Anti-FGFR2b antibody, antibody drug conjugate thereof and uses thereof

Technical Field

The present invention relates to antibodies specific binding fibroblast growth factor receptor 2b (FGFR2b), antibody-drug conjugates, and methods of use thereof, and compositions containing the same.

Background

The Fibroblast Growth Factor (FGF) Receptor Tyrosine Kinase (RTK) family consists of Fibroblast Growth Factor Receptor 1(FGFR1), FGFR2, FGFR3, and FGFR4, and encompasses high affinity receptors for up to 18 different FGF ligands. The receptor is a transmembrane tyrosine kinase, and FGFR signaling drives downstream pathways, including Mitogen-Activated Protein Kinase (MAPK) and AKT pathways critical to cell proliferation, differentiation, survival, and migration. Binding of FGF ligands to the receptor induces dimerization of the FGF: FGFR complex. Dimerization leads to kinase activation and autophosphorylation of multiple tyrosine residues in the cytoplasmic domain of the receptor, as well as activation of downstream signaling of the phosphoinositide 3-kinase (PI3K) -AKT and MAPK extracellular signal-regulated kinase (ERK) pathways. Alternative splicing of the IgIII loop in FGFR1-3 results in FGFRIIIb or FGFRIIIc isotype.

FGFR2 amplification has been reported in various cancers. FGFR2 amplification affects signaling without altering the endogenous kinase activity of the receptor. FGFR2 protein is overexpressed in about 3% of breast cancers, including triple negative breast cancers, and about 10% of gastric/esophageal cancers. FGFR2 overexpression has also been found in other cancers, including colon cancer, hepatocellular carcinoma, pancreatic cancer, ovarian cancer, uterine cancer, cervical cancer, endometrial cancer, bladder cancer, lung cancer, colon cancer, glioma cancer, and head and neck cancer. In addition, mutations in the FGFR2 gene have been reported in about 12% of endometrial cancers. FGFR2 overexpression has been associated with poor survival in gastric cancer patients.

Both preclinical and clinical studies demonstrate that FGFR-amplified tumors are sensitive to FGFR inhibition and thus sensitive to targeted therapy. It is reported that treatment of FGFR2 positive tumors with anti-FGFR2 therapy such as Bemarituzumab (FPA144) results in improvement in early survival and late-stage disease. “FPA144:A Therapeutic Antibody for Treating Patients with Gastric Cancers Bearing FGFR2 Gene Amplification” (*American cancer research institute (AACR)* Abstract ID 5446); Powers *et al.,* (2016) “FPA144, A Therapeutic Monoclonal Antibody Targeting the FGFR2b Receptor, Promotes Antibody Dependent Cell-Mediated Cytotoxicity and Stimulates Sensitivity to PD-1in the 4T1 Syngeneic Tumor Model” (*American cancer research institute* Abstract ID 1407); *Lee et al.,* (2016) “Antitumor Activity and Safety of FPA144,an ADCC-Enhanced, FGFR2b Isoform-Selective Monoclonal Antibody, in Patients with FGFR2b+Gastric Cancer and Advanced Solid Tumors” (*American Society of Clinical Oncology (ASCO)* Abstract ID 2502).

There remains an unmet medical need for improved anti-cancer drugs that are effective in FGFR2-expressing cancers.

Disclosure of Invention

The invention provides a novel anti-FGFR2b antibody or an antigen binding fragment thereof and an antibody drug conjugate, which have the advantages of high affinity and high specificity for human FGFR2b. The anti-FGFR2b antibodies or antigen-binding fragments thereof and anti-FGFR2b antibody drug conjugates provided herein can be used as an independent therapy or in combination with other therapies and/or other anti-cancer agents, for example, in the treatment of cancer/tumors.

In a first aspect, the invention relates to an anti-FGFR2b antibody or antigen binding fragment thereof capable of specifically binding FGFR2b.

In some embodiments, the invention provides an anti-FGFR2b antibody or antigen-binding fragment thereof that specifically binds FGFR2b, comprising:

(1) the 3 CDRs (HCDR1, HCDR2, HCDR3) comprised in the VH as shown in SEQ ID NO:7 or variants thereof; and/or the 3 CDRs (LCDR1, LCDR2, LCDR3) comprised in the VL as shown in SEQ ID NO:8 or variants thereof; or,

(2) the 3 CDRs (HCDR1, HCDR2, HCDR3) comprised in the VH as shown in SEQ ID NO:12 or variants thereof; and/or the 3 CDRs (LCDR1, LCDR2, LCDR3) comprised in the VL as shown in SEQ ID NO:13 or variants thereof.

In some embodiments, the invention provides an anti-FGFR2b antibody or antigen-binding fragment thereof that specifically binds FGFR2b, comprising:

1) HCDR1, comprising or consisting of the sequence as shown in SEQ ID NO:1;
HCDR2, comprising or consisting of the sequence as shown in SEQ ID NO:2;
HCDR3, comprising or consisting of the sequence as shown in SEQ ID NO:3;
LCDR1, comprising or consisting of the sequence as shown in SEQ ID NO:4;
LCDR2, comprising or consisting of the sequence as shown in SEQ ID NO:5; and
LCDR3, comprising or consisting of the sequence as shown in SEQ ID NO 6; or
2) HCDR1, comprising or consisting of the sequence as shown in SEQ ID NO:1;
HCDR2, comprising or consisting of the sequence as shown in SEQ ID NO:2;
HCDR3, comprising or consisting of the sequence as shown in SEQ ID NO:11;
LCDR1, comprising or consisting of the sequence as shown in SEQ ID NO:4;
LCDR2, comprising or consisting of the sequence as shown in SEQ ID NO:5; and
LCDR3, comprising or consisting of the sequence as shown in SEQ ID NO 6.

In some embodiments, the present invention provides anti-FGFR2b antibodies and antigen-binding fragments thereof that specifically bind FGFR2b, comprising a heavy chain variable region (VH), wherein:

(1) the heavy chain variable region comprises the amino acid sequence as shown in SEQ ID NO:7, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:7, or consists of SEQ ID NO:7; or

(2) the heavy chain variable region comprises the amino acid sequence as shown in SEQ ID NO:12, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:12, or consists of SEQ ID NO:12.

In some embodiments, the present invention provides anti-FGFR2B antibodies and antigen-binding fragments thereof that specifically bind FGFR2B, comprising a light chain variable region (VL), wherein:

(1) the light chain variable region comprises the amino acid sequence as shown in SEQ ID NO:8, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:8, or consists of SEQ ID NO:8; or

(2) the light chain variable region comprises the amino acid sequence as shown in SEQ ID NO:13, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:13, or consists of SEQ ID NO:13.

In other embodiments, the invention provides anti-FGFR2b antibodies and antigen-binding fragments thereof that specifically bind FGFR2b, comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein:

(1) the heavy chain variable region comprises the amino acid sequence as shown in SEQ ID NO:7, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:7, or consists of SEQ ID NO:7; the light chain variable region comprises the amino acid sequence as shown in SEQ ID NO:8, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:8, or consists of SEQ ID NO:8; or

(2) the heavy chain variable region comprises the amino acid sequence as shown in SEQ ID NO:12, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:12, or consists of SEQ ID NO:12; the light chain variable region comprises the amino acid sequence as shown in SEQ ID NO:13, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:13, or consists of SEQ ID NO:13.

In some embodiments, the antibody or antigen-binding fragment thereof further comprises heavy and/or light chain constant region sequences from a human antibody germline consensus sequence. The light chain constant region is preferably a human kappa or lambda chain constant region. The heavy chain constant region may be gamma, mu, alpha, delta, or epsilon chain, and in some embodiments, the heavy chain constant region is preferably derived from the constant region sequence of human IgG1, IgG2, IgG3, and IgG4. In some embodiments, the light chain constant region comprises the sequence as shown in SEQ ID NO:17, or consists of the sequence. In other embodiments, the heavy chain constant region comprises the sequence as shown in SEQ ID NO:16, or consists of the sequence.

It will be appreciated that sequence variants of these constant region domains, for example comprising one or more amino acid modifications, wherein the amino acid position is identified by the EU indexing system of Kabat *et al.,* (1991), may also be used.

In a specific embodiment, the invention provides anti-FGFR2b antibodies and antigen-binding fragments thereof that specifically bind FGFR2b, which comprises a heavy chain and a light chain, wherein:

1) the heavy chain comprises the amino acid sequence as shown in SEQ ID NO:9, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:9, or consists of SEQ ID NO:9; the light chain comprises an amino acid sequence as shown in SEQ ID NO:10, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:10, or consists of SEQ ID NO:10; or

2) the heavy chain comprises the amino acid sequence as shown in SEQ ID NO:14, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:14, or consists of SEQ ID NO:14; the light chain comprises an amino acid sequence as shown in SEQ ID NO:15, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:15, or consists of SEQ ID NO:15.

In a second aspect, the present invention provides an anti-FGFR2b antibody drug conjugate according to formula (I), an isomer thereof, a pharmaceutically acceptable salt thereof, or a mixture thereof:

Ab- (L-D)m [formula (I)]

Wherein,

L is a linker unit;

D is a cytotoxic small molecule drug;

m is an average number of L-D units conjugated to Ab, and m is selected from 1 to 10, preferably 2 to 8;

Ab is an anti-FGFR2b antibody or an antigen-binding fragment thereof comprising:

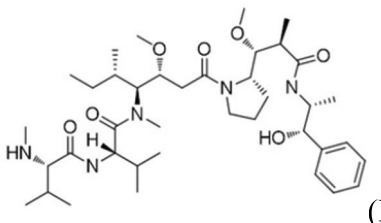
(1) a heavy chain variable region comprising HCDR1, HCDR2, and HCDR3 as shown in SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:11, respectively; and a light chain variable region comprising LCDR1, LCDR2, and LCDR3 as shown in SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively;

or

(2) a heavy chain variable region comprising HCDR1, HCDR2, and HCDR3 as shown in SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, respectively; and a light chain variable region comprising LCDR1, LCDR2, and LCDR3 as shown in SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively.

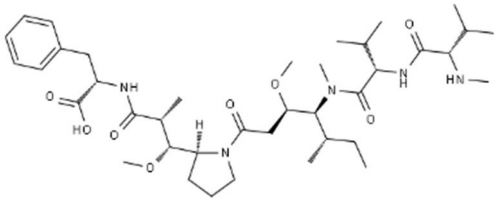
In some embodiments, the small molecule drug D of the present invention is a monomethyl auristatins, camptothecins or mertansine alkaloids.

In some embodiments, the monomethyl auristatins may be monomethyl auristatin E (MMAE)



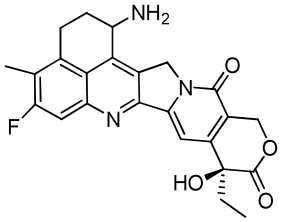
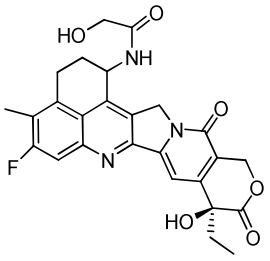
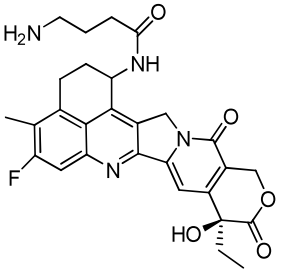
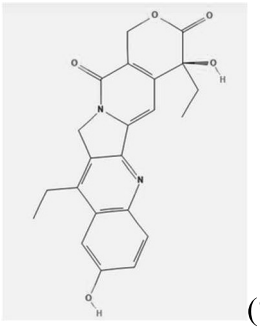
(MMAE)

or monomethyl auristatin F (MMAF)



(MMAF).

