ANTI-CLAUDIN 18.2 ANTIBODY DRUG CONJUGATES COMPRISING TOPOISOMERASE I INHIBITOR AND USES THEREOF

Related Applications

This application claims the benefit of priority to Korean Patent Application No. KR 10-2023-0136955, filed October 13, 2023, and Korean Patent Application No. KR 10-2024-0035926, filed March 14, 2024; the contents of which are hereby incorporated by reference in their entirety.

Background

Cancer refers to a disease caused by abnormally grown lumps due to autonomous overgrowth of body tissues, and is the result of uncontrolled cell growth in various tissues. Tumors in early stage can be removed by surgical and radio-therapeutic measures, and metastasized tumors are generally treated using chemotherapy.

Most chemotherapeutic agents administered parenterally may induce unwanted side effects and even serious toxicity, as a result of systemic administration. Accordingly, the focus of development has been on developing treatments to achieve increased efficacy and/or reduced minimal toxicity/side effects, for example through the selective application of these chemotherapeutic agents in tumor cells or immediately adjacent tissues. There remains a demand for development of antibody drug conjugates for effective therapeutic methods.

Summary

In certain aspects, the present disclosure provides antibody-drug conjugates comprising anti-Claudin 18 isoform 2 (CLDN18.2) antibody or antigen-binding fragment thereof that binds to CLDN18.2. In certain embodiments, the antibody disclosed herein binds to CLDN18.2 expressed in a tumor and may be used to deliver a topoisomerase I inhibitor (e.g., a camptothecin compound) to the tumor. In certain embodiments, the antibody drug conjugates disclosed herein have improved stability as compared to antibody drug conjugates known in the art.

In certain aspects, the present disclosure provides conjugates having a structure represented by General Formula I or a pharmaceutically acceptable salt thereof:

[General Formula I]

$Ab-[L^{Ab}-(B)_l]_m$

wherein,

Ab is an anti-Claudin 18 isoform 2 (CLDN18.2) antibody or antigen-binding fragment thereof comprising a heavy chain variable region and a light chain variable region, wherein

the heavy chain variable region comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 2, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6; and

the light chain variable region comprises a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 9, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 13;

each L^{Ab} is independently a linker;

each B is independently a topoisomerase I inhibitor (e.g., a camptothecin compound); and

l and m are each independently 1 to 20.

Brief Description of the Drawings

FIG. 1 shows the production process of ADC1 and structure thereof. Amino acid sequence CVIM is of SEQ ID NO: 22.

FIG. 2 shows the production process of ADC2 and structure thereof. Amino acid sequence CVIM is of SEQ ID NO: 22.

FIG. 3 shows the production process of ADC3 and structure thereof. Amino acid sequence CVIM is of SEQ ID NO: 22.

FIG. 4 shows that ADC1, ADC2, and ADC3 all exhibited different levels of hydrophobicity compared to the antibody and the prenylated antibody.

FIG. 5 shows that ADC3 exhibited significant tumor growth inhibition in the SNU-601 model of gastric cancer cell line compared to the control group.

FIG. 6 shows that in the SNU-601 model, ADC3 exhibited dose-dependent tumor growth inhibition compared to the control group. After day 33, ADC3 showed better tumor growth inhibition than ADC1 at the same dose (1.0 mg/kg, QDx1).

FIG. 7 shows that in the SNU-601 model, ADC1 and ADC3 showed tumor growth inhibition even at low dose (0.5 mg/kg, BIWx2). After day 26, ADC3 showed better tumor growth inhibition than ADC1 at the same dose (0.5 mg/kg, BIWx2).

FIG. 8 shows that in the PATU-8988s model of pancreatic cancer cell line, ADC3 showed excellent anti-cancer efficacy and dose-dependent tumor growth inhibition compared to the control group.

FIG. 9 shows the results of pharmacokinetic analysis in the rat model of ADC1 and ADC3.

Detailed Description

An antibody-drug conjugate (ADC) is a targeted technology for conjugating a toxin or a drug to an antibody that binds to an antigen, by which the toxin is released in a cell to cause death of cancer cells and the like. The ADC enables a drug to be accurately delivered to target cancer cells while minimally affecting healthy cells, and to be released only under specific conditions, and thus has excellent efficacy compared to antibody therapeutic agents themselves and can remarkably reduce the risk of side effects compared to existing anticancer agents.

The basic structure of these antibody-drug conjugates is "antibody-linker-small molecule drug (toxin)". In this structure, the linker plays a functional role in linking the antibody and the drug, but in some cases also ensures that the drug is released from the antibody at the appropriate time, for example after reaching target cells. That is, the stability of the linker can play a very important role in the efficacy and safety such as systemic toxicity of an antibody-drug conjugate (Discovery Medicine 2010, 10(53): 329-39).

The use of monoclonal antibodies for cancer treatment has had substantial success. For example, monoclonal antibodies are suitable for target-directed addressing of tumor tissue and tumor cells. Antibody-drug conjugates have become a novel and powerful option for the treatment of lymphomas and solid cancers, and immunomodulatory antibodies also have recently had considerable success in clinical trials. The development of therapeutic antibodies is based on deep understanding of cancer serology, protein engineering technology and the action thereof, mechanisms of resistance, and interactions between immune systems and cancer cells.

Antigens which are expressed on the surface of human cancer cells are defined as a broad range of targets which are over-expressed compared to normal tissues, mutated and selectively expressed. The key challenge is to identify antigens suitable for antibody-based therapies. These therapeutic agents mediate changes in antigen or receptor function (i.e.,

function as a stimulant or an antagonist), regulate the immune system through Fc and T cell activation, and exhibit efficacy through the delivery of specific drugs that bind to antibodies targeting specific antigens. Molecular techniques that can alter antibody pharmacokinetics, action function, size and immune stimulation are emerging as key factors in the development of novel antibody-based therapies. Evidence from clinical trials of therapeutic antibodies in cancer patients highlights the importance of approaches for selecting optimized antibodies, including affinity and binding of target antigens and antibodies, selection of an antibody structure, and therapeutic approaches (signaling blockade or immune function).

Human claudin 18 isoform 2 (CLDN18.2) is a transmembrane protein consisting of a full length of 261 amino acids with two extracellular loops and four transmembrane domains. CLDN18.2 differs in a part of the amino acid sequence up to extracellular loop domain 1, from CLDN18.1 expressed in some normal tissues.

CLDN18.2 belongs to the claudin family, which is an important component of tight junctions, and claudin proteins are expressed in various cancer tissues and are known to be potential targets for diagnostic and therapeutic modalities. CLDN18.2 is also known to play an important role in tumorigenesis and inflammatory responses, and changes in the expression of claudin proteins are known to cause dysfunction of tight junctions, affect signaling pathways, and have tumor-promoting action in some epithelial cancers. In addition, CLDN18.2 is specifically expressed in primary and metastatic gastric cancer, except for expression in the apical region of some gastric mucosa, is known as a cancer-specific antigen that is also associated with malignant transformation, and ectopic activation of CLDN18.2 has been reported in pancreatic cancer, esophageal cancer, ovarian cancer, lung cancer, and the like.

In certain aspects, the present disclosure provides antibody-drug conjugates comprising an anti-Claudin 18 isoform 2 (CLDN18.2) antibody or antigen-binding fragment thereof that binds to CLDN18.2. In certain embodiments, the antibody disclosed herein binds to CLDN18.2 expressed in a tumor and may be used to deliver a topoisomerase I inhibitor (e.g., a camptothecin compound) to the tumor. In certain embodiments, the antibody drug conjugates disclosed herein have improved stability as compared to antibody drug conjugates known in the art.

In certain aspects, the present disclosure provides conjugates having a structure represented by General Formula I or a pharmaceutically acceptable salt thereof:

[General Formula I]

$Ab\text{-}[L^{Ab}\text{-}(B)_l]_m$

wherein,

Ab is an anti-Claudin 18 isoform 2 (CLDN18.2) antibody or antigen-binding fragment thereof comprising a heavy chain variable region and a light chain variable region, wherein

the heavy chain variable region comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 2, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6; and

the light chain variable region comprises a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 9, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 13;

each L^{Ab} is independently a linker;

each B is independently a topoisomerase I inhibitor (e.g., a camptothecin compound); and

l and m are each independently 1 to 20.

In some embodiments, Ab comprises a heavy chain variable region comprising: the amino acid sequence of SEQ ID NO: 15; a sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 15 while maintaining the heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 2, the heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and the heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6; or a sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 15 while maintaining the heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 2, the heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and the heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6.

In some embodiments, Ab comprises a light chain variable region comprising: the amino acid sequence of SEQ ID NO: 16; a sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 16 while maintaining the light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 9, the light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and the light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 13; or a sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 16 while maintaining the light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 9, the light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and the light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 13.

In some embodiments, Ab comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15 and a light chain variable region comprising the amino acid sequences of SEQ ID NO: 16.

In some embodiments, Ab is a humanized antibody or a human antibody. In some embodiments, Ab is selected from a monoclonal antibody, a domain antibody (dAb), a single chain antibody (scAb), a Fab fragment, a Fab' fragment, a F(ab')2 fragment, an scFab fragment, an Fv fragment, a dsFv fragment, a single chain variable fragment (scFv), an scFv-Fc fragment, a single domain heavy chain antibody, a single domain light chain antibody, a variant antibody, a multimeric antibody, a minibody, a diabody, a bispecific antibody, and a multispecific antibody.

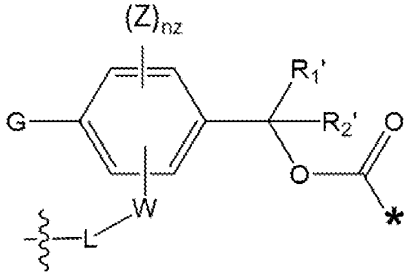
In some embodiments, Ab comprises an IgA, IgG, IgM, IgE, or IgD constant domain or is an IgA, IgG, IgM, IgE, or IgD antibody. In some embodiments, Ab comprises an IgG constant domain or is an IgG antibody. In some embodiments, Ab comprises an IgG1, IgG2, IgG3, or IgG4 constant domain or is an IgG1, IgG2, IgG3, or IgG4 antibody.

In some embodiments, Ab comprises LALA mutations in a heavy chain constant region. In some embodiments, the LALA mutations comprise L234A and L235A according to EU numbering convention. In some embodiments, Ab comprises LALA mutations in a heavy chain constant region in place of amino acids corresponding to amino acids 237-238 in SEQ ID NO: 17.

In some embodiments, each linker is cleavable. In some embodiments, each linker is cleavable by a glycosidase. In some embodiments, the glycosidase is β-glucuronidase or β-galactosidase. In some embodiments, the linker comprises a β-glucuronosyl or β-galactosyl moiety.

In some embodiments, each linker comprises a moiety independently represented by General Formula II or a pharmaceutically acceptable salt thereof:

[General Formula II]



wherein

G is a sugar moiety or a glucuronic acid moiety;

each R_1' and R_2' are independently hydrogen, C_1-C_8 alkyl or C_3-C_8 cycloalkyl; or R_1' and R_2', combine to complete a (C_3-C_8) cycloalkyl ring;

W is -C(O)-, -C(O)NR'-, -NR'C(O)-, -$(CH_2)_t$N(R')C(O)-, -C(O)O-, -$S(O_2)$N(R')-, -P(O)(R')'N(R'), -S(O)N(R')-, or -$P(O_2)$N(R')-, wherein the indicated C(O), N, CH_2, S, or P is directly bonded to the phenyl ring of Formula II;

R' and R'' are each independently hydrogen, C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{1-8} alkoxy, C_{1-8} alkylthio, mono- or di-C_{1-8} alkylamino, C_{3-20} heteroaryl, or C_{6-20} aryl;

each Z is independently C_{1-8} alkyl, halogen, cyano, or nitro;

nz is an integer from 0 to 3;

t is an integer from 1 to 3;

L is absent, C_1-C_{50} alkylene, C_1-C_{50} alkenylene or 1-50 atom heteroalkylene;

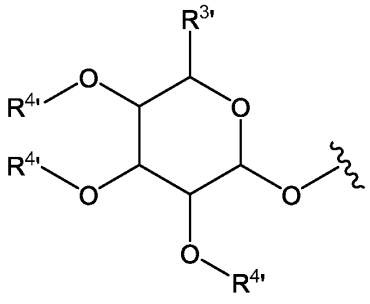
* represents a connection point to B; and

〰 represents a connection point to the antibody.

In some preferred embodiments, W is -C(O)-, -C(O)N(R')-, -$(CH_2)_t$N(R')C(O)-, -C(O)O-, -$S(O_2)$N(R')-, -P(O)R''N(R')-, -SON(R')-, or -PO_2N(R')-, wherein the C(O), S, or P is directly bonded to the phenyl ring of Formula II.

In some embodiments, G is a sugar moiety represented by General Formula (III)

[General Formula III]



;

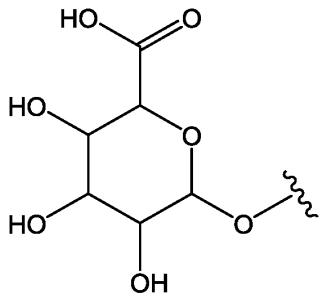
wherein

$R^{3'}$ is -CH_2OR^{3A}, or -CO_2R^{3B};

R^{3A} is H or a hydroxyl protecting group;

R^{3B} is H or a carboxyl protecting group; and

each $R^{4'}$ is, respectively and independently, a hydrogen or a hydroxyl protecting group.

In some embodiments, G is  ; and

W is -C(O)NR'-.

In some embodiments, L comprises:

A) a C_1-C_{50} alkylene or a 1 to 50 atom heteroalkylene comprising:

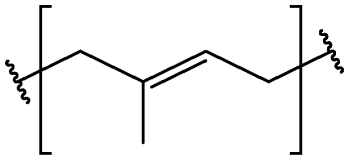
(i) one or more unsaturated bonds;

(ii) a heteroarylene (e.g., a heteroarylene in the alkylene or heteroalkylene chain); and/or

(iii) a C_{1-20} alkyl; and/or

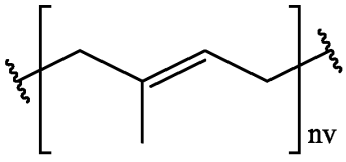
B) at least one isoprenyl group having the structure represented by General Formula IV:

[General Formula IV]



In some embodiments, the L comprises an isoprenyl group having a structure represented by General Formula IVa:

[General Formula IVa]



;

wherein nv is an integer from 1-20.

In some embodiments, wherein L comprises a peptide comprising at least one hydrophilic amino acid. In some embodiments, the hydrophilic amino acid comprises a side chain having a moiety that has electric charge in aqueous solution at neutral pH (e.g., an amine, guanidine, or carboxyl moiety). In some embodiments, the peptide comprises an amino acid selected from arginine, histidine, aspartate, asparagine, glutamate, glutamine, glycine, lysine, ornithine, proline, serine, and threonine.

In some embodiments, L is covalently connected to Ab by a sulfur atom in a cysteine residue of Ab (e.g., by a sulfur atom in the side chain of the cysteine residue) via a thioether bond. In some embodiments, Ab at the C-terminus comprises an amino acid motif that is recognized by an isoprenoid transferase. In some embodiments, the isoprenoid transferase is farnesyl protein transferase (FTase) or geranylgeranyl transferase (GGTase).

In some embodiments, L is covalently bonded to Ab by a thioether bond and the thioether bond comprises a sulfur atom of a cysteine of the amino acid motif. In some embodiments, the amino acid motif comprises a CYYX sequence, wherein: C is cysteine; each Y is an aliphatic amino acid; X is selected from glutamine, glutamate, serine, cysteine, methionine, alanine, and leucine. In some embodiments, each Y is independently selected from alanine, isoleucine, leucine, methionine, and valine.

In some embodiments, the amino acid motif comprises a CVIM (SEQ ID NO: 22) or CVLL sequence (SEQ ID NO: 23). In some embodiments, at least one of the 1 to 20 amino acids preceding the amino acid motif is glycine. In some embodiments, the amino acid motif has the sequence GGGGGGGCVIM (SEQ ID NO: 19).

In some embodiments, L comprises an oxime. In some embodiments, the oxygen atom of the oxime is on the side of L linked to W and the carbon atom of the oxime is on the side of L linked to Ab. In some embodiments, the carbon atom of the oxime is on the side of L linked to W and the oxygen atom of the oxime is on the side of L linked to Ab. In some embodiments, L is a 1-50 atom heteroalkylene comprising an oxime; and the oxygen atom of the oxime is on the side of L linked to W, and the carbon atom of the oxime is on the side of L linked to Ab; or the carbon atom of the oxime is on the side of L linked to W, and the oxygen atom of the oxime is on the side of L linked to Ab. In some embodiments, L comprises an oxime, and at least one isoprenyl unit covalently bonds the oxime to Ab (e.g., the at least one isoprenyl unit directly or indirectly bonds the oxime to Ab).

