ANTIBODY DRUG CONJUGATE, PREPARATION METHOD THEREFOR AND USE THEREOF

The application is based on and claims priority to CN application No. 202010410633.5 with filing date of May 15, 2020, the disclosure of which is hereby introduced into the application in its entirety.

FIELD OF THE INVENTION

The invention belongs to the technical field of biomedicine, specifically relating to an anti-Claudin 18.2 antibody-drug conjugate (ADC) and a composition comprising the ADC, and use thereof.

BACKGROUND OF THE INVENTION

The treatment of gastric cancer is predominated by surgical excision. For unresectable or recurrent gastric cancer, chemotherapy is the main therapy, but under the state of the art, it can only relieve symptoms and prolong survival. At present, there is no universally recognized standard chemotherapy regimen for advanced gastric cancer. In terms of bio-macromolecular drugs, except for Trastuzumab, Ramucirumab, Pembrolizumab, etc., which have been approved, most of other targeted drugs for gastric cancer are unsatisfactory in efficacy or still in the early stages of clinical research. The core weakness of existing drugs includes the unmet clinical needs of non-surgical treatment, very limited treatment options for advanced or recurrent gastric cancer, extremely poor prognosis, and high death rate. Due to the high incidence of gastric cancer, the current demands for gastric cancer drug are great.

Claudin 18.2 (CLDN 18.2) is a member of the Claudin protein family. Claudin family proteins are a type of proteins that mediate tight junctions between cells. Different Claudin subtypes are expressed in different tissues and are associated with different types of cancer. The expression of Claudin 18.2 in normal tissues is restricted in gastric mucosal cells. Claudin 18.2 is highly expressed in 70% of primary gastric adenocarcinoma and its metastases; it is also expressed in other cancers such as pancreatic cancer, esophageal cancer, and non-small cell lung cancer (NSCLC). The highly specific expression of Claudin 18.2 in tumor tissues makes Claudin 18.2 a very good target for tumor immunotherapy.

SUMMARY ~~OF THE INVENTION~~

The inventor of the present application described an antibody with high specificity and high affinity to Claudin 18.2 in PCT/CN2019/126495, the entire content of which is incorporated herein by reference and becomes a part of the present application. Through massive creative work, the inventor of the present application further invented Claudin 18.2 antibody-drug conjugate (ADC), which provides a safe and effective drug option for the treatment of tumors.

Specifically, the inventors conjugate an antibody with high affinity that specifically binds to Claudin 18.2 to a bioactive molecule through a linker, so as to obtain a class of Claudin 18.2 targeting ADCs and a composition comprising the ADC. The ADC or composition has high killing activity against some tumor cells expressed by Claudin 18.2. In animal experiments (*in vivo*), the ADC can effectively inhibit the growth of Claudin 18.2 positive tumors, especially gastric cancer or gastric adenocarcinoma, and is very safe in use.

In one aspect, the present disclosure provides an antibody-drug conjugate (ADC), the structure of which is shown in formula (I),

$$(D\text{-}L)_{\gamma}\text{-}A$$

formula (I)

wherein, D is a fragment of a bioactive molecule; L is a linker;

γ is an integer from 1 to 10; preferably, γ is an integer from 1 to 8 (for example, 1, 2, 3, 4, 5, 6, 7 or 8);

A is an antibody or antigen-binding fragment thereof that specifically binds to human CLDN18.2, and the antibody or antigen-binding fragment thereof comprises:

(1) the following VH and/or VL, wherein CDRs are defined according to the IMGT numbering system:

(1-1): VH comprising the following 3 CDRs: CDR-H1 having the sequence of SEQ ID No: 1, CDR-H2 having the sequence of SEQ ID No: 2 or 21, and CDR-H3 having the sequence of SEQ ID No: 3; and/or

VL comprising the following 3 CDRs: CDR-L1 having the sequence of SEQ ID No: 4, CDR-L2 having the sequence of SEQ ID No: 5, and CDR-L3 having the sequence of SEQ ID No: 6;

or

(1-2): compared with the VH or VL described in (1-1), at least one CDR contains a mutation, which is the substitution, deletion or addition of one or more amino acids, or any combination thereof (for example, the substitution, deletion or addition of 1, 2 or 3 amino acids, or any combination thereof); the antibody or antigen-binding fragment thereof containing the mutation is still capable of specifically binding to human CLDN 18.2;

or

(2) the following VH and/or VL, wherein CDRs are defined according to the AbM numbering system:

(2-1): VH comprising the following 3 CDRs: CDR-H1 having the sequence of SEQ ID No: 7, CDR-H2 having the sequence of SEQ ID No: 8 or 22, and CDR-H3 having the sequence of SEQ ID No: 9; and/or

VL comprising the following 3 CDRs: CDR-L1 having the sequence of SEQ ID No:10, CDR-L2 having the sequence of SEQ ID No:11, and CDR-L3 having the sequence of SEQ ID No: 12;

or

(2-2): compared with the VH or VL described in (2-1), at least one CDR contains a mutation, which is the substitution, deletion or addition of one or more amino acids, or any combination thereof (for example, the substitution, deletion or addition of 1, 2 or 3 amino acids, or any combination thereof); the antibody or antigen-binding fragment thereof containing the mutation is still capable of specifically binding to human CLDN 18.2;

preferably, the substitution is a conservative substitution;

preferably, the VH and/or VL of the antibody or antigen-binding fragment thereof include the framework regions (FRs) of a human or murine immunoglobulin.

In another aspect, the present disclosure provides a composition, comprising the ADC as described herein, and the molar ratio of the fragment of the bioactive molecule to the antibody or antigen-binding fragment thereof that specifically binds to CLDN18.2 (DAR value) is a decimal or integer from 1 to 10 (for example, a decimal or integer from 3 to 8, such as 1.0, 1.5, 2.0, 2.5, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7 , 3.79, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1 , 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 6.95, 7.0, 7.03, 7.1, 7.12, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9 or 8.0).

In another aspect, the present disclosure provides a pharmaceutical composition, comprising the ADC as described herein, or the composition as described herein, and a pharmaceutically acceptable carrier and/or excipient.

In another aspect, the present disclosure provides use of the ADC as described herein, the composition as described herein, or the pharmaceutical composition as described herein in the preparation of a medicament for the prevention and/or treatment and/or adjuvant treatment of tumors;

optionally, the medicament further includes an additional anti-tumor active ingredient; preferably, the ADC, composition or pharmaceutical composition is administered separately, simultaneously or sequentially with the additional anti-tumor active ingredient; preferably, the additional anti-tumor active ingredient is a bioactive polypeptide or an active fragment thereof, or a chemotherapeutic drug; more preferably, the bioactive polypeptide is selected from immune checkpoint inhibitors (e.g., PD-1 antibody, PD-L1 antibody, CTLA-4 antibody, and LAG-3 antibody), or cytokine (e.g., interferon, IL-2, IL-15, GM-CSF, IL-7, IL-12, IL-18, and IL-21); preferably, the chemotherapeutic drug is one or more selected from epirubicin, oxaliplatin, capecitabine, 5-fluorouracil, leucovorin, paclitaxel, and albumin-bound paclitaxel.