In some embodiments, the mertansine alkaloids may be DM1, DM3 or DM4.

In some embodiments, the camptothecins may be  (Exatecan),  (Dxd(1)),  (Dxd(2) or  (SN-38).

In some embodiments, the antibody-drug conjugate of the present invention comprises multiple D components, said multiple D components may be a combination of different therapeutic active substances or pharmaceutical active ingredients, or a combination of same therapeutic active substances or pharmaceutical active ingredients.

In some embodiments, the antibody-drug conjugate of the present invention has a drug/antibody ratio (DAR) of 1-15, for example a DAR of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15.

In some embodiments, the DAR is an average DAR.

In some embodiments, the average DAR is 1 to 15, for example 1 to 10.

As a specific embodiment, the average DAR of the antibody-drug conjugate of the present invention is preferably 2 to 10, for example 2 to 8.

As a specific embodiment, when D is a camptothecins, the average DAR of the antibody-drug conjugate of the present invention is preferably 2 to 8.

In some embodiments, a cytotoxin is covalently linked to an anti-FGFR2b antibody or an antigen-binding fragment thereof via a linker in a non-site-specific or site-specific manner.

The “linker”, “linker unit” and “connector” used in the present invention can be used interchangeably.

The “drug-containing linker” as used in the present invention refers to a compound obtained

from direct or indirect covalent bonding of a drug, for example the cytotoxic small molecule drug as described in D, with a linker.

In some embodiments, said L is a combination of one or more L'.

In some embodiments, said L' is selected from carbonyl, amino, amido, aminoacyl, -$(PEG)_n$-, -$(CH_2)_n$-, -$(CH_2)_n$- comprising a hetreoatom, -(C≡C)-, -(CH=CH)-, -O-, -S-, maleimido (mc), maleimide propionyl (MP), methylsulfonyl pyrimidinyl, valine-citrulline (val-cit), valine-alanine (val-ala), N-succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), alanine-phenylalanine (ala-phe), phenylalanine-lysine (phe-lys), p-aminobenzyloxycarbonyl (PAB), dimethyl ethanediamine (DMED), N-succinimidyl 4-(2-pyridinylthio)pentanoate (SPP), N-succinimidyl 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC), N-succinimidyl (4-iodo-acetyl)aminobenzoate (SIAB), N-succinimidyl 4-(2-pyridinyldithio)butanoate (SPDB), N-succinimidyl 3-(pyridin-2-yldithio)-propionate (SPDP), glycine-glycine-phenylalanine-glycine (GGFG), acetyl-lysine-valine-citrulline-p-aminobenzyloxycarbonyl (AcLys-vc-PABC).

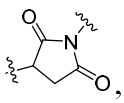
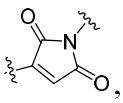
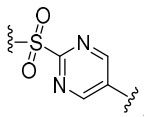
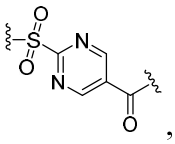
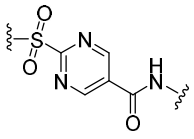
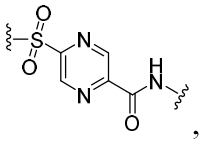
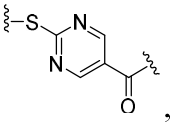
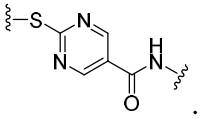
In some embodiments, n is independently selected from an integer of 1 to 20.

As a specific embodiment, when L' is selected from $(PEG)_n$, n is independently preferably 2, 4, 6 or 8.

As a specific embodiment, when L' is selected from $(CH_2)_n$, n is independently preferably an integer of 1 to 16.

As a specific embodiment, when L' is selected from $(CH_2)_n$ comprising a heteroatom, n is independently preferably an integer of 1 to 16; the heteroatoms is selected from N, O or S, the number of heteroatoms is independently preferably an integer of 1 to 8.

In some embodiments, said L may be J-L_1-L_2-X-L_3.

In some embodiments, said J is selected from , , , , , , , .

In some embodiments, L_1 is selected from a single bond, -$(PEG)_n$- or -$(CH_2)_n$-, said -$(CH_2)_n$- optionally comprises 1 to 6 heteroatoms selected from N, O, S; wherein n is independently selected from an integer of 1 to 20. Preferably, L_1 is selected from -$(PEG)_n$-, wherein n is independently 2, 4, 6 or 8.

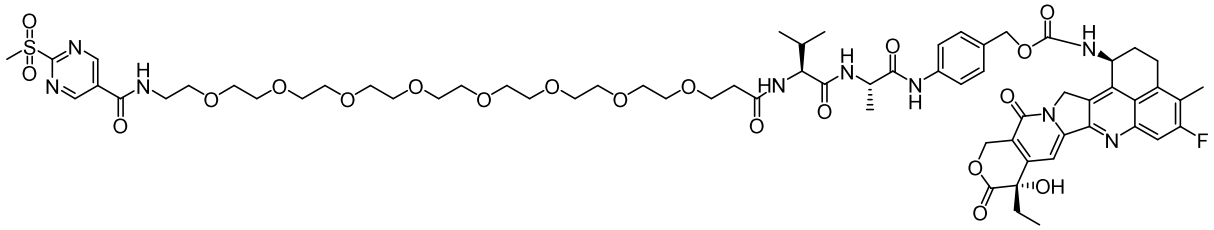
In some embodiments, L_2 is selected from a combination of one or more of a single bond, -O-, -S-, -CH_2-, -NH-, -C(O)-; wherein said -CH_2- and -NH- may be optionally substituted 1 to 3 times by $C_{1\sim6}$ alkyl or halogen.

In some embodiments, X is selected from a single bond or a combination of 1~4 X', said X' is independently selected from amino acid, for example glycine, alanine, phenylalanine, valine, lysine

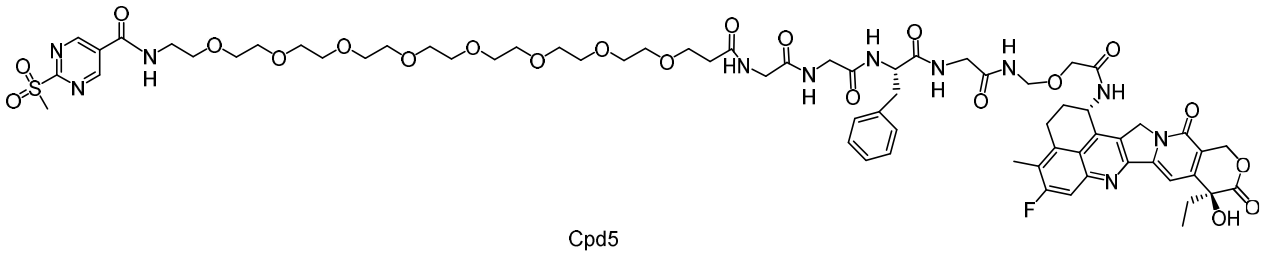
and citrulline. In some embodiments, X is selected from a single bond or valine-citrulline (val-cit), valine-alanine (val-ala), alanine-phenylalanine (ala-phe), phenylalanine-lysine (phe-lys), glycine-glycine-phenylalanine-glycine (GGFG).

In some embodiments, L_3 is selected from a combination of one or more of a single bond, -O-, -S-, -CH_2-, -NH-, -C(O)-, -phenyl-, -cyclopropyl-, -cyclobutyl-, -cyclohexyl-; wherein said -CH_2-, -NH-, -phenyl-, -cyclopropyl-, -cyclobutyl-, -cyclohexyl- may be optionally substituted 1 to 3 times by $C_{1\sim6}$ alkyl, halogen, cyano or hydroxy.

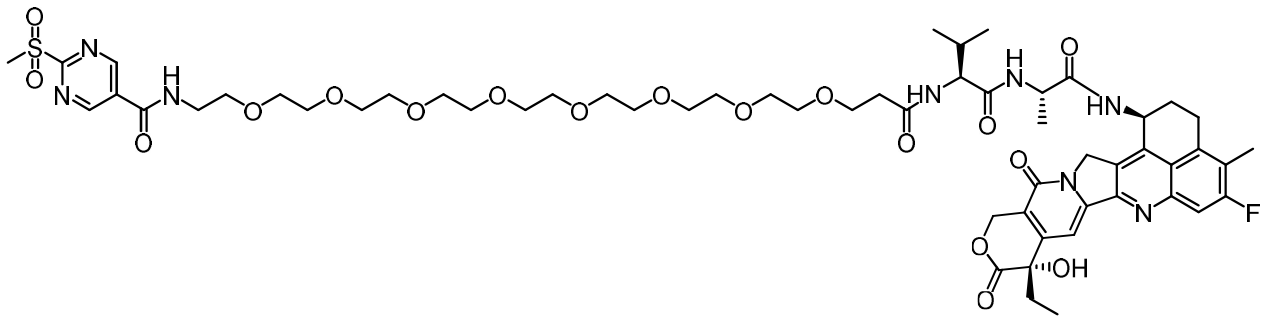
Further, the present invention provides three linker unit-cytotoxic small molecule drugs of formula (L-D), as shown in Formula Cpd3, Formula Cpd5 and Formula Cpd6:



Cpd3 ,



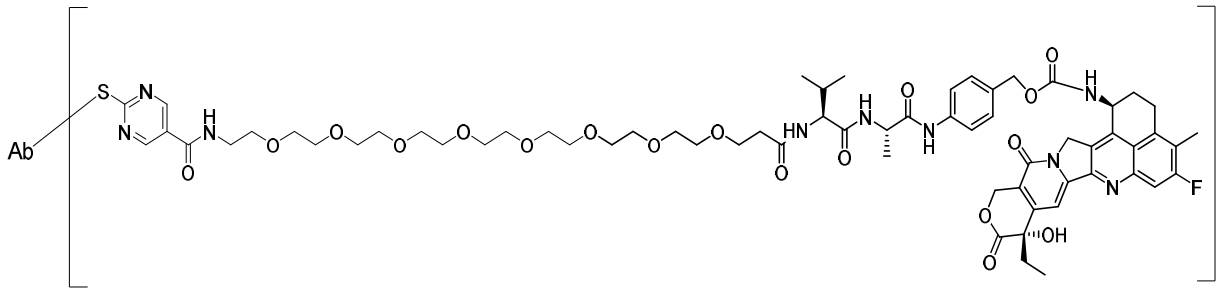
Cpd5 ,



Cpd6 .

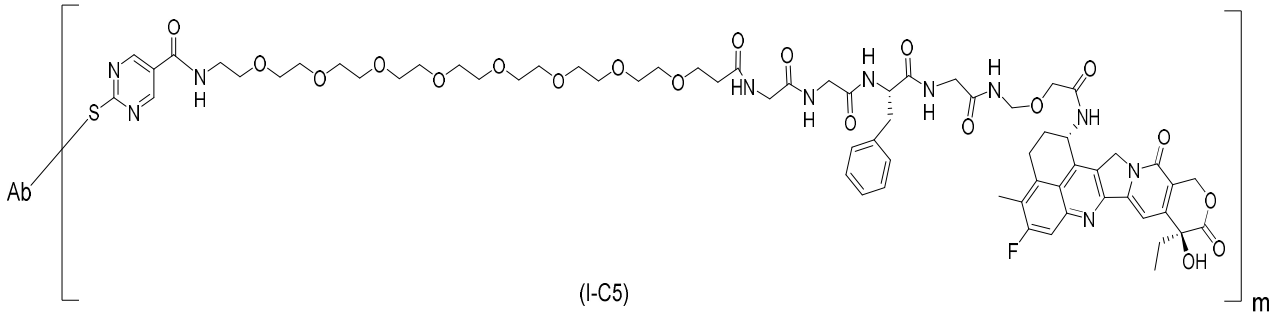
In some embodiments, the linker-toxin as shown in Formula Cpd3, Formula Cpd5 and Formula Cpd6 provided in the present invention are suitable for conjugating with anti-FGFR2b antibodies or the antigen-binding fragments thereof.