In some embodiments, L comprises a connecting unit represented by General Formula Va or Vb:

[General Formula Va]

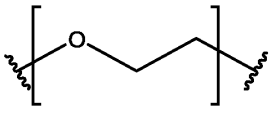
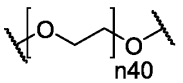
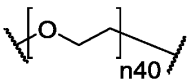
$-(CH_2)_r(V(CH_2)_p)_q-$

[General Formula Vb]

$-(CH_2CH_2X)_w-$

V is a single bond, -O-, -S-, - NR^{21}-, -C(O)NR^{22}-, -NR^{23}C(O)-, -$NR^{24}SO_2$-, or -SO_2NR^{25}-; X is -O-, C_{1-8} alkylene, or -NR^{21}-; R^{21} to R^{25} are each independently hydrogen, C_{1-6} alkyl, C_{1-6} alkyl C_{6-20} aryl, or C_{1-6} alkyl-C_{3-20} heteroaryl; r is an integer from 0 to 10; p is an

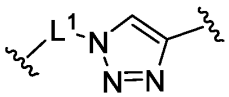
integer from 0 to 10; q is an integer from 1 to 20; and w is an integer from 1 to 20. In some embodiments, q is 1 to 10. In some embodiments, r is 1 or 2. In some embodiments, p is 1 or 2. In some embodiments, V is -O-. In some embodiments: q is an integer from 1 to 10; r and p are each an integer from 1 or 2; and V is -O-. In some embodiments, X is -O-.

In some embodiments, L comprises at least one polyethylene glycol monomer represented by . In some embodiments, L comprises a polyethylene glycol oligomer represented by  or , wherein n40 is an integer from 2 to 10.

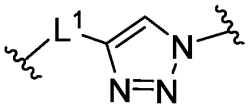
In some embodiments, L comprises an oxime, and the at least one polyethylene glycol unit covalently bonds the oxime to W. In some embodiments, L further comprises a binding unit formed by a reaction between an alkyne and an azide or between an aldehyde or ketone group and hydrazine or hydroxylamine.

In some embodiments, L further comprises a binding unit represented by General Formula VIa, VIb, VIc, VId, or VIe:

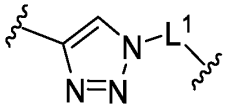
[General Formula VIa]



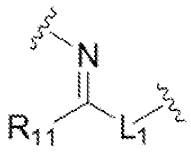
[General Formula VIb]



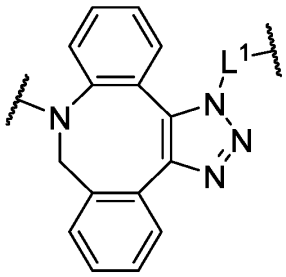
[General Formula VIc]



[General Formula VId]



[General Formula VIe]



wherein L^1 is each independently a single bond or C_{1-30} alkylene; and R^{11} is hydrogen or C_{1-10} alkyl.

In some embodiments, L is branched and comprises:

i) a branching unit covalently coupled to Ab by a primary linker;

ii) a first branch which couples a first B to the branching unit; and

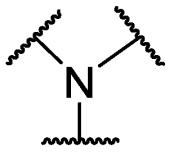
iiia) a second branch which couples a second B to the branching unit; or

iiib) a second branch, in which an alkyl or heteroalkyl (e.g., a polyethylene glycol monomer or a polyethylene glycol oligomer) is covalently coupled to the branching unit.

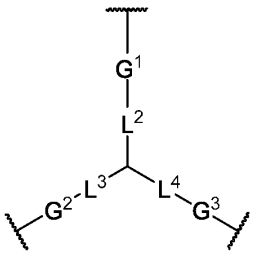
In some embodiments, L comprises a second branch which couples a second B, via a cleavage group, to the branching unit. In some embodiments, L comprises a second branch, in which an alkyl or heteroalkyl (*e.g.,* a polyethylene glycol monomer or a polyethylene glycol oligomer) is covalently coupled to the branching unit.

In some embodiments, the branching unit has a structure represented by General Formula VIIa, VIIb, VIIc, VIId, or VIIe:

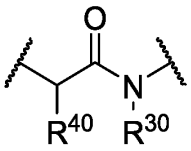
[General Formula VIIa]



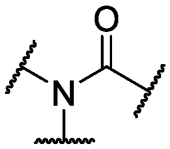
[General Formula VIIb]



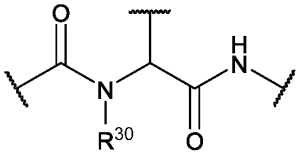
[General Formula VIIc]

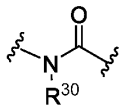
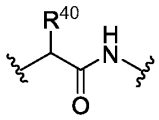
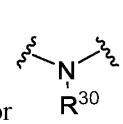


[General Formula VIId]



[General Formula VIIe]



wherein G^1, G^2, and G^3 each independently represents a bond, , ,  or ;

R^{30} is hydrogen or C_{1-30} alkyl;

R^{40} is hydrogen or L^5-$COOR^{50}$;

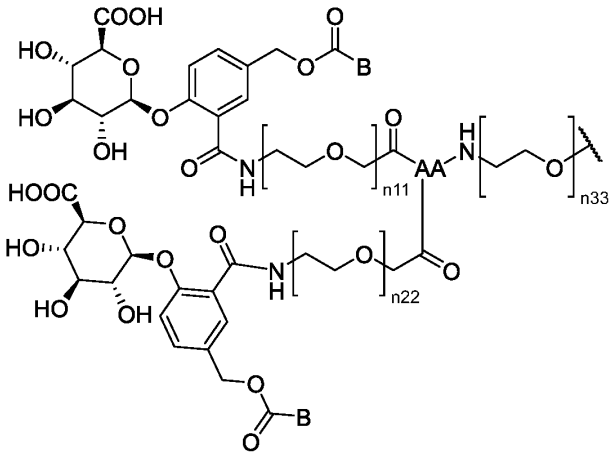
R^{50} is hydrogen or C_{1-30} alkyl;

L^2, L^3, L^4, and L^5 are each independently a bond or $-C_{n'}H_{2n'}-$; and

n' is an integer from1 to 10.

In some embodiments, at least one branched linker is covalently coupled to Ab; and at least two B are covalently coupled to the branched linker. In some embodiments, the conjugate comprises 1, 2, 3, or 4 branched linkers and each branched linker is covalently coupled to two B. In some embodiments, the branching unit comprises a lysine residue.

In some embodiments, the conjugate comprises a structure represented by:

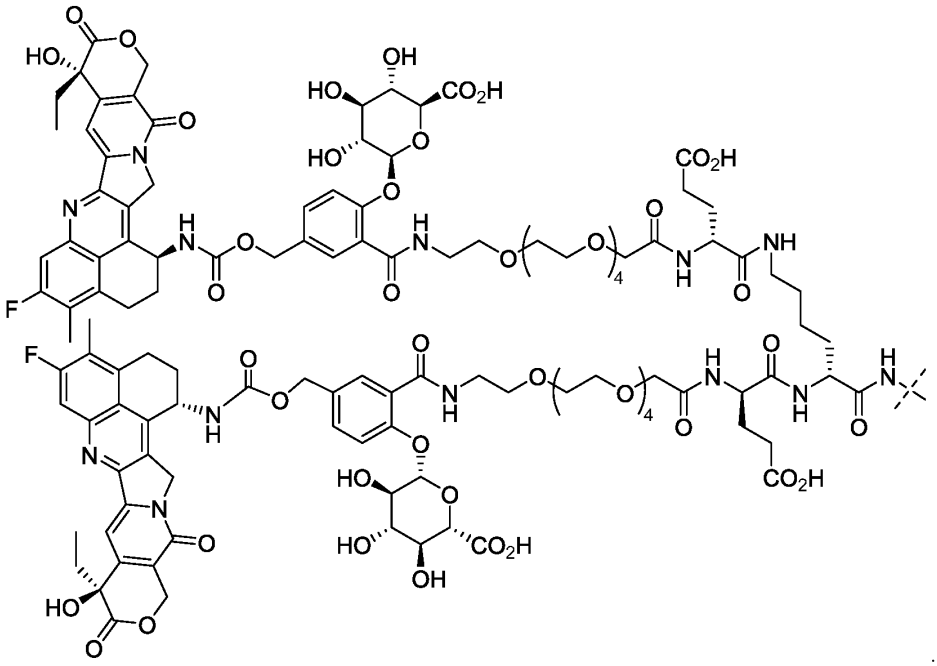


or a pharmaceutically acceptable salt thereof; wherein each B is, independently, a camptothecin compound; n11, n22, and n33 are each independently an integer from 0 to 30; and AA is a peptide comprising at least two amino acid residues.

In some embodiments, the camptothecin compound is camptothecin (CPT), hydroxycamptothecin, aminocamptothecin, exatecan, topotecan, belotecan, irinotecan, cositecan, rubitecan, lurtotecan, gimatecan, silatecan, namitecan, homocamptothecin (HomoCPT), diflomotecan, S39625, S38809, S38769, ZBH-01, ZBH-1207, BN80915, CPT211, Dxd, SN-38, or FL118. In some embodiments, the camptothecin compound is

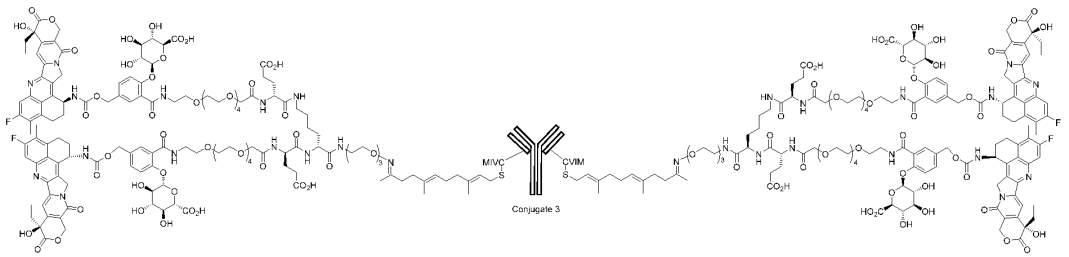
hydroxycamptothecin, aminocamptothecin, exatecan, belotecan, irinotecan, cositecan, rubitecan, lurtotecan, gimatecan, silatecan, namitecan, diflomotecan, Dxd, SN-38, or FL118. In some embodiments, the camptothecin compound is exatecan.

In some embodiments, the conjugate comprises a structure represented by:

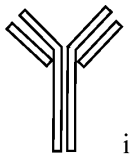


.

In some embodiments, the conjugate is:



or a salt thereof, wherein:



is Ab.

In some embodiments, Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 17 or 20, and a light chain comprising the amino acid sequence of SEQ ID NO: 18.

In certain aspects, the present disclosure provides pharmaceutical compositions comprising a conjugate disclosed herein and a pharmaceutically acceptable excipient.

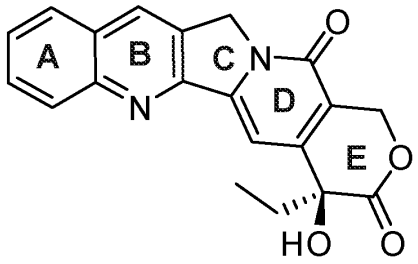
In certain aspects, the present disclosure provides pharmaceutical preparations comprising a conjugate disclosed herein and a pharmaceutically acceptable excipient, the pharmaceutical preparation being selected from injections, tablets, pills, powders, granules, capsules, troches, suspensions, liquids for internal use, emulsions, syrups, emulsions, freeze-dried preparations, and suppositories.

In certain aspects, the present disclosure provides methods of treating or preventing hyperplasia, cancer or an angiogenic disease in a subject in need thereof comprising administering a conjugate disclosed herein or a pharmaceutically acceptable salt thereof to the subject. In some embodiments, the method is a method of treating or preventing cancer. In some embodiments, the cancer is lung cancer, small-cell lung cancer, non-small-cell lung cancer, gastrointestinal cancer, colon cancer, intestinal cancer, bowel cancer, breast cancer, ovarian cancer, prostate cancer, testicular cancer, liver cancer, kidney cancer, bladder cancer, pancreatic cancer, brain cancer, sarcoma, osteosarcoma, Kaposi's sarcoma or melanoma.

Topoisomerase Inhibitors

The topoisomerase inhibitors described in the present disclosure are chemical compounds that block the action of topoisomerases, which are broken into two broad subtypes: type I topoisomerases (TopI) and type II topoisomerases (TopII). Topoisomerases play important roles in cellular reproduction and DNA organization, as they mediate the cleavage of single and double stranded DNA to relax supercoils, untangle catenanes, and condense chromosomes in eukaryotic cells. Topoisomerase inhibitors influence these essential cellular processes. Some topoisomerase inhibitors prevent topoisomerases from performing DNA strand breaks while others, deemed topoisomerase poisons, associate with topoisomerase-DNA complexes and prevent the re-ligation step of the topoisomerase mechanism. These topoisomerase-DNA-inhibitor complexes are cytotoxic agents, as the un-repaired single- and double stranded DNA breaks they cause can lead to apoptosis and cell death. Because of this ability to induce apoptosis, topoisomerase inhibitors have gained interest as therapeutics against infectious and cancerous cells. Blocking these enzymes may kill cancer cells. In some embodiments, the topoisomerase inhibitor is a topoisomerase I inhibitor. In some embodiments, the topoisomerase I inhibitor is a camptothecin compound (e.g., camptothecin or a camptothecin derivative or analogue).

Camptothecin (CPT) was first derived from the tree Camptotheca acuminata, native to southern China. CPT has a planar pentacyclic ring structure, that includes a pyrrolo[3,4-β]-quinoline moiety (rings A, B and C), conjugated pyridone moiety (ring D) and one chiral center at position 20 within the alpha-hydroxy lactone ring with (S) configuration (the E-ring). A structural feature of CPT is its planar pentacyclic ring system, which includes a lactone ring (the E-ring). The lactone ring is believed to create the active form of the drug. The discovery of CPT led to the synthesis of CPT derivatives, such as topotecan (TPT), irinotecan, belotecan, gimatecan and silatecan.



Camptothecin

Camptothecin Compounds: Camptothecin, and its derivatives, as further described below, form an extensively studied, and art-recognized, class of compounds known as "camptothecins" or "camptothecin compounds". For example, studies have shown that substitution at position 7, 9, 10 and 11 can have positive effect on CPT activity and physical properties, e.g. potency and metabolic stability. Enlargement of the lactone ring by one CH_2 unit also enhances properties, as in homocamptothecin. Substitution at position 12 or 14 leads to inactive derivatives.

A- and B-ring modification: Alkyl substitution

Alkyl substitution at position 7 has shown increased cytotoxicity, such as ethyl (C_2H_5) or chloromethyl (CH_2Cl). These groups are able to react with DNA in the presence of topoisomerase I which leads to more tumor activity. It has also been shown that increasing the length of the carbon chain (in position 7) leads to increased lipophilicity and consequently greater potency and stability in human plasma. Other 7-modified CPT analogues are silatecans and karenitecins. They are potent inhibitors on topoisomerase I and both have alkylsilyl groups in position 7 which make them lipophilic and more stable. Silatecans or 7-silylcampthothecins have shown reduced drug-HSA interactions which contributes to its blood stability and they can also cross the blood brain barrier. DB-67 is a 10-hydroxy derivative and is among the most active silatecans. BNP1350 which belongs to the series of karenitecins exhibits cytotoxic activity and ability to overcome drug resistance. Still another route to make CPT's lipophilic

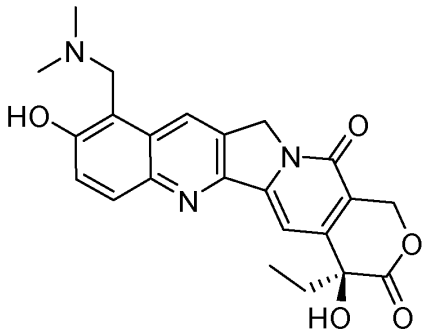
is to introduce lipophilic substituents, such as iminomethyl or oxyiminomethyl moieties. One of the most potent compounds is the oxyiminomethyl derivative ST1481 that has the advantage to overcome drug resistance caused by transport systems. Basic nitrogen in a carbon chain at position 7 makes the compound more hydrophilic and hence more water-soluble. For example, a derivate called CKD-602 is a potent topoisomerase I inhibitor and successfully overcomes the poor water solubility and toxicity seen with CPT. Considerably greater activity can be achieved by putting electron-withdrawing groups like amino, nitro, bromo or chloro at position 9 and 10 and hydroxyl group at position 10 or 11.

Hexacyclic CPT analogues

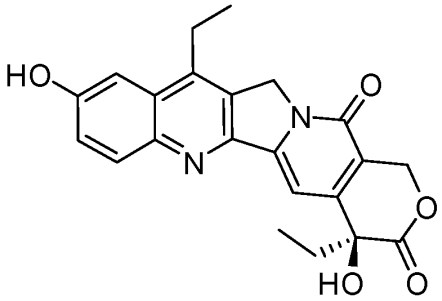
Hexacyclic CPT analogues have shown great potency. For example, a methylenedioxy or ethylenedioxy group connected between 10 and 11 forms a 5 or 6 membered ring which leads to more water-soluble derivates and increased potency. Adding amino or chloro group at 9th position or chloromethyl group at 7th position to these 10, 11-methylenedioxy or ethylenedioxy analogues results in compounds with even greater cytotoxicity but weaker solubility in water. To yield 10, 11-methylenedioxy or ethylenedioxy analogues with good water solubility a good way is to introduce a water solubilizing substituent at position 7. Lurtotecan meets those requirements; it is a 10, 11-ethylenedioxy analogue with a 4-methylpiperazino-methylene at position 7 and has shown a great potency in clinical researches.

