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(54) Title: METHODS FOR THE TREATMENT OF TROP2 POSITIVE DISEASES

(57) Abstract: Disclosed herein are methods for treating a disease comprising administering an anti-Trop2 antibody, or an antigen-binding fragment thereof, conjugated with a maytansinoid drug.

METHODS FOR THE TREATMENT OF TROP2 POSITIVE DISEASES

REFERENCE TO SEQUENCE LISTING

[0001] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 63CP-295027WO_SequenceListing, created on April 8, 2019, which is 10 kilobytes in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD

[0002] The present disclosure generally relates to methods of using compounds comprising antibodies, antigen-binding fragments thereof, polypeptides, and immunoconjugates that bind to Trop2 (TACSTD2) for treating diseases, such as malignancies.

BACKGROUND

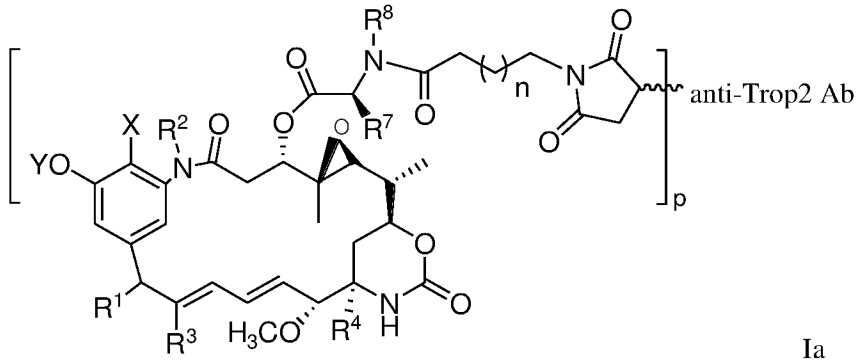
[0003] Human trophoblast cell surface antigen 2 (Trop-2), a 40-kDa transmembrane glycoprotein encoded by the TACSTD2 gene (*Cytogenet Cell Genet. 92*:164–65 (2001)), was first identified in human trophoblast and choriocarcinoma cell lines (*Proc Natl Acad Sci USA. 78*:5147–50 (1981)). Its short intracytoplasmic tail plays a key role in controlling several pathways that regulate cellular functions such as cell-cell adhesion, cell proliferation, and mobility (*Oncogene. 32*:1594–600 (2013); *Dev Dyn. 244*:99–109 (2015)). Trop-2 protein expression is often increased in a variety of epithelial cancers (*J Histochem Cytochem. 59*:701–10 (2011); *Oncogene. 32*:222–33 (2013)). A role as a marker of human prostate cancer stem cells has been proposed for it, particularly in cancer initiation and progression (*Proc Natl Acad Sci USA. 105*:20882–87 (2008)). Trop-2 overexpression has been correlated with an aggressive malignant phenotype and poor prognosis (*PLoS ONE. 9*:e96993 (2014); *PLoS ONE. 8*:e75864 (2013); *Clin Cancer Res. 12*:3057–63 (2006). The data reviewed above have made it an attractive diagnostic and prognostic marker candidate. Trop-2 is also currently being tested as a therapeutic target, since an anti-Trop-2 antibody-drug conjugate is being used to treat patients with several metastatic neoplasms, including triple-negative breast cancer (TNBC) and non-small-cell and small-cell lung cancer (*Oncotarget 6*:22496-512 (2015)).

[0004] Antibody drug conjugates (ADCs) are composed of three key elements: antibody, linker, and drug (cytotoxic payload). The antibody binds to specific markers (antigens or receptors) at the surface of the cancer cell, triggering internalization of the antibody-drug conjugate to the cancer cell, where the linker is degraded and the active drug is released.

[0005] A Trop-2-targeting ADC, sacituzumab govitecan, using the humanized RS7 antibody has been proposed as a potentially treatment for TNBC. This ADC not only has a high drug to monoclonal antibody (mAb) conjugation ratio of about 8:1 and but also is being administered to patients at a high dose of 10 mg/kg on Days 1 and 8 of 21-day cycles in a clinical trial (http://ClinicalTrials.gov number NCT02574455). Therefore, a large amount of the cytotoxic payload is released. There is a need to develop drugs against the Trop2 target with lower effective dose and administration frequency.

SUMMARY

[0006] Disclosed herein include embodiments of a method of treating a disease. In some embodiments, the method comprises: administering to a subject in need thereof an effective amount of an antibody drug conjugate (ADC) compound of Formula Ia:



Ia

or a pharmaceutically acceptable salt thereof,

wherein

X is hydrogen or halo;

Y is selected from the group consisting of hydrogen, C_1 - C_6 alkyl, C_3 - C_6 cycloalkyl, and -C(=O)R^5;

R^1 is selected from the group consisting of hydrogen, -OH, -OC(=O)R^5 and -OR^5;

R^2 is hydrogen or C_1 - C_6 alkyl;

R^3 is methyl, -CH_2OH, or -CH_2C(=O)R^6;

R^4 is -OH or -SH;

R^5 is C_1 - C_6 alkyl or benzyl;

R^6 is C_1 - C_6 alkyl, phenyl or benzyl;

R^7 is hydrogen, C_1 - C_6 alkyl or an amino acid side chain;

R^8 is hydrogen or C_1 - C_6 alkyl;

n is 0, 1, 2, 3, 4, 5, 6, 7 or 8;

p is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10; and

the anti-Trop2 Ab is an anti-Trop2 antibody, or an antigen-binding fragment thereof, which has specificity to a human Trop2 protein and comprises:

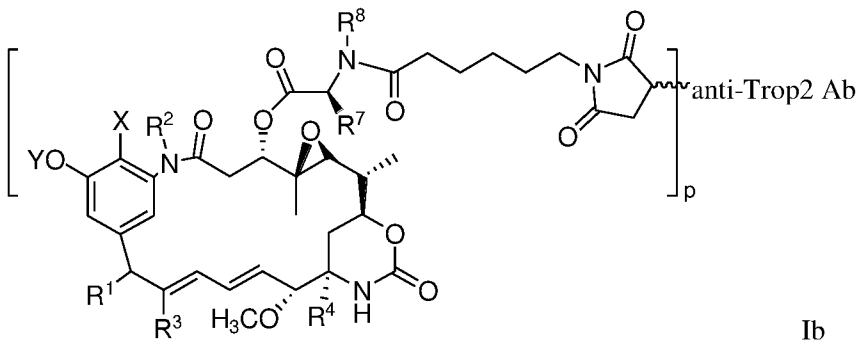
a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:1 or a polypeptide having at least 90% sequence identity to SEQ ID NO:1 and having a light chain complementary-determining region 1 (LCDR1) comprising the amino acid sequence of SEQ ID NO:2, a light chain complementary-determining region 2 (LCDR2) comprising the amino acid sequence of SEQ ID NO:3 and a light chain complementary-determining region 3 (LCDR3) comprising the amino acid sequence of SEQ ID NO:4; and

a heavy chain variant region (VH) comprising an amino acid sequence of SEQ ID NO:5 or a polypeptide having at least 90% sequence identity to SEQ ID NO:5 and having a heavy chain complementary-determining region 1 (HCDR1) comprising the amino acid sequence of SEQ ID NO:6, a heavy chain complementary-determining region 2 (HCDR2) comprising the amino acid sequence of SEQ ID NO:7 and a heavy chain complementary-determining region 3 (HCDR3) comprising the amino acid sequence of SEQ ID NO:8; and

wherein the effective amount is about 0.5 mg/kg to about 10 mg/kg.

[0007] In formula Ia, p represents the number of drug molecules conjugated to each anti-Trop2 Ab (drug antibody ratio or DAR) or the average DAR in the effective amount of ADC. In some embodiments, p is 1, 2, 3 or 4. In some embodiments, p is 2. In some embodiments, p is the average DAR, and is from about 1 to about 3, from about 1.8 to about 2.5, from about 2 to about 2.2, or about 2.1.

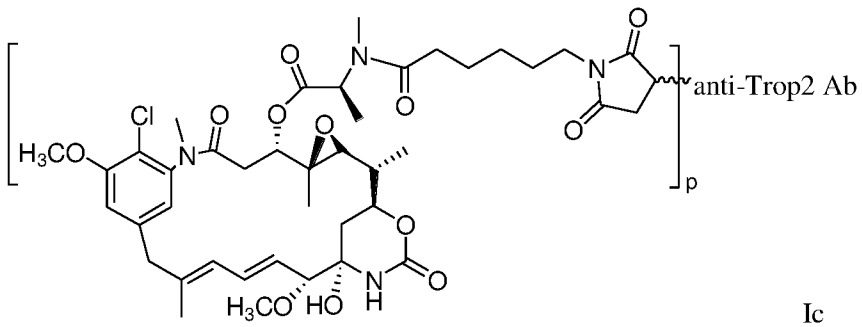
[0008] In some embodiments, the compound of Formula Ia is a compound of Formula Ib



Ib

or a pharmaceutically acceptable salt or solvate thereof.

[0009] The compound of Formula Ia can be a compound of Formula Ic



Ic

or a pharmaceutically acceptable salt thereof.

[0010] In formulae Ib and Ic, p represents DAR or the average DAR in the effective amount of ADC. In some embodiments, p is 1, 2, 3 or 4. In some embodiments, p is 2. In some embodiments, p is the average DAR, and is from about 1 to about 3, from about 1.8 to about 2.5, from about 2 to about 2.2, or about 2.1.

[0011] In some embodiments, the subject has a cancer characterized by Trop2 positive cells. The cancer can be triple negative breast cancer, glioblastoma, medulloblastoma, urothelial carcinoma, breast cancer, head and neck cancer, kidney cancer, ovarian cancer, Kaposi's sarcoma, pancreatic cancer and lung cancer. The cancer can be epithelial carcinoma. The cancer can be advanced epithelial carcinoma. In some embodiments, the cancer is refractory to or relapsed after at least one prior standard therapeutic regimen.

[0012] In some embodiments, the VL comprises the amino acid sequence of SEQ ID NO:1, and the VH comprises the amino acid sequence of SEQ ID NO:5. The anti-Trop2 antibody can comprise a light chain comprising the amino acid sequence of SEQ ID NO:9 and a heavy chain comprising the amino acid sequence of SEQ ID NO:10.

[0013] In some embodiments, the antibody or the antigen-binding fragment thereof is produced from a CHO-BAT-KF cell.

[0014] In some embodiments, at least about 80% of the anti-Trop2 antibody can comprise the G0 glycan at the asparagine residue 301 (Asn301) of SEQ ID NO:10, and/or at most about 2% of the anti-Trop2 antibody can comprise the Man5 glycan at the amino acid residue 301 of SEQ ID NO:10. In some embodiments, less than about 20% of the anti-Trop2 antibody comprise a fucosyl residue. In some embodiments, less than about 10% of the anti-Trop2 antibody comprise a fucosyl residue. In some

embodiments, less than about 5% of the anti-Trop2 antibody comprise a fucosyl residue. In some embodiments, less than about 1% of the anti-Trop2 antibody comprise a fucosyl residue.