In another aspect, the present disclosure provides the antibody-drug conjugate (ADC) as described herein, the composition as described herein, or the pharmaceutical composition as described herein for use in preventing and/or treating a tumor, and/or delaying tumor progression, and/or reducing or inhibiting tumor recurrence in a subject;

optionally, the antibody-drug conjugate (ADC) as described herein, the composition as described herein, or the pharmaceutical composition as described herein is for use with a second therapy, wherein the second therapy is selected from surgery, chemotherapy, radiotherapy, immunotherapy, gene therapy, DNA therapy, RNA therapy, nanotherapy, viral therapy, adjuvant therapy, and any combination thereof; the antibody-drug conjugate (ADC) as described herein, the composition as described herein, or the pharmaceutical composition as described herein, and the second therapy are to be applied separately, in combination, simultaneously, or sequentially;

preferably, a bioactive polypeptide is to be administered in the immunotherapy, and more preferably, the bioactive polypeptide is selected from immune checkpoint inhibitors (e.g., PD-1 antibody, PD-L1 antibody, CTLA-4 antibody, and LAG-3 antibody), or cytokine (e.g., interferon, IL-2, IL-15, GM-CSF, IL-7, IL-12, IL-18, and IL-21);

preferably, the chemotherapy is one or more selected from epirubicin, oxaliplatin, capecitabine, 5-fluorouracil, folinic acid, paclitaxel, and albumin-bound paclitaxel;

preferably, the tumor is selected from solid tumors, hematological tumors, and the metastatic, refractory or recurrent lesions of cancers;

preferably, the tumor or cancer is selected from esophageal cancer, gastrointestinal cancer, pancreatic cancer, thyroid cancer, colorectal cancer, kidney cancer, lung cancer (such as NSCLC), liver cancer, gastric cancer, gastric adenocarcinoma, gastroesophageal junction (GEJ) adenocarcinoma, head and neck cancer, bladder cancer, breast cancer, uterine cancer, cervical cancer, ovarian cancer, prostate cancer, testicular cancer, germ cell tumor, bone cancer, skin cancer, thymus cancer, cholangiocarcinoma, gallbladder cancer, melanoma, mesothelioma, lymphoma, myeloma (such as multiple myeloma), sarcoma, glioblastoma, and leukemia;

preferably, the tumor is selected from gastric cancer, gastric adenocarcinoma, gastroesophageal junction (GEJ) adenocarcinoma, esophageal cancer, gastrointestinal cancer, pancreatic cancer, lung cancer (such as NSCLC);

preferably, the tumor is gastric cancer, gastric adenocarcinoma or gastroesophageal junction (GEJ) adenocarcinoma, such as a locally advanced unresectable or metastatic tumor of gastric cancer, gastric adenocarcinoma or gastroesophageal junction (GEJ) adenocarcinoma;

preferably, the tumor is CLDN 18.2 positive, and more preferably, the tumor is HER2 negative.

In another aspect, the present disclosure provides use of the antibody-drug conjugate (ADC) as described herein, the composition as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for preventing and/or treating a tumor, and/or delaying tumor progression, and/or reducing or inhibiting tumor recurrence in a subject;

optionally, the medicament is to be applied with a second therapy, wherein the second therapy is selected from surgery, chemotherapy, radiotherapy, immunotherapy, gene therapy, DNA therapy, RNA therapy, nanotherapy, viral therapy, adjuvant therapy, and any combination thereof; the medicament and the second therapy are to be applied separately, in combination, simultaneously, or sequentially;

preferably, a bioactive polypeptide is to be administered in the immunotherapy, and more preferably, the bioactive polypeptide is selected from immune checkpoint inhibitors (e.g., PD-1 antibody, PD-L1 antibody, CTLA-4 antibody, and LAG-3 antibody), or cytokine (e.g., interferon, IL-2, IL-15, GM-CSF, IL-7, IL-12, IL-18, and IL-21);

preferably, the chemotherapy is one or more selected from epirubicin, oxaliplatin, capecitabine, 5-fluorouracil, folinic acid, paclitaxel, and albumin-bound paclitaxel;

preferably, the tumor is selected from solid tumors, hematological tumors, and the metastatic, refractory or recurrent lesions of cancers;

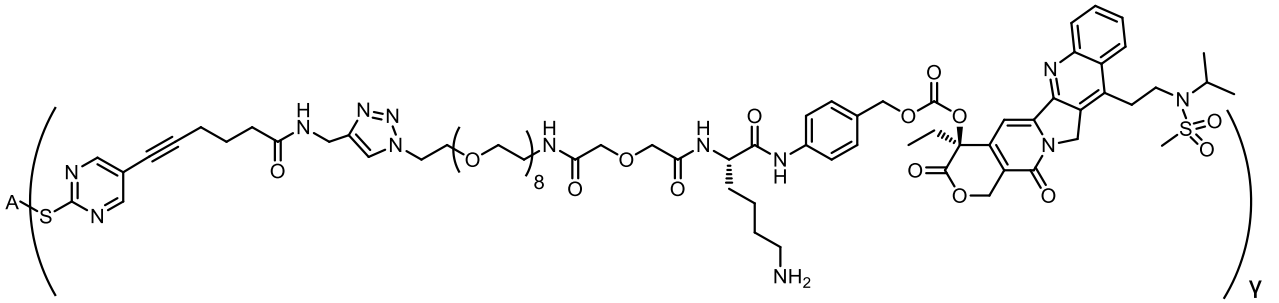
preferably, the tumor or cancer is selected from esophageal cancer, gastrointestinal cancer, pancreatic cancer, thyroid cancer, colorectal cancer, kidney cancer, lung cancer (such as NSCLC), liver cancer, gastric cancer, gastric adenocarcinoma, gastroesophageal junction (GEJ) adenocarcinoma, head and neck cancer, bladder cancer, breast cancer, uterine cancer, cervical cancer, ovarian cancer, prostate cancer, testicular cancer, germ cell tumor, bone cancer, skin cancer, thymus cancer, cholangiocarcinoma, gallbladder cancer, melanoma, mesothelioma, lymphoma, myeloma (such as multiple myeloma), sarcoma, glioblastoma, and leukemia;

preferably, the tumor is selected from gastric cancer, gastric adenocarcinoma, gastroesophageal junction (GEJ) adenocarcinoma, esophageal cancer, gastrointestinal cancer, pancreatic cancer, lung cancer (such as NSCLC);

preferably, the tumor is gastric cancer, gastric adenocarcinoma or gastroesophageal junction (GEJ) adenocarcinoma, such as a locally advanced unresectable or metastatic tumor of gastric cancer, gastric adenocarcinoma or gastroesophageal junction (GEJ) adenocarcinoma;

preferably, the tumor is CLDN 18.2 positive, and more preferably, the tumor is HER2 negative.