A ring can also be formed between position 7 and 9, like position 10 and 11. That gives new opportunities to make water-soluble derivatives. These hexacyclic CPT become more active when electron-withdrawing groups are put in position 11 and methyl or amino groups at 10. Exatecan is an example of hexacyclic CPT that has a 6 membered ring over position 7 and 9, and is 10-methyl, 11-fluoro substituted. It is water-soluble and more potent than topotecan.

In some embodiments, the camptothecin compound is camptothecin (CPT), hydroxycamptothecin, aminocamptothecin, exatecan, topotecan, belotecan, irinotecan, cositecan, rubitecan, lurtotecan, gimatecan, silatecan, namitecan, diflomotecan, homocamptothecin (HomoCPT), S39625, S38809, S38769, ZBH-01, ZBH-1207, BN80915, CPT211, Dxd, SN-38, or FL118.

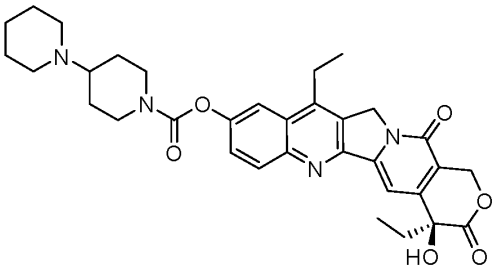


Topotecan



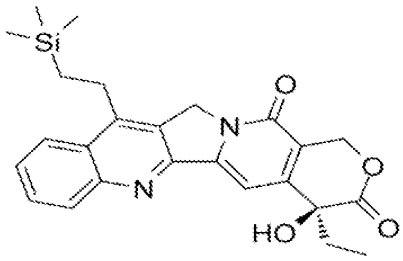
SN-38

7-Ethyl-10-hydroxycamptothecin

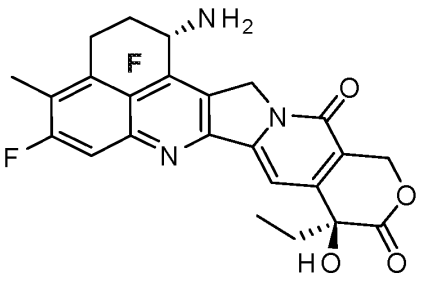


Irinotecan (prodrug of SN-38)

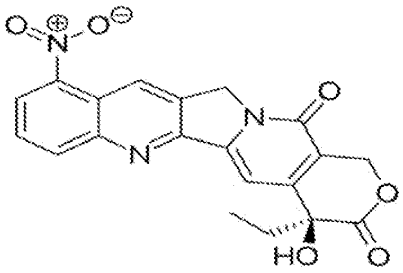
CTP-11



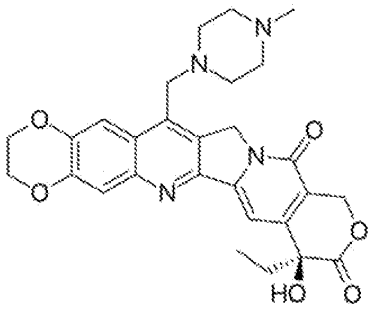
Cositecan (BNP1350, Karenitecin)



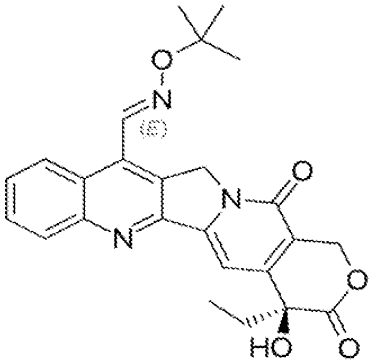
Exatecan (DX-8951)



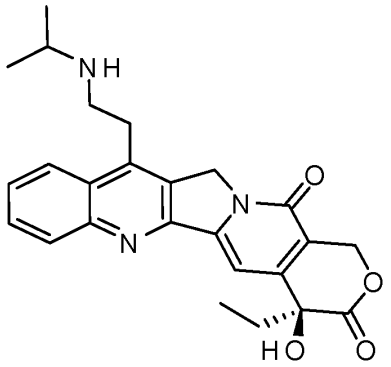
Rubitecan



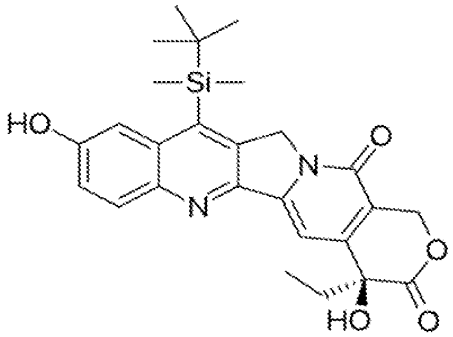
Lurtotecan



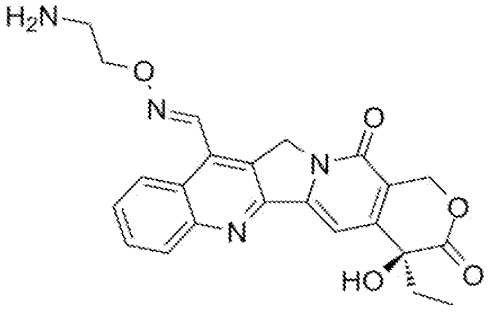
Gimatecan (ST1481)



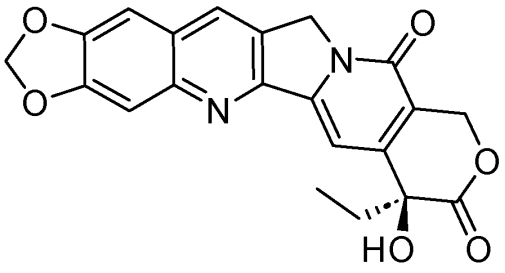
Belotecan (CKD-602)



Silatecan (DB-67, AR-67)

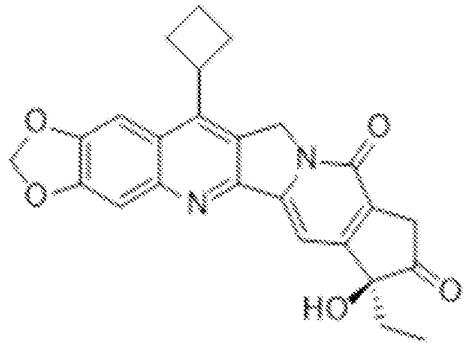


Namitecan (ST-1968)

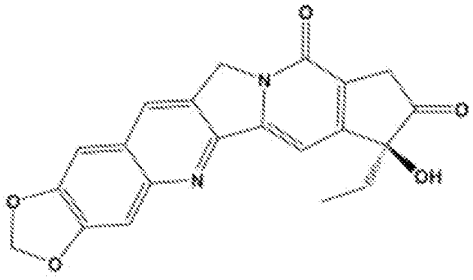


FL118

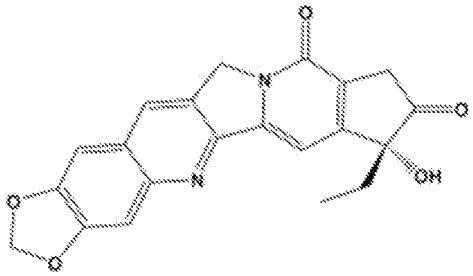
(10,11-(Methylenedioxy)-20(S)-camptothecin)



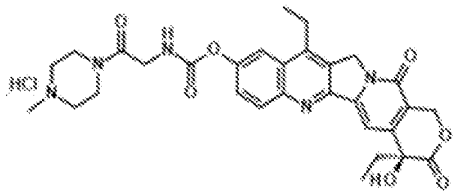
S39625



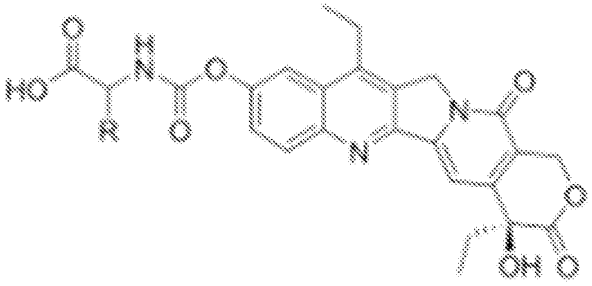
S38809



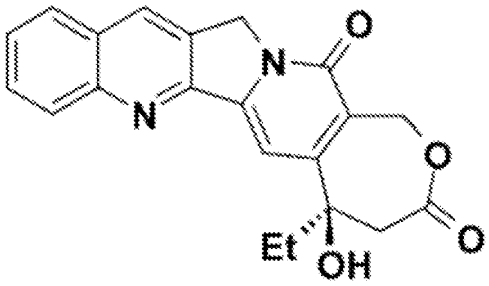
S38769



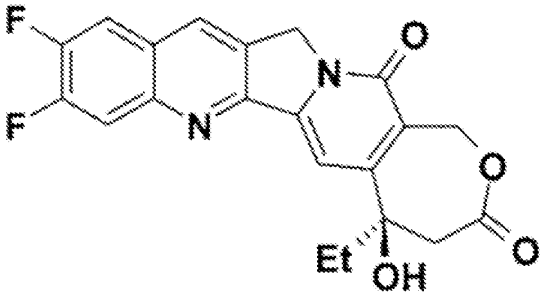
ZBH-01



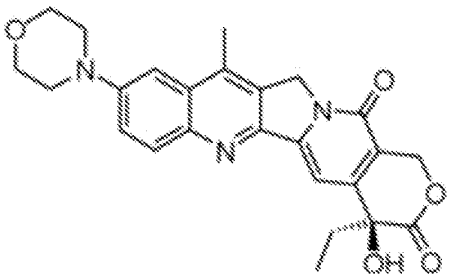
ZBH-1207 (R=L-CH_2COOH)



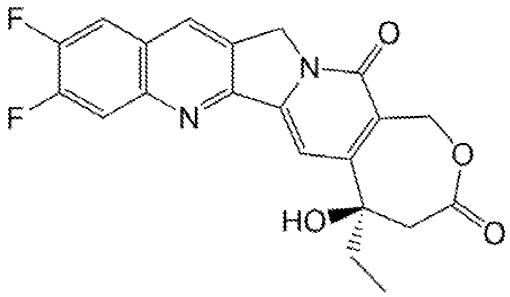
HomoCPT(hCPT)



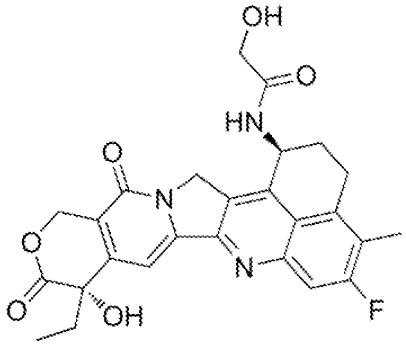
BN80915



CPT211



Diflomotecan (BN80905)



Dxd (Exatecan derivative)

In some preferred embodiments, the camptothecin compound is hydroxycamptothecin, aminocamptothecin, exatecan, belotecan, irinotecan, cositecan, rubitecan, lurtotecan, gimatecan, silatecan, namitecan, homocamptothecin (HomoCPT), diflomotecan, S39625, S38809, S38769, ZBH-01, ZBH-1207, BN80915, CPT211, Dxd, SN-38, or FL118. In some particularly preferred embodiments, the camptothecin compound is exatecan.

Antibody or Antigen-Binding Fragment thereof

The present disclosure provides an antibody that binds to Claudin 18 isoform 2 (CLDN18.2). As described herein, the antibody according to the present disclosure is a polypeptide comprising one or more complementarity-determining areas or regions (CDRs).

In some embodiments, the CDR is included in a "framework" region, and the framework orients the CDR(s) so that the CDR(s) can have appropriate antigen-binding properties.

In certain aspects, the present disclosure provides antibody-drug conjugates comprising an anti-CLDN18.2 antibody that binds to CLDN18.2. In certain embodiments, the antibody disclosed herein binds to CLDN18.2 expressed in a tumor and may be used to deliver a drug

to the tumor. In certain embodiments, the antibody drug conjugates disclosed herein have improved stability as compared to antibody drug conjugates known in the art.

In certain embodiments, the antibody comprises, but is not limited to, a monoclonal antibody, a bispecific antibody, a diabody, a multispecific antibody, a polyantibody, a minibody, a domain antibody, an antibody mimetic (or synthetic antibody), a chimeric antibody, a humanized antibody, a human antibody or an antibody fusion (or antibody conjugate), and a fragment thereof, and includes various forms of antibodies disclosed herein.

In certain embodiments, an antibody fragment of the antibody according to the present disclosure includes Fab, Fab', F(ab')2, scFab, Fv, dsFv, scFV, scFV-Fc, a minibody, a diabody, scAb, or dAb.

In certain embodiments, the antibody according to the present disclosure may consist of a polypeptide of only light chains or only heavy chains including the variable regions shown in Tables 1 to 2.

CDR sequences that may be included in the heavy and light chain variable regions of the antibody or antigen-binding fragment thereof according to an embodiment of the present disclosure are shown in Tables 1 to 2, respectively.

An antibody according to the present disclosure shares certain regions or sequences with other antibodies disclosed herein. In certain embodiments, the constant region of the antibody or antigen-binding fragment thereof may be shared. In certain embodiments, Fc regions may be shared. In certain embodiments, the frame of a variable region may be shared.

The heavy chain variable region and the light chain variable region according to the present disclosure may be linked to at least a part of a human constant region. The selection of a constant region may be determined partially by whether or not antibody-dependent cell-mediated cytotoxicity, antibody-dependent cellular phagocytosis, and/or complement-dependent cytotoxicity is required. For example, human isotypes IgG1 and IgG3 have complement-dependent cytotoxicity, and human isotypes IgG2 and IgG4 do not have such cytotoxicity. In addition, human IgG1 and IgG3 induce a cell-mediated effector function stronger than that of human IgG2 and IgG4. The light chain constant region may be lambda or kappa.

A variable region of an immunoglobulin chain generally has the same overall structure and includes a comparatively conserved framework region (FR) linked by three hypervariable regions called "complementarity determining areas or regions or domains" or complementarity determining regions (CDRs). The CDRs of a variable region derived from each chain including a heavy chain/light chain pair are typically aligned by a framework region to form a structure

specifically binding to a specific epitope of a target protein. These factors of naturally occurring light chain and heavy chain variable regions are typically included from the N-terminus to the C-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The position of amino acid sequences corresponding to each variable region may be determined by Kabat (Kabat et al., (1983) U.S. Dept, of Health and Human Services, "Sequences of Proteins of Immunological Interest"), Chothia (Chothia and Lesk, J. Mol. Biol. 196:901-917 (1987)) or in a manner related to the OPAL library (Hye Young Yang et. al., 2009 Mol. Cells 27: 225). The CDRs determined by each definition, when compared to each other, may be subsets which overlap or where one includes another. Those of ordinary skill in the art will be readily able to easily select CDR sequences according to the definitions above, given a variable region sequence of an antibody.

In certain embodiments, amino acid sequences of CDRs are defined according to Kabat definition. However, it is well known to those skilled in the art that CDRs of an antibody can be defined in the art according to a variety of methods, such as Chothia definition based on the location of a structural loop region (Al-Lazikani, B et al., J Mol Biol 273: 927-48 (1997)), and Kabat definition based on sequence variability (Kabat et al., "Sequences of proteins of immunological interest", fifth edition, National Institutes of Health, Bethesda, MD. (1991)).

In certain embodiments, amino acid residues in variable region sequences may also be determined using a Combined definition that incorporates both Kabat definition and Chothia definition. The Combined definition refers to the combination of the ranges of Kabat definition and Chothia definition. It should be understood by those skilled in the art that unless otherwise specified, the terms "CDR" and "complementarity determining region" of a given antibody or region thereof (e.g., a variable region) should be understood to encompass the complementarity determining region as defined according to any of the embodiments described in the present disclosure.

Although the scope of protection claimed in the claims of the present disclosure is based on the sequences defined according to Kabat definition, amino acid sequences defined according to other CDRs definitions should also fall within the scope of protection of the present disclosure.

In certain embodiments, the antibody according to the present disclosure is a humanized antibody. A humanized antibody refers to any antibody in which the constant region of a non-human antibody is completely substituted with a human form of the constant region, and at least a portion of the variable region of a non-human antibody, except for the three loops of an amino acid sequence outside each variable region that binds to a target structure, is completely

or partially substituted with the corresponding portion of a human antibody. In certain embodiments, the antibody according to the present disclosure is a human antibody.

Certain mutations may be introduced to the framework region to enhance the stability of antibodies while maintaining their antigen binding activity. Stabilization of therapeutic antibodies can result in improved serum half-life, lower dosage requirements, reduced side-effects, improved shelf-life and reduced shipping and storage costs.