[0015] In some embodiments, at least 50% of the anti-Trop2 antibody comprises the G0 glycan at an N-glycosylation site of a constant region of the heavy chain of the anti-Trop2 antibody. In some embodiments, at least 60% of the anti-Trop2 antibody comprises the G0 glycan at an N-glycosylation site of a constant region of the heavy chain of the anti-Trop2 antibody. In some embodiments, at least about 80% of the anti-Trop2 antibody can comprise the G0 glycan at the N-glycosylation site of the constant region of the heavy chain of the anti-Trop2 antibody. In some embodiments, at most 10% of the anti-Trop2 antibody can comprise the Man5 glycan at the N-glycosylation site of the constant region of the heavy chain of the anti-Trop2 antibody. In some embodiments, at most about 5% of the anti-Trop2 antibody can comprise the Man5 glycan at the N-glycosylation site of the constant region of the heavy chain of anti-Trop2 antibody. In some embodiments, at most about 2% of the anti-Trop2 antibody can comprise the Man5 glycan at the N-glycosylation site of the constant region of the heavy chain of anti-Trop2 antibody.

[0016] In some embodiments, the oligosaccharides of the Fc region of the anti-Trop2 antibodies in the effective amount of the ADC comprise no more than about 20% of fucosyl content. In some embodiments, the oligosaccharides of the Fc region of the anti-Trop2 antibodies in the effective amount of the ADC comprise no more than about 10% of fucosyl content. In some embodiments, the oligosaccharides of the Fc region of the anti-Trop2 antibodies in the effective amount of the ADC comprise no more than about 5% of fucosyl content. In some embodiments, the oligosaccharides of the Fc region of the anti-Trop2 antibodies in the effective amount of the ADC comprise no more than about 2% of fucosyl content. In some embodiments, the oligosaccharides of the Fc region of the anti-Trop2 antibodies in the effective amount of the ADC comprise no more than about 1% of fucosyl content. In some embodiments, the oligosaccharides of the Fc region of the anti-Trop2 antibodies in the effective amount of the ADC do not comprise any fucosyl residue.

[0017] In some embodiments, the characteristics of glycosylation mode satisfy one or more of the following preferred conditions:

The fucose content of the antibody is very low (e.g., 0–5%);

The galactose level of the antibody is low (e.g., $\leq$ 30%);

The mannose level of the antibody is low (e.g., $\leq$ 5%);

The high mannose level of the antibody is low (e.g., $\leq$ 5%);

The G0 level of the antibody is high (e.g., ≥ 60%).

[0018] In some embodiments, the antibody has a low galactose level, e.g., ≤ 5%.

[0019] In some embodiments, the antibody has a high G0 level, e.g., ≥ 80%.

[0020] In some embodiments, the glycosylation site of the antibody is an Asn residue on the heavy chain, such as Asn301 in SEQ ID NO 10.

[0021] In some embodiments, the effective amount is about 0.5 mg/kg to 10 mg/kg. The effective amount can be about 0.5 mg/kg to about 10 mg/kg about once every two to four weeks. The effective amount can be about 0.5 mg/kg to 10 mg/kg about once every three weeks. The compound can be administered for about four times or more. The effective amount can be about 0.5 mg/kg to 0.9 mg/kg, 0.7 mg/kg to 1.3 mg/kg, 1.5 mg/kg to 2.5 mg/kg, 3 mg/kg to 5 mg/kg, 5 mg/kg to 7 mg/kg, 7 mg/kg to 9 mg/kg, or 9 mg/kg to 10 mg/kg once every three weeks. The effective amount can be about 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, 8 mg/kg, or 10 mg/kg once every three weeks. The administration can be by intravenous infusion. The administration can be carried out for about one hour.

DETAILED DESCRIPTION

Definitions

[0022] As used herein, the following definitions shall apply unless otherwise indicated.

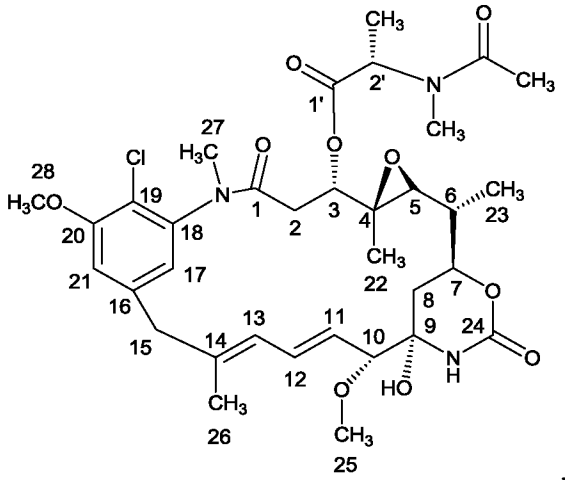
[0023] As used herein, unless otherwise stated, the singular forms "a," "an," and "the" include plural reference. Thus, for example, a reference to "a compound" includes a plurality of compounds.

[0024] As used herein, "about" will be understood by persons of ordinary skill in the art and will vary to some extent depending upon the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art, given the context in which it is used, "about" will mean up to plus or minus 10% or plus or minus 5%, or plus or minus 1% of the particular term. "About x" includes "x".

[0025] As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. For example, a composition consisting essentially of the elements as defined herein would not exclude other elements that do not materially affect the basic and novel characteristic(s) of the

claimed invention. "Consisting of" shall mean excluding more than trace amount of other ingredients and substantial method steps recited. Embodiments defined by each of these transition terms are within the scope of this disclosure.

[0026] As used herein, "maytansinoid" refers to a maytansine analogue, including stereoisomers thereof. Maytansine can be isolated from plants of the genus Maytenus U.S. Pat. No. 3,896,111). It is of the formula:



Maytansinoids are compounds having the ring structure of maytansine with one or more modifications of the substituents on the ring.

[0027] "Alkyl" refers to monovalent saturated aliphatic hydrocarbyl groups having from 1 to 10 carbon atoms and preferably 1 to 6 carbon atoms. C_v alkyl wherein v is an integer represents an alkyl having v carbons. This term includes, by way of example, linear and branched hydrocarbyl groups such as methyl (CH_3-), ethyl (CH_3CH_2-), *n*-propyl ($CH_3CH_2CH_2$-), isopropyl ($(CH_3)_2CH$-), *n*-butyl ($CH_3CH_2CH_2CH_2$-), isobutyl ($(CH_3)_2CHCH_2$-), *sec*-butyl ($(CH_3)(CH_3CH_2)CH$-), *t*-butyl ($(CH_3)_3C$-), *n*-pentyl ($CH_3CH_2CH_2CH_2CH_2$-), and neopentyl ($(CH_3)_3CCH_2$-). "Alkylene" is a divalent saturated aliphatic hydrocarbyl groups having from 1 to 10 carbon atoms and preferably 1 to 6 carbon atoms.

[0028] "Alkenyl" refers to straight or branched hydrocarbyl groups having from 2 to 6 carbon atoms and preferably 2 to 4 carbon atoms and having at least 1 and preferably from 1 to 2 sites of vinyl (>C=C<) unsaturation. Such groups are exemplified, for example, by vinyl, allyl, and but-3-en-1-yl. Included within this term are the *cis* and *trans* isomers or mixtures of these isomers.

[0029] "Alkynyl" refers to straight or branched monovalent hydrocarbyl groups having from 2 to 6 carbon atoms and preferably 2 to 3 carbon atoms and having at least 1 and preferably from 1 to 2 sites of acetylenic (-C≡C-) unsaturation. Examples of such alkynyl groups include acetylenyl (-C≡CH), and propargyl (-CH_2C≡CH).

[0030] "Amino" refers to the group -NR'R" where R' and R" are independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, and wherein R' and R" are optionally joined, together with the nitrogen bound thereto to form a heterocyclic or substituted heterocyclic group, provided that R' and R" are both not hydrogen, and wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein. When R' is hydrogen and R" is alkyl, the substituted amino group is sometimes referred to herein as alkylamino. When R' and R" are alkyl, the substituted amino group is sometimes referred to herein as dialkylamino. When referring to a monosubstituted amino, it is meant that either R' and R" is hydrogen but not both. When referring to a disubstituted amino, it is meant that neither R' and R" are hydrogen.

[0031] "Amino acid" refers any compound, whether natural, unnatural or synthetic, which includes both an amino group and a carboxy group. Examples of amino acid include, but are not limited to glycine (NH_2CH_2COOH), cysteine, alanine, N-methyl-L-alanine, including both the D and L optical isomers. "Amino acid side chain" refers to the substituent that replaces a hydrogen of the methylene group of glycine or glycine derivatives, such as N-alkylglycine or glycine esters. Examples of an amino acid side chain include, but are not limited to the side chains of the natural amino acids, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic.

[0032] "Aryl" or "Ar" refers to a monovalent aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (*e.g.*, phenyl) or multiple condensed rings (*e.g.*, naphthyl or anthryl) which condensed rings may or may not be aromatic (*e.g.*, 2-benzoxazolinone, 2H-1,4-benzoxazin-3(4H)-one-7-yl, and the like) provided that the point of attachment is at an aromatic carbon atom. Preferred aryl groups include phenyl and naphthyl.

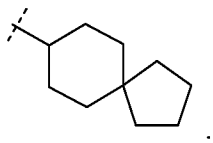
[0033] "Carbonyl" refers to the divalent group -C(O)- which is equivalent to -C(=O)-.

[0034] "Carboxy" or "carboxyl" refers to -COOH or CO_2H or salts thereof.

[0035] "Carboxylic acid" refers to a compound having at least one carboxy.

[0036] "Cyano" refers to the group -CN.

[0037] "Cycloalkyl" refers to cyclic alkyl groups of from 3 to 10 carbon atoms having single or multiple cyclic rings including fused, bridged, and spiro ring systems. One or more of the rings can be aryl, heteroaryl, or heterocyclic provided that the point of attachment is through the non-aromatic, non-heterocyclic ring carbocyclic ring. Examples of suitable cycloalkyl groups include, for instance, adamantyl, cyclopropyl, cyclobutyl, cyclopentyl, and cyclooctyl. Other examples of cycloalkyl groups include bicycle[2,2,2,]octanyl, norbornyl, and spirobicyclo groups such as spiro[4.5]dec-8-yl:



Cycloalkylene refers to a cyclic alkylene.

[0038] "Cycloalkenyl" refers to non-aromatic cyclic alkyl groups of from 3 to 10 carbon atoms having single or multiple cyclic rings and having at least one >C=C< ring unsaturation and preferably from 1 to 2 sites of >C=C< ring unsaturation.

[0039] "Halo" or "halogen" refers to fluoro, chloro, bromo and iodo and preferably is fluoro or chloro.

[0040] "Haloalkyl" refers to alkyl groups substituted with 1 to 5, 1 to 3, or 1 to 2 halo groups, wherein alkyl and halo are as defined herein.

[0041] "Hydroxy" or "hydroxyl" refers to the group -OH.