Further, the present invention provides an anti-FGFR2b antibody-drug conjugate, an isomer thereof, a pharmaceutical acceptable salt thereof, or a mixture thereof, wherein the structure of the anti-FGFR2B antibody-drug conjugate is as shown in Formula (I-C3), Formula (I-C5) and Formula (I-C6):



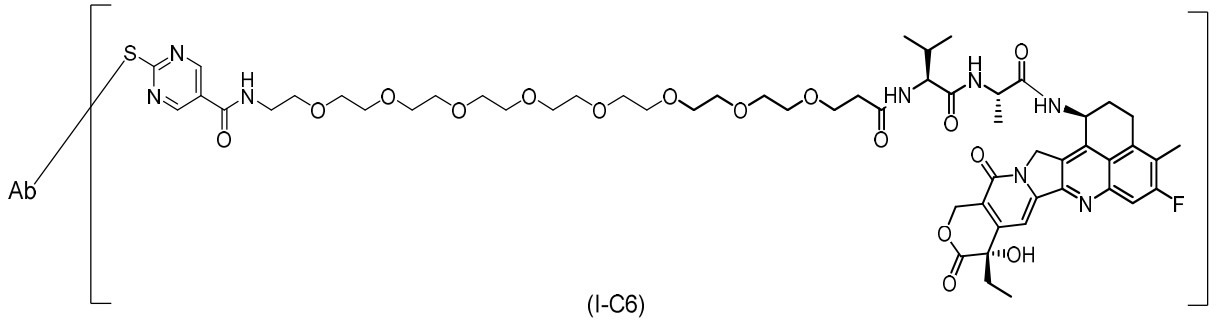
(I-C3)

,



(I-C5)

,



(I-C6)

;

wherein,

m is an average conjugation number, and m is independently selected from 1 to 10;

Ab is an anti-FGFR2b antibody or the antigen-binding fragment thereof comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprising the amino acid sequences HCDR1, HCDR2, and HCDR3 as shown in SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:11; and the light chain variable region comprising LCDR1, LCDR2, and LCDR3 as shown in SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6;

or

the heavy chain variable region comprising HCDR1, HCDR2, and HCDR3 as shown in SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; and the light chain variable region comprising LCDR1, LCDR2, and LCDR3 as shown in SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6.

In some embodiments, the anti-FGFR2b antibody or the antigen-binding fragment thereof of the invention comprises: a heavy chain variable region comprising the amino acid sequence as shown in SEQ ID NO:7 or an amino acid sequence having at least 95%, 96%, 97%, 98%, 99% identity thereto, and a light chain variable region comprising the amino acid sequence as shown in SEQ ID NO:8 or an amino acid sequence having at least 95%, 96%, 97%, 98%, 99% identity thereto.

In some embodiments, the anti-FGFR2b antibody or the antigen-binding fragment thereof of the invention comprises: a heavy chain variable region comprising the amino acid sequence as shown in SEQ ID NO:12 or an amino acid sequence having at least 95%, 96%, 97%, 98%, 99% identity thereto, and a light chain variable region comprising the amino acid sequence as shown in SEQ ID NO:13 or an amino acid sequence having at least 95%, 96%, 97%, 98%, 99% identity thereto.

In some embodiments, the anti-FGFR2b antibody or the antigen-binding fragment thereof of the invention comprises: a heavy chain comprising the amino acid sequence as shown in SEQ ID NO:9 or an amino acid sequence having at least 95%, 96%, 97%, 98%, 99% identity thereto, and a light chain comprising the amino acid sequence as shown in SEQ ID NO:10 or an amino acid sequence having at least 95%, 96%, 97%, 98%, 99% identity thereto.

In some embodiments, the anti-FGFR2b antibody or the antigen-binding fragment thereof of the invention comprises: a heavy chain comprising the amino acid sequence as shown in SEQ ID NO:14 or an amino acid sequence having at least 95%, 96%, 97%, 98%, 99% identity thereto, and a light chain comprising the amino acid sequence as shown in SEQ ID NO:15 or an amino acid sequence having at least 95%, 96%, 97%, 98%, 99% identity thereto.

In some embodiments, the anti-FGFR2b antibody or the antigen-binding fragment thereof of the invention comprises: a heavy chain variable region comprising the amino acid sequence as shown in SEQ ID NO:7 and a light chain variable region comprising an amino acid sequence as shown in SEQ ID NO:8.

In some embodiments, the anti-FGFR2b antibody or the antigen-binding fragment thereof of the invention comprises: a heavy chain variable region comprising the amino acid sequence as shown in SEQ ID NO:12 and a light chain variable region comprising an amino acid sequence as shown in SEQ ID NO:13.

In some embodiments, the anti-FGFR2b antibody or the antigen-binding fragment thereof of the invention comprises: a heavy chain comprising the amino acid sequence as shown in SEQ ID NO:9 and a light chain comprising an amino acid sequence as shown in SEQ ID NO:10.

In some embodiments, the anti-FGFR2b antibody or the antigen-binding fragment thereof of the invention comprises: a heavy chain comprising the amino acid sequence as shown in SEQ ID NO:14 and a light chain comprising an amino acid sequence as shown in SEQ ID NO:15.

In some embodiments, the antibody or the antigen binding fragment of the invention is a murine antibody, a chimeric antibody, a humanized antibody, or a fully human antibody.

As a specific embodiment, the fragment of SEQ ID:12 of the present invention is a humanized antibody.

As a specific embodiment, the fragment of SEQ ID:13 of the present invention is a humanized antibody.

As a specific embodiment, the fragment of SEQ ID:14 of the present invention is a humanized antibody.

As a specific embodiment, the fragment of SEQ ID:15 of the present invention is a humanized antibody.

In some embodiments, the anti-FGFR2b antibody or the antigen-binding fragment thereof of the invention is a Fab, Fab', Fab'-SH, Fv, scFv, F(ab')2, or sdAb.

In some embodiments, the anti-FGFR2b antibodies or antigen-binding fragments thereof of the

invention is of any IgG subtype, such as IgG1, IgG2, IgG3, IgG4; preferably, the antibody is low or non-fucosylated.

In some embodiments, the Ab of the present invention comprises 3 complementarity determining regions (HCDRs) from the heavy chain variable region, HCDR1, HCDR2, and HCDR3.

In some embodiments, the Ab of the present invention comprises 3 complementarity determining regions (LCDRs) from the light chain variable region, LCDR1, LCDR2, and LCDR3.

In some embodiments, the Ab of the present invention comprises 3 complementarity determining regions (HCDRs) from the heavy chain variable region and 3 complementarity determining regions (LCDRs) from the light chain variable region.

In some embodiments, the Ab comprises a heavy chain variable region (VH).

In some embodiments, the heavy chain variable region comprises 3 complementarity determining regions (HCDRs) from the heavy chain variable region.

In some embodiments, the Ab comprises a light chain variable region (VL).

In some embodiments, the light chain variable region comprises 3 complementarity determining regions (LCDRs) from the light chain variable region.

In some embodiments, the Ab comprises heavy chain variable region (VH) and light chain variable region (VL).

In some embodiments, the heavy chain variable region of the present invention:

（i）comprises or consists of the amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to the amino acid sequence of SEQ ID NO:7;

（ii）comprises or consists of the amino acid sequence of SEQ ID NO:7; or

（iii）comprises or consists of the amino acid sequence having one or more (preferably not more than 10, more preferably not more than 5, 4, 3, 2, 1) amino acid alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) compared to the amino acid sequence of SEQ ID NO:7, preferably the amino acid sequence alterations do not occur in the CDR regions.

In some embodiments, the heavy chain variable region of the invention:

（i）comprises or consists of the amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to the amino acid sequence of SEQ ID NO:12;

（ii）comprises or consists of the amino acid sequence of SEQ ID NO 12; or

（iii）comprises or consists of the amino acid sequence having one or more (preferably not more than 10, more preferably not more than 5, 4, 3, 2, 1) amino acid alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) compared to the amino acid sequence of SEQ ID NO:12, preferably the amino acid sequence alterations do not occur in the CDR regions.

In some embodiments, the light chain variable region of the invention:

（i）comprises or consists of the amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to the amino acid sequence of SEQ ID NO:8;

（ii）comprises or consists of the amino acid sequence of SEQ ID NO 8; or

（iii）comprises or consists of the amino acid sequence having one or more (preferably not more than 10, more preferably not more than 5, 4, 3, 2, 1) amino acid alterations (preferably amino

acid substitutions, more preferably amino acid conservative substitutions) compared to the amino acid sequence of SEQ ID NO:8, preferably the amino acid sequence alterations do not occur in the CDR regions.

In some embodiments, the light chain variable region of the invention:

（i）comprises or consists of the amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to the amino acid sequence of SEQ ID NO:13;

（ii）comprises or consists of the amino acid sequence of SEQ ID NO 13; or

（iii）comprises or consists of the amino acid sequence having one or more (preferably not more than 10, more preferably not more than 5, 4, 3, 2, 1) amino acid alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) as compared to the amino acid sequence of SEQ ID NO:13, preferably the amino acid sequence alterations do not occur in the CDR regions.

In some embodiments, the 3 complementarity determining regions HCDR1, HCDR2, and HCDR3 of the VH of the present invention are:

（i）three complementarity determining regions HCDR1, HCDR2, and HCDR3 comprised in the VH as shown in SEQ ID NO:7; or

（ii）a sequence comprising in total at least one and no more than 5, 4, 3, 2 or 1 amino acid alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) in the single HCDR region compared to the sequence of (i).

In some embodiments, the 3 complementarity determining regions HCDR1, HCDR2, and HCDR3 of the VH of the present invention are:

（i）three complementarity determining regions HCDR1, HCDR2, and HCDR3 comprised in the VH as shown in SEQ ID NO:12; or

（ii）a sequence comprising in total at least one and no more than 5, 4, 3, 2 or 1 amino acid alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) in the single HCDR region compared to the sequence of (i).

In some embodiments, the 3 complementarity determining regions LCDR1, LCDR2, and LCDR3 of the VL of the present invention are:

（i）three complementarity determining regions LCDR1, LCDR2, and LCDR3 comprised in the VL as shown in SEQ ID NO:8; or

（ii）a sequence comprising in total at least one and no more than 5, 4, 3, 2 or 1 amino acid alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) in the single LCDR region compared to the sequence of (i).

In some embodiments, the 3 complementarity determining regions LCDR1, LCDR2, and LCDR3 of the VL of the present invention are:

（i）three complementarity determining regions LCDR1, LCDR2, and LCDR3 comprised in the VL as shown in SEQ ID NO:13; or

（ii）a sequence comprising in total at least one and no more than 5, 4, 3, 2 or 1 amino acid alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) in the single LCDR region compared to the sequence of (i).

In some embodiments, the HCDR1 comprises or consists of the amino acid sequence of SEQ ID

NO:1, or the HCDR1 comprises an amino acid sequence having one, two or three alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) compared to the sequence of SEQ ID NO:1.