In certain embodiments, the present disclosure discloses one or more amino acid sequences having substantial sequence identity to one or more amino acid sequences disclosed herein. Substantial identity means that the effects disclosed herein are maintained in the presence of sequence variations. In certain embodiments, the amino acid sequence has about 90% identity, about 95% identity, or about 99% identity to the heavy chain variable regions shown in Tables 1 to 2. In another embodiment, the amino acid sequence has about 90% identity, about 95% identity, or about 99% identity to the light chain variable regions shown in Tables 1 to 2. For example, in the case of variants exhibiting 90% identity, 95% identity, or 99% identity to the sequence of the antibody or antigen-binding fragment thereof according to the present disclosure, any mutation occurs in the framework of the variable region rather than the CDRs.

In certain embodiments, a nucleic acid encoding the antibody or fragment thereof according to the present disclosure is a nucleic acid encoding a full-length antibody including the CDRs disclosed herein, the variable region including the CDRs, and the variable region, and the constant region. Once the amino acid sequence is determined, the nucleic acid sequence may be easily determined in consideration of a known reverse transcription program, codon usage, and the like.

Antigen Specificity and Affinity for Antibody

In certain preferred embodiments, the antibody or antigen-binding fragment thereof according to the present disclosure has specificity to a human CLDN18.2 antigen and affinity suitable for use as an antibody therapeutic/diagnostic agent. In certain embodiments, the affinity for aggregates may be KD < 1,000 nM, < 100 nM, < 10 nM, < 1 nM, < 0.1 nM, < 0.01 nM, or < 0.001 nM, and may be, for example, 10^{-6} M to 10^{-12} M.

Production of Antibody

In the present disclosure, a non-human antibody may be derived from, for example, any antibody-producing animal, for example, a mouse, a rat, a rabbit, a goat, a donkey, or non-

human primates (e.g., monkeys such as cynomolgus or rhesus monkey) or apes (e.g., chimpanzees). A non-human antibody may be produced by immunizing an animal by using a method known in the art. For a method for producing a humanized antibody, according to an embodiment of the present disclosure, refer to US Patent No. 15/532598. A fully human antibody may be produced by administering an antigen to a transformed animal including a human immunoglobulin gene locus, or by treating a phage display library expressing a human antibody repertory with an antigen, and then selecting the target antibody. The antibody may be polyclonal or monoclonal, or may be synthesized within a cell host through the expression of recombinant DNA.

A monoclonal antibody (mAb) may be produced using a conventional monoclonal antibody method, for example, a standard somatic hybridization technique in the literature (Kohler and Milstein, 1975, Nature 256:495).

Method for Expressing Antibody

The antibody disclosed herein may be expressed in a hybridoma cell line or an expression cell line other than a hybridoma. An expression construct encoding the antibody may be used to transform a mammalian, an insect or a microbial host cell. A construct such as a plasmid may be produced, as described in the foregoing description, using any of various known methods for introducing a polynucleotide into a host cell. The specific method may vary according to the type of host cell. Methods for introducing a heterogeneous polynucleotide into a mammalian cell are widely known in the art, and include, but are not limited to, for example, dextran-mediated transfer, calcium phosphate precipitation, polybrene-mediated transfer, protoplast fusion, electrophoresis, capsulation of a transferred polynucleotide using liposomes, mixing of a nucleic acid and a positively charged lipid, and direct microinjection of DNA into the nucleus.

Use of Anti-Human CLDN18.2 Antibody Drug-Conjugates for Therapeutic Purposes

The expression of CLDN18.2 in cancer is associated with unfavorable prognosis of cancer patients and is known to also affect cancer metastasis. For example, CLDN18.2 is overexpressed in lung cancer, small cell lung cancer, gastrointestinal cancer, colon cancer, bowel cancer, breast cancer, ovarian cancer, prostate cancer, testicular cancer, liver cancer, kidney cancer, bladder cancer, pancreatic cancer, brain cancer, sarcoma, osteosarcoma, Kaposi's sarcoma, melanoma, and the like. For anticancer antibody treatment, for example, the anti-human CLDN18.2 antibody may, as described herein, be used in a form linked to various

topoisomerase inhibitors via a linker to remove CLDN18.2-overexpressing cancer cells. Accordingly, an antibody binding to CLDN18.2 may be used in a form bonded to an isomerase inhibitor, and thus can be used as a targeted therapeutic agent for directing to CLDN18.2-expressing cells.

Treatment Method: Pharmaceutical Formulation and Administration Route

An embodiment of the present disclosure also provides a treatment method using an antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof. In certain embodiments, the antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof is provided to a patient. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof inhibits cancer cell metastasis by binding to human CLDN18.2 expressed on the surface of cancer cells. In certain embodiments, the antibody binds to human CLDN18.2 expressed on the surface of cancer cells in a form bonded to a topoisomerase I inhibitor (e.g., a camptothecin compound), thereby specifically delivering the topoisomerase I inhibitor (e.g., the camptothecin compound) bonded to the antibody to cancer cells, to induce the death of the cancer cells. In certain embodiments, the antibody binds to human CLDN18.2 expressed on the surface of cancer cells in the form of an antibody specific to the same target or another target, thereby increasing specificity of multiple antibodies for cancer cells or inducing connections between cancer cells and other types of cells such as immune cells, to induce the death of the cancer cells.

Pharmaceutical compositions

Provided is also a pharmaceutical composition including a therapeutically effective amount of the antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof, and a pharmaceutically acceptable diluent, a carrier, a solubilizer, an emulsifier, a preservative, and/or an adjuvant. Also, for example, a method of treating a cancer patient by administering such a pharmaceutical composition is provided. The term "patient" includes human patients.

The pharmaceutical composition may include a pharmaceutically acceptable carrier. The carrier is used as a meaning including an excipient, a diluent, or an adjuvant. The carrier may be selected from the group consisting of, for example, lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starch, acacia rubber, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methyl cellulose, polyvinyl pyrrolidone, water, saline, buffer such as PBS, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium

stearate, and mineral oil. The composition may include a filler, an anticoagulant, a lubricant, a wetting agent, a flavoring agent, an emulsifier, a preservative, or a combination thereof.

The pharmaceutical composition may be prepared as any formulation according to general methods. The composition may be formulated into formulations for oral administration (e.g., powders, tablets, capsules, syrups, pills or granules) or for parenteral administration (for example, injections). In addition, the composition may be prepared as a systemic or local formulation.

The pharmaceutical composition may include an effective amount of the antibody or antigen-binding fragment thereof, an anticancer agent, or a combination thereof. The term "effective amount" refers to an amount sufficient to exhibit preventive or therapeutic effects when administered to an individual requiring prevention or treatment. The effective amount may be appropriately selected depending on a cell or individual that is selected by those or ordinary skill in the art. The effective amount may be determined according to factors including the severity of the disease, the age, body weight, health and gender of a patient, sensitivity of a patient to the drug, administration time, administration routes, excretion rate, treatment period, and drugs used in combination or simultaneously with the used composition, and other factors well known in the medical field.

The dosage of the pharmaceutical composition may range, for example, from 10 μg/kg to about 30 mg/kg, optionally from 0.1 mg/kg to about 30 mg/kg, or alternatively from 0.3 mg/kg to about 20 mg/kg per adult. The pharmaceutical composition may be administered once a day, multiple times a day, once every 1 to 4 weeks, or once to 12 times a year.

Hereinafter, the present disclosure will be described in more detail with reference to examples and experimental examples.

The following examples and experimental Examples are intended to aid in understanding of the present disclosure and are not intended to limit the scope of the present disclosure.

Definitions

Unless otherwise defined herein, scientific and technical terms used in this application shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclature used in connection with, and techniques of, chemistry, cell and tissue culture, molecular biology, cell and cancer biology, neurobiology, neurochemistry, virology, immunology, microbiology, pharmacology, genetics and protein and nucleic acid chemistry, described herein, are those well-known and commonly used in the art.

The methods and techniques of the present disclosure are generally performed, unless otherwise indicated, according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout this specification. See, e.g. "Principles of Neural Science", McGraw-Hill Medical, New York, N.Y. (2000); Motulsky, "Intuitive Biostatistics", Oxford University Press, Inc. (1995); Lodish et al., "Molecular Cell Biology, 4th ed.", W. H. Freeman & Co., New York (2000); Griffiths et al., "Introduction to Genetic Analysis, 7th ed.", W. H. Freeman & Co., N.Y. (1999); and Gilbert et al., "Developmental Biology, 6th ed.", Sinauer Associates, Inc., Sunderland, MA (2000).

Chemistry terms used herein, unless otherwise defined herein, are used according to conventional usage in the art, as exemplified by "The McGraw-Hill Dictionary of Chemical Terms", Parker S., Ed., McGraw-Hill, San Francisco, C.A. (1985).

All of the above, and any other publications, patents and published patent applications referred to in this application are specifically incorporated by reference herein. In case of conflict, the present specification, including its specific definitions, will control.

The term "agent" is used herein to denote a chemical compound (such as an organic or inorganic compound, a mixture of chemical compounds), a biological macromolecule (such as a nucleic acid, an antibody, including parts thereof as well as humanized, chimeric and human antibodies and monoclonal antibodies, a protein or portion thereof, e.g., a peptide, a lipid, a carbohydrate), or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues. Agents include, for example, agents whose structure is known, and those whose structure is not known.

A "patient," "subject," or "individual" are used interchangeably and refer to either a human or a non-human animal. These terms include mammals, such as humans, primates, livestock animals (including bovines, porcines, etc.), companion animals (e.g., canines, felines, etc.) and rodents (e.g., mice and rats).

"Treating" a condition or patient refers to taking steps to obtain beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

The term "preventing" is art-recognized, and when used in relation to a condition, such as a local recurrence (e.g., pain), a disease such as cancer, a syndrome complex such as heart

failure or any other medical condition, is well understood in the art, and includes administration of a composition which reduces the frequency of, or delays the onset of, symptoms of a medical condition in a subject relative to a subject which does not receive the composition. Thus, prevention of cancer includes, for example, reducing the number of detectable cancerous growths in a population of patients receiving a prophylactic treatment relative to an untreated control population, and/or delaying the appearance of detectable cancerous growths in a treated population versus an untreated control population, e.g., by a statistically and/or clinically significant amount.

"Administering" or "administration of" a substance, a compound or an agent to a subject can be carried out using one of a variety of methods known to those skilled in the art. For example, a compound or an agent can be administered, intravenously, arterially, intradermally, intramuscularly, intraperitoneally, subcutaneously, ocularly, sublingually, orally (by ingestion), intranasally (by inhalation), intraspinally, intracerebrally, and transdermally (by absorption, e.g., through a skin duct). A compound or agent can also appropriately be introduced by rechargeable or biodegradable polymeric devices or other devices, e.g., patches and pumps, or formulations, which provide for the extended, slow or controlled release of the compound or agent. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

Appropriate methods of administering a substance, a compound or an agent to a subject will also depend, for example, on the age and/or the physical condition of the subject and the chemical and biological properties of the compound or agent (e.g., solubility, digestibility, bioavailability, stability and toxicity). In some embodiments, a compound or an agent is administered orally, e.g., to a subject by ingestion. In some embodiments, the orally administered compound or agent is in an extended release or slow release formulation, or administered using a device for such slow or extended release.

As used herein, the phrase "conjoint administration" refers to any form of administration of two or more different therapeutic agents such that the second agent is administered while the previously administered therapeutic agent is still effective in the body (e.g., the two agents are simultaneously effective in the patient, which may include synergistic effects of the two agents). For example, the different therapeutic compounds can be administered either in the same formulation or in separate formulations, either concomitantly or sequentially. Thus, an individual who receives such treatment can benefit from a combined effect of different therapeutic agents.

A "therapeutically effective amount" or a "therapeutically effective dose" of a drug or agent is an amount of a drug or an agent that, when administered to a subject will have the intended therapeutic effect. The full therapeutic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a therapeutically effective amount may be administered in one or more administrations. The precise effective amount needed for a subject will depend upon, for example, the subject's size, health and age, and the nature and extent of the condition being treated, such as cancer or MDS. The skilled worker can readily determine the effective amount for a given situation by routine experimentation.

As used herein, the terms "optional" or "optionally" mean that the subsequently described event or circumstance may occur or may not occur, and that the description includes instances where the event or circumstance occurs as well as instances in which it does not. For example, "optionally substituted alkyl" refers to the alkyl may be substituted as well as where the alkyl is not substituted.

It is understood that substituent and substitution patterns on the compounds of the present disclosure can be selected by one of ordinary skilled person in the art to result chemically stable compounds which can be readily synthesized by techniques known in the art, as well as those methods set forth below, from readily available starting materials. If a substituent is itself substituted with more than one group, it is understood that these multiple groups may be on the same carbon or on different carbons, so long as a stable structure results.

As used herein, the term "optionally substituted" refers to the replacement of one to six hydrogen radicals in a given structure with the radical of a specified substituent including, but not limited to: hydroxyl, hydroxyalkyl, alkoxy, halogen, alkyl, nitro, silyl, acyl, acyloxy, aryl, cycloalkyl, heterocyclyl, amino, aminoalkyl, cyano, haloalkyl, haloalkoxy, -OCO-CH_2-O-alkyl, -OP(O)(O-alkyl)$_2$ or –CH_2-OP(O)(O-alkyl)$_2$. Preferably, "optionally substituted" refers to the replacement of one to four hydrogen radicals in a given structure with the substituents mentioned above. More preferably, one to three hydrogen radicals are replaced by the substituents as mentioned above. It is understood that the substituent can be further substituted.

The term "conjugates" as used herein refers to cell binding agents that are covalently bonded to one or more molecules of a cytotoxic compound. In this regard, "cell binding agent" is a molecule having affinity for a biological target, and may be, for example, an antibody, particularly a monoclonal antibody, or an antibody fragment, and the binding agent functions to direct a biologically active compound to a biological target. In certain embodiments, the conjugate may be designed to target tumor cells through cell surface antigens. The antigen may

be a cell surface antigen that is overexpressed or expressed in an abnormal cell type. Specifically, the target antigen may be expressed only on proliferative cells (e.g., tumor cells). The target antigen may be selected on the basis of different expression, usually between proliferative tissues and normal tissues. In the present disclosure, the antibody is bonded to the linker.

In the present disclosure, a "variant" of a polypeptide, for example, an antigen-binding fragment, a protein, or an antibody, is a polypeptide in which insertion, deletion, addition, and/or substitution have occurred at one or more amino acid residues compared to other polypeptide sequences, and includes fusion polypeptides. Protein variants also include those modified by protein enzymatic cleavage, phosphorylation or other post-translational modifications, but retaining the biological activity of the antibody disclosed herein, such as binding and specificity to CLDN18.2. Variants may have about 99% identity, about 98% identity, about 97% identity, about 96% identity, about 95% identity, about 94% identity, about 93% identity, about 92% identity, about 91% identity, about 90% identity, about 89% identity, about 88% identity, about 87% identity, about 86% identity, about 85% identity, about 84% identity, about 83% identity, about 82% identity, about 81% identity, or about 80% identity to the sequence of the antibody or antigen-binding fragment thereof according to the present disclosure. Percent identity (%) or homology may be calculated by methods known in the art.

In certain embodiments, the percent homology or identity can be calculated by 100X[(same position)/min(TGA, TGB)], wherein TGA and TGB are the sum of the number of residues and internal gap positions in sequences A and B to be compared (Russell et al., J. Mol Biol., 244: 332-350 (1994).

The term "derivative" of a polypeptide as used herein refers to a polypeptide that has chemical modification at one or more residues through conjugation with other chemical moieties, different from insertion, deletion, addition or substitution variants.

The term "percent sequence identity" or "percent identity" between two polynucleotide or polypeptide sequences refers to the number of identical matched positions shared by the sequences over a comparison window, taking into account additions or deletions (i.e., gaps) that must be introduced for optimal alignment of the two sequences. A matched position is any position where an identical nucleotide or amino acid is presented in both the target and reference sequence. Gaps presented in the target sequence are not counted since gaps are not nucleotides or amino acids. Likewise, gaps presented in the reference sequence are not counted since target sequence nucleotides or amino acids are counted, not nucleotides or amino acids from the reference sequence. The percentage of sequence identity is calculated by determining

the number of positions at which the identical amino-acid residue or nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. The comparison of sequences and determination of percent sequence identity between two sequences can be accomplished using readily available software programs. Suitable software programs are available from various sources, and for alignment of both protein and nucleotide sequences. One suitable program to determine percent sequence identity is bl2seq, part of the BLAST suite of program available from the U.S. government's National Center for Biotechnology Information BLAST web site (at world wide web at blast.ncbi.nlm.nih.gov). Bl2seq performs a comparison between two sequences using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. Other suitable programs are, e.g., Needle, Stretcher, Water, or Matcher, part of the EMBOSS suite of bioinformatics programs and also available from the European Bioinformatics Institute (EBI) at world wide web at ebi.ac.uk/Tools/psa.

As used herein, "homology" with respect to a peptide, polypeptide or antibody sequence refers to the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms known in the art needed to achieve maximal alignment over the full length of the sequences being compared.

The term "affinity" or "avidity" refers to the strength of interaction between an antibody or its antigen-binding fragment and an antigen, determined by characteristics of the antigen such as size, shape, and/or charge, and the CDR sequences of the antibody or antigen-binding fragment. Methods for determining such affinities are known in the art and may also be referenced herein.