[0042] "Heteroaryl" refers to an aromatic group of from 6 to 10 carbon atoms and 1 to 4 heteroatoms selected from the group consisting of oxygen, nitrogen and sulfur within the ring. Such heteroaryl groups can have a single ring (e.g., pyridinyl or furyl) or multiple condensed rings (e.g., indolizinyl or benzothienyl) wherein the condensed rings may or may not be aromatic and/or contain a heteroatom provided that the point of attachment is through an atom of the aromatic heteroaryl group. In one embodiment, the nitrogen and/or the sulfur ring atom(s) of the heteroaryl group are optionally oxidized to

provide for the N-oxide (N→O), sulfinyl, or sulfonyl moieties. Preferred heteroaryls include pyridinyl, pyrrolyl, indolyl, thiophenyl, and furanyl.

[0043] "Heterocycle" or "heterocyclic" or "heterocycloalkyl" or "heterocyclyl" refers to a saturated or partially saturated, but not aromatic, group having from 3 to 10 ring carbon atoms and from 1 to 4 ring heteroatoms selected from the group consisting of nitrogen, sulfur, or oxygen. Heterocycle encompasses single ring or multiple condensed rings, including fused bridged and spiro ring systems. In fused ring systems, one or more the rings can be cycloalkyl, aryl, or heteroaryl provided that the point of attachment is through the non-aromatic ring. In one embodiment, the nitrogen and/or sulfur atom(s) of the heterocyclic group are optionally oxidized to provide for the N-oxide, sulfinyl, or sulfonyl moieties.

[0044] Examples of heterocycle and heteroaryls include, but are not limited to, azetidine, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, dihydroindole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, phthalimide, 1,2,3,4-tetrahydroisoquinoline, 4,5,6,7-tetrahydrobenzo[b]thiophene, thiazole, thiazolidine, thiophene, benzo[b]thiophene, morpholinyl, thiomorpholinyl (also referred to as thiamorpholinyl), 1,1-dioxothiomorpholinyl, piperidinyl, pyrrolidine, and tetrahydrofuranyl.

[0045] "Substituted alkyl," "substituted alkenyl," "substituted alkynyl," "substituted cycloalkyl," "substituted cycloalkenyl," "substituted aryl," "substituted heteroaryl" or "substituted heterocyclic" refers to alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, heteroaryl or heterocyclic groups, respectively, which are substituted with 1 to 5, preferably 1 to 3, or more preferably 1 to 2 substituents selected from the group consisting of alkyl, halo alkyl, -O-R^{20}, -S-R^{20}, alkenyl, alkynyl, -C(=O)R^{20}, -C(=S)R^{20}, -C(=O)OR^{20}, -NR^{20}C(=O)R^{21}, -OC(=O)R^{21}, -$NR^{20}R^{20}$, -C(=O)$NR^{20}R^{20}$, -C(=S)$NR^{20}R^{20}$, -NR^{20}C(=O)$NR^{20}R^{20}$, -NR^{20}C(=S)$NR^{20}R^{20}$, -OC(=O)$NR^{20}R^{20}$, -$SO_2NR^{20}R^{20}$, -$OSO_2NR^{20}R^{20}$, -$NR^{20}SO_2NR^{20}R^{20}$, -C(=NR^{20})$NR^{20}R^{20}$, aryl, -NR^{20}C(=O)OR^{21}, -OC(=O)OR^{21}, cyano, cycloalkyl, cycloalkenyl, -NR^{20}C(=NR^{20})$NR^{20}R^{20}$, halo, hydroxy, heteroaryl, heterocyclic, nitro, -SO_3H, -SO_2R^{21}, and -OSO_2R^{21}, wherein each R^{20} is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkenyl, heteroaryl, and heterocyclic or two R^{20} with the atom(s) bound thereto form a heterocyclic ring, and R^{21} is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkenyl, heteroaryl, and heterocyclic.

[0046] "Nitro" refers to the group -NO_2.

[0047] "Oxo" refers to the atom (=O) or (-O^-).

[0048] "Spiro ring systems" refers to bicyclic ring systems that have a single ring carbon atom common to both rings.

[0049] "Thiol" refers to the group -SH.

[0050] "Thiocarbonyl" refers to the divalent group -C(S)- which is equivalent to -C(=S)-.

[0051] "Thione" refers to the atom (=S).

[0052] "Compound" or "compounds" as used herein is meant to include the stereoiosmers and tautomers of the indicated formulas.

[0053] "Stereoisomer" or "stereoisomers" refer to compounds that differ in the chirality of one or more stereocenters. Stereoisomers include enantiomers and diastereomers.

[0054] "Tautomer" refer to alternate forms of a compound that differ in the position of a proton, such as enol-keto and imine-enamine tautomers, or the tautomeric forms of heteroaryl groups containing a ring atom attached to both a ring -NH- moiety and a ring =N- moiety such as pyrazoles, imidazoles, benzimidazoles, triazoles, and tetrazoles.

[0055] "Solvate" refer to an association of a solvent with a compound, in the crystalline form. The solvent association is typically due to use of the solvent in the synthesis, crystallization, and/or recrystallization of the compound. "Solvate" includes hydrate which is an association of water with a compound, in the crystalline form.

[0056] "Patient" or "subject" refers to mammals and includes humans and non-human mammals. In some embodiments, the term refers to humans. In some embodiments, the term refers to non-human mammals, such as wild, domestic, and farm animals. In yet other embodiments, the term refers to dogs, cats, mice, rats, rabbits, guinea pigs, or primates such as chimpanzees.

[0057] "Pharmaceutically acceptable salt" refers to pharmaceutically acceptable salts of a compound, which salts are derived from a variety of organic and inorganic counter ions well known in the art and include, by way of example only, when the molecule contains anacidic functionality, salts of organic or inorganic bases, such as sodium, potassium, calcium, magnesium, ammonium, isopropylamine,

trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and tetraalkylammonium, and the like; and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, and oxalate. Other non-limiting examples of acids include sulfuric acid, nitric acid, phosphoric acid, propionic acid, glycolic acid, pyruvic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicyclic acid and the like.

[0058] "Treating" or "treatment" of a disease in a patient refers to (1) preventing the disease from occurring in a patient that is predisposed or does not yet display symptoms of the disease; (2) inhibiting the disease or arresting its development; or (3) ameliorating or causing regression of the disease.

[0059] "Effective amount" is intended to mean an amount of an active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue, system, animal, individual or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes treating a disease.

[0060] "Administering" a composition may be accomplished by oral administration, injection, infusion, parenteral, intravenous, mucosal, sublingual, intramuscular, intradermal, intranasal, intraperitoneal, intraarterial, subcutaneous absorption or by any method in combination with other known techniques. In some embodiments, administration occurs systemically.

[0061] As used herein, the phrase "in need thereof" means that the subject has been identified as having a need for the particular method or treatment. In some embodiments, the identification can be by any means of diagnosis. In any of the methods and treatments described herein, the subject can be in need thereof.

[0062] Provided herein is use of an anti-Trop2 antibody drug conjugate in methods for treating a disease.

Anti-Trop2 Antibody-Drug Conjugates

[0063] In some embodiments, the anti-Trop2 antibody drug conjugate is a maytansinoid conjugated to an anti-Trop2 antibody via a linker that is not acid labile, not peptidase cathepsin sensitive, and that is stable in circulation while being able to release the cytotoxic drug inside the cells. In some embodiments,

the anti-Trop2 antibody drug conjugate is an antibody drug conjugate in which the drug is specifically linked at an artificial cysteine site located on the heavy chain of the antibody, and the antibody drug conjugate has an average drug load of 2 molecules per antibody. A maytansinoid can be conjugated to an anti-Trop2 antibody as described in PCT Publication No. WO/2019/029715.

[0064] Maytansinoids suitable for attaching the linking group include maytansinol and maytansinol analogues which can be isolated from natural sources according to known methods, produced using biotechnologies (*see e.g.,* Yu *et al., PNAS 99*:7968-7973 (2002)), or prepared synthetically according to known methods (*see e.g.,* Cassady *et al., Chem. Pharm. Bull. 52(1)*:1-26 (2004)).

[0065] Certain examples of suitable maytansinol analogues include:

(1) C-19-dechloro (U.S. Pat. No. 4,256,746) (prepared by LAH reduction of ansamytocin P2);

(2) C-20-hydroxy (or C-20-demethyl)+/-C-19-dechloro (U.S. Pat. Nos. 4,361,650 and 4,307,016) (prepared by demethylation using Streptomyces or Actinomyces or dechlorination using lithium aluminium hydride (LAH));

(3) C-20-demethoxy, C-20-acyloxy (-OCOR), +/-dechloro (U.S. Pat. No. 4,294,757) (prepared by acylation using acyl chlorides);

(4) C-9-SH (U.S. Pat. No. 4,424,219) (prepared by the reaction of maytansinol with H_2S or P_2S_5);

(5) C-14-hydroxymethyl (CH_2OH) or acyloxymethyl ($CH_2OC(=O)$phenyl or $CH_2OC(=O)(C_1$ -C_5 alkyl)) (U.S. Pat. No. 4,331,598) (prepared from Nocardia);

(6) C-15-hydroxy/acyloxy (U.S. Pat. No. 4,364,866) (prepared by the conversion of maytansinol by Streptomyces);

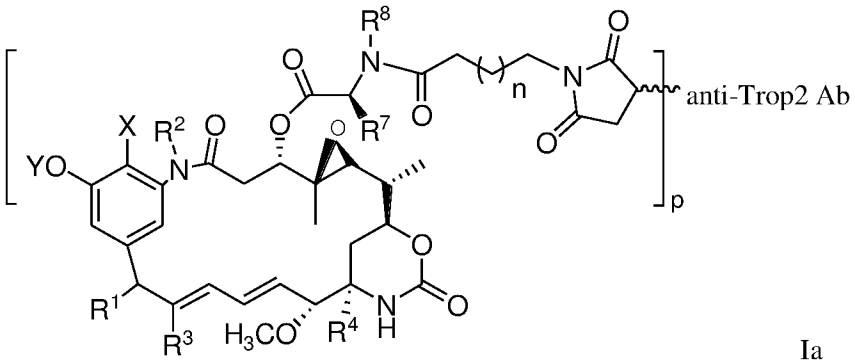
(7) C-15-methoxy (U.S. Pat. Nos. 4,313,946 and 4,315,929) (isolated from Trewia nudlflora);

(8) C-18-N-demethyl (U.S. Pat. Nos. 4,362,663 and 4,322,348) (prepared by the demethylation of maytansinol by Streptomyces); and

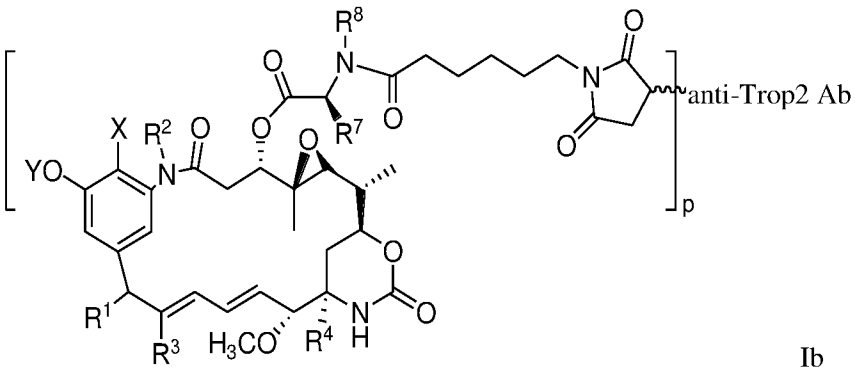
(9) 4,5-deoxy (U.S. Pat. No. 4,371,533) (prepared by the titanium trichloride/LAH reduction of maytansinol).