In another aspect, the present disclosure provides an antibody-drug conjugate (ADC), wherein the conjugate is



wherein A is an antibody that comprises a VH and a VL; wherein:

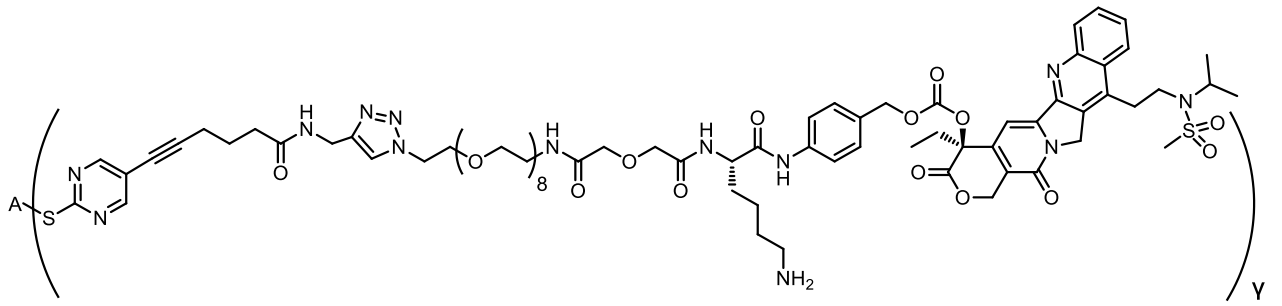
(i) the VH comprises a CDR-H1 having the sequence of SEQ ID No: 1, a CDR-H2 having the sequence of SEQ ID No: 2 or 21, and a CDR-H3 having the sequence of SEQ ID No: 3; and the VL comprises a CDR-L1 having the sequence of SEQ ID No: 4, a CDR-L2 having the sequence of SEQ ID No: 5, and a CDR-L3 having the sequence of SEQ ID No: 6; or

(ii) the VH comprises a CDR-H1 having the sequence of SEQ ID No: 7, a CDR-H2 having the sequence of SEQ ID No: 8 or 22, and a CDR-H3 having the sequence of SEQ ID No: 9; and the VL comprises a CDR-L1 having SEQ ID No:10, a CDR-L2 having the sequence of SEQ ID No:11, and a CDR-L3 having the sequence of SEQ ID No: 12;

and

wherein γ is an integer from 1 to 10.

In another aspect, the present disclosure provides an antibody-drug conjugate (ADC), wherein the conjugate is



wherein A comprises a heavy chain sequence as set forth in SEQ ID NO: 19, and a light chain sequence as set forth in SEQ ID NO: 20, wherein A is linked via one or more thiol group(s) to form the conjugate, and γ is an integer from 1 to 10.

In another aspect, the present disclosure provides a pharmaceutical composition comprising one or more of the conjugate as described herein, and one or more pharmaceutical excipients.

Antibody-drug conjugates (ADCs)

CLAIMS

1. An antibody-drug conjugate (ADC), the structure of which is shown in formula (I),

$$(D\text{-}L)_{\gamma}\text{-}A$$

formula (I)

wherein, D is a fragment of a bioactive molecule; L is a linker;

γ is an integer from 1 to 10; preferably, γ is an integer from 1 to 8 (for example, 1, 2, 3, 4, 5, 6, 7 or 8);

A is an antibody or antigen-binding fragment thereof that specifically binds to human CLDN18.2, and the antibody or antigen-binding fragment thereof comprises:

(1) the following VH and/or VL, wherein CDRs are defined according to the IMGT numbering system:

(1-1): VH comprising the following 3 CDRs: CDR-H1 having the sequence of SEQ ID No: 1, CDR-H2 having the sequence of SEQ ID No: 2 or 21, and CDR-H3 having the sequence of SEQ ID No: 3; and/or

VL comprising the following 3 CDRs: CDR-L1 having the sequence of SEQ ID No: 4, CDR-L2 having the sequence of SEQ ID No: 5, and CDR-L3 having the sequence of SEQ ID No: 6;

or

(1-2): compared with the VH or VL described in (1-1), at least one CDR contains a mutation, which is the substitution, deletion or addition of one or more amino acids, or any combination thereof (for example, the substitution, deletion or addition of 1, 2 or 3 amino acids, or any combination thereof); the antibody or antigen-binding fragment thereof containing the mutation is still capable of specifically binding to human CLDN 18.2;

or

(2) the following VH and/or VL, wherein CDRs are defined according to the AbM numbering system:

(2-1): VH comprising the following 3 CDRs: CDR-H1 having the sequence of SEQ ID No: 7, CDR-H2 having the sequence of SEQ ID No: 8 or 22, and CDR-H3 having the sequence of SEQ ID No: 9; and/or

VL comprising the following 3 CDRs: CDR-L1 having the sequence of SEQ ID No:10, CDR-L2 having the sequence of SEQ ID No:11, and CDR-L3 having the sequence of SEQ ID No: 12;

or

(2-2): compared with the VH or VL described in (2-1), at least one CDR contains a mutation, which is the substitution, deletion or addition of one or more amino acids, or any combination thereof (for example, the substitution, deletion or addition of 1, 2 or 3 amino acids, or any combination thereof); the antibody or antigen-binding fragment thereof containing the mutation

is still capable of specifically binding to human CLDN 18.2;

preferably, the substitution is a conservative substitution;

preferably, the VH and/or VL of the antibody or antigen-binding fragment thereof include the framework regions (FRs) of a human or murine immunoglobulin.

2. The ADC according to claim 1, wherein:

(1) the antibody or antigen-binding fragment thereof comprises the VH as set forth in SEQ ID NO: 13 or 14; and/or the VL as set forth in SEQ ID NO: 15;

(2) compared with the VH described in (1), the VH comprised in the antibody or antigen-binding fragment thereof has at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity; and/or, compared with the VL described in (1), the VL compirsed in the antibody or antigen-binding fragment thereof has at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity;

or

(3) compared with the VH described in (1), the VH comprised in the antibody or antigen-binding fragment thereof has the substitution, deletion or addition of one or more amino acids, or any combination thereof (for example, the substitution, deletion or addition of 1, 2, 3, 4 or 5 amino acids, or any combination thereof); and/or, compared with the VL described in (1), the VL comprised in the antibody or antigen-binding fragment thereof has the substitution, deletion or addition of one or more amino acids, or any combination thereof (for example, the substitution, deletion or addition of 1, 2, 3, 4 or 5 amino acids, or any combination thereof); preferably, the substitution is a conservative substitution.

3. The ADC according to claim 1 or 2, wherein the antibody comprises:

(1) the CH (heavy chain constant region) of human immunoglobulin or a variant thereof, wherein the variant comprises a substitution, deletion or addition of one or more amino acids compared with the wild type sequence from which it is derived (for example, the substitution, deletion or addition of up to 20, up to 15, up to 10, or up to 5 amino acids; for example, the substitution, deletion or addition of 1, 2, 3, 4, or 5 amino acids); and/or

(2) the CL (light chain constant region) of human immunoglobulin or a variant thereof, wherein the variant comprises a substitution, deletion or addition of one or more amino acids compared with the wild type sequence from which it is derived (for example, the substitution, deletion or addition of up to 20, up to 15, up to 10, or up to 5 amino acids; for example, the substitution, deletion or addition of 1, 2, 3, 4 or 5 amino acids);

preferably, the CH is an IgG heavy chain constant region, such as an IgG1, IgG2, IgG3 or IgG4 heavy chain constant region;

preferably, the antibody comprises the heavy chain constant region of human IgG1;

preferably, the antibody comprises the CH as set forth in SEQ ID NO: 16, or a variant thereof which has a conservative substitution of up to 20 amino acids compared with SEQ ID NO: 16 (for example, a conservative substitution of up to 15, 10 or 5 amino acids; for example,

a conservative substitution of 1, 2, 3, 4, or 5 amino acids);

preferably, the CL is a constant region of light chain κ;

preferably, the antibody comprises the CL as set forth in SEQ ID NO: 17, or a variant thereof which has a conservative substitution of up to 20 amino acids compared with SEQ ID NO: 17 (for example, a conservative substitution of up to 15, 10 or 5 amino acids; for example, a conservative substitution of 1, 2, 3, 4, or 5 amino acids);

more preferably, the antibody comprises a CH as set forth in SEQ ID NO: 16 and/or a CL as set forth in SEQ ID NO: 17.