Antibodies or their antigen-binding fragments used in the present invention are said to "specifically bind" to the target, such as the antigen, when the dissociation constant (KD) is $\leq 10^{-6}$ M. Antibodies bind "with high affinity" to the target when KD is $\leq 1x\ 10^{-8}$ M.

As used in the present disclosure, the "antigen-binding fragment" of a chain (heavy chain or light chain) of an antibody or immunoglobulin includes a part of an antibody which lacks some amino acids compared to a full-length chain, but can specifically bind to an antigen. This fragment can be considered as having biological activity, in that the fragment can specifically bind to a target antigen, or can compete with other antibodies or antigen binding fragments thereof to bind to a specific epitope. In certain embodiments, such a fragment includes at least one CDR present in a full-length light chain or heavy chain, and in some embodiments, includes a short-chain heavy chain and/or light chain, or part thereof. This biological active fragment may be produced by a recombinant DNA technique or may be produced, for example, by cleaving an intact antibody enzymatically or chemically. An immunologically functional immunoglobulin fragment includes, but is not limited to, Fab, Fab, F(ab)2, scFab, dsFv, Fv, scFV, scFV-Fc, diabody, minibody, 35cab, and dAb, and may be derived from any mammal, including, but not being limited to, a human, a mouse, a rat, a camelid, or a rabbit. The functional parts of antibodies such as the one or more CDRs disclosed in the present disclosure may be linked with a secondary protein or a small compound by a covalent bond, and thereby used as a targeted therapeutic agent for a specific target.

In the present disclosure, the "Fc" region includes two heavy chain fragments including CH2 and CH3 domains of an antibody. These two heavy chain fragments are linked to each other by hydrophobic interaction of two or more of disulfide bonds and a CH3 domain.

In the present disclosure, the "Fab fragment" consists of one light chain and one heavy chain including a variable region and CH1 only. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule. In an scFab, two molecules of Fab are linked by a flexible linker.

In the present disclosure, the "Fab′ fragment" includes a Fab fragment and additionally a region between CH1 and CH2 domains of a heavy chain. A disulfide bond may form between two heavy chains of Fab′ fragments of two molecules, forming a F(ab′)2 molecule.

In the present disclosure, as described above, the "F(ab′)2 fragment" includes two light chains and two heavy chains including a variable region CH1 and part of a constant region between the CH1 and CH2 domains, with an inter-chain disulfide bond formed between the two heavy chains. Accordingly, a F(ab′)2 fragment consists of two Fab′ fragments, and the two Fab′ fragments are joined to each other by the disulfide bond therebetween.

In the present disclosure, the "Fv region" is a fragment of an antibody which includes each variable region of a heavy chain and a light chain, but does not include constant regions. In an sdFV, a heavy chain and a light chain are linked by a disulfide bond. In an scFc, the Fv

is linked by a flexible linker. In a scFv-Fc, a Fc is linked to a scFV. In a minibody, CH3 is linked to a scFV. A diabody includes the scFVs of two molecules.

In the present disclosure, the "single chain Fv" or "scFv" antibody fragment includes the VH and VL domains of an antibody, and these domains are present within a single polypeptide chain. An Fv polypeptide may additionally include a polypeptide linker between a Vh domain which enables the scFv to form the target structure for antigen binding, and a VL domain.

In the present disclosure, the "short-chain antibody (36cab)" is a single polypeptide chain including one constant region of a heavy chain or a light chain constant region in which heavy chain and light chain variable regions are linked by a flexible linker. For a short-chain antibody, U.S. Pat. No. 5,260,203 may be referred to, and short-chain antibody is disclosed herein by reference.

In the present disclosure, the "domain antibody (dAb)" is an immunologically functional immunoglobulin fragment including only a variable region of a heavy chain or a variable region of a light chain. In certain embodiments, two or more VH regions are linked by a covalent bond via a peptide linker, to form a bivalent domain antibody. Two VH regions of this bivalent domain antibody may target the same or different antigens.

In the present disclosure, "complementarity determining region" (CDR; that is, CDR1, CDR2, and CDR3) denotes amino acid residues of the variable region of an antibody, which are necessary for binding to antigen. Each variable region typically has three CDR domains, identified as CDR1, CDR2, and CDR3.

In the present disclosure, the "framework region" (FR) is a variable region residue other than the CDR residues. Each variable region typically has four FRs, identified as FR1, FR2, FR3, and FR4.

In the present disclosure, the "bivalent antigen-binding protein" or "bivalent antibody" includes two antigen-binding sites. The two antigen-binding sites included in a bivalent antibody may have the same antigen specificity, or may be a bispecific antibody where the antigen-biding sites bind to different antigens.

In the present disclosure, the "multispecific antigen-binding protein" or "multispecific antibody" targets two or more antigens or epitopes.

As used herein, a "chimeric antibody" refers to an antibody (immunoglobulin) 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(are) 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 exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison et al., Proc. Nat'l Acad. Sci. USA, 81:6851-55 (1984)). Chimeric antibodies of interest herein include PRIMATIZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, e.g., immunizing macaque monkeys with an antigen of interest.

"Humanized" forms of non-human (e.g., murine) antibodies, are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an CDR of the recipient are replaced by residues from an CDR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a 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 sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, and the like. The number of these amino acid substitutions in the FR is typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, e.g., Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). See also, for example, Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1:105-115 (1998); Harris, Biochem. Soc. Transactions 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994); and U.S. Patent Nos. 6,982,321 and 7,087,409.

A "human antibody" is one that possesses an amino-acid sequence corresponding to that of an antibody, produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-

display libraries. Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner et al., J. Immunol., 147(1):86-95 (1991). See also van Dijk and van de Winkel, Curr. Opin. Pharmacol. 5:368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, e.g., immunized xenomice (see, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 regarding XENOMOUSETM technology). See also, for example, Li et al., Proc. Nat'l Acad. Sci. USA, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

A "human consensus framework" is a framework that represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). Examples include for the VL, the subgroup may be subgroup kappa I, kappa II, kappa III or kappa IV as in Kabat et al., supra. Additionally, for the VH, the subgroup may be subgroup I, subgroup II, or subgroup III as in Kabat et al., supra.

An "affinity-matured" antibody is one with one or more alterations in one or more CDRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). In one embodiment, an affinity-matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks et al., Bio/Technology 10:779-783 (1992) describes affinity maturation by VH- and VL-domain shuffling. Random mutagenesis of CDR and/or framework residues is described by, for example: Barbas et al. Proc Nat. Acad. Sci. USA 91:3809-3813 (1994); Schier et al. Gene 169:147- 155 (1995); Yelton et al. J. Immunol. 155:1994-2004 (1995); Jackson et al., J. Immunol. 154(7):3310-9 (1995); and Hawkins et al, J. Mol. Biol. 226:889-896 (1992).

In the present disclosure, "linker" refers to a compound which covalently bonds a cytotoxic compound to an antibody.

In the present disclosure, "unsubstituted or substituted" is used to refer to a parent group which may be unsubstituted or substituted, "substituted" refers to a parent group having at least

one substituent, and a substituent refers to a chemical moiety covalently bonded to or fused with a parent group.

In the present disclosure, "halo" refers to fluorine, chlorine, bromine, iodine, and the like.

As used herein, the term "alkyl" refers to saturated aliphatic groups, including but not limited to C_1-C_{10} straight-chain alkyl groups or C_1-C_{10} branched-chain alkyl groups. Preferably, the "alkyl" group refers to C_1-C_6 straight-chain alkyl groups or C_1-C_6 branched-chain alkyl groups. Most preferably, the "alkyl" group refers to C_1-C_4 straight-chain alkyl groups or C_1-C_4 branched-chain alkyl groups. Examples of "alkyl" include, but are not limited to, methyl, ethyl, 1-propyl, 2-propyl, n-butyl, sec-butyl, tert-butyl, 1-pentyl, 2-pentyl, 3-pentyl, neo-pentyl, 1-hexyl, 2-hexyl, 3-hexyl, 1-heptyl, 2-heptyl, 3-heptyl, 4-heptyl, 1-octyl, 2-octyl, 3-octyl or 4-octyl and the like. The "alkyl" group may be optionally substituted.

The term "acyl" is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)-, preferably alkylC(O)-.

The term "acylamino" is art-recognized and refers to an amino group substituted with an acyl group and may be represented, for example, by the formula hydrocarbylC(O)NH-.

The term "acyloxy" is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)O-, preferably alkylC(O)O-.

The term "alkoxy" refers to an alkyl group having an oxygen attached thereto. Representative alkoxy groups include methoxy, ethoxy, propoxy, tert-butoxy and the like.

The term "alkoxyalkyl" refers to an alkyl group substituted with an alkoxy group and may be represented by the general formula alkyl-O-alkyl.

The term "alkyl" refers to saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl-substituted cycloalkyl groups, and cycloalkyl-substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C_{1-30} for straight chains, C_{3-30} for branched chains), and more preferably 20 or fewer.

Moreover, the term "alkyl" as used throughout the specification, examples, and claims is intended to include both unsubstituted and substituted alkyl groups, the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone, including haloalkyl groups such as trifluoromethyl and 2,2,2-trifluoroethyl, etc.

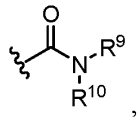
The term "C_{x-y}" or "C_x-C_y", when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups that contain from x

to y carbons in the chain. C_0alkyl indicates a hydrogen where the group is in a terminal position, a bond if internal. A C_{1-6}alkyl group, for example, contains from one to six carbon atoms in the chain.

The term "alkylamino", as used herein, refers to an amino group substituted with at least one alkyl group.

The term "alkylthio", as used herein, refers to a thiol group substituted with an alkyl group and may be represented by the general formula alkylS-.

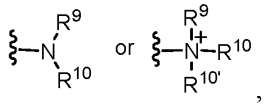
The term "amido", as used herein, refers to a group



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wherein R^9 and R^{10} each independently represent a hydrogen or hydrocarbyl group, or R^9 and R^{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines and salts thereof, e.g., a moiety that can be represented by



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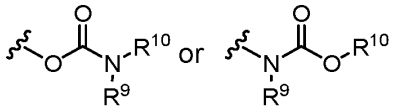
wherein R^9, R^{10}, and $R^{10'}$ each independently represent a hydrogen or a hydrocarbyl group, or R^9 and R^{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term "aminoalkyl", as used herein, refers to an alkyl group substituted with an amino group.

The term "aralkyl", as used herein, refers to an alkyl group substituted with an aryl group.

The term "aryl" as used herein include substituted or unsubstituted single-ring aromatic groups in which each atom of the ring is carbon. Preferably the ring is a 5- to 7-membered ring, more preferably a 6-membered ring. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Aryl groups include benzene, naphthalene, phenanthrene, phenol, aniline, and the like.

The term "carbamate" is art-recognized and refers to a group



wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl group.

The term "carbocyclylalkyl", as used herein, refers to an alkyl group substituted with a carbocycle group.

The term "carbocycle" includes 5-7 membered monocyclic and 8-12 membered bicyclic rings. Each ring of a bicyclic carbocycle may be selected from saturated, unsaturated and aromatic rings. Carbocycle includes bicyclic molecules in which one, two or three or more atoms are shared between the two rings. The term "fused carbocycle" refers to a bicyclic carbocycle in which each of the rings shares two adjacent atoms with the other ring. Each ring of a fused carbocycle may be selected from saturated, unsaturated and aromatic rings. In an exemplary embodiment, an aromatic ring, e.g., phenyl, may be fused to a saturated or unsaturated ring, e.g., cyclohexane, cyclopentane, or cyclohexene. Any combination of saturated, unsaturated and aromatic bicyclic rings, as valence permits, is included in the definition of carbocyclic. Exemplary "carbocycles" include cyclopentane, cyclohexane, bicyclo[2.2.1]heptane, 1,5-cyclooctadiene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]oct-3-ene, naphthalene and adamantane. Exemplary fused carbocycles include decalin, naphthalene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]octane, 4,5,6,7-tetrahydro-1H-indene and bicyclo[4.1.0]hept-3-ene. "Carbocycles" may be substituted at any one or more positions capable of bearing a hydrogen atom.

The term "carbocyclylalkyl", as used herein, refers to an alkyl group substituted with a carbocycle group.

The term "carbonate" is art-recognized and refers to a group $-OCO_2-$.

The term "carboxy", as used herein, refers to a group represented by the formula $-CO_2H$.

The term "cycloalkyl" includes substituted or unsubstituted non-aromatic single ring structures, preferably 4- to 8-membered rings, more preferably 4- to 6-membered rings. The term "cycloalkyl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is cycloalkyl and the substituent (e.g., R^{100}) is attached to the cycloalkyl ring, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole,

oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, pyrimidine, denzodioxane, tetrahydroquinoline, and the like.

The term "ester", as used herein, refers to a group -C(O)OR^9 wherein R^9 represents a hydrocarbyl group.

The term "ether", as used herein, refers to a hydrocarbyl group linked through an oxygen to another hydrocarbyl group. Accordingly, an ether substituent of a hydrocarbyl group may be hydrocarbyl-O-. Ethers may be either symmetrical or unsymmetrical. Examples of ethers include, but are not limited to, heterocycle-O-heterocycle and aryl-O-heterocycle. Ethers include "alkoxyalkyl" groups, which may be represented by the general formula alkyl-O-alkyl.

The terms "halo" and "halogen" as used herein means halogen and includes chloro, fluoro, bromo, and iodo.

The terms "hetaralkyl" and "heteroaralkyl", as used herein, refers to an alkyl group substituted with a hetaryl group.

The terms "heteroaryl" and "hetaryl" include substituted or unsubstituted aromatic single ring structures, preferably 5- to 7-membered rings, more preferably 5- to 6-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms "heteroaryl" and "hetaryl" also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heteroaromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, and pyrimidine, and the like.

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, and sulfur.

The term "heterocyclylalkyl", as used herein, refers to an alkyl group substituted with a heterocycle group.

The terms "heterocyclyl", "heterocycle", and "heterocyclic" refer to substituted or unsubstituted non-aromatic ring structures, preferably 3- to 10-membered rings, more preferably 3- to 7-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms "heterocyclyl" and "heterocyclic" also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heterocyclic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls,

cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heterocyclyl groups include, for example, piperidine, piperazine, pyrrolidine, morpholine, lactones, lactams, and the like.

The term "hydrocarbyl", as used herein, refers to a group that is bonded through a carbon atom that does not have a =O or =S substituent, and typically has at least one carbon-hydrogen bond and a primarily carbon backbone, but may optionally include heteroatoms. Thus, groups like methyl, ethoxyethyl, 2-pyridyl, and even trifluoromethyl are considered to be hydrocarbyl for the purposes of this application, but substituents such as acetyl (which has a =O substituent on the linking carbon) and ethoxy (which is linked through oxygen, not carbon) are not. Hydrocarbyl groups include, but are not limited to aryl, heteroaryl, carbocycle, heterocycle, alkyl, alkenyl, alkynyl, and combinations thereof.

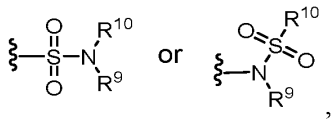
The term "hydroxyalkyl", as used herein, refers to an alkyl group substituted with a hydroxy group.

The term "lower" when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups where there are ten or fewer atoms in the substituent, preferably six or fewer. A "lower alkyl", for example, refers to an alkyl group that contains ten or fewer carbon atoms, preferably six or fewer. In certain embodiments, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy substituents defined herein are respectively lower acyl, lower acyloxy, lower alkyl, lower alkenyl, lower alkynyl, or lower alkoxy, whether they appear alone or in combination with other substituents, such as in the recitations hydroxyalkyl and aralkyl (in which case, for example, the atoms within the aryl group are not counted when counting the carbon atoms in the alkyl substituent).

The terms "polycyclyl", "polycycle", and "polycyclic" refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls) in which two or more atoms are common to two adjoining rings, e.g., the rings are "fused rings". Each of the rings of the polycycle can be substituted or unsubstituted. In certain embodiments, each ring of the polycycle contains from 3 to 10 atoms in the ring, preferably from 5 to 7.

The term "sulfate" is art-recognized and refers to the group $-OSO_3H$, or a pharmaceutically acceptable salt thereof.

The term "sulfonamido" is art-recognized and refers to the group represented by the general formulae



wherein R^9 and R^{10} independently represents hydrogen or hydrocarbyl.

The term "sulfoxide" is art-recognized and refers to the group–S(O)-.

The term "sulfonate" is art-recognized and refers to the group SO_3H, or a pharmaceutically acceptable salt thereof.

The term "sulfone" is art-recognized and refers to the group $–S(O)_2-$.

The term "substituted" refers to moieties having substituents replacing a hydrogen on one or more carbons of the backbone. It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic substituents of organic compounds. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. Substituents can include any substituents described herein, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate.

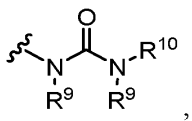
The term "thioalkyl", as used herein, refers to an alkyl group substituted with a thiol group.

The term "thioester", as used herein, refers to a group $-C(O)SR^9$ or $–SC(O)R^9$

wherein R^9 represents a hydrocarbyl.