[0066] Many positions on maytansinol can be useful as the linkage position, depending upon the type of linker used. For example, for forming an ester linkage, the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group and the C-20 position having a hydroxyl group are all suitable. In some embodiments, the linkage position is the C-3 position.

[0067] In some embodiments, provided is a compound of Formula Ia or Ib



Ia



Ib

or a pharmaceutically acceptable salt or solvate thereof,

wherein

X is hydrogen or halo;

Y is selected from the group consisting of hydrogen, C_1 - C_6 alkyl, C_3 - C_6 cycloalkyl, and -C(=O)R^5;

R^1 is selected from the group consisting of hydrogen, -OH, -OC(=O)R^5 and -OR^5;

R^2 is hydrogen or C_1 - C_6 alkyl;

R^3 is methyl, -CH_2OH, or -CH_2C(=O)R^6;

R^4 is -OH or -SH;

R^5 is C_1 - C_6 alkyl or benzyl;

R^6 is C_1 - C_6 alkyl, phenyl or benzyl;

R^7 is hydrogen, C_1 - C_6 alkyl or an amino acid side chain;

R^8 is hydrogen or C_1 - C_6 alkyl;

n is 0, 1, 2, 3, 4, 5, 6, 7 or 8;

p is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10; and

the anti-Trop2 Ab is an anti-Trop2 antibody, or an antigen binding fragment thereof, which has specificity to a human Trop2 protein.

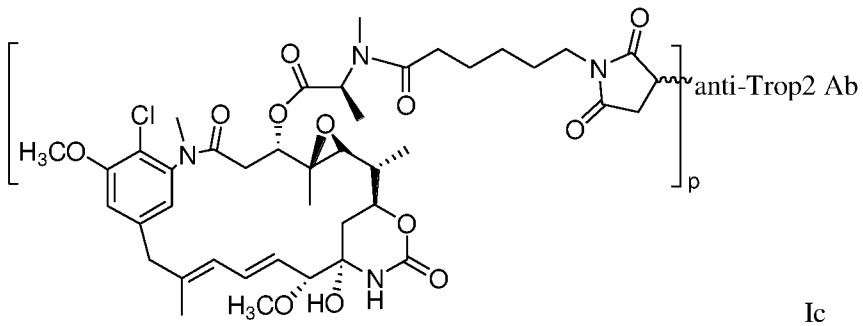
[0068] In formulae Ia and Ib, p represents the number of drug molecules conjugated to each anti-Trop2 Ab (drug antibody ratio or DAR). In some embodiments, p is 1, 2, 3 or 4. In some embodiments, p is 2. In some embodiments, p is the average DAR of the ADC molecules in the effective amount, and is from about 1 to about 3, from about 1.8 to about 2.5, from about 2 to about 2.2, or about 2.1.

[0069] In some embodiments, the Anti-Trop2 Ab is an antibody, or an antigen-binding fragment thereof, which has specificity to a human Trop2 protein and comprises:

a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:1 or a polypeptide having at least 90% sequence identity to SEQ ID NO:1 and having a light chain complementary-determining region 1 (LCDR1) comprising the amino acid sequence of SEQ ID NO:2, a light chain complementary-determining region 2 (LCDR2) comprising the amino acid sequence of SEQ ID NO:3 and a light chain complementary-determining region (LCDR3) comprising the amino acid sequence of SEQ ID NO:4; and

a heavy chain variant region (VH) comprising an amino acid sequence of SEQ ID NO:5 or a polypeptide having at least 90% sequence identity to SEQ ID NO:5 and having a heavy chain complementary-determining region 1 (HCDR1) comprising the amino acid sequence of SEQ ID NO:6, a heavy chain complementary-determining region 2 (HCDR2) comprising the amino acid sequence of SEQ ID NO:7 and a heavy chain complementary-determining region 3 (HCDR3) comprising the amino acid sequence of SEQ ID NO:8.

[0070] In some embodiments, the compound of Formula Ia is a compound of Formula Ic:



Ic

or a pharmaceutically acceptable salt or solvate thereof, wherein the anti-Trop2 Ab is an anti-Trop2 (TACSTD2) antibody.

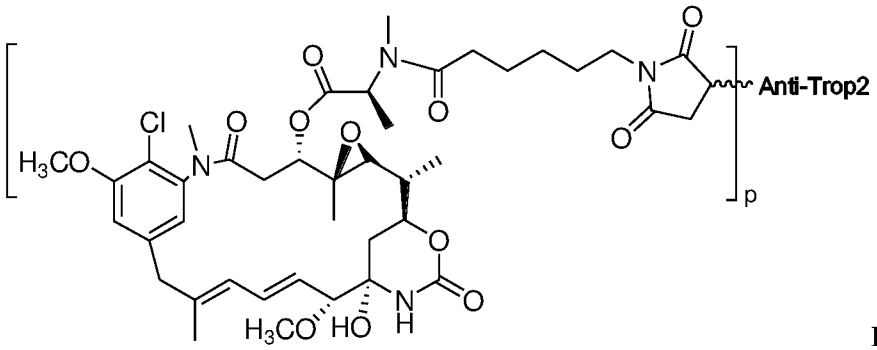
[0071] In some embodiments, the drug is specifically linked to an artificial cysteine site located on the heavy chain of the antibody. In some embodiments, at least 50% of the ADC molecules in the effective amount have a DAR of 2 and the two drug molecules are specifically linked to an artificial cysteine site located on the heavy chain of the antibody. In some embodiments, at least 60% of the ADC molecules in the effective amount have a DAR of 2 and the two drug molecules are specifically linked to an artificial cysteine site located on the heavy chain of the antibody. In some embodiments, at least 70% of the ADC molecules in the effective amount have a DAR of 2 and the two drug molecules are specifically linked to an artificial cysteine site located on the heavy chain of the antibody. In some embodiments, at least 80% of the ADC molecules in the effective amount have a DAR of 2 and the two drug molecules are specifically linked to an artificial cysteine site located on the heavy chain of the antibody. In some embodiments, at least 90% of the ADC molecules in the effective amount have a DAR of 2 and the two drug molecules are specifically linked to an artificial cysteine site located on the heavy chain of the antibody. In some embodiments, at least 95% of the ADC molecules in the effective amount have a DAR of 2 and the two drug molecules are specifically linked to an artificial cysteine site located on the heavy chain of the antibody.

[0072] In some embodiments, the anti-Trop2 antibody is expressed through an expression vector by CHO-BAT-KF (Accession No.: CCTCC NO: C2017127). The cell line was created by knocking out α-(1,6)-fucosyltransferase, characterized by enhanced ADCC function. An anti-Trop2 antibody, or an antigen binding fragment thereof, can be expressed and purified as described in PCT Publication No. WO/2019/029715. For example, an anti-Trop antibody can be expressed using the CHO-BAT-KF cell line as the host cell. Purification can be carried out by centrifuging the cell suspension and harvesting thesupernatant, which can be further cleared by centrifuging. Protein A affinity columns, such as Mab

Select SuRe (GE Healthcare), and ion exchange, such as Capto S (GE), can be used to purify the expressed antibodies.

[0073] In some embodiments, the antibody-drug conjugates disclosed herein utilize a stable linker, which may contribute to a longer MRT and lower dosing frequency. Thus, site-specific conjugate technology can not only improve the ADC homogeneity, but also result in better pharmacokinetics (PK) performance.

[0074] In some embodiments, the compound is ADC1 which is:



Ic

or a pharmaceutically acceptable salt thereof, wherein

p is average DAR and is 1.5 to 2.5;

Anti-Trop2 is an anti-Trop2 antibody, which comprises a light chain comprising the amino acid sequence of SEQ ID NO:9 and a heavy chain comprising the amino acid sequence of SEQ ID NO:10.

[0075] In some embodiments, p is from about 1.8 to about 2.4, from about 2 to about 2.2, or p is about 2.1.

[0076] In some embodiments, the anti-Trop2 antibodies in the effective amount of the ADC1 are glycosylated as described herein. In some embodiments, the oligosaccharides of the anti-Trop2 antibodies in the effective amount of the ADC1 comprise no more than about 20% of fucosyl content. In some embodiments, the oligosaccharides of the anti-Trop2 antibodies in the effective amount of the ADC1 comprise no more than about 10% of fucosyl content. In some embodiments, the oligosaccharides of the anti-Trop2 antibodies in the effective amount of the ADC1 comprise no more than about 5% of fucosyl content. In some embodiments, the oligosaccharides of the anti-Trop2 antibodies in the effective amount of the ADC1 comprise no more than about 2% of fucosyl content. In some embodiments, the oligosaccharides of the anti-Trop2 antibodies in the effective amount of the ADC1 comprise no more than

about 1% of fucosyl content. In some embodiments, the oligosaccharides of the anti-Trop2 antibodies in the effective amount of the ADC1 do not comprise any fucosyl residue.

[0077] In some embodiments, the oligosaccharides of Fc region of the antibodies in the effective amount of ADC1 have a fucose content of 0-5%.

[0078] In some embodiments, the anti-TROP2 antibody is expressed by an expression vector by CHO-BAT-KF. In some embodiments, the host cell line is knocked out the α-(1,6)-fucosyltransferase, which is characterized by the expressed antibody has a fucose content of 0-5% and an ADCC-enhanced effect. In some embodiments, the host cell is a cell that was deposited at the China Type Culture Collection with Accession No.: CCTCC NO: C2017127.

[0079] In some embodiments, at least 50% of ADC1 comprise ADC molecules wherein DAR is 2 and wherein the drug is specifically linked to cysteine 122 on the heavy chain of the antibody. In some embodiments, at least 60% of ADC1 comprise ADC molecules wherein DAR is 2 and wherein the drug is specifically linked to cysteine 122 on the heavy chain of the antibody. In some embodiments, at least 70% of ADC1 comprise ADC molecules wherein DAR is 2 and wherein the drug is specifically linked to cysteine 122 on the heavy chain of the antibody. In some embodiments, at least 80% of ADC1 comprise ADC molecules wherein DAR is 2 and wherein the drug is specifically linked to cysteine 122 on the heavy chain of the antibody. In some embodiments, at least 90% of ADC1 comprise ADC molecules wherein DAR is 2 and wherein the drug is specifically linked to cysteine 122 on the heavy chain of the antibody. In some embodiments, at least 95% of ADC1 comprise ADC molecules wherein DAR is 2 and wherein the drug is specifically linked to cysteine 122 on the heavy chain of the antibody.

Anti-Trop2 Antibody

[0080] Trop-2 is a type-I transmembrane protein that has been cloned from both human (Fornaro *et al., Int J Cancer* 62:610-8 (1995)) and mouse cells (Sewedy *et al., Int J Cancer 75*:324-30 (1998)). In addition to its role as a tumor-associated calcium signal transducer (Ripani *et al., Int J Cancer 76*:671-6 (1998)), the expression of human Trop-2 was shown to be necessary for tumorigenesis and invasiveness of colon cancer cells, which could be effectively reduced by administering a polyclonal antibody against the extracellular domain of Trop-2 (Wang *et al., Mol Cancer Ther* 7:280-5 (2008)).