4. The ADC according to any one of claims 1to 3, wherein the antibody is:

(1) the heavy chain comprising the VH of the sequence as set forth in SEQ ID NO: 13 and the CH of the sequence as set forth in SEQ ID NO: 16, and the light chain comprising the VL of the sequence as set forth in SEQ ID NO: 15 and the CL of the sequence as set forth in SEQ ID NO: 17;

or

(2) the heavy chain comprising the VH of the sequence as set forth in SEQ ID NO: 14 and the CH of the sequence as set forth in SEQ ID NO: 16, and the light chain comprising the VL of the sequence as set forth in SEQ ID NO: 15 and the CL of the sequence as set forth in SEQ ID NO: 17.

5. The ADC according to any one of claims 1 to 4, wherein the antibody or antigen-binding fragment thereof comprises:

(1) a heavy chain, comprising an amino acid sequence selected from the group consisting of:

(1-1) the sequence as set forth in SEQ ID NO: 18 or SEQ ID NO: 19;

(1-2) a sequence having the substitution, deletion or addition of one or more amino acids (e.g., the substitution, deletion or addition of 1, 2, 3, 4 or 5 amino acids) compared to the sequence as set forth in SEQ ID NO: 18 or SEQ ID NO: 19; or

(1-3) a sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the sequence as set forth in SEQ ID NO: 18 or SEQ ID NO: 19;

and

(2) a light chain, comprising an amino acid sequence selected from the group consisting of:

(2-1) the sequence as set forth in SEQ ID NO:20;

(2-2) a sequence having the substitution, deletion or addition of one or more amino acids (e.g., the substitution, deletion or addition of 1, 2, 3, 4 or 5 amino acids) compared to the sequence as set forth in SEQ ID NO: 20; or

(2-3) a sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%

identity to the sequence as set forth in SEQ ID NO: 20;

preferably, the substitutions described in (1-2) and (2-2) are conservative substitutions.

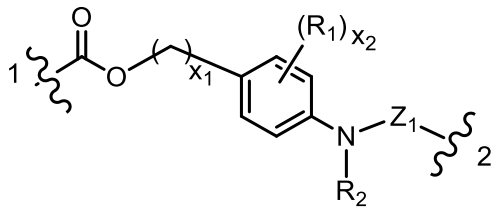
6. The ADC according to any one of claims 1 to 5, wherein the antibody or antigen-binding fragment thereof is selected from ScFv, Fab, Fab', $(Fab')_2$, Fv fragment, disulfide bond-linked Fv(dsFv), diabody, bispecific antibody, and multispecific antibody.

7. The ADC according to any one of claims 1 to 6, which has a structure as shown in formula (II),

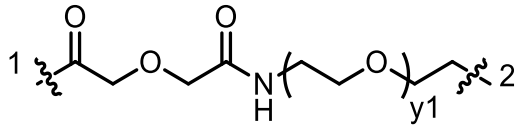
$$\{D-[L_1-(L_2)_{m1}-(L_3)_{m2}-(L_4)_{m3}-E]\}_{\gamma}-A$$

Formula (II)

wherein,

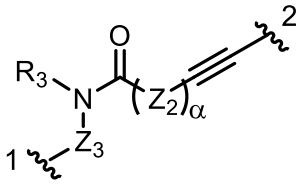


L_1 is , wherein R_1 and R_2 each independently are hydrogen (such as protium or deuterium), halogen, carboxylic acid group, sulfonic acid group, cyano, C_{1-6} alkyl, halogenated C_{1-6} alkyl, cyano- substituted C_{1-6} alkyl (e.g., $-CH_2CN$), C_{1-6} alkoxy, C_{2-10} alkenyl or C_{2-10} alkynyl; Z_1 is an amino acid or a peptide composed of 2-10 amino acids; x_1 and x_2 each independently are 0, 1, 2, 3, 4, 5 or 6; L_1 is connected to D at position 1, and connected to L_2 at position 2;

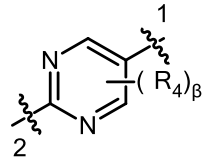


L_2 is , wherein y_1 is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10; L_2 is connected to L_1 at position 1, and connected to L_3 at position 2;

L_3 is selected from a 5-12 membered aromatic heterocycle;



L_4 is , wherein Z_2 is selected from C_{1-6} alkylene, C_{2-10} alkenylene, C_{2-10} alkynylene, and C_{3-8} cycloalkylene; R_3 is selected from hydrogen (such as protium or deuterium) and C_{1-6} alkyl; Z_3 does not exist or is selected from C_{1-6} alkylene; alternatively, R_3 and Z_3 together with the nitrogen atom to which they are attached form a 4-8 membered heterocyclyl; α is 0,1,2,3,4,5 or 6, and L_4 is connected to E at position 2 ,and connected to L_3 at position 1;



E is , wherein each R_4 is hydrogen (such as protium or deuterium), β is 0, 1, 2, 3, 4, 5 or 6, and E is connected to A at position 2, and connected to L_4 at position 1;

m_1, m_2 and m_3 are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

A is as defined in any one of claims 1 to 6;

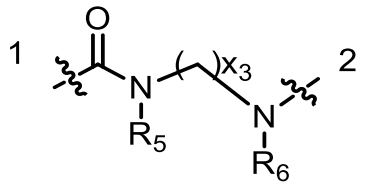
D and γ are as defined in claim 1.

8. The ADC according to any one of claims 1 to 6, which has a structure as shown in formula (III):

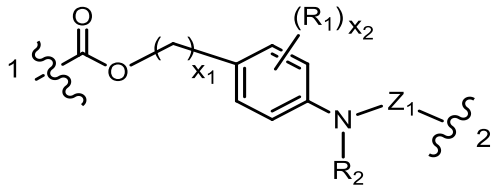
$$\{D\text{-}[(L_1')_{m4}\text{-}L_1\text{-}(L_5)_{m5}\text{-}(L_3)_{m2}\text{-}(L_4)_{m3}\text{-}E]\}_{\gamma}\text{-}A$$

Formula (III)

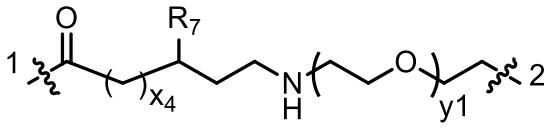
wherein



L_1' is , wherein R_5 and R_6 each independently are hydrogen (such as protium or deuterium) or C_{1-6} alkyl; x_3 is 1, 2, 3, 4, 5 or 6; and if L_1' is present, it connects with D at position 1 and connects with L_1 at position 2;