In some embodiments, the HCDR2 comprises or consists of the amino acid sequence of SEQ ID NO:2, or the HCDR2 comprises an amino acid sequence having one, two or three alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) compared to the sequence of SEQ ID NO:2.

In some embodiments, the HCDR3 comprises or consists of the amino acid sequence of SEQ ID NO:3, or the HCDR3 comprises an amino acid sequence having one, two or three alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) compared to the sequence of SEQ ID NO:3.

In some embodiments, the LCDR1 comprises or consists of the amino acid sequence of SEQ ID NO:4, or the LCDR1 comprises an amino acid sequence having one, two or three alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) compared to the sequence of SEQ ID NO:4.

In some embodiments, the LCDR2 comprises or consists of the amino acid sequence of SEQ ID NO:5, or the LCDR2 comprises an amino acid sequence having one, two or three alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) compared to the sequence of SEQ ID NO:5.

In some embodiments, the LCDR3 comprises or consists of the amino acid sequence of SEQ ID NO:6, or the LCDR3 comprises an amino acid sequence having one, two or three alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) compared to the sequence of SEQ ID NO:6.

In some embodiments, the HCDR3 comprises or consists of the amino acid sequence of SEQ ID NO:11, or the HCDR3 comprises an amino acid sequence having one, two or three alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) compared to the sequence of SEQ ID NO:11.

In some embodiments, the Ab of the present invention comprises a heavy chain constant region.

In some embodiments, the Ab of the present invention comprises a light chain constant region.

In some embodiments, the Ab of the present invention comprises a heavy chain constant region and a light chain constant region.

In some embodiments, the heavy chain constant region is a heavy chain constant region of IgG1, IgG2, IgG3, or IgG4.

In some embodiments, a heavy chain of the invention

（i）comprises or consists of the amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO:9;

（ii）comprises or consists of the amino acid sequence of SEQ ID NO:9; or

（iii）comprising or consisting of the amino acid sequence having one or more (preferably not more than 20 or 10, more preferably not more than 5, 4, 3, 2, 1) amino acid alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) compared to the amino acid sequence of SEQ ID NO:9.

In some embodiments, a heavy chain of the invention

（i）comprises or consists of the amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO:14

（ii）comprises or consists of the amino acid sequence of SEQ ID NO:14; or

（iii）comprising or consisting of the amino acid sequence having one or more (preferably not more than 20 or 10, more preferably not more than 5, 4, 3, 2, 1) amino acid alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) compared to the amino acid sequence of SEQ ID NO:14.

In some embodiments, a light chain of the invention

（i）comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO:10;

（ii）comprises or consists of the amino acid sequence of SEQ ID NO:10; or

（iii）comprising or consisting of the amino acid sequence having one or more (preferably not more than 20 or 10, more preferably not more than 5, 4, 3, 2, 1) amino acid alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) compared to the amino acid sequence of SEQ ID NO:10.

In some embodiments, the Ab described in the present invention specifically binds to FGFR2b, and comprises the three complementarity determining regions HCDR1, HCDR2, and HCDR3 comprised in the VH as shown in SEQ ID NO:7, and/or the three complementarity determining regions LCDR1, LCDR2, and LCDR2 comprised in the VL as shown in SEQ ID NO:8.

In some embodiments, a light chain of the invention

（i）comprises or consists of the amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO:15;

（ii）comprises or consists of the amino acid sequence of SEQ ID NO:15; or

（iii）comprising or consisting of the amino acid sequence having one or more (preferably not more than 20 or 10, more preferably not more than 5, 4, 3, 2, 1) amino acid alterations (preferably amino acid substitutions, more preferably amino acid conservative substitutions) compared to the amino acid sequence of SEQ ID NO:15.

In some embodiments, the Ab described in the present invention specifically binds to FGFR2b, and comprises the three complementarity determining regions HCDR1, HCDR2, and HCDR3 comprised in the VH as shown in SEQ ID NO:7, and/or the three complementarity determining regions LCDR1, LCDR2, and LCDR2 comprised in the VL as shown in SEQ ID NO:8.

In some embodiments, the Ab described in the present invention specifically binds to FGFR2b, and comprises the three complementarity determining regions HCDR1, HCDR2, and HCDR3 comprised in the VH as shown in SEQ ID NO:12, and/or the three complementarity determining regions LCDR1, LCDR2, and LCDR2 comprised in the VL as shown in SEQ ID NO:13.

In some embodiments, the Ab of the present invention comprises

VH comprising or consisting of the amino acid sequence as shown in SEQ ID NO:7 or the amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity thereto, and/or

VL comprising or consisting of the amino acid sequence as shown in SEQ ID NO:8 or the

amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity thereto.

In some embodiments, the Ab of the present invention comprises

VH comprising or consisting of the amino acid sequence as shown in SEQ ID NO:12 or the amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity thereto, and/or

VL comprising or consisting of the amino acid sequence as shown in SEQ ID NO:13 or the amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity thereto.

The invention provides an antibody drug conjugate targeting human FGFR2b, an isomer thereof, a pharmaceutically acceptable salt thereof or a mixture thereof, the antibody drug conjugate has the following advantages:

(1) binding to target cells expressing human FGFR2b with high affinity;

(2) entering into cells through endocytosis and killing target cells; in some embodiments, the ADC of the present invention having high endocytosis efficiency;

(3) treating, preventing, ameliorating a disorder associated with aberrant function or expression of FGFR2b (e.g., cancer) in a subject, or treating, preventing, ameliorating one or more symptoms of the disease;

(4) reducing or inhibiting tumor growth or progression in a subject having a tumor that expresses FGFR2b;

(5) inducing regression (e.g., long-term regression) of a tumor expressing FGFR2b;

(6) exerting cytotoxic activity in cells expressing FGFR2b;

In a third aspect, the invention provides an isolated polynucleotide molecule encoding any one of the antibodies or antigen-binding fragments thereof of the first aspect.

In a fourth aspect, the invention provides a vector comprising the nucleic acid molecule of the third aspect. In some embodiments, the vector is an expression vector.

In a fifth aspect, the invention provides a host cell comprising the vector of the fourth aspect or the nucleic acid molecule of the third aspect. In some embodiments, the host cell is prokaryotic, such as *Escherichia coli*. In other embodiments, the host cell is eukaryotic, such as a HEK293 cell, a CHO cell, a yeast cell, or a plant cell.

In a sixth aspect, the present invention provides a pharmaceutical composition comprising the anti-FGFR2b antibody or the antigen-binding fragment thereof described in the first aspect; or the anti-FGFR2b antibody drug conjugate, an isomer thereof, a pharmaceutically acceptable salt thereof, or a mixture thereof in the second aspect, and a pharmaceutically acceptable excipient described.

In a seventh aspect, the invention provides use of an anti-FGFR2b antibody or the antigen-binding fragment thereof described in the first aspect; or the anti-FGFR2b antibody drug conjugate, an isomer thereof, a pharmaceutically acceptable salt thereof, or a mixture thereof described in the second aspect; or the pharmaceutical composition described in the sixth aspect in the manufacture of a medicament for treating and/or preventing a disease or disorder mediated by FGFR2b.

In one embodiment, the invention provides an anti-FGFR2b antibody or the antigen-binding fragment thereof described in the first aspect; or the anti-FGFR2b antibody drug conjugate, an isomer thereof, a pharmaceutically acceptable salt thereof, or a mixture thereof described in the second aspect; or a pharmaceutical composition described in the sixth aspect for use in therapy and/or prophylaxis. In a specific embodiment, the invention provides an anti-FGFR2b antibody or the antigen-binding fragment thereof described in the first aspect; or the anti-FGFR2b antibody drug conjugate, an isomer thereof, a pharmaceutically acceptable salt thereof, or a mixture thereof described in the second aspect; or the pharmaceutical composition described in the sixth aspect for use in the treatment and/or prevention of a FGFR2b mediated disease or disorder.

In an eighth aspect, the invention provides a method of treating and/or preventing a FGFR2b-mediated disease or disorder, comprising administering to a subject in need thereof an effective amount of the anti-FGFR2b antibody or the antigen-binding fragment thereof described in the first aspect; or the antibody drug conjugate, an isomer thereof, a pharmaceutically acceptable salt thereof, or a mixture thereof described in the second aspect; or a pharmaceutical composition described in the six aspect.

In some embodiments, the FGFR2b-mediated disease or disorder described in the various aspects above is a cancer or tumor.

In some embodiments, the cancer or tumor is selected from the group consisting of lung cancer, lung squamous carcinoma, lung adenocarcinoma, ovarian cancer, endometrial cancer, breast cancer, triple-negative breast cancer, intrahepatic cholangiocarcinoma, bladder cancer, colon cancer, prostate cancer, cervical cancer, colorectal cancer, pancreatic cancer, gastric cancer, esophageal cancer, hepatocellular carcinoma, renal cell carcinoma, head and neck cancer, mesothelioma, melanoma, sarcoma, brain tumor, gastroesophageal adenocarcinoma, malignant uterine tumor, gastroesophageal junction adenocarcinoma, bile duct cancer, gallbladder cancer, intrahepatic bile duct cancer, oral mucosal cancer, and urothelial cancer.

In a ninth aspect, the present invention provides a pharmaceutical combination comprising an anti-FGFR2b antibody or the antigen-binding fragment thereof described in the first aspect; or the anti-FGFR2b antibody drug conjugate, an isomer thereof, a pharmaceutically acceptable salt thereof, or a mixture thereof described in the second aspect; or a pharmaceutical composition described in the six aspect, and one or more other therapeutic agents.

In a tenth aspect, the invention provides a kit comprising an anti-FGFR2b antibody or antigen-binding fragment thereof described in the first aspect; or the anti-FGFR2b antibody drug conjugate, an isomer thereof, a pharmaceutically acceptable salt thereof, or a mixture thereof described in the second aspect; or a pharmaceutical composition described in the six aspect.

Drawings

FIG. 1: the anti-FGFR2b antibody drug conjugate inhibits proliferation of SNU-5 cells.

FIG. 2: the anti-FGFR2b antibody drug conjugate inhibits proliferation of SNU-16 cells.

FIG. 3: effect of HC18 antibody drug conjugates on tumor volume in SNU-16 mouse subcutaneous transplantation tumor model.

FIG. 4: effect of HC18 antibody drug conjugates on mouse body weight in SNU-16 mouse subcutaneous transplantation tumor model.

FIG. 5: effect of different HC18-01 antibody drug conjugates on tumor volume in SNU-16 mouse subcutaneous transplantation tumor model.

FIG. 6: effect of different HC18-01 antibody drug conjugates on mouse body weight in SNU-16 mouse subcutaneous transplantation tumor model.

FIG. 7: anti-tumor efficacy of different doses of HC18-01-ADC1 in SNU-16 human gastric cancer transplantation mouse tumor model.

FIG. 8: effect of different doses of HC18-01-ADC1 on body weight in SNU-16 human gastric cancer transplantation mouse tumor model.

FIG. 9: anti-tumor efficacy of different doses of HC18-01-ADC1 in KATO-III human gastric cancer transplantation mouse tumor model.

FIG. 10: effect of different doses of HC18-01-ADC on body weight in KATO-III human gastric cancer transplantation mouse tumor model.

FIG. 11: effect of HC18-01-ADC1 on average tumor volume of xKATO-III subcutaneous transplantation mice model.

FIG. 12: effect of HC18-01-ADC1 on tumor weight in xKATO-III subcutaneous transplantation mice model.