The term "thioether", as used herein, is equivalent to an ether, wherein the oxygen is replaced with a sulfur.

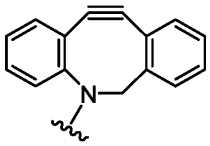
The term "urea" is art-recognized and may be represented by the general formula



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wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl.

The term "DBCO" used herein refers to an optionally substituted dibenzocyclooctyne moiety, e.g., the following structure:



The term "sugar moiety" used herein refers to a naturally occurring sugar or a modified sugar which is part of a larger molecule and is connected to the remainder of the larger molecule through, for example, one of the hydroxyl groups present on the sugar. For example, in some embodiments the sugar is connected to the remainder of the larger molecule via the hydroxyl group on the anomeric carbon. Examples of sugar moieties include, but are not limited to, glucuronosyl and galactosyl.

A "glycosidase" is an enzyme that breaks down glycosidic bonds in carbohydrates, glycoproteins, and glycolipids. Examples of glycosidases are β-glucuronidase and β-galactosidase. β-glucuronidase is a type of glucuronidase that catalyzes hydrolysis of β-D-glucuronic acid or β-glucuronosyl residues. β-Galactosidase is a glycoside hydrolase enzyme that catalyzes hydrolysis of terminal non-reducing β-D-galactose or β-galactosyl residues in β-D-galactosides.

The term "modulate" as used herein includes the inhibition or suppression of a function or activity (such as cell proliferation) as well as the enhancement of a function or activity.

The term "cleavage group" refers to a chemical moiety which dissociates when subjected to a stimulus, such as acidic conditions, basic conditions, reducing conditions, oxidizing conditions, light, or heat, or an enzyme, such as an esterase.

Examples

The invention now being generally described, it will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention and are not intended to limit the invention.

Preparation Example 1: Production of Exemplified Human CLDN18.2 (claudin 18 isoform 2) Specific Antibodies

CLDN18.2 (claudin 18 isoform 2) is widely expressed in tumors. An exemplified anti-CLDN18.2 antibody clone PR002726, which specifically binds to CLDN18.2, was produced by the method described in U.S. Patent Publication No. US 2022/0332814, the entirety of which is herein included in the present specification by reference. The amino acid sequences of the antibody clone PR002726 are shown in **Table 1** below. The amino acid sequences of the CDRs below are defined according to the Kabat definition.

Table 1. Amino Acid Sequences of Antibody Clone PR002726

PR002726 heavy chain		SEQ ID NO:
FR1	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	1
CDR1	SFVMS	2
FR2	WVRQAPGKGLEWVS	3
CDR2	TISGSGRSTYYADSVKG	4
FR3	RFTISRDNSKNTLHLQMNSLRAEDTAVYYCAK	5
CDR3	DAAAAGTKFDY	6
FR4	WGQGTLVTVSS	7
VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFVMSWVRQAPGKGLEWVSTISGSGRSTYYADSVKGRFTISRDNSKNTLHLQMNSLRAEDTAVYYCAKDAAAAGTKFDYWGQGTLVTVSS	15
HC	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFVMSWVRQAPGKGLEWVSTISGSGRSTYYADSVKGRFTISRDNSKNTLHLQMNSLRAEDTAVYYCAKDAAAAGTKFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**LL**GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	17
PR002726 light chain		SEQ ID NO:
FR1	EIVLTQSPATLSLSPGERATLSC	8
CDR1	RASQSVSSYLA	9
FR2	WYQQKPGQAPRLLIY	10

CDR2	DASNRAT	11
FR3	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC	12
CDR3	QQRSNWPLT	13
FR4	FGGGTKVEIKRTV	14
VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPLTFGGGTKVEIKRTV	16
LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	18

Additionally, an exemplified anti-CLDN18.2 antibody clone PR301839 was constructed by introducing a CaaX peptide moiety (GGGGGGGCVIM, SEQ ID NO: 19) to the C-terminus of the light chain of the antibody clone PR002726 with the method disclosed in U.S. Patent No. US 10,583,197, the entirety of which is herein included in the present specification by reference. And LALA mutations (L237A/L238A) were introduced into the heavy chain constant region of the antibody clone PR002726. The antibody clone PR301839 produced was used in ADC synthesis. The amino acid sequences of the antibody clone PR301839 are shown in **Table 2** below.

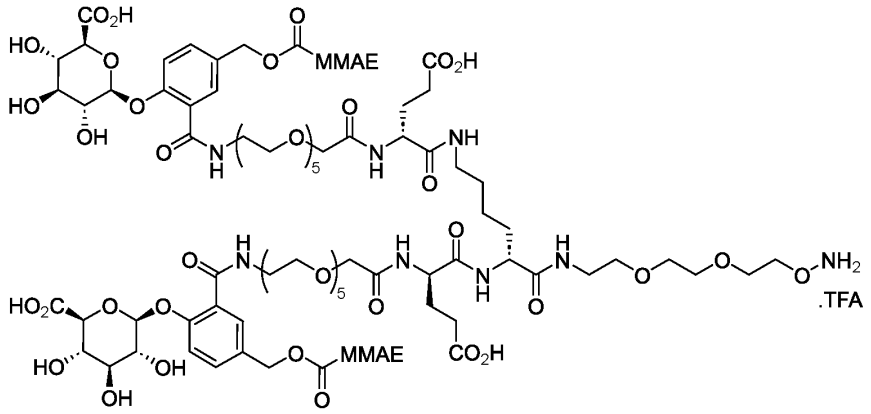
Table 2. Amino Acid Sequences of Antibody Clone PR301839

PR301839 heavy chain		SEQ ID NO:
FR1	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	1
CDR1	SFVMS	2
FR2	WVRQAPGKGLEWVS	3
CDR2	TISGSGRSTYYADSVKG	4
FR3	RFTISRDNSKNTLHLQMNSLRAEDTAVYYCAK	5
CDR3	DAAAAGTKFDY	6
FR4	WGQGTLVTVSS	7
VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFVMSWVRQAPGKGLEWVSTISGSGRSTYYADSVKGRFTISRDNSKNTLHLQMNSLRAEDTAVYYCAKDAAAAGTKFDYWGQGTLVTVSS	15

HC	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFVMSWVRQ APGKGLEWVSTISGSGRSTYYADSVKGRFTISRDNSKNTL HLQMNSLRAEDTAVYYCAKDAAAAGTKFDYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**A** **A**GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG	20
PR301839 light chain		SEQ ID NO:
FR1	EIVLTQSPATLSLSPGERATLSC	8
CDR1	RASQSVSSYLA	9
FR2	WYQQKPGQAPRLLIY	10
CDR2	DASNRAT	11
FR3	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC	12
CDR3	QQRSNWPLT	13
FR4	FGGGTKVEIKRTV	14
VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPG QAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDF AVYYCQQRSNWPLTFGGGTKVEIKRTV	16
LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPG QAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDF AVYYCQQRSNWPLTFGGGTKVEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC<u>GGGGGGGCVIM</u>	21

Preparation Example 2: Synthesis of Exemplified Compounds of the Disclosure

Production of Compound 1

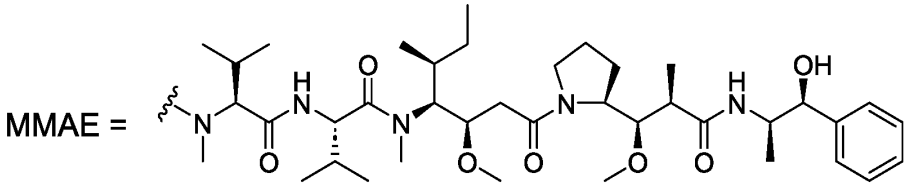


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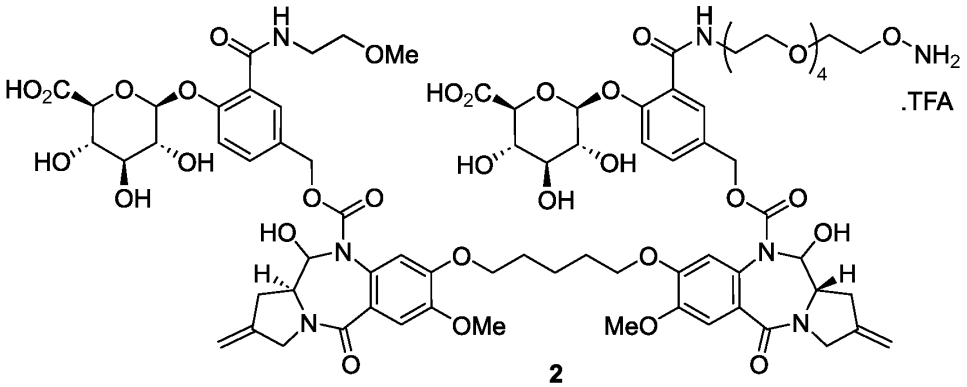
Compound 1 was produced with the method disclosed in U.S. Patent No. 11,173,214, the entirety of which is herein included in the present specification by reference.

EI-MS m/z: $[½M+H]^+$ 1623.6, $[⅓M+H]^+$ 1082.8.

The structure of MMAE (Monomethyl auristatin E) in Compound 1 is as follows:



Production of Compound 2

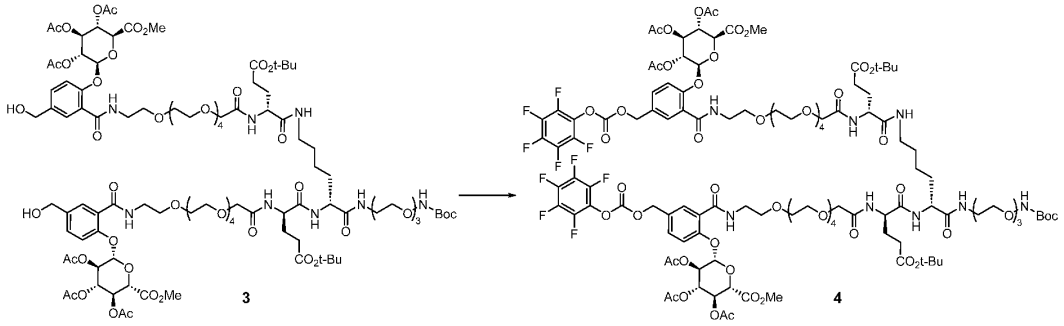


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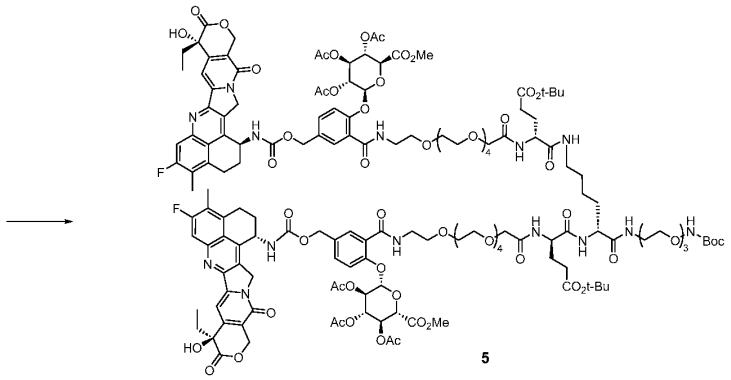
Compound 2 was produced with the method disclosed in U.S. Patent No. 11,654,197, the entirety of which is herein included in the present specification by reference.

EI-MS m/z: $[M+H]^+$ 1698.2, $[½M+H]^+$ 849.6.

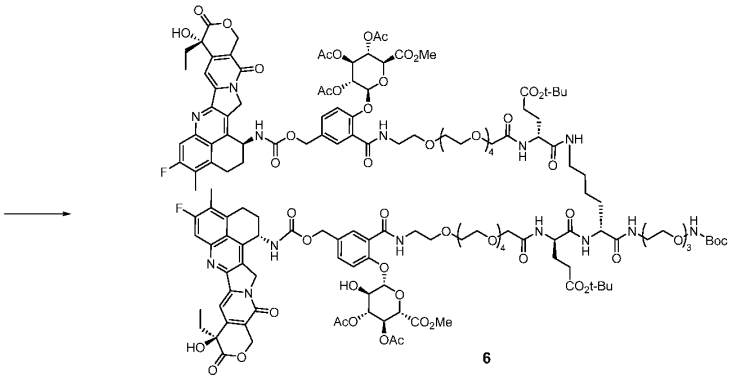
Production of Compound 7



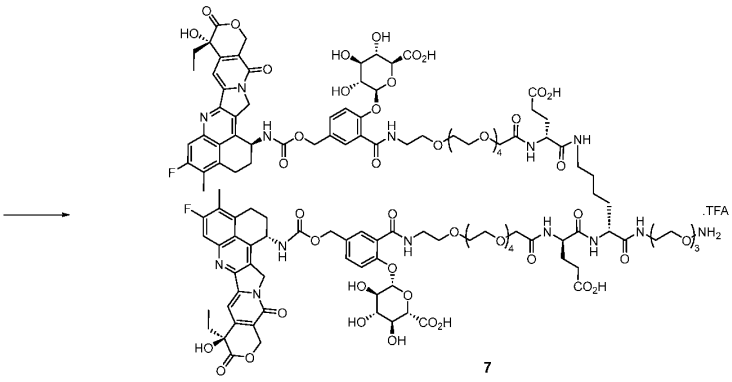
3 4



5



6



7

Production of Compound 4

Compound 3 (International Patent Application No. PCT/KR2023/018766, the entirety of which is incorporated herein by reference) (1.5 g, 0.67 mmol) was dissolved in (24 mL) of dichloromethane. Then, 0.22 mL of pyridine (2.68 mmol) and 0.79 g of bis(pentafluorophenyl) carbonate (2.01 mmol) were added at room temperature under a nitrogen atmosphere and stirred for 16 hours. The reaction solution was diluted with 80 mL of ethyl acetate, rinsed with 50 mL x 2 of saturated aqueous sodium hydrogen carbonate, followed by 50 mL of brine, and dehydrated using anhydrous sodium sulfate. Compound 4 (2.06 g) was obtained after filtering and concentration under reduced pressure, without additional purification, and was subsequently utilized in the next process.

EI-MS m/z: $[M+H]^+$ 2670.3, $[½M+H]^+$ 1335.7, $[⅓M+H]^+$ 890.8.

Production of Compound 5

Compound 4 (100 mg, 0.04 mmol) was dissolved in N,N-dimethylformamide (3 mL), followed by sequential addition of exatecan mesylate (44 mg, 0.08 mmol), N,N-diisopropylethylamine (0.05 mL, 0.28 mmol), and 1-hydroxy-7-benzotriazole (HOAt, 2.55 mg, 0.02 mmol) at room temperature under nitrogen atmosphere with stirring for 3 hours. The reaction mixture was diluted with ethyl acetate (40 mL), washed successively with saturated ammonium chloride aqueous solution (30 mL), saturated sodium bicarbonate aqueous solution (30 mL), and brine (30 mL), then dried over anhydrous sodium sulfate. Compound 5 (140 mg) was obtained after filtering and concentration under reduced pressure, without additional purification.

EI-MS m/z: $[½M+H]^+$ 1587.5, $[⅓M+H]^+$ 1058.8.

Production of Compound 6

Compound 5 (550 mg, 0.17 mmol) was dissolved in methanol (7 mL), tetrahydrofuran (7 mL) and then a solution of lithium hydroxide (65.8 mg, 1.57 mmol) dissolved in distilled water (8 mL) was added gradually at -40°C, under nitrogen atmosphere. Then the mixture was agitated for 2 hours as the temperature of the reaction was incrementally increased to 0°C. The reaction mixture was neutralized using acetic acid, condensed under reduced pressure, purified via HPLC, and then freeze-dried to provide Compound 6 (236 mg, 44%) in the form of a yellow solid.

EI-MS m/z: $[½M+H]^+$ 1446.7, $[⅓M+H]^+$ 964.9.

Production of Compound 7

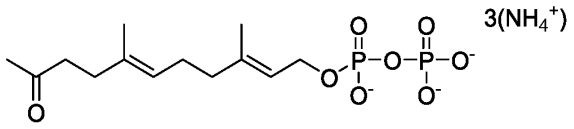
Compound 6 (236 mg, 0.08 mmol) was dissolved in acetonitrile (10 mL) and phosphoric acid (85%, 5 mL) was added at 0°C. After stirring at room temperature for 4 hours,

the reaction mixture was purified by HPLC, followed by freeze-drying to afford yellow solid Compound 7 (138 mg, 61%).

EI-MS m/z: $[½M+H]^+$ 1340.5, $[⅓M+H]^+$ 894.1.

Preparation Example 3: Synthesis of Exemplified ADCs of the Disclosure

Exemplified ADCs were produced with the below two steps. LCB14-0606 was produced with the method disclosed in U.S. Patent No. 9,669,107, the entirety of which is herein included in the present specification by reference. The structural formula of LCB14-0606 is as follows:



LCB14-0606

Step 1: Production of Prenylated Antibody with LCB14-0606

An exemplified anti-CLDN18.2 antibody clone PR301839 was prepared according to Preparation Example 1. A mixture comprising antibody clone PR301839 for the prenylation reaction was prepared and reacted at 30°C for 16 hours. The reaction mixture contained a total of 24 μM of the antibody clone PR301839, 600 nM FTase (Genscript), and 144 μM LCB14-0606 in a buffer solution (50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 10 μM $ZnCl_2$, 0.25 mM DTT). After the reaction, the prenylated antibody was desalted using a G25 Sepharose column (AKTA purifier, GE healthcare) equilibrated with PBS buffer.