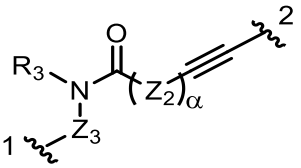


L_1 is , wherein R_1 and R_2 each independently are hydrogen (such as protium or deuterium), halogen, carboxylic acid group, sulfonic acid group, cyano, C_{1-6} alkyl, halogenated C_{1-6} alkyl, cyano- substituted C_{1-6} alkyl (e.g., $-CH_2CN$), C_{1-6} alkoxy, C_{2-10} alkenyl or C_{2-10} alkynyl; Z_1 is an amino acid or a peptide composed of 2-10 amino acids; x_1 and x_2 each independently are 0, 1 , 2, 3, 4, 5 or 6; and, at position 1, L_1 is connected to L_1' (when L_1' exists) at position 1, or to D (when L_1' does not exist) at position 1; L_1 is connected to L_5 at position 2;



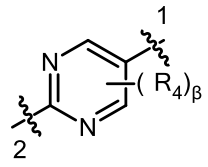
L_5 is , wherein R_7 is hydrogen or C_{1-6} alkyl, or R_7 is connected to the N atom on the γ-C thereof to form a 5-6 membered heterocyclyl; x_4 is 1, 2, 3, 4, 5 or 6; y_1 is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10; and L_5 is connected to L_1 at position 1, and connected to L_3 at position 2;

L_3 is selected from a 5-12 membered aromatic heterocycle;



L_4 is , wherein Z_2 is selected from C_{1-6} alkylene, C_{2-10} alkenylene, C_{2-10} alkynylene and C_{3-8} cycloalkylene; R_3 is selected from hydrogen (such as protium and deuterium) and C_{1-6} alkyl; Z_3 does not exist or is selected from C_{1-6} alkylene; or, R_3 and Z_3 together with the

nitrogen atom to which they are attached form a 4-8 membered heterocyclic radical; α is 0, 1, 2, 3, 4, 5 or 6; and L_4 is connected to E at position 2, and connected to L_3 at position 1;

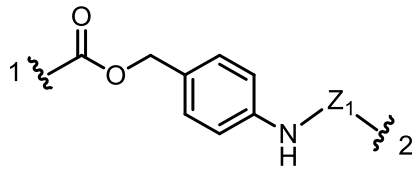
E is , wherein each R_4 independently is hydrogen (such as protium or deuterium), β is 0, 1, 2, 3, 4, 5, or 6, and E is connected to A at position 2, and connected to L_4 at position 1;

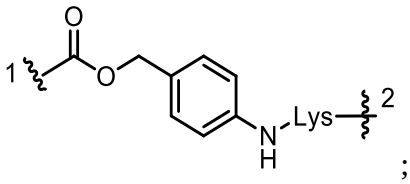
m_1, m_2, m_3 and m_4 each independently are 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

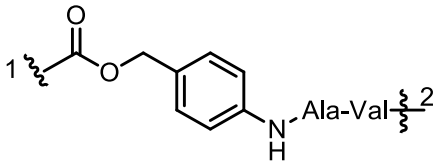
A is as defined in any one of claims 1 to 6;

D and γ are as defined in claim 1.

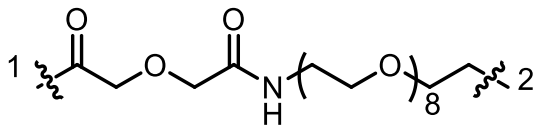
9. The ADC according to any one of claims 7 to 8, wherein L_1 is

, wherein Z_1 is an amino acid or a peptide composed of 2-5 amino acids, wherein the amino acid is selected from Lys, Cit, Val, D-Val, Phe, Leu, Gly, Ala, and Asn; preferably, Z_1 is selected from Cit , Lys, Cit-Val, and Ala-Val;

preferably, L_1 is ;

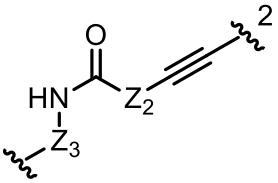
preferably, L_1 is .

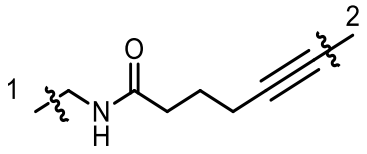
10. The ADC according to any one of claims 7 to 9, wherein L_2 is

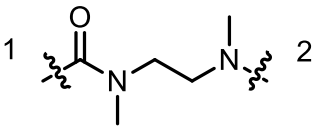
, and m_1 is 1.

11. The ADC according to any one of claims 7 to 10, wherein L_3 is a 5-6 membered aromatic heterocycle, and m_2 is 1;

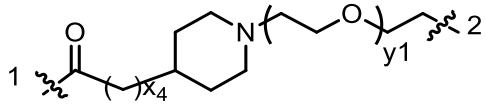
preferably, L_3 is triazole, and m_2 is 1.

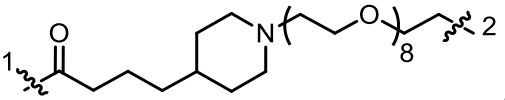
12. The ADC according to any one of claims 7 to 11, wherein L_4 is , Z_2 is C_{1-6} alkylene, Z_3 is C_{1-6} alkylene; and m_3 is 1;

preferably, L_4 is , and m_3 is 1.

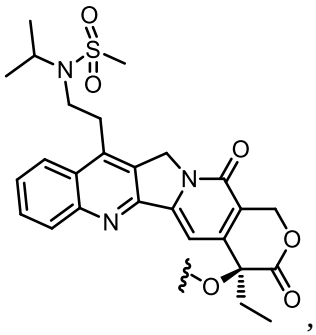
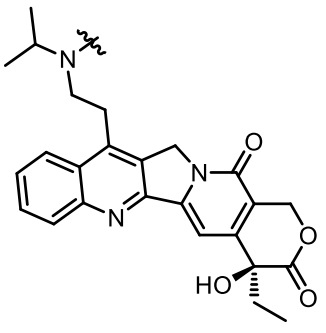
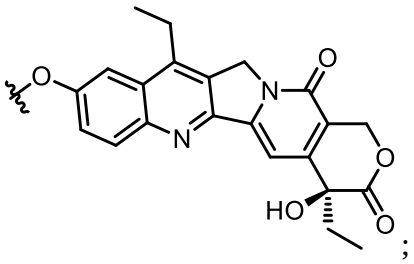
13. The ADC according to any one of claims 8 to 12, wherein L_1' is .

14. The ADC according to any one of claims 8 to 13, wherein L_5 is

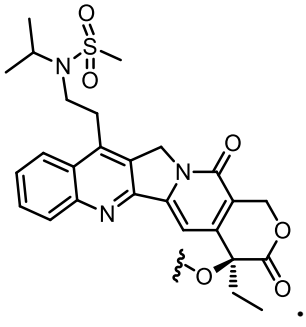
, wherein x_4 is 1, 2, 3, 4, 5 or 6; y_1 is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

preferably, L_5 is .