FIG. 13: effect of HC18-01-ADC1 on average tumor volume in human lung cancer tumor xenograft mouse model.

FIG. 14: effect of HC18-01-ADC1 on tumor weight in human lung cancer tumor xenograft mouse models.

Definition

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the technical scope of the art.

In order that the present invention may be more readily understood, certain technical and scientific terms are specifically defined as follows. Unless otherwise defined herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. For definitions and terminology in this field, the skilled person can refer in particular to Current protocol Molecular Biology (Ausubel). Abbreviations for amino acid residues are standard 3-letter and/or 1-letter codes used in the art to refer to one of the 20 commonly used L-amino acids. As used herein, including the claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise.

The term "about" when used in conjunction with a numerical value is intended to encompass the numerical value within a range having a lower limit that is 5% less than the stated numerical value and an upper limit that is 5% greater than the stated numerical value.

The term "and/or" should be understood to mean any one of the options or a combination of any two or more of the options.

The term "FGFR2b" refers to any recombinant or naturally occurring form of fibroblast growth factor receptor 2b (FGFR2b), variants or homologs thereof that maintain, e.g., at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of the activity of FGFR2b. The variant or homologue has at least

90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the naturally occurring FGFR2 protein in the complete sequence or in partial sequence (e.g., 50, 100, 150, or 200 contiguous amino acid portions).

The term "antibody" is used herein in the broadest sense and encompasses a variety of antibody constructs, including, but not limited to, monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments, so long as they exhibit the desired antigen-binding activity. A complete antibody will typically comprise at least two full length heavy chains and two full length light chains, but in some cases may comprise fewer chains, for example an antibody naturally occurring in a camel may comprise only heavy chains.

The term "anti-FGFR2b antibody" refers to an antibody molecule that specifically binds FGFR2b, which is capable of inhibiting FGFR2b activity. The anti-FGFR2b antibody is capable of inhibiting FGFR2b activity relative to the absence of FGFR2b antibody, e.g., by at least partially or completely blocking stimulation by FGFR2b, reducing, preventing or delaying activation of FGFR2b, or inactivating, desensitizing or downregulating the signaling, activity or amount of FGFR2b. In some embodiments, the antibody may inhibit FGFR2b activity by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more compared to a control.

The term "antibody fragment" refers to a molecule distinct from an intact antibody, which comprises a portion of an intact antibody and is capable of binding to the antigen to which the intact antibody binds. Examples of antibody fragments include, but are not limited to, Fv, Fab, Fab', Fab'-SH, F(ab')2; a diabody; a linear antibody; single chain antibodies (e.g., scFv); a single domain antibody; a bivalent or bispecific antibody or fragment thereof; camelid antibodies (heavy chain antibodies); and multispecific antibodies (e.g., bispecific antibodies) formed from antibody fragments.

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding of the antibody to an antigen. The variable domains of the heavy and light chains of natural antibodies typically have similar structures, wherein each domain comprising four conserved Framework Regions (FRs) and three Complementarity Determining Regions (CDRs) (see, e.g., Kindt et al., Kuby Immunology, 6th ed., W.H.Freeman and Co. page 91, (2007)). A single VH or VL domain is sufficient to confer antigen binding specificity.

"Complementarity determining region" or "CDR" is a region of an antibody variable domain that is highly variable in sequence and forms structurally defined loops ("hypervariable loops") and/or contains antigen-contacting residues ("antigen-contacting points"). The CDRs are primarily responsible for binding to an antigenic epitope. The CDRs in the variable domain are commonly referred to as CDR1, CDR2 and CDR3, numbered sequentially from the N-terminus. The precise amino acid sequence boundaries of the CDRs within a given variable region amino acid sequence can be determined using any one or combination of a number of well-known antibody CDR assignment systems, including, for example: Chothia (Chothia et al., (1989) Nature 342: 877-883, Al-Lazikani et al., “Standard conformations for the canonical structures of immunoglobulins”, Journal of Molecular Biology, 273, 927-948(1997)), based on the three-dimensional structure of the antibody and the topology of the CDR loops, Kabat (Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed, U.S. Department of Health and Human Services, National instruments of Health (1987)), based on antibody sequence variability, AbM (University of Bath), Contact (University College London), the international ImMunoGeneTics database (IMGT) (http://imgt.cines.fr/), and the North CDR definition

based on affinity propagation clustering using a large number of crystal structures.

It should be noted that the boundaries of the CDRs of the variable regions of the same antibody obtained based on different assignment systems may differ. That is, the CDR sequences of the same antibody variable region defined under different assignment systems are different. Thus, in reference to defining an antibody with a particular CDR sequence as defined herein, the scope of the antibody also encompasses antibodies whose variable region sequences comprise the particular CDR sequence, but whose claimed CDR boundaries differ from the particular CDR boundaries as defined herein due to the application of different schemes (e.g., different assignment systems or combinations).

Unless otherwise indicated, in the present invention, the term "CDR" or "CDR sequence" encompasses CDR sequences determined in any of the ways described above.

CDRs may also be determined based on having the same AbM numbering position as a reference CDR sequence (e.g., any of the CDRs exemplified herein). In some embodiments, the CDRs of an antibody of the invention are positioned according to the AbM numbering scheme.

Unless otherwise indicated, in the present invention, when referring to residue positions in antibody variable regions and CDRs (including heavy chain variable region residues), it refers to the numbered positions according to the AbM numbering system.

As used herein, the term "comprising" or "comprises" is intended to mean that the stated element, integer or step is included, but not to the exclusion of any other element, integer or step. When the term "comprising" or " comprises " is used herein, unless otherwise indicated, it also encompasses the case where it consists of the stated elements, integers or steps. For example, when referring to an antibody variable region "comprising" a particular sequence, it is also intended to encompass antibody variable regions consisting of that particular sequence.

The term "chimeric antibody" is an antibody having the variable domains of a first antibody and the constant domains of a second antibody, wherein the first and second antibodies are from different species. Typically, the variable domains are obtained from an antibody of a rodent or the like ("parent antibody"), while the constant domain sequences are obtained from a human antibody, such that the resulting chimeric antibody is less likely to induce an adverse immune response in a human subject as compared to the parent rodent antibody.

The term "humanized antibody" refers to antibody forms containing sequences from human and non-human (e.g., mouse, rat) antibodies. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the Framework Regions (FR) are those of a human immunoglobulin sequence. The humanized antibody optionally may comprise at least a portion of a human immunoglobulin constant region (Fc).

The term "fully human antibody" refers to an antibody comprising only human immunoglobulin protein sequences. If produced in a mouse, in a mouse cell, or in a hybridoma derived from a mouse cell, the fully human antibody may contain mouse carbohydrate chains. Likewise, "mouse antibody" refers to an antibody comprising only mouse immunoglobulin sequences. Alternatively, if produced in rats, in rat cells, or in hybridomas derived from rat cells, the fully human antibodies may contain rat carbohydrate chains. Likewise, "rat antibody" refers to an antibody comprising only rat immunoglobulin sequences.

The term "Fc region" is used to define the C-terminal region of an immunoglobulin heavy chain comprising at least a portion of a constant region. The term includes native sequence Fc regions and variant Fc regions. In some embodiments, the human IgG heavy chain Fc region extends from Cys226 or Pro230 to the carboxy-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present (numbering in this paragraph is according to the EU numbering system, also known as the EU index, e.g. Rabat et al., Sequences of Proteins of Immunological Interest,5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991).

The term "affinity" or "binding affinity" refers to the intrinsic binding affinity that reflects the interaction between members of a binding pair. The affinity of a molecule X for its partner Y can be generally represented by the equilibrium dissociation constant (KD), which is the ratio of the dissociation and association rate constants (kdis and kon, respectively). Affinity can be measured by common methods known in the art. One particular method for measuring affinity is the ForteBio kinetic binding assay herein.

The term "not binding" to a protein or cell means that it does not bind to the protein or cell, or does not bind to it with high affinity, that is, the KD for binding to the protein or cell is 1.0×10-^{6}M or higher, more preferably 1.0×10^{-5}M or higher, more preferably 1.0×10^{-4}M or higher, 1.0×10^{-3}M or higher, more preferably 1.0×10^{-2}M or higher.

The term "high affinity" for IgG antibodies means a KD for the antigen of 1.0×10^{-6}M or less, preferably 5.0×10^{-8}M or less, more preferably 1.0×10^{-8}M or less, 5.0×10^{-9}M or less, more preferably 1.0×10^{-9}M or less. For other antibody subtypes, "high affinity" binding may vary. For example, "high affinity" binding of an IgM subtype means a KD of 10^{-6}M or less, preferably 10^{-7}M or less, more preferably 10^{-8}M or less.

As used herein, the term "binding" or "specific binding" means that the binding is selective for the antigen and can be distinguished from unwanted or non-specific interactions. The ability of an antigen binding site to bind to a particular antigen can be determined by enzyme-linked immunosorbent assay (ELISA) or conventional binding assays known in the art such as by Radioimmunoassay (RIA) or biofilm thin layer interferometry or MSD assay or Surface Plasmon Resonance (SPR).

The term "median effective concentration (EC50)" refers to the concentration of a drug, antibody or toxin that induces a response that is 50% between baseline and maximum after a specified exposure time.

The term "therapeutic agent" herein encompasses any substance effective in preventing or treating tumors, such as cancer, including chemotherapeutic agents, cytokines, angiogenesis inhibitors, cytotoxic agents, other antibodies, small molecule drugs, or immunomodulatory agents (e.g., immunosuppressive agents).

The term "antibody drug conjugate" or "ADC" refers to an antibody or antibody fragment covalently conjugated to a therapeutically active substance or active pharmaceutical ingredient such that the therapeutically active substance or active pharmaceutical ingredient is targeted to the binding target of the antibody to exhibit its pharmacological function. The therapeutically active substance or active pharmaceutical ingredient may be a cytotoxin capable of killing a cell, preferably a cancer cell, targeted by the ADC. Covalent attachment of the therapeutically active substance, active pharmaceutical ingredient or cytotoxin can be performed in a non-site-specific manner using a linker, or in a site-specific manner.

The term "site-specific conjugation " refers to a means of attachment that specifically links a therapeutically active substance or active pharmaceutical ingredient to a specific site of an antibody. In some embodiments, the conjugation is accomplished via a linker.

The term "cytotoxic agent" is used interchangeably with "cytotoxin" and in the present invention refers to a substance that inhibits or disrupts cellular function and/or causes cell death or destruction.

The terms "linker ", "linker unit" and “connector” are used interchangeably herein and refer to a chemical moiety that covalently links an antibody to a therapeutically active substance or active pharmaceutical ingredient in an ADC. In some embodiments, the linker may comprise amino acid residues that link the antigen to the payload. The amino acid residues may form dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide or dodecapeptide units. Amino acid residues include those that occur naturally as well as non-naturally occurring amino acid analogs, such as citrulline or a beta-amino acid, such as beta-alanine, or an omega-amino acid such as 4-amino-butyric acid.

Depending on the nature classification, linkers suitable for use in the present invention may be cathepsin degradable linkers, such as valine-citrulline (val-cit) linkers, cBu-Cit linkers and CX linkers; a non-cleavable linker such as a SMCC linker or an MD linker; acid sensitive linkers, linkers of silicone structure, disulfide-carbamate linkers, MC-GGFG linkers, TRX linkers, galactoside containing linkers, pyrophosphate linkers, near-infrared-sensitive linkers, UV-sensitive linkers such as PC4AP.