[0081] The anti-Trop2 antibodies used herein show high affinity specific to Trop2 which is highly expressed in a range of solid tumors including the breast cancer, cervical cancer, colorectal cancer, esophageal cancer, gastric cancer, lung cancer, oral squamous cell carcinoma, ovarian cancer, prostate

cancer, pancreatic cancer, thyroid cancer, urinary bladder cancer, ovarian cancer, glioma, porta hepatis bile duct cancer, kidney cancer, colorectal cancer, T cell lymphoma, epithelial carcinoma, advanced epithelial carcinoma, and so on, but rarely or even not expressed in normal tissue cells.

[0082] In some embodiments, the anti-Trop2 antibody, or an antigen-binding fragment thereof, has specificity to a human Trop2 protein and comprises: a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:1 or a polypeptide having at least 90% sequence identity to SEQ ID NO:1 and having a light chain complementary-determining region 1 (LCDR1) comprising the amino acid sequence of SEQ ID NO:2, a light chain complementary-determining region 2 (LCDR2) comprising the amino acid sequence of SEQ ID NO:3 and a light chain complementary-determining region 3 (LCDR3) comprising the amino acid sequence of SEQ ID NO:4; and a heavy chain variant region (VH) comprising an amino acid sequence of SEQ ID NO:5 or a polypeptide having at least 90% sequence identity to SEQ ID NO:5 and having a heavy chain complementary-determining region 1 (HCDR1) comprising the amino acid sequence of SEQ ID NO:6, a heavy chain complementary-determining region 2 (HCDR2) comprising the amino acid sequence of SEQ ID NO:7 and a heavy chain complementary-determining region 3 (HCDR3) comprising the amino acid sequence of SEQ ID NO:8.

[0083] In some embodiments, the VL comprises a polypeptide having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values, sequence identity to SEQ ID NO:1 (See Table 1, where LCDR1, LCDR2, and LCDR3 sequences are underlined and bolded). In some embodiments, the VH comprises a polypeptide having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values, sequence identity to SEQ ID NO:5 (See Table 1, where HCDR1, HCDR2, and HCDR3 sequences are underlined and bolded). In some embodiments, the VL comprises the amino acid sequence of SEQ ID NO:1, and/or the VH comprises the amino acid sequence of SEQ ID NO:5. The anti-Trop2 antibody can comprise a light chain comprising the amino acid sequence of SEQ ID NO:9 and/or a heavy chain comprising the amino acid sequence of SEQ ID NO:10.

Table 1. Sequences

Name	Sequences (CDR underlined in VL/VH)	SEQ ID NO:
VL	DIQLTQSPSS LSASVGDRVS ITC**KASQDVS** **IAVA**WYQQKP GKAPKLLIY**S** **ASYRYT**GVPD RFSGSGSGTD FTLTISSLQP EDFAVYYC**QQ** **HYITPLT**FGA GTKVEIKRTV	1

Name	Sequences (CDR underlined in VL/VH)	SEQ ID NO:
LCDR1	KASQDVSIAV A	2
LCDR2	SASYRYT	3
LCDR3	QQHYITPLT	4
VH	QVQLQQSGSE LKKPGASVKV SCKASGYTFT **NYGMN**WVKQA PGQGLKWMG**W** **INTYTGEPTY** **TDDFKG**RFAF SLDTSVSTAY LQISSLKADD TAVYFCAR**GG** **FGSSYWYFDV** WGQGTLVTVS S	5
HCDR1	NYGMN	6
HCDR2	WINTYTGEPT YTDDFKG	7
HCDR3	GGFGSSYWYF DV	8
Light chain	DIQLTQSPSS LSASVGDRVS ITCKASQDVS IAVAWYQQKP GKAPKLLIYS ASYRYTGVPD RFSGSGSGTD FTLTISSLQP EDFAVYYCQQ HYITPLTFGA GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC	9
Heavy chain	QVQLQQSGSE LKKPGASVKV SCKASGYTFT NYGMNWVKQA PGQGLKWMGW INTYTGEPTY TDDFKGRFAF SLDTSVSTAY LQISSLKADD TAVYFCARGG FGSSYWYFDV WGQGTLVTVS SCSTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKKVE PKSCDKTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K	10

[0084] In some embodiments, the antibody or the antigen-binding fragment thereof is produced from a CHO-BAT-KF cell.

[0085] In some embodiments, the oligosaccharides of the anti-Trop2 antibodies in the effective amount of the ADC comprise no more than about 20% of fucosyl content. In some embodiments, the oligosaccharides of the anti-Trop2 antibodies in the effective amount of the ADC comprise no more than about 10% of fucosyl content. In some embodiments, the oligosaccharides of the anti-Trop2 antibodies in the effective amount of the ADC comprise no more than about 5% of fucosyl content. In some embodiments, the oligosaccharides of the anti-Trop2 antibodies in the effective amount of the ADC comprise no more than about 2% of fucosyl content. In some embodiments, the oligosaccharides of the anti-Trop2 antibodies in the effective amount of the ADC comprise no more than about 1% of fucosyl content. In some embodiments, the oligosaccharides of the anti-Trop2 antibodies in the effective amount of the ADC do not comprise any fucosyl residue.

[0086] In some embodiments, the characteristics of glycosylation mode satisfy one or more of the following preferred conditions:

The fucose content of the antibody is very low (e.g., 0–5%);

The galactose level of the antibody is low (e.g., ≤ 30%);

The mannose level of the antibody is low (e.g., ≤ 5%);

The high mannose level of the antibody is low (e.g., ≤ 5%);

The G0 level of the antibody is high (e.g., ≥ 60%).

[0087] In some embodiments, the antibody has a low galactose level, e.g., ≤ 5%.

[0088] In some embodiments, the antibody has a high G0 level, e.g., ≥ 80%.

[0089] In some embodiments, the glycosylation site of the antibody is an Asn residue, such as Asn301, on the heavy chain.

[0090] In some embodiments, at least, or at least about, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or a number or a range between any two of these values, of the anti-Trop2 antibody can comprise the G0 glycan at the N-glycosylation site of the constant region of the heavy chain of the anti-Trop2 antibody. In some embodiments, at most, or at most about, 0.001%, 0.01%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, or a number or a range between any two of these values, of the anti-Trop2 antibody can comprise any glycans other than the G0 glycan at the N-glycosylation site of the constant region of the heavy chain of the anti-Trop2 antibody.

[0091] In some embodiments, at most, or at most about, 0.001%, 0.01%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, or a number or a range between any two of these values, of the anti-Trop2 antibody can comprise the G0-GN glycan at the N-glycosylation site of the constant region of the heavy chain of the anti-Trop2 antibody. In some embodiments, at most, or at most about, 0.001%, 0.01%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, or a number or a range between any two of these values, of the anti-Trop2 antibody can comprise the Man5 glycan at the N-glycosylation site of the constant region of the heavy chain of the anti-Trop2 antibody. In some embodiments, at most, or at most

about, 0.001%, 0.01%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, or a number or a range between any two of these values, of the anti-Trop2 antibody can comprise the G1 glycan at the N-glycosylation site of the constant region of the heavy chain of the anti-Trop2 antibody. In some embodiments, at most, or at most about, 0.001%, 0.01%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, or a number or a range between any two of these values, of the anti-Trop2 antibody can comprise the G1' glycan at the N-glycosylation site of the constant region of the heavy chain of the anti-Trop2 antibody. In some embodiments, at most, or at most about, 0.001%, 0.01%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, or a number or a range between any two of these values, of the anti-Trop2 antibody can comprise the G2 glycan at the N-glycosylation site of the constant region of the heavy chain of the anti-Trop2 antibody.

[0092] In some embodiments, the fucose content of the antibody is very low. For example, the fucose content of the antibody is, is about, or is at most, 0.001%, 0.01%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, or a number or a range between any two of these values. In some embodiments, the galactose level of the antibody is low. For example, the galactose level of the antibody is, is about, or is at most, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, or a number or a range between any two of these values. In some embodiments, the mannose content of the antibody is very low. For example, the mannose content of the antibody is, is about, or is at most, 0.001%, 0.01%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, or a number or a range between any two of these values. In some embodiments, the high mannose content of the antibody is very low. For example, the mannose content of the antibody is, is about, or is at most, 0.001%, 0.01%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, or a number or a range between any two of these values. In some embodiments, the G0 level of the antibody is high. For example, the G0 level of the antibody is, is about, or is at least, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or a number or a range between any two of these values.

Conjugation of a Drug to an Anti-Trop2 Antibody

[0093] As discussed, a drug (*e.g.,* a maytansinoid drug derivative) can be conjugated to an anti-Trop2 antibody through a linker. In one embodiment, the anti-Trop2 antibody can be modified with an appropriate bifunctional modifying agent. In some embodiments, a group comprising a thiol (SH) group (also referred to as thio-comprising group) can be introduced to the side-chain of an amino acid residue, such as the side-chain of a lysine, on the anti-Trop2 antibody. For example, the amino group of a lysine residue on the anti-Trop2 antibody can be converted to a thiol-comprising group by reaction with 2-iminothiolane (Traut's Reagent), or with N-succinimidyl 3-(2-pyridyldithio)propanoate (SPDP), N-succinimidyl 4-(2-pyridyldithio) butanoate (SPDB), etc. and followed by reduction with a reducing reagent, such as 2-mercaptoethanol, dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine (TCEP). In some embodiments, to allow proper coupling, the anti-Trop2 antibody can be engineered with a cysteine, via mutagenesis, or inserted in a specific location that results minimal effect on the antibody activities including affinity, specificity, ADCC, CDC, ADCP, and immunogenicity.

[0094] Non-limiting examples of thiol-comprising group that can replace the side-chain amino group of a lysine residue include -NHC(=NH)$(CH_2)_n$SH and -NHC(O)$(CH_2)_n$SH, wherein n is 1, 2, 3, 4, 5 or 6. When a thiol-comprising group is introduced to an amino acid residue, the amino acid residue is referred to as thiolated amino acid. For example, when the side-chain amino group of a lysine residue is converted to a thio-comprising group, the lysine residue is referred to as thiolated lysine. The number of free thiol (SH) group introduced in an anti-Trop2 antibody may vary, such as between 1 and about 20, or 1 to 10, and or 1 to 5. The linkers or drug-linkers can form bonds with the free thiol (SH) group of a cysteine residue or a thiolated lysine residue on the anti-Trop2 antibody. In some embodiments, the number of linkers or drug-linkers that form bonds with cysteine residues in the anti-Trop2 antibody is between 1 and about 10. In some embodiments, the number of such formed bonds is at least 1, or alternatively at least 2, or 3, or 4, or 5. In some embodiments, the number of such formed bonds is no more than 10, or alternatively no more than 9, or 8, 7, 6, 5, or 4. In some embodiments, each anti-Trop2 antibody, on average, is conjugated with 1-4 drug molecules, more specifically, with an average of 2 drug molecules.

