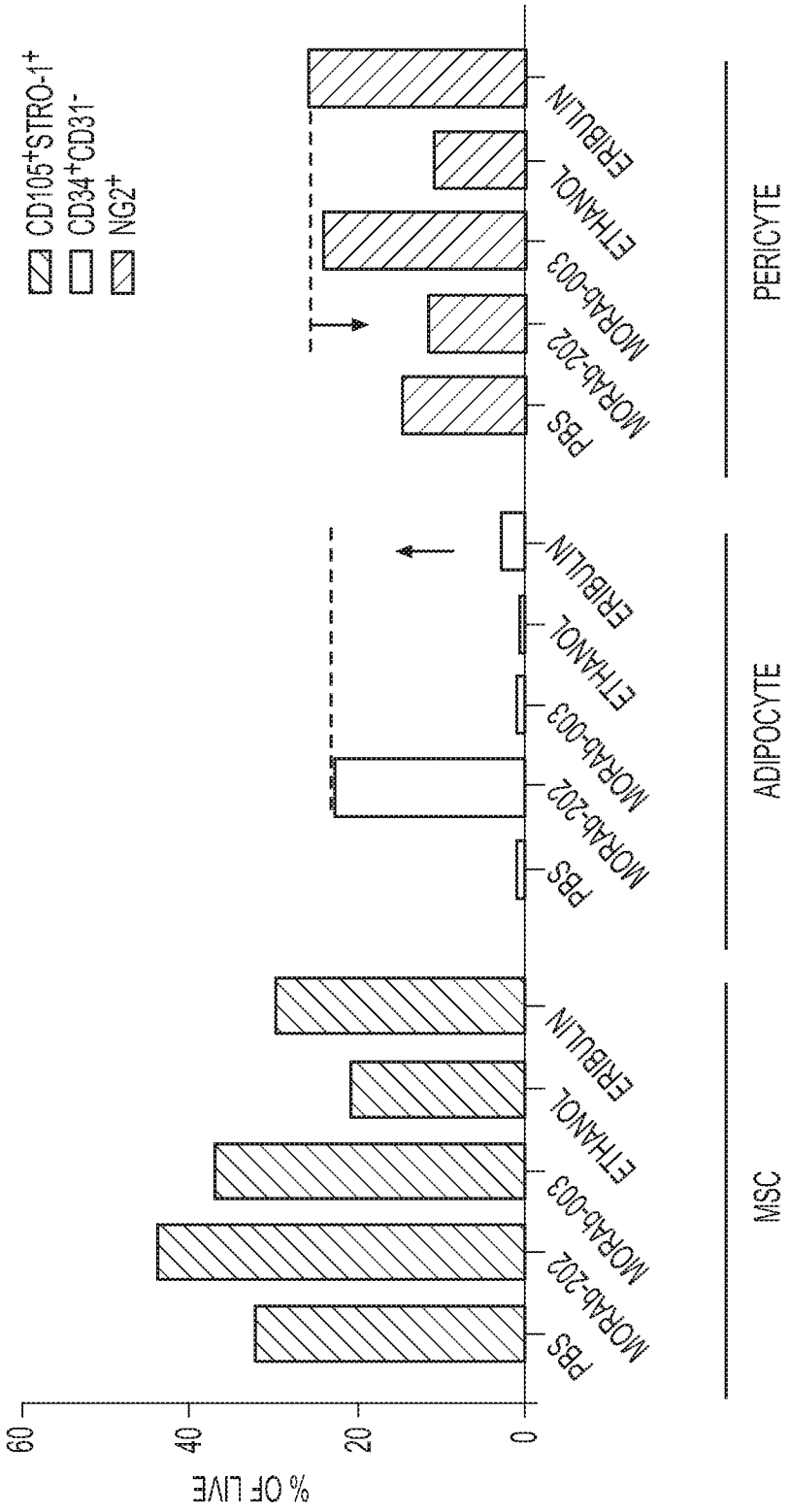
CD105+STRO-1+
CD34+CD31-
NG2+
% OF LIVE
60
40
20
0
PBS
MORAb-202
MORAb-003
ETHANOL
ERIBULIN
MSC
ADIPOCYTE
PERICYTE