The linker of the invention may also be a combination of one or more linkers, e.g., a cathepsin degradable linker may be combined with other types of linkers to form a new linker. Thus, the term "linker" as used herein encompasses a single type of linker, or a combination of different types of linkers, as long as it is capable of conjugation the antibody of the invention to a drug.

The term "loading" or "drug loading" or "payload" refers to the average number of payloads per antibody within an ADC molecule (herein "payload" is used interchangeably with "therapeutically active substance or active pharmaceutical ingredient"). The drug load may range from 1 to 20 therapeutically active substances or active pharmaceutical ingredients per antibody. The term "drug/antibody ratio" or "DAR" refers to the ratio of therapeutically active substance or active pharmaceutical ingredient (D) conjugated to an antibody to the antibody. The ADCs described herein typically have a DAR of 1-20, and in certain embodiments 1-8, 2-8, 2-6, 2-5, 2-18, 4-16, 5-12, 6-10, 3-8, 4-6, 6-10, and 2-4. Representative DAR values are 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15, typically expressed as a combination of the letters D and a number, where the number represents the value of the DAR, e.g. D2 represents the drug/antibody ratio for a DAR value of 2. In some embodiments, the DAR is an average DAR, i.e., the total ratio of the small molecule drug moiety (D) coupled to the Ab moiety described herein to the Ab moiety in the product as characterized by a detection method (e.g., by conventional methods such as UV/visible spectroscopy, mass spectrometry, ELISA assay, and HPLC). The DAR may be limited by the number of attachment sites on the antibody. For example, in the case where the attachment site is a cysteine thiol, the antibody may have only one or a few cysteine thiol groups or may have only one or a few sufficiently reactive thiol groups (through which the linker unit can be attached).

The term "FGFR2b-mediated disease or disorder" refers to a disease state that is caused or promoted by aberrant signaling (e.g., genetic mutation, overexpression, or aberrant activation) of

fibroblast growth factor receptor 2b (FGFR2b). When FGFR2b signaling is abnormal, it can lead to a variety of pathological conditions, such as cancer (e.g., gastric cancer, breast cancer, and lung cancer), developmental diseases, fibrosis, and proliferative diseases. In a specific embodiment of the invention, the term "FGFR2b-mediated disease or disorder" refers to a cancer or tumor associated with aberrant expression, mutation, or dysfunction of a FGFR2b gene or protein.

The term "treating" refers to slowing, interrupting, arresting, relieving, stopping, reducing, or reversing the progression or severity of an existing symptom, disorder, condition, or disease. Desirable therapeutic effects include, but are not limited to, preventing the occurrence or recurrence of disease, alleviating symptoms, reducing any direct or indirect pathological consequences of the disease, preventing metastasis, reducing the rate of disease progression, improving or palliating the disease state, and alleviating or improving prognosis. In some embodiments, the antibodies of the invention are used to delay disease development or to slow the progression of disease.

The term "preventing" includes the inhibition of the onset or development of a disease or disorder or symptoms of a particular disease or disorder. In some embodiments, subjects with a family history of cancer are candidates for a prophylactic regimen. Generally, in the context of cancer, the term "preventing" refers to the administration of a drug prior to the onset of signs or symptoms of cancer, particularly in a subject at risk for cancer.

The term "effective amount" refers to an amount or dose of an antibody or conjugate or composition of the invention that produces the desired effect in a patient in need of treatment or prevention following administration to the patient in a single or multiple dose. An effective amount can be readily determined by the attending physician, as one skilled in the art, by considering a number of factors including: a species such as a mammal; body weight, age, and general health; the specific diseases involved; the degree or severity of the disease; the response of the individual patient; the specific antibody administered; a mode of administration; bioavailability characteristics of the administered formulation; a selected dosing regimen; and the use of any concomitant therapies.

The term "therapeutically effective amount" refers to an amount that is effective to achieve the desired therapeutic outcome at the desired dosage and for the desired period of time. A therapeutically effective amount of an antibody or antibody fragment, or conjugate or composition thereof, may vary according to factors such as the disease state, the age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or deleterious effects of the antibody or antibody fragment or conjugate or composition thereof are less than the therapeutically beneficial effects. A "therapeutically effective amount" preferably inhibits a measurable parameter (e.g., tumor growth rate, tumor volume, etc.) by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 50%, 60%, or 70%, and still more preferably by at least about 80% or 90%, relative to an untreated subject. The ability of a compound to inhibit a measurable parameter (e.g., cancer) can be evaluated in an animal model system predictive of efficacy in human tumors.

The term "prophylactically effective amount" refers to an amount effective to achieve the desired preventive result at the required dosage and for the required period of time. Typically, since a prophylactic dose is used in a subject prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

The term "pharmaceutical composition" refers to a composition that is present in a form that

allows for the biological activity of the active ingredients contained therein to be effective, and that contains no additional ingredients that have unacceptable toxicity to the subject to which the composition is administered.

The term "in combination" refers to administration of one or more additional therapeutic agents including simultaneous (co-) administration and sequential administration in any order.

The term "pharmaceutically acceptable salt" refers to a salt of the drug conjugate of the present application. Such salts may be safe and/or effective when used in mammals and may have the required biological activity. The antibody drug conjugate of the present application may form a salt with an acid.

The term "pharmaceutically acceptable carrier" refers to an ingredient of a pharmaceutical formulation or composition other than an active ingredient that is not toxic to a subject.

The term "subject" includes any human or non-human animal. The term "non-human animal" includes all vertebrates, e.g., mammals and non-mammals, such as non-human primates, sheep, dogs, cats, horses, cattle, chickens, amphibians, reptiles, and the like.

Detailed Description

The following examples further illustrate the present invention, however, it is to be understood that the examples are described by way of illustration and not limitation, and that various modifications may be made by those skilled in the art. The examples, in which specific conditions are not specified, were carried out according to conventional conditions or conditions recommended by the manufacturer. The reagents and instruments used without indicating the manufacturer are all conventional products which are commercially available.

In some embodiments, the invention provides a method of preparing an anti-FGFR2b antibody, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the anti-FGFR2b antibody or an expression vector comprising the nucleic acid under conditions suitable for expression of the nucleic acid encoding the anti-FGFR2b antibody, and optionally isolating the anti-FGFR2b antibody. In a certain embodiment, the method further comprises recovering the anti-FGFR2b antibody from the host cell (or host cell culture medium).

To recombinantly produce the anti-FGFR2b antibodies of the invention, the nucleic acid encoding the anti-FGFR2b antibodies of the invention is first isolated and inserted into a vector for further cloning and/or expression in a host cell. Such nucleic acids are readily isolated and sequenced using conventional procedures, e.g., by using oligonucleotide probes that are capable of binding specifically to nucleic acids encoding the anti-FGFR2b antibodies of the invention.

The anti-FGFR2b antibodies of the invention prepared as described herein can be purified by known prior art techniques such as high performance liquid chromatography, ion exchange chromatography, gel electrophoresis, affinity chromatography, size exclusion chromatography, and the like. The actual conditions used to purify a particular protein also depend on net charge, hydrophobicity, hydrophilicity, etc., and these will be apparent to those skilled in the art. The purity of the anti-FGFR2b antibodies of the invention can be determined by any of a variety of well-known analytical methods, including size exclusion chromatography, gel electrophoresis, high performance liquid chromatography, and the like.

The prior art describes various methods for conjugation cytotoxic or other therapeutic agents to

antibodies. For example, conjugation can occur in an antibody through the amino group of the lysine side chain and the amino group at the N-terminus of the antibody, aspartic acid, glutamic acid, and the carboxyl group at the C-terminus or activated cysteine thiol group.

The practice of the present invention will employ, unless explicitly indicated to the contrary, conventional methods of chemistry, biochemistry, organic chemistry, molecular biology, microbiology, recombinant DNA techniques, genetics, immunology and cell biology, which are within the skill of the art.

The structures of the compounds disclosed herein can be confirmed by conventional methods well known to those skilled in the art. If the disclosure involves the absolute configuration of the compounds, the absolute configuration can be confirmed by conventional technical means in the art.

Compounds were named according to conventional naming principles in the art or using ChemDraw® software, and commercially available compounds were given the supplier catalog name.

Example 1: humanization of murine FGFR2b antibody and preparation of FGFR2b antibody

The murine antibodies of the invention are prepared by the method disclosed in WO2023185778A1, which may be referred to simply as "HC18". WO2023185778A 1 is incorporated in its entirety herein by reference.

The control antibody FPA144 is prepared referring to WO2015017600A1 which is incorporated in its entirety herein by reference.

The VH and VL sequences of the murine antibody HC18 were aligned with the known human antibody databases, respectively, to find the VH and VL sequences of the human germline genes with the highest homology to the murine VH and VL sequences, respectively.

The humanization design and PTM analysis method used by the invention comprises the following steps:

1. The variable region sequences of the selected antibodies were analyzed to distinguish the antigen binding domains (CDRs) from the Framework Regions (FRs).

2. Homology modeling is performed to obtain a modeled structure of the parent antibody. Hydrophilicity of the framework regions was calculated and amino acids of the antibody located on the framework regions were determined (i.e. hydrophilicity < 15%).

3. A humanized framework sequence was selected for each VH and VL based on sequence homology. The CDRs of the parent antibody were grafted onto a humanized framework, resulting in the sequences of the grafted antibody, which were defined as VH1 and VL1.

4. VH1 and VL1 were compared to the parent VH and VL sequences. All key residues that differ between the grafted and parental antibody framework sequences (i.e., possible reverse mutation sites) were identified

5. One or more reverse mutations were stepwise made in the VH1 and VL1 sequences according to the following principles:

a key amino acids for antibody loop interactions.

b. amino acids at light and heavy chain interfaces.

c. key amino acids for antibody core formation.

6. Multiple humanized sequences were designed for each antibody.

7. Analyzing post-translational modifications and potential degradation sites in the sequence,

including deamidation, isomeric oxidation, glycosylation, and the like.

8. PTM risk sites on the surface, such as N-glycosylation sites, deamidation sites, isomerization sites, oxidation sites, unpaired cysteine residues, etc., were identified in the structural model, which may affect the binding activity and downstream production of the antibody.

9. If high risk PTM sites are present in the CDR regions of the antibody, the risk sites will be considered removed.

From the above analysis, it was found that there is a high risk of isomerization sites DG on HCDR3 of murine antibody HC18, and that substitution DG with DA by PTM removal did not have a significant effect on antibody binding and druggability.

The murine antibody HC18 was humanized to obtain humanized antibody HC18-01, and the CDR sequences are shown in Table 1. The humanized antibody was constructed, expressed, purified, and identified by SDS-PAGE and SEC-HPLC. The results show that the band of each antibody on the SDS-PAGE non-reduced gel is about 150kD, the band on the SDS-PAGE reduced gel is about 50kD and about 25 kD, which were in line with the expected size. The SEC monomer purity of all antibodies was greater than 95%.