Step 2: Drug-Conjugation Method

Oxime Bond Reaction of ADC 1 (conjugation by oxime bond formation) MMAE

The oxime bond formation reaction between the prenylated antibody and the linker-drug was carried out by mixing 100 mM sodium acetate buffer (pH 5.2), 10% DMSO, 24 μM of prenylated antibody, and 10 equivalents of the linker-drug (Compound 1 of Preparation Example 2) (240 μM) at 30°C with stirring at 500 to 600 rpm for 24 hours. After the reaction, excess low molecular weight compounds were removed by FPLC (Fast protein liquid chromatography) (AKTA purifier, GE healthcare), and the protein fraction was collected and concentrated. The production process and structure thereof are shown in **FIG. 1**.

Oxime Bond Reaction of ADC2 (conjugation by oxime bond formation) PBD

The oxime bond formation reaction between the prenylated antibody and the linker-drug was carried out by mixing 100 mM sodium acetate buffer (pH 5.2), 10% DMSO, 20 μM of prenylated antibody, and 10 equivalents of the linker-drug (Compound 2 of Preparation

Example 2) (200 μM) at 30°C with stirring at 500 to 600 rpm for 6 hours. After the reaction, excess low molecular weight compounds were removed by FPLC (AKTA purifier, GE healthcare), and the protein fraction was collected and concentrated. The production process and structure thereof are shown in **FIG. 2**.

Oxime Bond Reaction of ADC3 (conjugation by oxime bond formation) Exatecan

The oxime bond formation reaction between the prenylated antibody and the linker-drug was carried out by mixing 100 mM sodium acetate buffer (pH 5.2), 10% DMSO, 48 μM of prenylated antibody, and 6 equivalents of the linker-drug (Compound 7 of Preparation Example 2) (288 μM) at 30°C with stirring at 500 to 600 rpm for 24 hours. After the reaction, excess low molecular weight compounds were removed by FPLC (AKTA purifier, GE healthcare), and the protein fraction was collected and concentrated. The production process and structure thereof are shown in **FIG. 3**.

To confirm the characteristic of the three ADCs (ADC1-3), hydrophobic interaction chromatography (HIC) was conducted using an HPLC instrument with an HIC column, employing a mobile phase prepared with ammonium sulfate, acetonitrile, and potassium phosphate, and the results are shown in **FIG. 4**.

As shown in **FIG. 4**, ADC1, ADC2, and ADC3 all exhibited different levels of hydrophobicity, with the hydrophobicity confirmed to be in the order of ADC1 > ADC2 ≒ ADC3.

Table 3. Exemplified ADCs

ADC	Antibody	Linker-toxin
ADC1	PR301839	Compound 1
ADC2		Compound 2
ADC3		Compound 7

Experimental Example 1: Evaluation of Cancer Cell Binding Affinity of Anti-CLDN18.2 Antibody

The cell binding affinity of the anti-CLDN18.2 antibody clone PR301839 produced in Preparation Example 1 was confirmed using gastric cancer cell line (SNU-601), and pancreatic cancer cell line (PATU-8988s).

Specifically, the above cancer cell lines were cultured, and the cells were divided into two groups: control IgG antibody-treated group and anti-CLDN18.2 antibody clone PR301839-treated group. Subsequently, the cells were incubated in a phosphate-buffered saline solution containing 2% fetal bovine serum at 4°C for 1 hour to remove non-specific binding of

antibodies. Next, the control antibody and anti-CLDN18.2 antibody clone PR301839 were incubated with each group of the cell lines at 4°C for 1 hour to allow the antibodies to bind to the CLDN18.2 proteins on the cancer cell surface. Subsequently, antibodies that did not bind to the cell surface were removed using a phosphate-buffered saline solution containing 2% fetal bovine serum. Next, the cells were incubated with fluorescent-labeled secondary antibodies that bound to control IgG antibody and anti-CLDN18.2 antibody clone PR301839 at 4°C for 1 hour. Following the removal of secondary antibodies that did not bind to the antibodies using a phosphate-buffered saline solution, the fluorescence intensity of the cells was measured using a flow cytometer to calculate the fluorescence intensity of cell lines in the anti-CLDN18.2 antibody clone PR301839 group compared to those in the control IgG group, confirming the binding affinity of the antibodies to the cancer cell lines. The results are presented in **Table 4** below.

Table 4. Fluorescence Intensity of SNU-601 and PATU-8988s

Mean FITC-A (Fold Value)		
Sample	**SNU-601**	**PATU-8988s**
Anti-CLDN18.2 antibody clone PR301839/control IgG	23	16

Experimental Example 2: *In vitro* Cell Toxicity Evaluation

The cell proliferation inhibitory activity of the three ADCs produced in Preparation Example 3 against cancer cell lines was measured.

Commercially available human gastric cancer cell line (SNU-601), and pancreatic cancer cell line (PATU-8988s) were used. Additionally, each cancer cell line was seeded at 5,000 cells per well in a 96-well plate and cultured for 24 hours. Subsequently, the cells were treated with ADCs at concentrations ranging 0.256 pM to 100 nM (5-fold serial dilution). After 144 hours, the number of viable cells was quantified using SRB (Sulforhodamine B) dye, and the results are shown in **Table 5** below.

Table 5. 50% Cytotoxicity Concentrations of ADCs in Cancer Cell Lines

	CC_{50} (nM)	
	SNU-601	**PATU-8988s**
ADC1	1.69	4.62
ADC2	-	0.76
ADC3	0.81	7.42

"-": not measured.

As shown in Table 5, the cytotoxicity assay results for ADC comprising exatecan (ADC3) showed stronger cytotoxicity compared to ADC comprising MMAE (ADC1) against gastric cancer cell line. Additionally, ADC comprising MMAE (ADC1), ADC comprising pyrrolobenzodiazepine (PBD) (ADC2) and ADC comprising exatecan (ADC3) all exhibited superior cytotoxicity against pancreatic cancer cell line.

The cell proliferation inhibitory activity of the three ADCs produced in Preparation Example 3 against normal cell lines was measured.

Commercially available human skin cell line (HaCaT), and hematopoietic stem cells ($CD34^+$ HSC) were used. Additionally, each cancer cell line was seeded at 5,000 cells per well in a 96-well plate and cultured for 24 hours. Subsequently, the cells were treated with ADCs at concentrations ranging 0.256 pM to 100 nM (5-fold serial dilution). After 144 hours, the number of viable cells was quantified using SRB (Sulforhodamine B) dye, and the results are shown in **Table 6** below.

Table 6. 50% Cytotoxicity Concentrations of ADCs in Normal Cell Lines

	CC_{50} (nM)	
	HaCaT	**$CD34^+$ HSC**
ADC1	79.2	-
ADC2	-	32.79
ADC3	490	> 500

"-": not measured.

As shown in Table 5, ADC3 showed weaker cytotoxicity compared ADC1 and ADC2 in normal cell lines. The safety of ADCs was shown in the order of ADC3 > ADC1 > ADC2.

The therapeutic index (TI; also referred to as therapeutic ratio) is a quantitative measurement of the relative safety of a drug. It is a comparison of the amount of a therapeutic agent that causes toxicity to the amount that causes the therapeutic effect. The lager the TI means the safer drug. The results of calculating the TI of the three ADCs produced in Preparation Example 3 are shown in **Table 7** below. The TI value for each ADC is the CC_{50} of the normal cell line divided by the CC_{50} of the cancer cell line.

Table 7. Therapeutic Index (TI) of ADCs

	TI		
	HaCaT/ SNU-601	**HaCaT/ PATU-8988s**	**$CD34^+$ HSC/ PATU-8988s**
ADC1	46.86	-	17.14
ADC2	-	-	43.14

ADC3	604.94	66.03	67.39

"-": not measured.

As shown in Table 7, the TI value was highest for ADC3, which means that among ADC1, ADC2, and ADC3, ADC3 is the safest drug.

Experimental Example 3: *In vivo* Anti-Cancer Efficacy Assessment

The tumor growth inhibitory efficacy of ADCs produced in Preparation Example 3 was analyzed in a tumor xenograft mouse model.

Specifically, gastric cancer cell line (SNU-601), and pancreatic cancer cell line (PATU-8988s) were used for cell culture and 2,000,000 to 5,000,000 cells were mixed with 100 μl of PBS solution and 100 μl of Matrigel. Subsequently, these mixtures were subcutaneously implanted into Balb/c-nude mice to generate tumor xenograft mouse models. ADCs were intravenously injected according to the dosage and administration schedule shown in Table 7 when the tumor size reached 100-300 mm^3. The tumor size was measured immediately before the initial administration (Day 1) and periodically for a certain period (up to 50 days), and the results are shown in **FIGs. 5 to 7**.

Table 8. ADC administration Schedule

	SNU-601	PATU-8988s
ADC1	1.0 mg/kg (QDx1), 0.5 mg/kg (BIWx2)	-
ADC3	1.0, 2.0, 4.0 mg/kg (QDx1) 0.5 mg/kg (BIWx2)	2.0, 4.0 mg/kg (BIWx2)

"-": not measured.

As shown in **FIG. 5**, ADC3 exhibited significant tumor growth inhibition in the SNU-601 model of gastric cancer cell line compared to the control group. As shown in **FIG. 6**, in the SNU-601 model, ADC3 exhibited dose-dependent tumor growth inhibition compared to the control group. After day 33, ADC3 showed better tumor growth inhibition than ADC1 at the same dose (1.0 mg/kg, QDx1). As shown in **FIG. 7**, in the SNU-601 model, ADC1 and ADC3 showed tumor growth inhibition even at low dose (0.5 mg/kg, BIWx2). After day 26, ADC3 showed better tumor growth inhibition than ADC1 at the same dose (0.5 mg/kg, BIWx2).

Additionally, as shown in **FIG. 8**, in the PATU-8988s model of pancreatic cancer cell line, ADC3 showed excellent anti-cancer efficacy and dose-dependent tumor growth inhibition compared to the control group.

Consistent with *in vitro* effectiveness, ADC3 showed superior *in vivo* efficacy in different tumor mouse models. Taken together, anti CLDN18.2 antibody and exatecan conjugate described herein have strongly competitive profiles of high anti-tumor activity, but low toxicity.

Experimental Example 4: Analysis of Pharmacokinetics in Rat Model

To analyze the pharmacokinetics of ADCs produced in Preparation Example, ADC1 and ADC3 were intravenously administered to SD rat at a single dose of 3 mg/kg. Plasma samples were taken at various time points and stored frozen for analysis. Specifically, plasma samples were collected at 0 minutes, 3 minutes, 3 hours, 1-, 2-, 3-, 7-, 9-, and 14-days post-administration to measure the concentrations of ADCs. The plasma concentrations of the ADC1 and ADC3 at the indicated time points were measured using a LC-MS/MS analysis method.

FIG. 9 shows the results of pharmacokinetic analysis in the rat model of ADC1 and ADC3 that ADC1 and ADC3 had different half-life. ADC3 has an excellent half-life, more than twice that of ADC1.

Incorporation by Reference

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

Equivalents

While specific embodiments of the disclosure have been discussed, the above specification is illustrative and not restrictive. Many variations of the disclosure will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the disclosure should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

We claim:

1. A conjugate having a structure represented by General Formula I or a pharmaceutically acceptable salt thereof:

[General Formula I]

$$Ab\text{-}[L^{Ab}\text{-}(B)_l]_m$$

wherein,

Ab is an anti-Claudin 18 isoform 2 (CLDN18.2) antibody or antigen-binding fragment thereof comprising a heavy chain variable region and a light chain variable region, wherein

the heavy chain variable region comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 2, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6; and

the light chain variable region comprises a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 9, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 13;

each L^{Ab} is, independently, a linker;

each B is, independently, a topoisomerase I inhibitor (e.g., a camptothecin compound); and

l and m are each independently 1 to 20.

2. The conjugate of claim 1, wherein Ab comprises a heavy chain variable region comprising:

the amino acid sequence of SEQ ID NO: 15;

a sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 15 while maintaining the heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 2, the heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and the heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6; or

a sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 15 while maintaining the heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 2, the heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and the heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6.

3. The conjugate of claim 1 or 2, wherein Ab comprises a light chain variable region comprising:

the amino acid sequence of SEQ ID NO: 16;

a sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 16 while maintaining the light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 9, the light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and the light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 13; or

a sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 16 while maintaining the light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 9, the light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and the light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 13.

4. The conjugate of claim 1, wherein Ab comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15 and a light chain variable region comprising the amino acid sequences of SEQ ID NO: 16.

5. The conjugate of any one of claims 1-4, wherein Ab is a humanized antibody or a human antibody.

6. The conjugate of any one of claims 1-5, wherein Ab is selected from a monoclonal antibody, a domain antibody (dAb), a single chain antibody (scAb), a Fab fragment, a Fab' fragment, a F(ab')2 fragment, an scFab fragment, an Fv fragment, a dsFv fragment, a single chain variable fragment (scFv), an scFv-Fc fragment, a single domain heavy chain antibody, a single domain light chain antibody, a variant antibody, a multimeric antibody, a minibody, a diabody, a bispecific antibody, and a multispecific antibody.

7. The conjugate of any one of claims 1-6, wherein Ab comprises an IgA, IgG, IgM, IgE, or IgD constant domain or is an IgA, IgG, IgM, IgE, or IgD antibody.

8. The conjugate of claim 7, wherein Ab comprises an IgG constant domain or is an IgG antibody.

9. The conjugate of claim 8, wherein Ab comprises an IgG1, IgG2, IgG3, or IgG4 constant domain or is an IgG1, IgG2, IgG3, or IgG4 antibody.

10. The conjugate of any one of claims 7-9, wherein Ab comprises LALA mutations in a heavy chain constant region.

11. The conjugate of claim 10, wherein the LALA mutations comprise L234A and L235A according to EU numbering convention.

12. The conjugate of any one of claims 1-9, wherein Ab comprises LALA mutations in a heavy chain constant region in place of amino acids corresponding to amino acids 237-238 in SEQ ID NO: 17.

13. The conjugate of any one of claims 1-12, wherein each linker is cleavable.

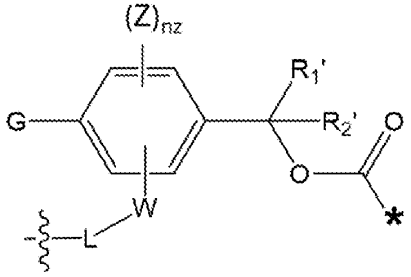
14. The conjugate of claim 13, wherein each linker is cleavable by a glycosidase.

15. The conjugate of claim 14, wherein the glycosidase is β-glucuronidase or β-galactosidase.

16. The conjugate of claim 15, wherein the linker comprises a β-glucuronosyl or β-galactosyl moiety.

17. The conjugate of claim 14 or 15, wherein each linker comprises a moiety independently represented by General Formula II or a pharmaceutically acceptable salt thereof:

[General Formula II]



wherein

G is a sugar moiety or a glucuronic acid moiety;

each R_1' and R_2' are independently hydrogen, C_1-C_8 alkyl or C_3-C_8 cycloalkyl; or R_1' and R_2', combine to complete a (C_3-C_8) cycloalkyl ring;

W is -C(O)-, -C(O)N(R')-, -N(R')C(O)-, -$(CH_2)_t$N(R')C(O)-, -C(O)O-, -$S(O_2)$N(R')-, -P(O)(R'')N(R')-, -S(O)N(R')-, or -$P(O_2)$N(R')-, wherein the indicated C(O), N, CH_2, S, or P is directly bonded to the phenyl ring of Formula II;

R' and R'' are each independently hydrogen, C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{1-8} alkoxy, C_{1-8} alkylthio, mono- or di-C_{1-8} alkylamino, C_{3-20} heteroaryl, or C_{6-20} aryl;

each Z is independently C_{1-8} alkyl, halogen, cyano, or nitro;

nz is an integer from 0 to 3;

t is an integer from 1 to 3;

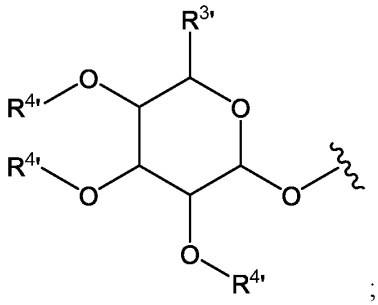
L is absent, C_1-C_{50} alkylene, C_1-C_{50} alkenylene or 1-50 atom heteroalkylene;

* represents a connection point to B; and

〰 represents a connection point to the antibody.

18. The conjugate of claim 17, wherein G is a sugar moiety represented by General Formula III:

[General Formula III]



;

wherein

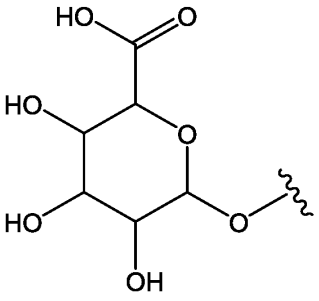
$R^{3'}$ is $-CH_2OR^{3A}$, or $-CO_2R^{3B}$;

R^{3A} is H or a hydroxyl protecting group;

R^{3B} is H or a carboxyl protecting group; and

each $R^{4'}$ is, respectively and independently, a hydrogen or a hydroxyl protecting group.