[0095] In another embodiment, a drug-linker can be conjugated to an anti-Trop2 antibody by binding to the thiol group of a cysteine residue. Each anti-Trop2 antibody typically contains multiple cysteines, but many, if not all, of them form disulfite bonds between each other, and thus are not available for such conjugation. In some embodiments, therefore, one or more of the disulfide bonds of the anti-Trop2 antibody can be broken to form free thiol (SH) groups by reaction with a reducing reagent, such as 2-mercaptoethanol, dithiothreitol (DTT) or tris(2-carboxyethyl) phosphine (TCEP), for instance. The

reaction can be monitored and/or controlled so that a sufficient number of disulfite bonds are broken to allow conjugation while maintaining a sufficient number of disulfide bonds to keep the structure stability of the anti-Trop2 antibody.

[0096] In some embodiments, the number of bonds formed between the drug-linker and cysteine residue on the anti-Trop2 antibody is from 1 to 10. In one embodiment, the number of such bonds is at least 1, or alternatively at least 2, or 4. In some embodiments, the number of such formed bonds is no more than 10, or alternatively no more than 9, or 8, 7, 6, 5, or 4. In one embodiment, each anti-Trop2 antibody, on average, is conjugated with 2-2.5 drug molecules through cysteines.

[0097] In some embodiments, drug molecules are conjugated to the anti-Trop2 antibody through a mixture of lysine and cysteine residues.

[0098] An anti-Trop2 antibody can be modified, by way of, *e.g.*, site-specific mutagenesis, to introduce additional thiolated lysine or cysteine residues to allow suitable conjugation. Amino acid modification methods are well known in the art. Modified anti-Trop2 antibody can then be experimentally examined for their stability and antigen binding capability. In one embodiment, at least one thiolated lysine or cysteine residue is introduced by such modification. In another embodiment, at least two thiolated lysine or cysteine residues are introduced by such modification. In another embodiment, the Fc portion of the anti-Trop2 antibody is engineered with increased ADCC activity.

Drug Load

[0099] The drug load on an anti-Trop2 antibody may vary depending on many factors, such as the potency of the drug, the size, stability of the anti-Trop2 antibody, conjugatable groups available on the anti-Trop2 antibody, etc. In some embodiments, 1 to 10 maytansinoid drug molecules are conjugated with 1 anti-Trop2 antigen binding unit (e.g., an anti-Trop2 antibody). In some embodiments, an average of 2 to 4 maytansinoid drug molecules are conjugated with 1 anti-Trop2 antigen binding unit. In some embodiments, an average of 2 maytansinoid drug molecules are conjugated with 1 anti-Trop2 antigen binding unit.

[0100] In some embodiments, 1 drug molecule is site-specifically conjugated to a specific artificial cysteine residue on each heavy chain of the antibody, and therefore each antibody is conjugated to 2 drug molecules. The site-specific conjugation improves the ADC homogeneity, which may result in better pharmacokinetics (PK) performance. Low drug to antibody ratio also leads to lower amount of cytotoxic drug being released once the ADC is administered.

Methods of Treatment

[0101] Disclosed herein include embodiments of a method of treating a disease. In some embodiments, the method comprises: administering to a subject in need thereof an effective amount of a compound of any one of Formulae Ia-Ic, wherein the effective amount is about 0.5 mg/kg to about 10 mg/kg. In another aspect, provided herein is a method of treating a proliferative, inflammatory or immunologic disease or condition in a patient in need thereof comprising administering an effective amount of one or more compounds as described herein, for example, a compound of any one of Formulae Ia-Ic.

[0102] In some embodiments, provided is use of a compound of any one of Formulae Ia-Ic in the manufacture of a medicament in the treatment of a disease as described herein, wherein the compound is for administration to a patient at an amount of about 0.5 mg/kg to about 10 mg/kg.

[0103] In some embodiments, provided is a kit comprising a compound of any one of Formulae Ia-Ic for the treatment of a disease as described herein, and instructions to administer the compound to a patient in need thereof at an amount of about 0.5 mg/kg to about 10 mg/kg.

[0104] The compounds can be formulated as pharmaceutical compositions and administered to the patient in a variety of forms adapted to the chosen route of administration, *i.e.,* orally or parenterally, by intravenous (I.V.), intramuscular, topical or subcutaneous routes. The amount of the compounds will vary depend on the nature of the drug, linker, drug load, degree of cell surface triggered the internalization, trafficking, and release of the drug, the disease being treated, the conditions of the patient, such as age, gender, weight, etc. and can be determined by methods known to the art, for example, see U.S. Pat. No. 4,938,949, and will be ultimately at the discretion of the attendant physician or clinician.

[0105] In some embodiments, the amount of the compound administered each time is, or is about, 0.5 mg/kg to 20 mg/kg. For example, the amount of the compound administered each time can be, or can be about, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, 13 mg/kg, 14 mg/kg, 15 mg/kg, 16 mg/kg, 17 mg/kg, 18 mg/kg, 19 mg/kg, 20 mg/kg, or a number or a range between any two of these values. For example, the amount of the compound administered each time can be about 0.5 mg/kg to 10 mg/kg. For example, the effective amount can be about 0.5 mg/kg to 0.9 mg/kg, 0.7 mg/kg to 1.3 mg/kg, 1.5 mg/kg to 2.5 mg/kg, 3 mg/kg to 5 mg/kg, 5 mg/kg to 7 mg/kg, 7 mg/kg to 9 mg/kg, or 9 mg/kg to 10 mg/kg.

[0106] In some embodiments, the compound can be administered, or administered about once every two weeks, once every three weeks, once every four weeks, once every five weeks, once every six weeks, once every seven weeks, once every eight weeks, once every nine weeks, once every ten weeks, or a number or a range between any two of these values. For example, the effective amount administered can be about 0.5 mg/kg to about 10 mg/kg about once every two to four weeks. The effective amount administered can be about 0.5 mg/kg to 10 mg/kg about once every three weeks, such as about 0.5 mg/kg to 0.9 mg/kg, 0.7 mg/kg to 1.3 mg/kg, 1.5 mg/kg to 2.5 mg/kg, 3 mg/kg to 5 mg/kg, 5 mg/kg to 7 mg/kg, 7 mg/kg to 9 mg/kg, or 9 mg/kg to 10 mg/kg every three weeks. The effective amount administered can be about 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, 8 mg/kg, or 10 mg/kg once every three weeks.

[0107] In some embodiments, provided is a method of treating a disease comprising administering to a subject in need thereof an effective amount of a compound of any one of Formulae Ia-Ic, wherein the effective amount is about 35 mg to about 1000 mg once every two to four weeks.

[0108] In some embodiments, provided is use of a compound of any one of Formulae Ia-Ic in the manufacture of a medicament in the treatment of a disease as described herein, wherein the compound is for administration to a patient at an amount of about 35 mg to about 1000 mg once every two to four weeks.

[0109] In some embodiments, provided is a kit comprising a compound of any one of Formulae Ia-Ic for the treatment of a disease as described herein, and instructions to administer the compound to a patient in need thereof at an amount of about 35 mg to about 1000 mg once every two to four weeks.

[0110] In some embodiments, the amount is about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 75 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, or about 100 mg, or a number or a range between any two of these values, once every two to four weeks. In some embodiments, the amount is about 35 mg, about 70 mg, about 100 mg, about 140 mg, about 150 mg, about 200 mg, about 210 mg, about 280 mg, about 300 mg, about 350 mg, about 400 mg, about 420 mg, about 450 mg, about 490 mg, about 500 mg, about 550 mg, about 560 mg, about 600 mg, about 630 mg, about 650 mg, or about 700 mg, or a number or a range between any two of these values, once every three weeks.

[0111] In some embodiments, the total number of administration of the compound can be, or can be about, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or a number or a range between any two of these values. For example, the compound can be administered for about four times.

[0112] In some embodiments, the duration of an administration can be, or can be about, 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, or a number or a range between any two of these values. For example, the administration can be carried out for about one hour.

[0113] In some embodiments, provided is a method of treating a cancer comprising administering to a human patient in need thereof an effective amount of a compound of Formula Ic, such as ADC1, wherein the effective amount is about 0.5 mg/kg to about 10 mg/kg once every three weeks and wherein the compound of Formula Ic is administered by I.V. infusion. In some embodiments, the compound is administered in an amount of about 0.5, 1, 2, 4, 6, 8 or 10 mg/kg once every three weeks by I.V. infusion.

[0114] In some embodiments, provided is use of a compound of Formula Ic, such as ADC1, in the manufacture of a medicament in the treatment of a disease as described herein, wherein the compound is for administration to a patient at an amount of about 0.5 mg/kg to about 10 mg/kg once every three weeks by I.V. infusion. In some embodiments, the compound is for administration in an amount of about 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, 8 mg/kg or 10 mg/kg once every three weeks by I.V. infusion.

[0115] In some embodiments, provided is a kit comprising a compound of Formula Ic, such as ADC1, for the treatment of a disease as described herein, and instructions that the compound is to be administered to a human patient in need thereof at an amount of about 0.5 mg/kg to about 10 mg/kg once every three weeks by I.V. infusion. In some embodiments, the compound is to be administered in an amount of about 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, 8 mg/kg or 10 mg/kg once every three weeks by I.V. infusion.

[0116] In some embodiments, provided is a method of treating a cancer comprising administering to a human patient in need thereof an effective amount of a compound of Formula Ic, such as ADC1, wherein the effective amount is about 35 mg to about 1000 mg once every three weeks and wherein the compound of Formula Ic is administered by I.V. infusion. In some embodiments, the compound is administered in an amount of about 35 mg, 70 mg, 140 mg, 280 mg, 420 mg, 560 mg or 700 mg once every three weeks by I.V. infusion.

[0117] In some embodiments, provided is use of a compound of Formula Ic, such as ADC1, in the manufacture of a medicament in the treatment of a disease as described herein, wherein the compound is for administration to a patient at an amount of about 35 mg to about 1000 mg once every three weeks by

I.V. infusion. In some embodiments, the compound is for administration in an amount of about 35 mg, 70 mg, 140 mg, 280 mg, 420 mg, 560 mg or 700 mg once every three weeks by I.V. infusion.

[0118] In some embodiments, provided is a kit comprising a compound of Formula Ic, such as ADC1, for the treatment of a disease as described herein, and instructions that the compound is to be administered to a human patient in need thereof at an amount of about 35 mg to about 1000 mg once every three weeks by I.V. infusion. In some embodiments, the compound is to be administered in an amount of about 35 mg, 70 mg, 140 mg, 280 mg, 420 mg, 560 mg or 700 mg once every three weeks by I.V. infusion.

[0119] In some embodiments, the compounds are administered in conjunction with another therapy. For example, the compounds can be co-administered with another therapy for treating cancer, for example, radiation therapy or another anticancer agent known in the art.

Pharmaceutical Compositions

[0120] In a further aspect, provided are pharmaceutical compositions comprising one or more compounds as described herein, for example, a compound of any one of Formulae Ia-Ic, and one or more pharmaceutically acceptable carriers. Such compositions may contain 0.1% or more of active compound. The percentage of the compositions may vary and may be between about 2 to about 90% of the weight of a given dosage form. The amount of active compound in such therapeutically useful compositions is such that an effective amount can be administered.