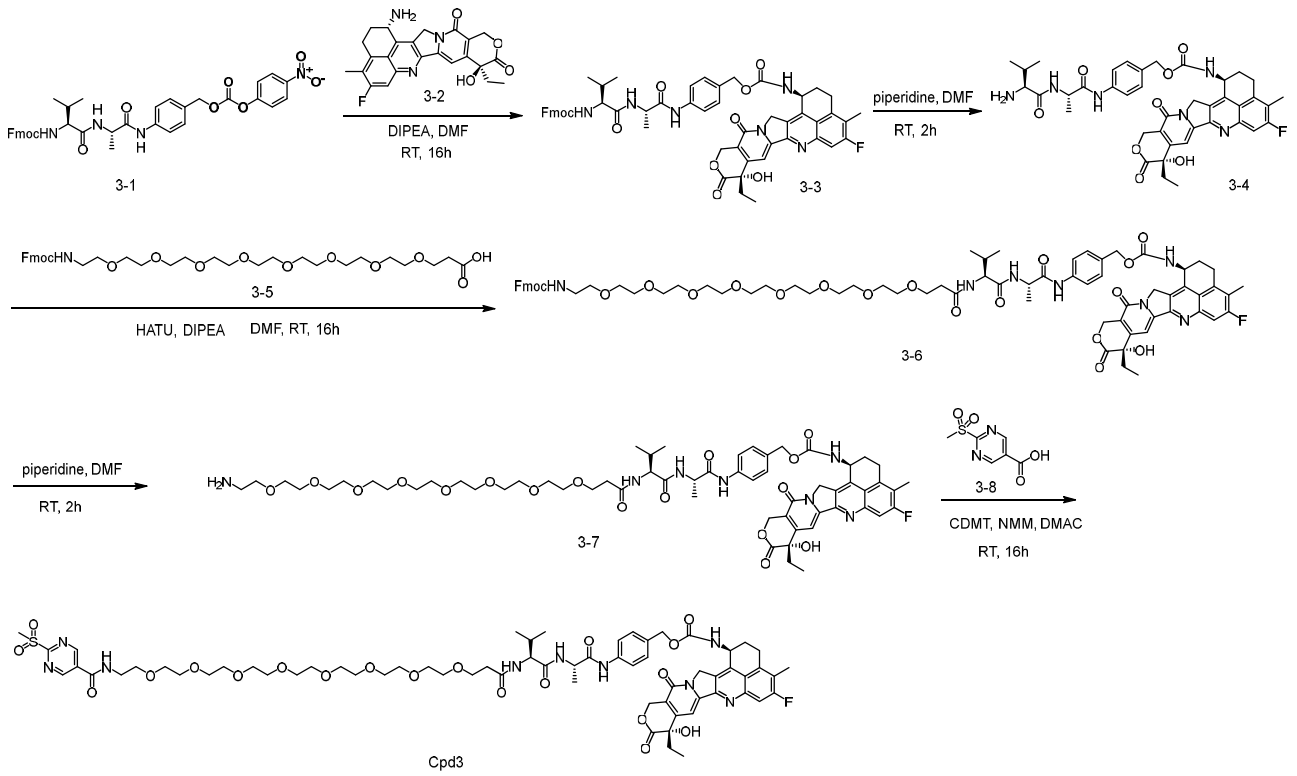
TABLE 1. amino acid sequences of CDR region of humanized antibodies

Clone number	**SEQ ID NO:**	**Sequence**	
HC18-01	1	HCDR1	SYWMH
	2	HCDR2	SIYPENSDTSYNQKFKG
	11	HCDR3	YHGYDADY
	4	LCDR1	SASSSVSYMY
	5	LCDR2	STSNLAS
	6	LCDR3	QQRSSYPYT

Example 2: preparation method of linker-payload

The preparation of the linkers and payloads used in the present invention, such as deruxtecan, GGFG-Dxd, Dxd, GGFG-Exd, and Val-Ala-PAB, refers to WO2014057687, WO1997046260, and CN101795711, which are incorporated herein by reference in their entirety.

Linker-payload Cpd3 used in the present invention and the preparation method thereof are as follows:



Step 1: (9H-fluoren-9-yl)methyl ((S)-3-methyl-1-(((S)-1-((4-((((4-nitrophenoxy)carbonyl) oxy)methyl)phenyl) amino)-1-oxopropan-2-yl)amino)-1-oxobutan-2-yl)carbamate (**3-1**, 2.5 g, 3.6 mmol), (1S,9S)-1-amino-9- ethyl-5-fluoro-9-hydroxy-4-methyl-1,2,3,9,12,15-hexahydro-10H,13H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13-dione (**3-2**, 1.6 g, 3.6 mmol), N,N-diisopropylethylamine (965 mg, 7.5 mmol) were dissloved in anhydrous N,N-dimethylformamide (20 mL), the reaction solution was stirred at room temperature for 16 hours. LCMS monitored the reaction was complete, 4-((S)-2-((S)-2-((((9H-fluoren-9-yl) methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl ((1S,9S)-9-ethyl-5-fluoro-9-hydroxy- 4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7] indolizino[1,2-b]quinolin-1-yl)carbamate (**3-3**) was obtained. LCMS (ESI) m/z: 977.3 $[M+H]^+$.

Step 2: To the reaction solution of step 1 was added hexahydropyridine (464 mg, 5.4 mmol) directly, the reaction solution was stirred at room temperature for 2 hours. LCMS monitored the reaction of the raw material was complete, the reaction solution was separated and purified by C18 column chromatography directly to obtain 4-((S)-2-((S)-2-amino-3-methylbutanamido) propanamido)benzyl ((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl- 10,13-dioxo-2,3,9,10,13,15 -hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl) carbamate (**3-4**) (1.8g). LCMS (ESI) m/z: 755.2 $[M+H]^+$.

Step 3: 1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13,16,19,22,25,28-nonaoxa-4-azahentriacontan-31-oic acid (**3-5**, 1.8 g, 2.38 mmol), 4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)benzyl ((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl- 10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de] pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamate (**3-4**, 1.8 g, 2.38 mmol), N,N-diisopropylethylamine (614 mg, 4.76 mmol) were dissloved in anhydrous N,N-dimethylformamide (10 mL), then the reaction was stirred at room temperature for 3 h. LCMS monitored the reaction was complete, 4-((33S,36S)-1-(9H-fluoren-9-yl)-33-isopropyl-

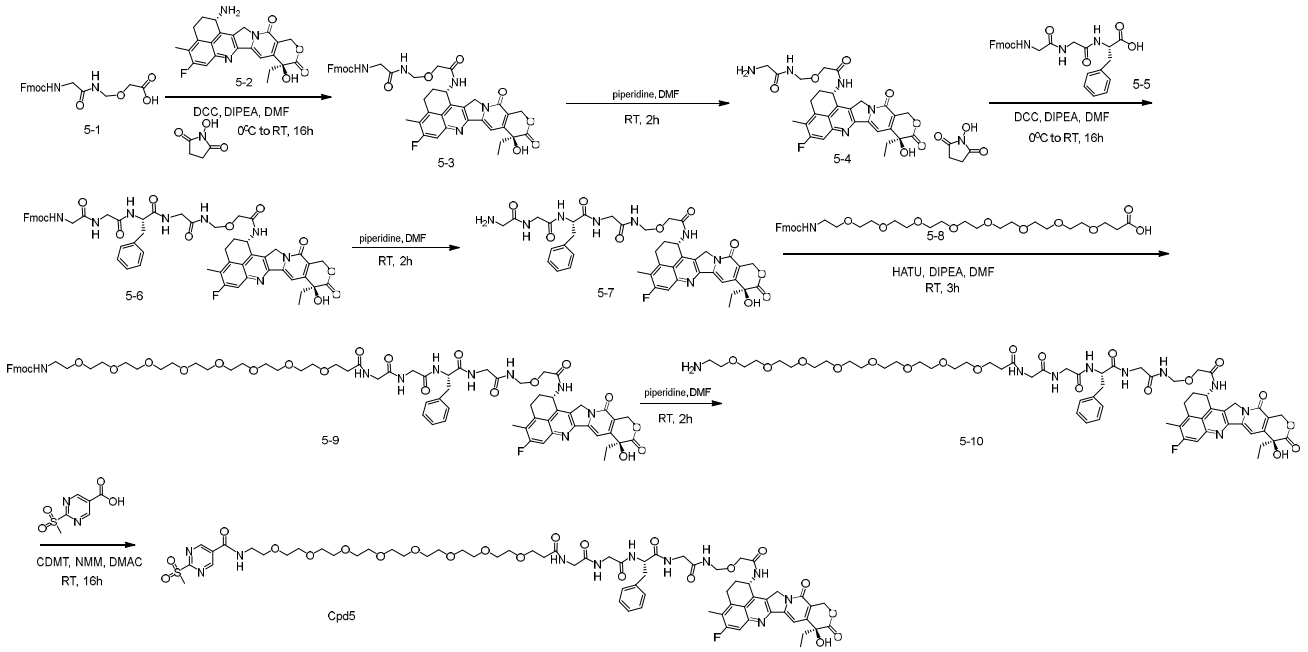
36-methyl-3,31,34-trioxo-2,7,10,13,16,19,22,25,28-nonaoxa-4,32,35-triazaheptatriacontan-37-amido)benzyl ((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo- 2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamate (**3-6**) was obtained. LCMS (ESI) m/z: 1400.5 $[M+H]^+$.

Step 4: To the reaction solution of step 3 was added hexahydropyridine (409 mg, 4.76 mmol) directly, the reaction solution was stirred at room temperature for 2 hours. LCMS monitored the reaction of the raw material was complete, the reaction solution was separated and purified by C18 column chromatography directly to obtain 4-((29S,32S)-1-amino-29-isopropyl-32-methyl-27,30 -dioxo-3,6,9,12,15,18,21,24-octaoxa-28,31-diaza-tritriacontan-33-amido)benzyl((1S,9S)-9-ethyl-5-fl uoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7] indolizino[1,2-b]quinolin-1-yl)carbamate (**3-7**) (1.2g). LCMS (ESI) m/z: 1178.4 $[M+H]^+$.

Step 5: 4-((29S,32S)-1-amino-29-isopropyl-32-methyl-27,30-dioxo-3,6,9,12,15,18,21,24-octaoxa-28,31-diaza-tritriacontan-33-amido)benzyl ((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15- hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino [1,2-b]quinolin-1-yl)carbamate (**3-7**, 20 mg, 0.017 mmol), 2-(methylsulfonyl)pyrimidine-5-carboxylic acid (**3-8**, 13.7 mg, 0.068 mmol), 4-(4,6-dimethoxytriazin-2-yl)-4-methylmorpholinium tetrafluoroborate (22.3 mg, 0.068 mmol) were dissloved in anhydrous N,N-dimethylacetamide (3 mL), the reaction solution was stirred at room temperature for 16 hours. The reaction was separated and purified by C18 column chromatography to obtain 4-((31S,34S)-31-isopropyl-34-methyl-1-(2-(methylsulfonyl)pyrimidin-5-yl)-1,29,32-trioxo-5,8,11,14,17,20,23,26- octaoxa-2,30,33-triazapentatriacontan-35-amido)benzyl ((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13- dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamate (i.e., **Cpd3**, 2.3 mg, yield: 10%).

^{1}H NMR (400 MHz, DMSO-d6) δ 9.95 (s, 1H), 9.37 (s, 1H), 9.14 (s, 1H), 8.46 (s, 1H), 8.21 (d, *J* = 6.8 Hz, 1H), 8.06 (d, *J* = 7.8 Hz, 1H), 7.89 (d, *J* = 8.3 Hz, 1H), 7.78 (d, *J* = 10.9 Hz, 1H), 7.60 (d, *J* = 8.4 Hz, 2H), 7.36 (d, *J* = 8.1 Hz, 2H), 7.31 (s, 1H), 6.53 (s, 1H), 5.45 (s, 2H), 5.28 (s, 3H), 5.08 (s, 2H), 4.42 – 4.34 (m, 1H), 4.22 – 4.17 (m, 1H), 3.62 – 3.53 (m, 10H), 3.52 – 3.46 (m, 28H), 2.38 (s, 3H), 2.33 (s, 1H), 2.20 (s, 3H), 2.01 – 1.81 (m, 4H), 1.30 (d, *J* = 7.0 Hz, 3H), 0.90 – 0.80 (m, 9H) .