19. The conjugate of claim 17 or 18, wherein

G is  ; and

W is -C(O)NR'-.

20. The conjugate of any one of claims 17-19, wherein L comprises:

A) a C_1-C_{50} alkylene or a 1 to 50 atom heteroalkylene comprising:

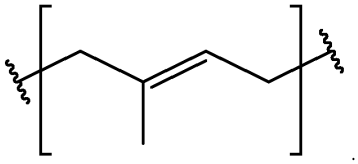
(i) one or more unsaturated bonds;

(ii) a heteroarylene (e.g., a heteroarylene in the alkylene or heteroalkylene chain); and/or

(iii) a C_{1-20} alkyl; and/or

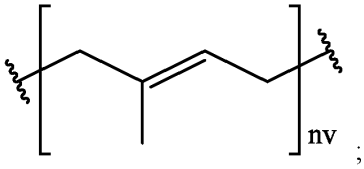
B) at least one isoprenyl group having the structure represented by General Formula IV:

[General Formula IV]



21. The conjugate of claim 20, wherein the L comprises an isoprenyl group having a structure represented by General Formula IVa:

[General Formula IVa]



;

wherein nv is an integer from 1-20.

22. The conjugate of any one of claims 17-21, wherein L comprises a peptide comprising at least one hydrophilic amino acid.

23. The conjugate of claim 22, wherein the hydrophilic amino acid comprises a side chain having a moiety that has electric charge in aqueous solution at neutral pH (e.g., an amine, guanidine, or carboxyl moiety).

24. The conjugate of claim 22 or 23, wherein the peptide comprises an amino acid selected from arginine, histidine, aspartate, asparagine, glutamate, glutamine, glycine, lysine, ornithine, proline, serine, and threonine.

25. The conjugate of any one of claims 17-24, wherein L is covalently connected to Ab by a sulfur atom in a cysteine residue of Ab (e.g., by a sulfur atom in the side chain of the cysteine residue) via a thioether bond.

26. The conjugate of any one of claims 17-25, wherein Ab at the C-terminus comprises an amino acid motif that is recognized by an isoprenoid transferase.

27. The conjugate of claim 26, wherein the isoprenoid transferase is farnesyl protein transferase (FTase) or geranylgeranyl transferase (GGTase).

28. The conjugate of claim 26 or 27, wherein L is covalently bonded to Ab by a thioether bond and the thioether bond comprises a sulfur atom of a cysteine of the amino acid motif.

29. The conjugate of any one of claims 26-28, wherein the amino acid motif comprises a CYYX sequence, wherein:
C is cysteine;
each Y is an aliphatic amino acid;
X is selected from glutamine, glutamate, serine, cysteine, methionine, alanine, and leucine.

30. The conjugate of claim 29, wherein
each Y is independently selected from alanine, isoleucine, leucine, methionine, and valine.

31. The conjugate of any one of claims 26-30, wherein the amino acid motif comprises a CVIM (SEQ ID NO:22) or CVLL sequence (SEQ ID NO:23).

32. The conjugate of any one of claims 26-31, wherein at least one of the 1 to 20 amino acids preceding the amino acid motif is glycine.

33. The conjugate of any one of claims 26-32, wherein the amino acid motif has the sequence GGGGGGGCVIM (SEQ ID NO: 19).

34. The conjugate of any one of claims 17-33, wherein L comprises an oxime.

35. The conjugate of claim 34, wherein the oxygen atom of the oxime is on the side of L linked to W and the carbon atom of the oxime is on the side of L linked to Ab.

36. The conjugate of claim 34, wherein the carbon atom of the oxime is on the side of L linked to W and the oxygen atom of the oxime is on the side of L linked to Ab.

37. The conjugate of any one of claims 17-36, wherein

L is a 1-50 atom heteroalkylene comprising an oxime; and
the oxygen atom of the oxime is on the side of L linked to W, and
the carbon atom of the oxime is on the side of L linked to Ab; or
the carbon atom of the oxime is on the side of L linked to W, and
the oxygen atom of the oxime is on the side of L linked to Ab.

38. The conjugate of any one of claims 17-37, wherein L comprises an oxime, and at least one isoprenyl unit covalently bonds the oxime to Ab (e.g., the at least one isoprenyl unit directly or indirectly bonds the oxime to Ab).

39. The conjugate of any one of claims 17-38, wherein L comprises a connecting unit represented by General Formula Va or Vb:

[General Formula Va]

$-(CH_2)_r(V(CH_2)_p)_q-$

[General Formula Vb]

$-(CH_2CH_2X)_w-$

V is a single bond, -O-, -S-, - NR^{21}-, -C(O)NR^{22}-, -NR^{23}C(O)-, -$NR^{24}SO_2$-, or -SO_2NR^{25}-;
X is -O-, C_{1-8} alkylene, or -NR^{21}-;
R^{21} to R^{25} are each independently hydrogen, C_{1-6} alkyl, C_{1-6} alkyl C_{6-20} aryl, or C_{1-6} alkyl-C_{3-20} heteroaryl;
r is an integer from 0 to 10;
p is an integer from 0 to 10;
q is an integer from 1 to 20; and
w is an integer from 1 to 20.

40. The conjugate of claim 39, wherein q is 1 to 10.

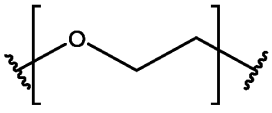
41. The conjugate of claim 39 or 40, wherein r is 1 or 2.

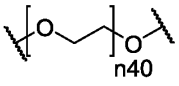
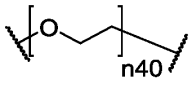
42. The conjugate of any one of claims 39-41, wherein p is 1 or 2.

43. The conjugate of any one of claims 39-42, wherein V is -O-.

44. The conjugate of claim 39, wherein:
q is an integer from 1 to 10;
r and p are each an integer from 1 or 2; and
V is -O-.

45. The conjugate of any one of claims 39-44, wherein X is -O-.

46. The conjugate of any one of claims 39-45, wherein L comprises at least one polyethylene glycol monomer represented by .

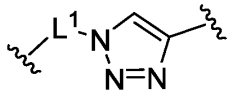
47. The conjugate of claim 46, wherein L comprises a polyethylene glycol oligomer represented by  or , wherein n40 is an integer from 2 to 10.

48. The conjugate of claim 46 or 47, wherein L comprises an oxime, and the at least one polyethylene glycol unit covalently bonds the oxime to W.

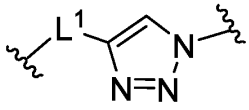
49. The conjugate of any one of claims 17-48, wherein L further comprises a binding unit formed by a reaction between an alkyne and an azide or between an aldehyde or ketone group and hydrazine or hydroxylamine.

50. The conjugate of any one of claims 17-49, wherein L further comprises a binding unit represented by General Formula VIa, VIb, VIc, VId, or VIe:

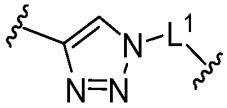
[General Formula VIa]



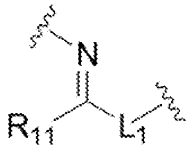
[General Formula VIb]



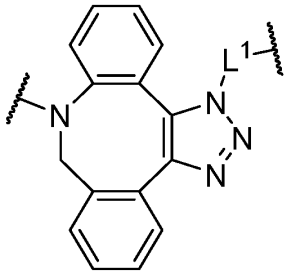
[General Formula VIc]



[General Formula VId]



[General Formula VIe]



wherein

L^1 is each independently a single bond or $C_{1\text{-}30}$ alkylene; and

R^{11} is hydrogen or $C_{1\text{-}10}$ alkyl.

51. The conjugate of any one of claims 17-50, wherein L is branched and comprises:

i) a branching unit covalently coupled to Ab by a primary linker;

ii) a first branch which couples a first B to the branching unit; and

iiia) a second branch which couples a second B to the branching unit; or

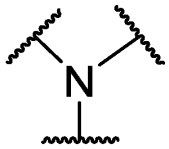
iiib) a second branch, in which an alkyl or heteroalkyl (e.g., a polyethylene glycol monomer or a polyethylene glycol oligomer) is covalently coupled to the branching unit.

52. The conjugate of claim 51, wherein L comprises a second branch which couples a second B, via a cleavage group, to the branching unit.

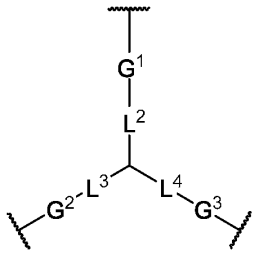
53. The conjugate of claim 51, wherein L comprises a second branch, in which an alkyl or heteroalkyl (*e.g.,* a polyethylene glycol monomer or a polyethylene glycol oligomer) is covalently coupled to the branching unit.

54. The conjugate of claim 51, wherein the branching unit has a structure represented by General Formula VIIa, VIIb, VIIc, VIId, or VIIe:

[General Formula VIIa]



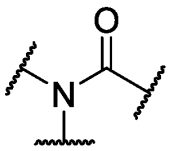
[General Formula VIIb]



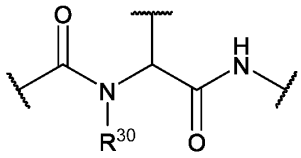
[General Formula VIIc]



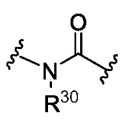
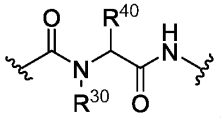
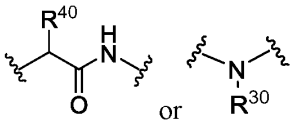
[General Formula VIId]



[General Formula VIIe]



wherein

G^1, G^2, and G^3 each independently represents a bond, , ,  or ;

R^{30} is hydrogen or $C_{1\text{-}30}$ alkyl;

R^{40} is hydrogen or L^5-$COOR^{50}$;

R^{50} is hydrogen or $C_{1\text{-}30}$ alkyl

L^2, L^3, L^4, and L^5 are each independently a bond or $-C_{n'}H_{2n'}-$; and

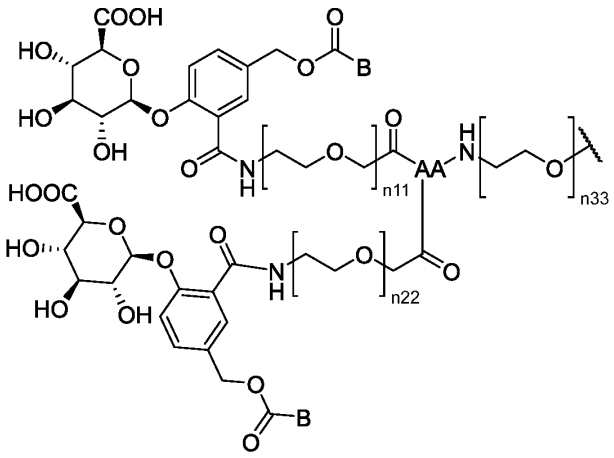
n' is an integer from1 to 10.

55. The conjugate of any one of claims 51-54, wherein:
at least one branched linker is covalently coupled to Ab; and
at least two B are covalently coupled to the branched linker.

56. The conjugate of claim 55, wherein the conjugate comprises 1, 2, 3, or 4 branched linkers and each branched linker is covalently coupled to two B.

57. The conjugate of any one of claims 51-56, wherein the branching unit comprises a lysine residue.

58. The conjugate of any one of claims 1-57, wherein the conjugate comprises a structure represented by:



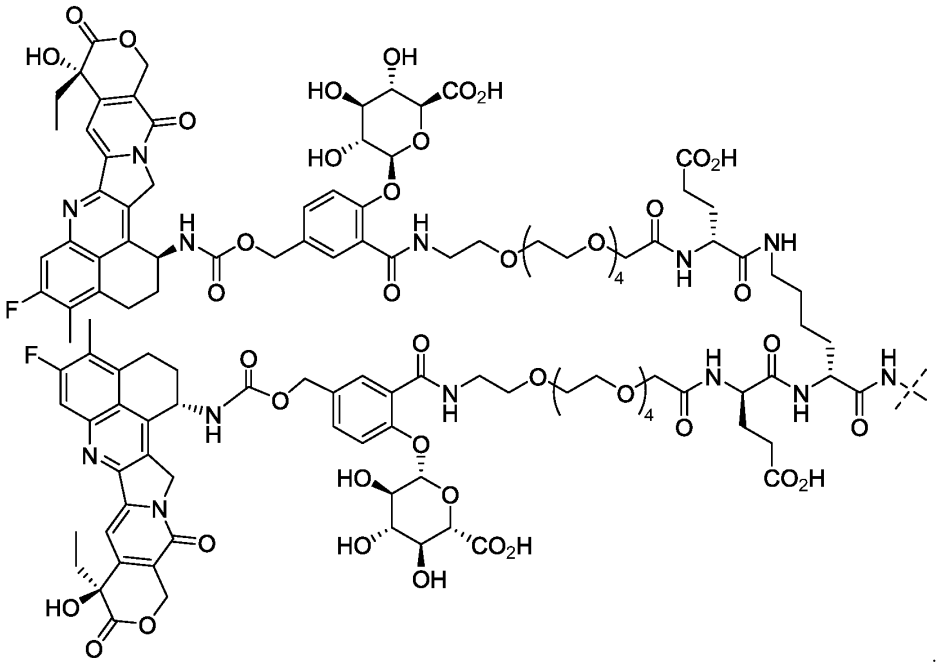
or a pharmaceutically acceptable salt thereof; wherein
each B is, independently, a camptothecin compound;
n11, n22, and n33 are each independently an integer from 0 to 30; and
AA is a peptide comprising at least two amino acid residues.

59. The conjugate of any one of claims 1-58, wherein the camptothecin compound is camptothecin (CPT), hydroxycamptothecin, aminocamptothecin, exatecan, topotecan, belotecan, irinotecan, cositecan, rubitecan, lurtotecan, gimatecan, silatecan, namitecan, homocamptothecin (HomoCPT), diflomotecan, S39625, S38809, S38769, ZBH-01, ZBH-1207, BN80915, CPT211, Dxd, SN-38, or FL118.

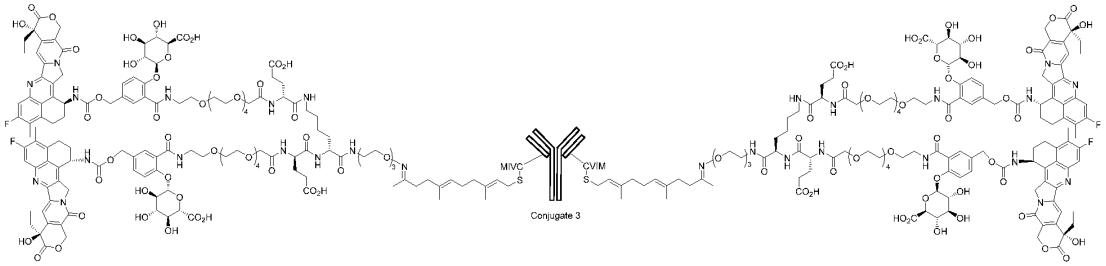
60. The conjugate of claim 59, wherein the camptothecin compound is hydroxycamptothecin, aminocamptothecin, exatecan, belotecan, irinotecan, cositecan, rubitecan, lurtotecan, gimatecan, silatecan, namitecan, diflomotecan, Dxd, SN-38, or FL118.

61. The conjugate of claim 60, wherein the camptothecin compound is exatecan.

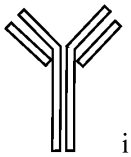
62. The conjugate of any one of claims 1-61, wherein the conjugate comprises a structure represented by:



63. The conjugate of claim 1, wherein the conjugate is:



or a salt thereof, wherein:



is Ab.

64. The conjugate of claim 63, wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 17 or 20, and a light chain comprising the amino acid sequence of SEQ ID NO: 18.

65. A pharmaceutical composition comprising the conjugate of any one of claims 1-64 and a pharmaceutically acceptable excipient.

66. A pharmaceutical preparation comprising the conjugate of any one of claims 1-64 and a pharmaceutically acceptable excipient, the pharmaceutical preparation being selected from injections, tablets, pills, powders, granules, capsules, troches, suspensions, liquids for internal use, emulsions, syrups, emulsions, freeze-dried preparations, and suppositories.

67. A method of treating or preventing hyperplasia, cancer or an angiogenic disease in a subject in need thereof comprising administering a conjugate of any one of claims 1-64 or a pharmaceutically acceptable salt thereof to the subject.

68. The method of claim 67, wherein the method is a method of treating or preventing cancer.

69. The method of claim 67 or 68, wherein the cancer is lung cancer, small-cell lung cancer, non-small-cell lung cancer, gastrointestinal cancer, colon cancer, intestinal cancer, bowel cancer, breast cancer, ovarian cancer, prostate cancer, testicular cancer, liver cancer, kidney cancer, bladder cancer, pancreatic cancer, brain cancer, sarcoma, osteosarcoma, Kaposi’s sarcoma or melanoma.