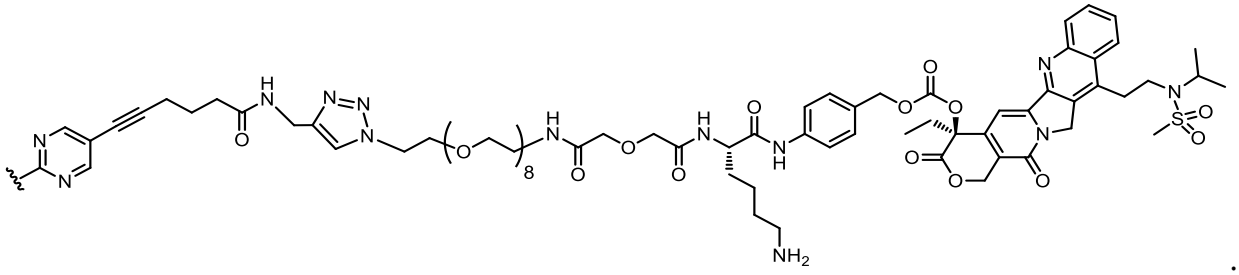
15. The ADC according to any one of claims 1 to 14, wherein D is

,  or ;

preferably, D is

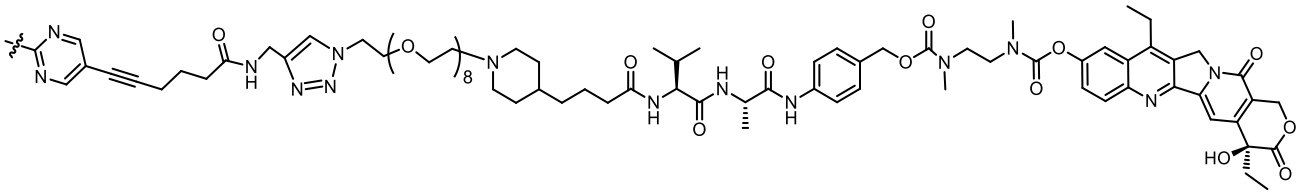
.

16. The ADC according to claim 7, wherein the D-[L_1-$(L_2)_{m1}$-$(L_3)_{m2}$-$(L_4)_{m3}$-E]- in formula (II) is

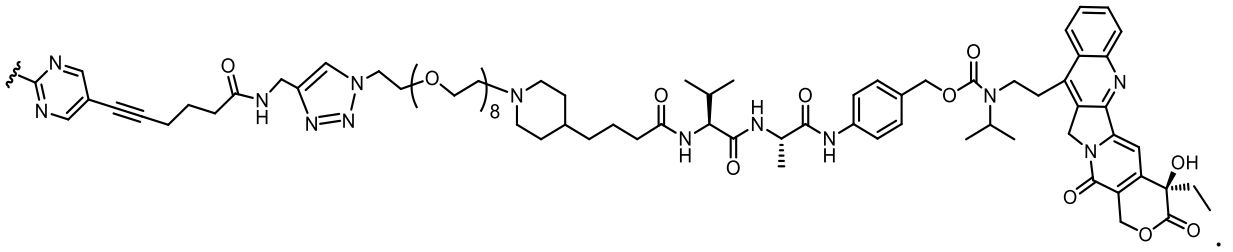


.

17. The ADC according to claim 8, wherein the D-[$(L_1')_{m4}$-L_1-$(L_5)_{m5}$-$(L_3)_{m2}$-$(L_4)_{m3}$-E]- in formula (III) is

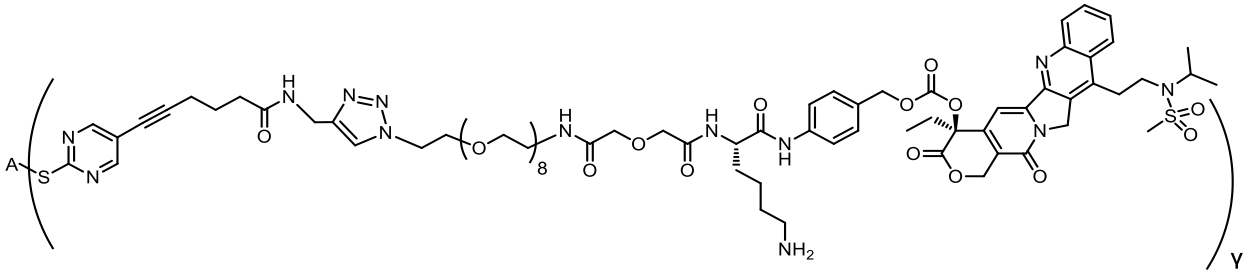


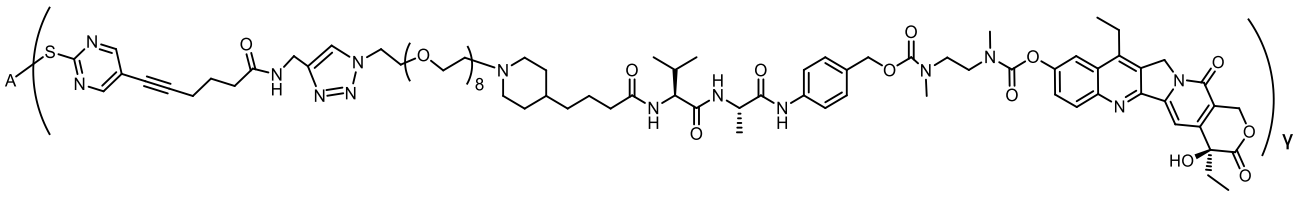
or

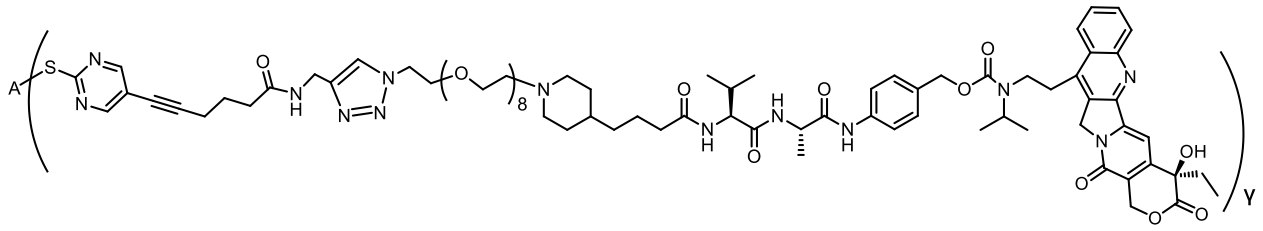


.

18. The ADC according to any one of claims 1 to 17, which is selected from:







wherein γ is an integer from 1 to 10; for example, γ is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

19. The ADC according to any one of claims 1 to 18, wherein the antibody or antigen-binding fragment thereof has a label; preferably, the antibody or antigen-binding fragment thereof has a detectable label, such as enzymes (i.e., horseradish peroxidase), radionuclides, fluorescent dyes, luminescent substances (e.g., chemiluminescent substances) or biotin.

20. A composition, comprising the ADC according to any one of claims 1 to 19, and the molar ratio of the fragment of the bioactive molecule to the antibody or antigen-binding fragment thereof that specifically binds to CLDN18.2 (DAR value) is a decimal or integer from 1 to 10 (for example, a decimal or integer from 3 to 8, such as 1.0, 1.5, 2.0, 2.5, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7 , 3.79, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1 , 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 6.95, 7.0, 7.03, 7.1, 7.12, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9 or 8.0).

21. A pharmaceutical composition, comprising the ADC according to any one of claims 1 to 19, or the composition according to claim 20, and a pharmaceutically acceptable carrier and/or excipient.