[0121] Examples of pharmaceutical compositions suitable for injection or infusion can include sterile aqueous solutions or dispersions in a pharmaceutically acceptable liquid carrier or vehicle, or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. Other forms of pharmaceutical compositions include topical formulations, such as gel, ointments, creams, lotions or transdermal patches, etc. The pharmaceutical compositions include using techniques well known to those in the art. Suitable pharmaceutically-acceptable carriers, outside those mentioned herein, are known in the art; for example, see Remington, The Science and Practice of Pharmacy, 20th Edition, 2000, Lippincott Williams & Wilkins, (Editors: Gennaro, A. R., et al.).

[0122] In a further aspect, provided are methods of producing a pharmaceutical composition comprising admixing a compound as described herein, for example, a compound of any one of Formulae Ia-Ic, and a pharmaceutically acceptable carrier. Methods of admixing an active ingredient with a pharmaceutically

acceptable carrier are generally known in the art, for example, uniformly mixing the active compound(s) with liquids or finely divided solid carriers, or both, in the required proportions, and then, if necessary, forming the resulting mixture into a desired shape.

[0123] In some embodiments, a compound of any one of Formula Ia-Ic is formulated as an injectable, for example, at a concentration of 2-50 mg/mL in an aqueous solution comprising 4-10 mg/mL sodium chloride and/or 5-12 mg/mL sodium acetate, or alternatively at a concentration of 2-50 mg/mL in an aqueous solution comprising 5-10 mg/mL sodium chloride, 1-5 mg/mL sodium phosphate dibasic heptahydrate, 0.1-0.5 mg/mL sodium phosphate monobasic monohydrate.

[0124] Other examples of formulations of a compound of any one of Formulae Ia-Ic include an injectable formulation having a concentration of 2-100 mg/mL of the compound in an aqueous solution comprising 0.5-1.0% sodium chloride, 0.05-0.10% monobasic sodium phosphate dihydrate, 1.0-2.0% dibasic sodium phosphate dihydrate, 0.01-0.05% sodium citrate, 0.10-0.20% citric acid monohydrate, 1.0-2.0% mannitol, 0.1%-0.2 polysorbate 80, and Water for Injection, USP. Sodium hydroxide added as necessary to adjust pH.

Methods

[0125] The compounds useful herein can be prepared from readily available starting materials using the following general methods and procedures. It will be appreciated that where typical or preferred process conditions (*i.e.*, reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc) are given, other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

[0126] Additionally, as will be apparent to those skilled in the art, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. Suitable protecting groups for various functional groups as well as suitable conditions for protecting and deprotecting particular functional groups are well known in the art. For example, numerous protecting groups are described in T. W. Greene and G. M. Wuts, *Protecting Groups in Organic Synthesis*, Third Edition, Wiley, New York, 1999, and references cited therein.

[0127] Furthermore, the compounds useful herein may contain one or more chiral centers. Accordingly, if desired, such compounds can be prepared or isolated as pure stereoisomers, i.e., as individual enantiomers or diastereomers, or as stereoisomer-enriched mixtures. All such stereoisomers (and

enriched mixtures) are included within the scope of this disclosure, unless otherwise indicated. Pure stereoisomers (or enriched mixtures) may be prepared using, for example, optically active starting materials or stereoselective reagents well-known in the art. Alternatively, racemic mixtures of such compounds can be separated using, for example, chiral column chromatography, chiral resolving agents and the like.

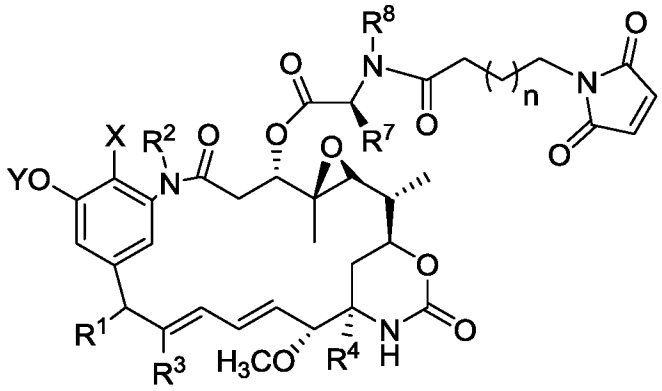
[0128] The starting materials for the following reactions are generally known compounds or can be prepared by known procedures or obvious modifications thereof. For example, many of the starting materials are available from commercial suppliers such as Aldrich Chemical Co. (Milwaukee, Wisconsin, USA), Bachem (Torrance, California, USA), Emka-Chemce or Sigma (St. Louis, Missouri, USA). Others may be prepared by procedures, or obvious modifications thereof, described in standard reference texts such as Fieser and Fieser's *Reagents for Organic Synthesis*, Volumes 1-15 (John Wiley and Sons, 1991), Rodd's *Chemistry of Carbon Compounds*, Volumes 1-5 and Supplementals (Elsevier Science Publishers, 1989), *Organic Reactions*, Volumes 1-40 (John Wiley and Sons, 1991), March's *Advanced Organic Chemistry*, (John Wiley and Sons, 4th Edition), and Larock's *Comprehensive Organic Transformations* (VCH Publishers Inc., 1989).

[0129] The various starting materials, intermediates, and compounds useful herein may be isolated and purified where appropriate using conventional techniques such as precipitation, filtration, crystallization, evaporation, distillation, and chromatography. Characterization of these compounds may be performed using conventional methods such as by melting point, mass spectrum, nuclear magnetic resonance, and various other spectroscopic analyses.

[0130] Coupling reagents include carbodiimide, amininum and phosphonium based reagents. Carbodiimide type reagents include dicyclohexylcarbodiimide (DCC), diisopropylcarbodiimide (DIC), and 1-ethyl-3-(3-dimethylaminopropyl)-dicarbodiimide (EDC), etc. Aminium salts include N-[(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridine-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide (HATU), N-[(1H-benzotriazol-1-yl)(dimethylamino)methylene]-N-methylmethanaminium hexafluorophosphate N-oxide (HBTU), N-[(1H-6-chlorobenzotriazol-1-yl)(dimethylamino)methylene]-N-methylmethanaminium hexafluorophosphate N-oxide (HCTU), N-[(1H-benzotriazol-1-yl)(dimethylamino)methylene]-N-methylmethanaminium tetrafluoroborate N-oxide (TBTU), and N-[(1H-6-chlorobenzotriazol-1-yl)(dimethylamino)methylene]-N-methylmethanaminium tetrafluoroborate N-oxide (TCTU). Phosphonium salts include 7-azabenzotriazol-1-yl-N-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP) and benzotriazol-1-yl-N-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP). Amide formation step may be conducted in

a polar solvent such as dimethylformamide (DMF) and may also include an organic base such as diisopropylethylamine (DIEA) or dimethylaminopyridine (DMAP).

[0131] For example, compounds of Formula Ia or Ib can be prepared by contacting a compound of Formulae A or B, respectively, wherein the variables are as defined herein, with an antibody in a suitable solvent, such as a buffer. Compounds of Formulae A and B can be synthesized as described in PCT Publication No. WO/2019/029715.



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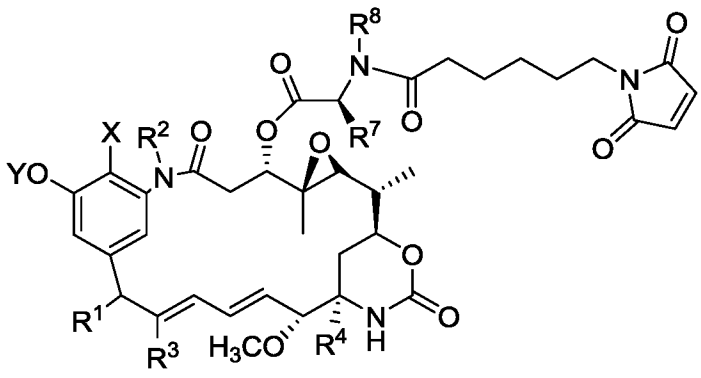


B

EXAMPLE

Clinical Study

[0132] This clinical study evaluates treatment of patients with advanced epithelial carcinoma by ADC1 described herein.

[0133] Inclusion criteria:

- Between 18 and 75 years old;

- Standard treatment of the disease is ineffective or unacceptable, or non-existent, or disease is refractory to or relapsed after at least one prior standard therapeutic regimen;
- The disease has been confirmed histologically;
- Positive expression of Trop2 confirmed by immunohistochemistry;
- At least one measurable tumor according to RECIST 1.1;
- An Eastern Cooperative Oncology Group (ECOG) performance status of between 0 and -1;
- An expected survival of three or more months;
- Have appropriate laboratory test indicators;
- Left ventricular ejection fraction (LVEF) of at least 50% by echocardiography;
- At least 4 weeks after termination of previous treatment for the disease, if any, and substantial recovery from the adverse effects of the previous treatment (e.g., according to CTCAE version 5.0 criteria).

[0134] Exclusion criteria:

- Active hepatitis B or hepatitis C or syphilis;
- History of immunodeficiency;
- Clinically significant active infection;
- Other concurrent, severe, or uncontrollable systemic diseases (such as clinically significant metabolic diseases, poor wound healing, ulcers, etc.);
- History of moderate or severe dyspnea at rest, or current continuous oxygen therapy, or current interstitial lung disease (ILD) or pneumonia;
- Clinically significant cardiovascular diseases within the past six months prior to the treatment;
- Metastasis symptoms of the disease to the brain or the central nervous system;
- Grade two or more of peripheral neuropathy (CTCAE version 5.0 classification);
- Participated in and received a clinical trial treatment within four weeks prior to treatment with ADC1;
- Not fully recovered form a major surgical treatment within four weeks prior to treatment with ADC1;
- Known or suspected to be allergic to the ADC1 or any component of a composition comprising ADC1;
- Pregnant or lactating.

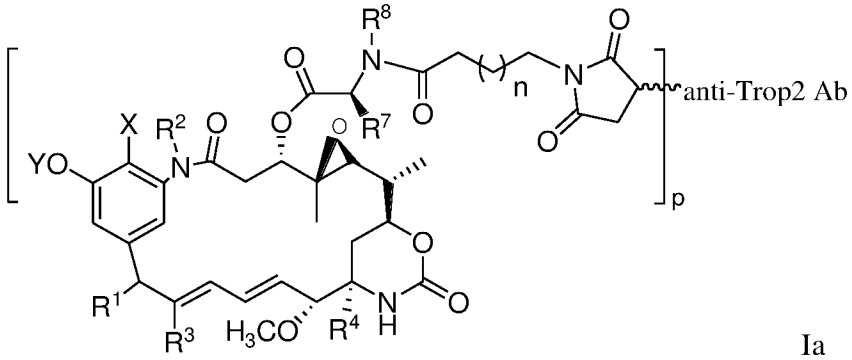
[0135] The patients enrolled are divided into 7 groups, each receiving ADC1 at a dose of 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, 8 mg/kg, or 10 mg/kg once every three weeks via I.V. infusion. Safety and tolerance will be evaluated after the first treatment and clinical pharmacokinetics and

immunogenicity will be evaluated after four treatments. Preliminary effectiveness is determined throughout the treatment. After receiving the treatment, the patient may achieve a complete response, a partial response, or stable disease.