FIG. 22

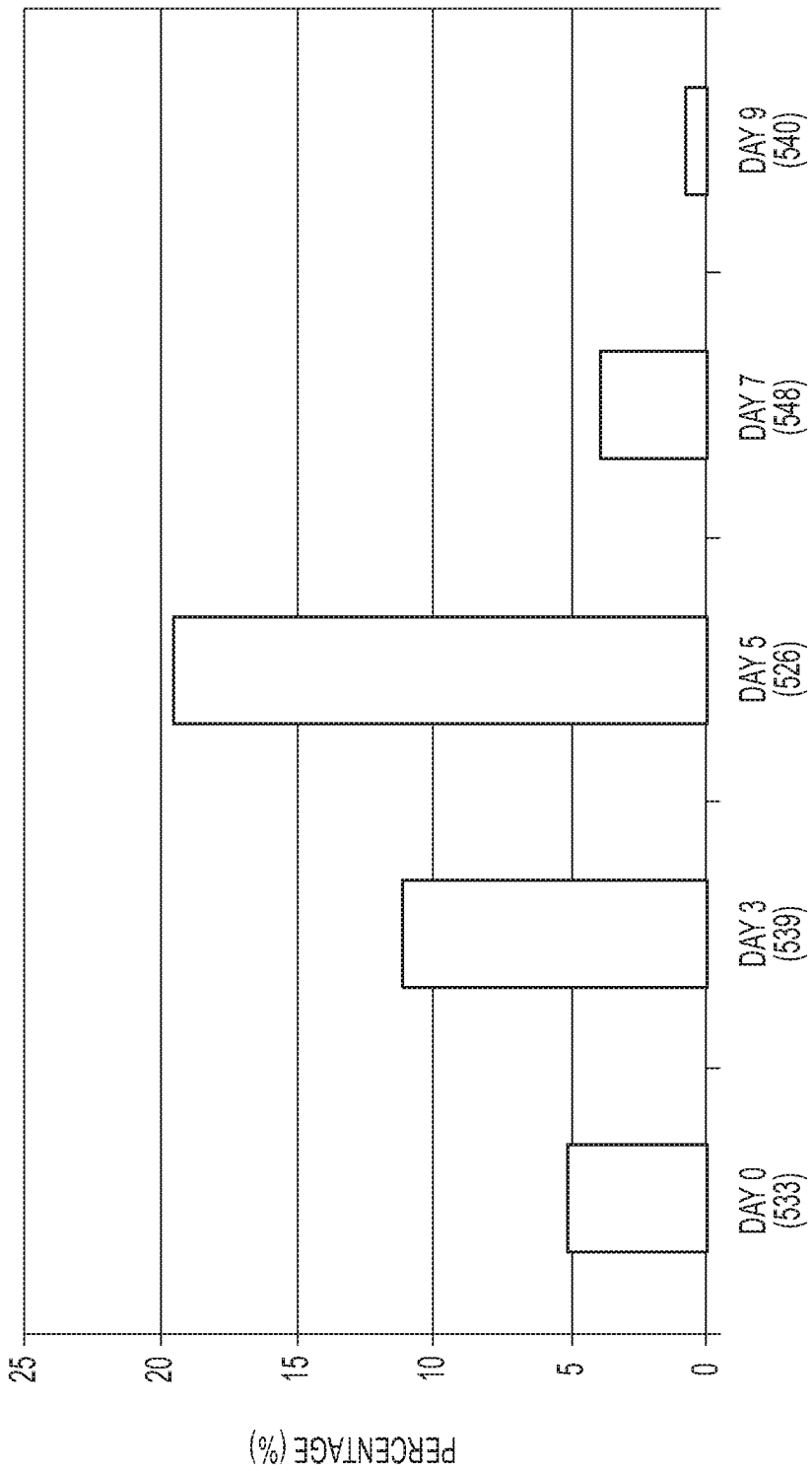
25
20
15
10
5
0
PERCENTAGE (%)
DAY 0 (533)
DAY 3 (539)
DAY 5 (526)
DAY 7 (548)
DAY 9 (540)

FIG. 23