22. Use of the ADC according to any one of claims 1 to 19, the composition according to claim 20, or the pharmaceutical composition according to claim 21 in the preparation of a medicament for the prevention and/or treatment and/or adjuvant treatment of tumors;

optionally, the medicament further includes an additional anti-tumor active ingredient; preferably, the ADC, composition or pharmaceutical composition is administered separately, simultaneously or sequentially with the additional anti-tumor active ingredient; preferably, the additional anti-tumor active ingredient is a bioactive polypeptide or an active fragment thereof, or a chemotherapeutic drug; more preferably, the bioactive polypeptide is selected from immune checkpoint inhibitors (e.g., PD-1 antibody, PD-L1 antibody, CTLA-4 antibody, and LAG-3 antibody), or cytokine (e.g., interferon, IL-2, IL-15, GM-CSF, IL-7, IL-12, IL-18, and IL-21); preferably, the chemotherapeutic drug is one or more selected from epirubicin, oxaliplatin, capecitabine, 5-fluorouracil, leucovorin, paclitaxel, and albumin-bound paclitaxel.

23. Use according to claim 22, wherein the tumor is selected from solid tumors, hematological tumors, or metastatic, refractory or recurrent lesions of cancers;

preferably, the tumor or cancer is selected from esophageal cancer, gastrointestinal cancer, pancreatic cancer, thyroid cancer, colorectal cancer, kidney cancer, lung cancer (such as non-small cell lung cancer, NSCLC), liver cancer, gastric cancer, gastric adenocarcinoma, gastroesophageal junction (GEJ) adenocarcinoma, head and neck cancer, bladder cancer, breast cancer, uterine cancer, cervical cancer, ovarian cancer, prostate cancer, testicular cancer, germ cell tumor, bone cancer, skin cancer, thymus cancer, cholangiocarcinoma, gallbladder cancer, melanoma, mesothelioma, lymphoma, myeloma (such as multiple myeloma), sarcoma, glioblastoma, and leukemia;

preferably, the tumor is selected from gastric cancer, gastric adenocarcinoma, gastroesophageal

junction (GEJ) adenocarcinoma, esophageal cancer, gastrointestinal cancer, pancreatic cancer, and lung cancer (such as NSCLC);

preferably, the tumor is gastric cancer, gastric adenocarcinoma or gastroesophageal junction (GEJ) adenocarcinoma, such as a locally advanced unresectable or metastatic tumor of gastric cancer, gastric adenocarcinoma or gastroesophageal junction (GEJ) adenocarcinoma;

preferably, the tumor is CLDN 18.2 positive, and more preferably, the tumor is HER2 negative.

24. ~~A method of~~The antibody-drug conjugate (ADC) according to any one of claims 1 to 19, the composition according to claim 20, or the pharmaceutical composition according to claim 21 for use in preventing and/or treating a tumor, and/or delaying tumor progression, and/or reducing or inhibiting tumor recurrence in a subject~~, wherein the method comprises a step of administering to a subject in need thereof an effective amount of the antibody-drug conjugate (ADC) according to any one of claims 1 to 19, the composition according to claim 20, or the pharmaceutical composition according to claim 21~~;

optionally, the antibody-drug conjugate (ADC) according to any one of claims 1 to 19, the composition according to claim 20, or the pharmaceutical composition according to claim 21 is for use with a second therapy~~method further comprises a step of applying a second therapy to the subject~~, wherein the second therapy is selected from surgery, chemotherapy, radiotherapy, immunotherapy, gene therapy, DNA therapy, RNA therapy, nanotherapy, viral therapy, adjuvant therapy, and any combination thereof; the antibody-drug conjugate (ADC) according to any one of claims 1 to 19, the composition according to claim 20, or the pharmaceutical composition according to claim 21,~~method~~ and the second therapy are to be applied separately, in combination, simultaneously, or sequentially;

preferably, ~~the immunotherapy includes the administration of~~ a bioactive polypeptide is to be administered in the immunotherapy, and more preferably, the bioactive polypeptide is selected from immune checkpoint inhibitors (e.g., PD-1 antibody, PD-L1 antibody, CTLA-4 antibody, and LAG-3 antibody), or cytokine (e.g., interferon, IL-2, IL-15, GM-CSF, IL-7, IL-12, IL-18, and IL-21);

preferably, the chemotherapy is one or more selected from epirubicin, oxaliplatin, capecitabine, 5-fluorouracil, folinic acid, paclitaxel, and albumin-bound paclitaxel;

preferably, the tumor is selected from solid tumors, hematological tumors, and the metastatic, refractory or recurrent lesions of cancers;

preferably, the tumor or cancer is selected from esophageal cancer, gastrointestinal cancer, pancreatic cancer, thyroid cancer, colorectal cancer, kidney cancer, lung cancer (such as NSCLC), liver cancer, gastric cancer, gastric adenocarcinoma, gastroesophageal junction (GEJ) adenocarcinoma, head and neck cancer, bladder cancer, breast cancer, uterine cancer, cervical cancer, ovarian cancer, prostate cancer, testicular cancer, germ cell tumor, bone cancer, skin cancer, thymus cancer, cholangiocarcinoma, gallbladder cancer, melanoma, mesothelioma, lymphoma, myeloma (such as multiple myeloma), sarcoma, glioblastoma, and leukemia;

preferably, the tumor is selected from gastric cancer, gastric adenocarcinoma, gastroesophageal junction (GEJ) adenocarcinoma, esophageal cancer, gastrointestinal cancer, pancreatic cancer, lung cancer (such as NSCLC);

preferably, the tumor is gastric cancer, gastric adenocarcinoma or gastroesophageal junction (GEJ) adenocarcinoma, such as a locally advanced unresectable or metastatic tumor of gastric cancer, gastric adenocarcinoma or gastroesophageal junction (GEJ) adenocarcinoma;

preferably, the tumor is CLDN 18.2 positive, and more preferably, the tumor is HER2 negative.

25. Use of the antibody-drug conjugate (ADC) according to any one of claims 1 to 19, the composition according to claim 20, or the pharmaceutical composition according to claim 21 in the manufacture of a medicament for preventing and/or treating a tumor, and/or delaying tumor progression, and/or reducing or inhibiting tumor recurrence in a subject;

optionally, the medicament is to be applied with a second therapy, wherein the second therapy is selected from surgery, chemotherapy, radiotherapy, immunotherapy, gene therapy, DNA therapy, RNA therapy, nanotherapy, viral therapy, adjuvant therapy, and any combination thereof; the medicament and the second therapy are to be applied separately, in combination, simultaneously, or sequentially;

preferably, a bioactive polypeptide is to be administered in the immunotherapy, and more preferably, the bioactive polypeptide is selected from immune checkpoint inhibitors (e.g., PD-1 antibody, PD-L1 antibody, CTLA-4 antibody, and LAG-3 antibody), or cytokine (e.g., interferon, IL-2, IL-15, GM-CSF, IL-7, IL-12, IL-18, and IL-21);

preferably, the chemotherapy is one or more selected from epirubicin, oxaliplatin, capecitabine, 5-fluorouracil, folinic acid, paclitaxel, and albumin-bound paclitaxel;

preferably, the tumor is selected from solid tumors, hematological tumors, and the metastatic, refractory or recurrent lesions of cancers;