What is claimed is:

1. A method for treating a disease comprising:

administering to a subject in need thereof an effective amount of a compound of Formula Ia:



Ia

or a salt thereof,

wherein

X is hydrogen or halo;

Y is selected from the group consisting of hydrogen, C_1 - C_6 alkyl, C_3 - C_6 cycloalkyl, and -C(=O)R^5;

R^1 is selected from the group consisting of hydrogen, -OH, -OC(=O)R^5 and -OR^5;

R^2 is hydrogen or C_1 - C_6 alkyl;

R^3 is methyl, -CH_2OH, or -CH_2C(=O)R^6;

R^4 is -OH or -SH;

R^5 is C_1 - C_6 alkyl or benzyl;

R^6 is C_1 - C_6 alkyl, phenyl or benzyl;

R^7 is hydrogen, C_1 - C_6 alkyl or an amino acid side chain;

R^8 is hydrogen or C_1 - C_6 alkyl;

n is 0, 1, 2, 3, 4, 5, 6, 7 or 8;

p is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10; and

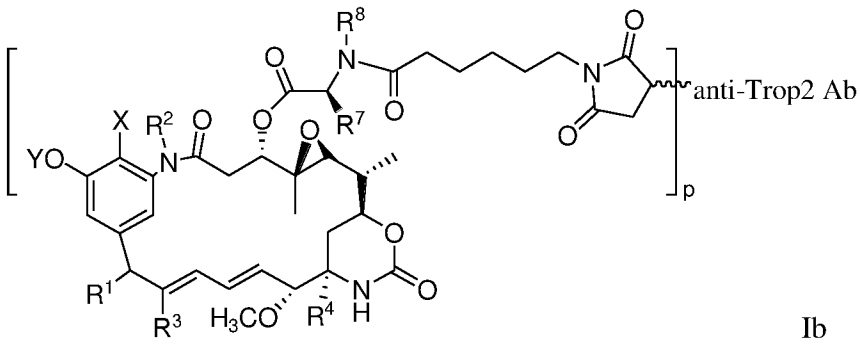
the anti-Trop2 Ab is an anti-Trop2 antibody, or an antigen-binding fragment thereof, which has specificity to a human Trop2 protein and comprises:

a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:1 or a polypeptide having at least 90% sequence identity to SEQ ID NO:1 and having a LCDR1 comprising the amino acid sequence of SEQ ID NO:2, a LCDR2 comprising the amino acid sequence of SEQ ID NO:3 and a LCDR3 comprising the amino acid sequence of SEQ ID NO:4; and

a heavy chain variant region (VH) comprising an amino acid sequence of SEQ ID NO:5 or a polypeptide having at least 90% sequence identity to SEQ ID NO:5 and having a HCDR1 comprising the amino acid sequence of SEQ ID NO:6, a HCDR2 comprising the amino acid sequence of SEQ ID NO:7 and a HCDR3 comprising the amino acid sequence of SEQ ID NO:8; and

wherein the effective amount is about 0.5 mg/kg to about 10 mg/kg.

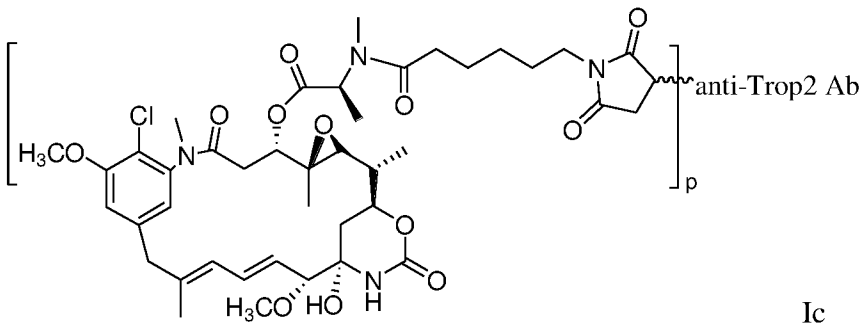
2. The method of claim 1, wherein the compound of Formula Ia is a compound of Formula Ib



Ib

or a pharmaceutically acceptable salt or solvate thereof.

3. The method of claim 1, wherein the compound of Formula Ia is a compound of Formula Ic



Ic

or a pharmaceutically acceptable salt thereof.

4. The method of any one of claims 1-3, wherein the subject has a cancer characterized by Trop2 positive cells.

5. The method of claim 4, wherein the cancer is triple negative breast cancer, glioblastoma, medulloblastoma, urothelial carcinoma, breast cancer, head and neck cancer, kidney cancer, ovarian cancer, Kaposi's sarcoma, pancreatic cancer and lung cancer.

6. The method of claim 4, wherein the cancer is epithelial carcinoma.

7. The method of claim 6, wherein the cancer is advanced epithelial carcinoma.

8. The method of any one of claims 1-7, wherein the cancer is refractory or to or relapsed after at least one prior standard therapeutic regimen.

9. The method of any one of claims 1-8, wherein the VL comprises the amino acid sequence of SEQ ID NO:1, and wherein the VH comprises the amino acid sequence of SEQ ID NO:5.

10. The method of any one of claims 1-9, wherein the anti-Trop2 antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:9 and a heavy chain comprising the amino acid sequence of SEQ ID NO:10.

11. The method of claim 10, wherein at least about 80% of the anti-Trop2 antibody comprises the G0 glycan at the asparagine residue 301 (Asn301) of SEQ ID NO:10, and wherein at most about 2% of the anti-Trop2 antibody comprises the Man5 glycan at the amino acid residue 301 of SEQ ID NO:10.

12. The method of any one of claims 1-10, wherein at least 50% of the anti-Trop2 antibody comprises the G0 glycan at an N-glycosylation site of a constant region of the anti-Trop2 antibody.

13. The method of claim 12, wherein at least about 80% of the anti-Trop2 antibody comprises the G0 glycan at the N-glycosylation site of the constant region of the anti-Trop2 antibody.

14. The method of any one of claims 12-13, wherein at most 10% of the anti-Trop2 antibody comprises the Man5 glycan at the N-glycosylation site of the constant region of the anti-Trop2 antibody.

15. The method of any one of claims 12-14, wherein at most about 2% of the anti-Trop2 antibody comprises the Man5 glycan at the N-glycosylation site of the constant region of the anti-Trop2 antibody.

16. The method of any one of claims 1-15, wherein the fucose content of the anti-Trop2 antibody is very low, the galactose level of the anti-Trop2 antibody is low, the mannose level of the anti-Trop2 antibody is low, the high mannose level of the anti-Trop2 antibody is low, and/or the G0 level of the anti-Trop2 antibody is high.

17. The method of any one of claims 1-16, wherein the fucose content of the anti-Trop2 antibody is at most about 5%, the galactose level of the anti-Trop2 antibody is at most 30%, the mannose level of the anti-Trop2 antibody is at most 5%, the high mannose level of the anti-Trop2 antibody is at most 5%, and/or the G0 level of the anti-Trop2 antibody is at least 60%.

18. The method of any one of claims 1-17, wherein p is 1, 2, 3 or 4.

19. The method of claim 18, wherein p is 2.

20. The method of any one of claims 1-19, wherein the anti-Trop2 antibody, or the antigen-binding fragment thereof, is produced from a CHO-BAT-KF cell.

21. The method of any one of claims 1-19, wherein of the effective amount is about 0.5 mg/kg to 10 mg/kg.

22. The method of any one of claims 1-21, wherein of the effective amount is about 0.5 mg/kg to about 10 mg/kg about once every two to four weeks.

23. The method of claim 22, wherein the effective amount is about 0.5 mg/kg to 10 mg/kg about once every three weeks.

24. The method of any one of claims 1-22, wherein the compound is administered for about four or more times.

25. The method of claim 24, wherein the effective amount is about 0.5 mg/kg to 0.9 mg/kg, 0.7 mg/kg to 1.3 mg/kg, 1.5 mg/kg to 2.5 mg/kg, 3 mg/kg to 5 mg/kg, 5 mg/kg to 7 mg/kg, 7 mg/kg to 9 mg/kg, or 9 mg/kg to 10 mg/kg once every three weeks.

26. The method of claim 24, wherein the effective amount is about 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, 8 mg/kg, or 10 mg/kg once every three weeks.

27. The method of any one of claims 1-26, wherein the administration is by intravenous injection.

28. The method of any one of claims 1-27, wherein the administration is carried out for about one hour.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2020/095753

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/30(2006.01)i; A61K 31/5365(2006.01)i; A61P 35/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K; A61K; A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS, SIPOABS, DWPI, CNTXT, EPTXT, WOTXT, USTXT, CNKI, ISI, NCBI, baidu, genbank, STN, applications/inventors, maytansinoid, maytansine, anti-trop 2, antibody drug conjugates, bat0808, G0 glycan, Asn 301, SEQ ID NOs:1-8

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 109078181 A (BIO-THERA SOLUTIONS, LTD.) 25 December 2018 (2018-12-25) claims 5-7, 9, description page 2 paragraph 9, page 4 paragraphs 31-32, page 5 paragraphs 39-41, page 16 paragraphs 163-164, SEQ ID NOs:3-4, figures 7-9	1-10, 18-28
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Y	CN 109096399 A (BIO-THEREA SOLUTIONS, LTD.) 28 December 2018 (2018-12-28) abstract, claim 6, description page 1 paragraph 3	11-28
PX	CN 110526978 A (BIO-THERA SOLUTIONS, LTD.) 03 December 2019 (2019-12-03) claims 1-9, description page 16 paragraphs 164-166, page 26 paragraphs 223-224	1-10, 18-28
PY	CN 110526978 A (BIO-THERA SOLUTIONS, LTD.) 03 December 2019 (2019-12-03) claims 1-9, description page 16 paragraphs 164-166, page 26 paragraphs 223-224	11-28
A	WO 2018090045 A1 (CHO PHARMA, INC. et al.) 17 May 2018 (2018-05-17) the whole document	1-28

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 August 2020	**23 September 2020**
Name and mailing address of the ISA/CN **National Intellectual Property Administration, PRC** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088** **China** Facsimile No. **(86-10)62019451**	Authorized officer **CHEN,Hao** Telephone No. **86-(10)-53962068**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2020/095753

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	TANG, W. et al. "Abstract P6-20-16:BAT8003, a potent anti-trop-2 antibody-drug conjugate, for the treatment of triple negative breast cancer." *CANCER RESEARCH.*, Vol. 79, No. 4 Suppl., 28 February 2019 (2019-02-28), The whole document	1-28
A	MARIA, P.C. et al. "The target invites a foe: antibody–drug conjugates in gynecologic oncology." *Current opinion in obstetrics & gynecology.*, Vol. 30, No. 1, 01 February 2018 (2018-02-01), pages 44-50	1-28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2020/095753

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **1-28**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Claims 1-28 direct to a method of treatment of the patients, and do not meet the criteria set out in PCT Rules 39.1(iv). The search has been carried out and based on the use of the compound of any one of claims 1-28 for the manufacturing of a medicament for the treatment of diseases.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2020/095753

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2020/095753

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