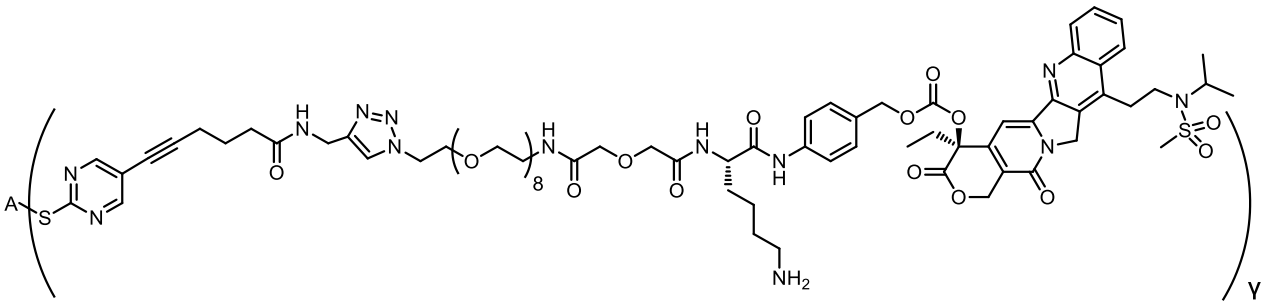
preferably, the tumor or cancer is selected from esophageal cancer, gastrointestinal cancer, pancreatic cancer, thyroid cancer, colorectal cancer, kidney cancer, lung cancer (such as NSCLC), liver cancer, gastric cancer, gastric adenocarcinoma, gastroesophageal junction (GEJ) adenocarcinoma, head and neck cancer, bladder cancer, breast cancer, uterine cancer, cervical cancer, ovarian cancer, prostate cancer, testicular cancer, germ cell tumor, bone cancer, skin cancer, thymus cancer, cholangiocarcinoma, gallbladder cancer, melanoma, mesothelioma, lymphoma, myeloma (such as multiple myeloma), sarcoma, glioblastoma, and leukemia;

preferably, the tumor is selected from gastric cancer, gastric adenocarcinoma, gastroesophageal junction (GEJ) adenocarcinoma, esophageal cancer, gastrointestinal cancer, pancreatic cancer, lung cancer (such as NSCLC);

preferably, the tumor is gastric cancer, gastric adenocarcinoma or gastroesophageal junction (GEJ) adenocarcinoma, such as a locally advanced unresectable or metastatic tumor of gastric cancer, gastric adenocarcinoma or gastroesophageal junction (GEJ) adenocarcinoma;

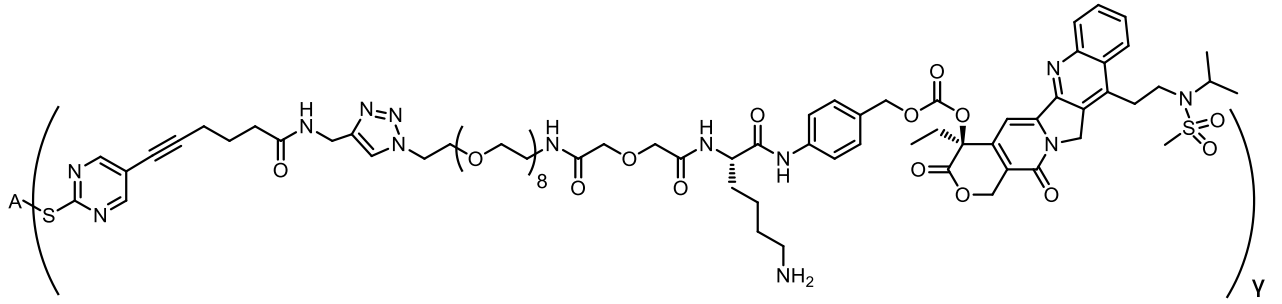
preferably, the tumor is CLDN 18.2 positive, and more preferably, the tumor is HER2 negative.

26. The ADC according to claim 1, wherein the conjugate is



wherein γ is an integer from 1 to 10, and A is an antibody that specifically binds to human CLDN18.2.

27. An antibody-drug conjugate (ADC), wherein the conjugate is



wherein A is an antibody that comprises a VH and a VL; wherein:

(i) the VH comprises a CDR-H1 having the sequence of SEQ ID No: 1, a CDR-H2 having the sequence of SEQ ID No: 2 or 21, and a CDR-H3 having the sequence of SEQ ID No: 3; and the VL comprises a CDR-L1 having the sequence of SEQ ID No: 4, a CDR-L2 having the sequence of SEQ ID No: 5, and a CDR-L3 having the sequence of SEQ ID No: 6; or

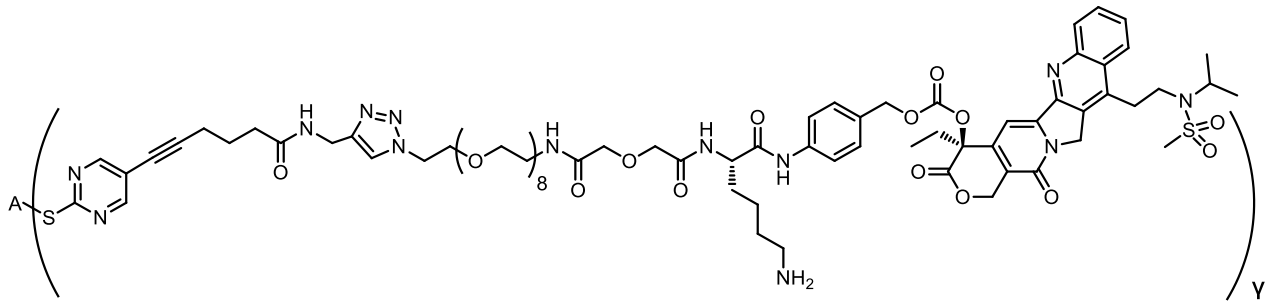
(ii) the VH comprises a CDR-H1 having the sequence of SEQ ID No: 7, a CDR-H2 having the sequence of SEQ ID No: 8 or 22, and a CDR-H3 having the sequence of SEQ ID No: 9; and the VL comprises a CDR-L1 having SEQ ID No:10, a CDR-L2 having the sequence of SEQ ID No:11, and a CDR-L3 having the sequence of SEQ ID No: 12;

and

wherein γ is an integer from 1 to 10.

28. The ADC according to claim 26, wherein A comprises a VH having the sequence of SEQ ID NO: 14; and a VL having the sequence of SEQ ID NO: 15, wherein γ is an integer from 1 to 10.

29. An antibody-drug conjugate (ADC), wherein the conjugate is



wherein A comprises a heavy chain sequence as set forth in SEQ ID NO: 19, and a light chain sequence as set forth in SEQ ID NO: 20, wherein A is linked via one or more thiol group(s) to form the conjugate, and γ is an integer from 1 to 10.

30. A pharmaceutical composition comprising one or more of the conjugate of any one of claims 27-29, and one or more pharmaceutical excipients.

31. The pharmaceutical composition of claim 30, which has a DAR value of from 3 to 8.

32. The pharmaceutical composition of claim 30, which has a DAR value of about 3.8.

33. The pharmaceutical composition of claim 30, which has a DAR value of about 7.1.

34. The pharmaceutical composition of claim 30, which has a DAR value of about 7.4.