Linker-payload Cpd5 used in the present invention and the preparation method thereof are as follows:



Step 1: 1-(9H-fluoren-9-yl)-3,6-dioxo-2,9-dioxa-4,7-diazaundecan-11-oic acid (**5-1**, 440 mg, 1.15 mmol), (1S,9S)-1-amino-9-ethyl-5-fluoro-9-hydroxy-4-methyl-1,2,3,9,12,15-hexahydro-10H,13H-benzo[de] pyrano [3',4':6,7]indolizino[1,2-b]quinoline-10,13-dione (500 mg, 1.15 mmol) were dissloved in anhydrous N,N-dimethylformamide (20 mL), N,N'-dicyclohexyl carbodiimide (308 mg, 1.5 mmol), N-hydroxysuccinimide (132 mg, 1.15 mmol) and N,N-diisopropylethylamine (148 mg, 1.15 mmol) were added under an ice-bath, then the reaction solution was stirred at room temperature for 16 hours. LCMS monitored the reaction was complete, the reaction was extracted with ethyl acetate and water, the organic phase was concentrated, the residue was purified on silica gel column with methanol and dichloromethane (1: 20) to obtain (9H-fluoren-9-yl)methyl (2-(((2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12 H-benzo[de] pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2-oxoethoxy)methyl)amino)-2-oxoethyl)carbamate (**5-3**, 650 mg). LCMS (ESI) m/z: 802.3 $[M+H]^+$.

Step 2: (9H-fluoren-9-yl)methyl (2-(((2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo- 2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b] quinolin-1-yl)amino)-2-oxoethoxy)methyl)amino)-2-oxoethyl)carbamate (**5-3**, 650 mg, 0.81 mmol) was dissloved in N,N-dimethylformamide (10 mL), then hexahydropyridine (69 mg, 5.4 mmol) was added, the reaction solution was stirred at room temperature for 2 hours. LCMS monitored the reaction of the raw material was complete, the reaction solution was seperated and purified by C18 column chromatography directly to obtain 2-amino-N-((2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydr o-1H,12H- benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2-oxoethoxy)methyl) acetamide (**5-4**, 420 mg). LCMS (ESI) m/z: 580.2 $[M+H]^+$.

Step 3: 2-Amino-N-((2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-

2,3,9,10,13,15- hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl) amino)-2-oxoethoxy)methyl)- acetamide (**5-4**, 420 mg, 0.73 mmol), (((9H-fluoren-9-yl)methoxy)carbonyl)glycylglycyl-L-phenylalanine (**5-5**, 370 mg, 0.73 mmol) were dissloved in anhydrous N,N-dimethylformamide (10 mL), N,N'-dicyclohexyl carbodiimide (195 mg, 0.95 mmol), N-hydroxysuccinimide (84 mg, 0.73 mmol) and N,N-diisopropylethylamine (94 mg, 0.73 mmol) were added under an ice-bath, then the reaction solution was stirred at room temperature for 16 hours. LCMS monitored the reaction was complete, the reaction was extracted with ethyl acetate and water, the organic phase was concentrated, the residue was purified on silica gel column with methanol and dichloromethane (1: 15) to obtain (9H-fluoren-9-yl)methyl ((S)-10-benzyl-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1,6,9,12,15- pentaoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-yl)carbamate (**5-6**, 550 mg). LCMS (ESI) m/z: 1063.5 $[M+H]^+$.

Step 4: (9H-fluoren-9-yl)methyl ((S)-10-benzyl-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl- 10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino [1,2-b]quinolin-1-yl)amino)- 1,6,9,12,15-pentaoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-yl) carbamate (**5-6**, 550 mg, 0.52 mmol) was dissloved in N,N-dimethylformamide (5 mL), then hexahydropyridine (44 mg, 0.52 mmol) was added, the reaction solution was stirred at room temperature for 2 hours. LCMS monitored the reaction of the raw material was complete, the reaction solution was seperated and purified by C18 column chromatography directly to obtain ((S)-10-benzyl-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H- benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1,6,9,12,15-pentaoxo-3-oxa-5,8,11,14-tetraaza- hexadecan-16-yl)amine (**5-7**, 400 mg). LCMS (ESI) m/z: 841.4 $[M+H]^+$.

Step 5: 1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13,16,19,22,25,28-nonaoxa-4-aza-hentriacontan-31-oic acid (347 mg, 0.52 mmol), HATU (274 mg, 0.72 mmol) and N,N-diisopropylethylamine (124 mg, 0.96 mmol) were dissloved in anhydrous N,N-dimethylformamide (5 mL), then the reaction was stirred at room temperature for 1 h, then ((S)-10-benzyl-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H- benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1,6,9,12,15-pentaoxo-3-oxa-5,8,11,14-tetraaza- hexadecan-16-yl)amine (**5-7**, 400 mg, 0.48 mmol) was added, then the reaction was stirred at room temperature for 16 h. LCMS monitored the reaction was complete, the reaction solution was purified by C18 column chromatography directly to obtain (9H-fluoren-9-yl)methyl ((S)-10-benzyl-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H- benzo[de]pyrano[3',4':6,7]indolizino[1,2-b] quinolin-1-yl)amino)-1,6,9,12,15,18-hexaoxo- 3,21,24,27,30,33,36,39,42-nonaoxa-5,8,11,14,17-pentaaza-tetratetracontan-44-yl)carbamate (**5-9**, 350 mg). LCMS (ESI) m/z: 1486.6 $[M+H]^+$.

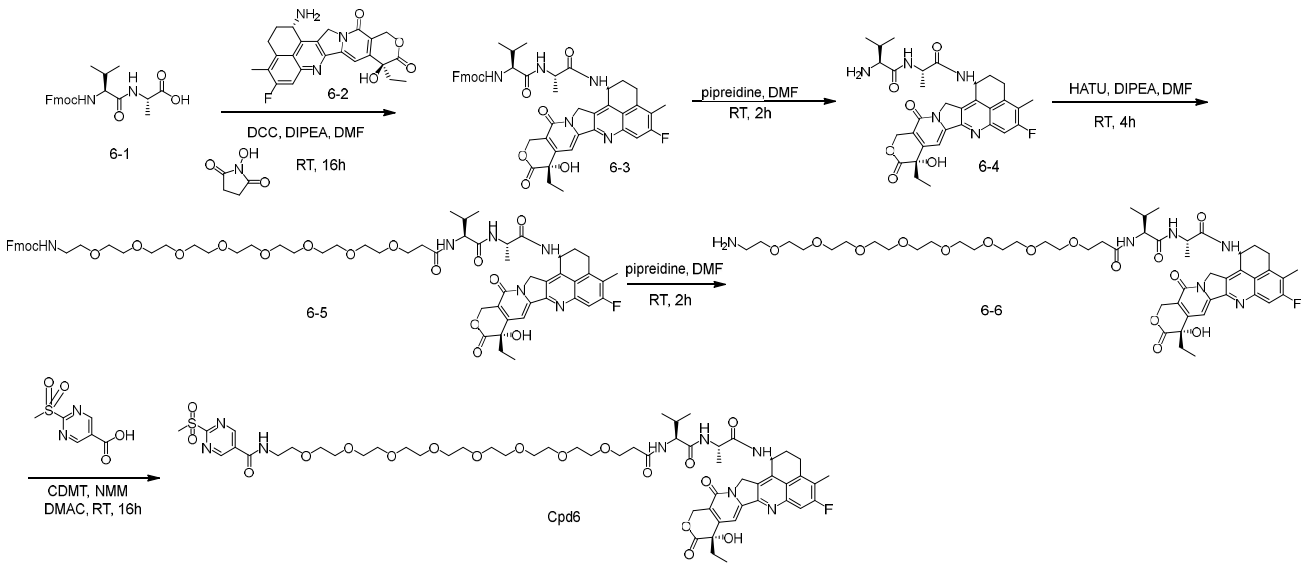
Step 6: (9H-fluoren-9-yl)methyl ((S)-10-benzyl-1-(((1S,9S)-9-ethyl-5-fluoro-

9-hydroxy-4-methyl- 10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7] indolizino[1,2-b]quinolin-1-yl)amino)- 1,6,9,12,15,18-hexaoxo-3,21,24,27,30,33,36,39,42-nonaoxa-5,8,11,14,17-pentaazatetratetracontan-44-yl)carbamate (**5-9**, 350 mg, 0.24 mmol) was dissloved in N,N-dimethylformamide (5 mL), then hexahydropyridine (20 mg, 0.24 mmol) was added, the reaction solution was stirred at room temperature for 2 hours. LCMS monitored the reaction of the raw material was complete, the reaction solution was seperated and purified by C18 column chromatography directly to obtain 1-amino-N-((S)-10-benzyl-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo- 2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7] indolizino[1,2-b]quinolin-1-yl)amino)-1,6,9,12,15- pentaoxo-3-oxa-5,8,11,14-tetraaza-hexadecan-16-yl)-3,6,9,12,15,18,21,24-octaoxa-heptacosan-27-amide (**5-10**, 200 mg). LCMS (ESI) m/z: 1264.6 $[M+H]^+$.

Step 7: 1-Amino-N-((S)-10-benzyl-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo- 2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino [1,2-b]quinolin-1-yl)amino)-1,6,9,12,15- pentaoxo-3-oxa-5,8,11,14-tetraaza-hexadecan-16-yl) -3,6,9,12,15,18,21,24-octaoxa-heptacosan-27-amide (**5-10**, 100 mg, 0.079 mmol), 2-(methylsulfonyl)pyrimidine-5-carboxylic acid (64 mg, 0.3 mmol), 2-chloro-4,6-dimethoxy-1,3,5-triazine (53 mg, 0.3 mmol), N-methylmorpholine (182 mg, 1.8 mmol) were dissloved in anhydrous N,N-dimethylacetamide (5 mL), the reaction solution was stirred at room temperature for 16 hours. Separated and purified by C18 column chromatography to obtain N-((S)-10-benzyl-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexa hydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1,6,9,12,15,18-hexa oxo- 3,21,24,27,30,33,36,39,42-nonaoxa-5,8,11,14,17-pentaaza-tetratetracontan-44-yl)-2-(methylsulfonyl)pyrimidine-5-carboxamide (i.e. **Cpd5**, 5mg). LCMS (ESI) m/z: 1448.6 $[M+H]^+$.

^{1}H NMR (400 MHz, DMSO-d6) δ 9.37 (d, *J* = 2.1 Hz, 2H), 9.12 (s, 1H), 8.63 (s, 1H), 8.51 (d, *J* = 8.6 Hz, 1H), 8.29 (s, 1H), 8.21 – 8.07 (m, 2H), 7.99 (s, 1H), 7.78 (d, *J* = 10.9 Hz, 1H), 7.31 (s, 1H), 7.21 (t, *J* = 15.2 Hz, 5H), 6.52 (s, 1H), 5.60 (s, 1H), 5.42 (s, 2H), 5.20 (s, 2H), 4.64 (d, *J* = 6.3 Hz, 2H), 4.47 (s, 1H), 4.02 (s, 2H), 3.76 – 3.66 (m, 4H), 3.59 – 3.53 (m, 8H), 3.51 – 3.45 (m, 29H), 2.39 (s, 5H), 2.18 (s, 2H), 2.00 (d, *J* = 7.9 Hz, 1H), 1.84 (dd, *J* = 15.4, 7.8 Hz, 2H), 1.33 – 1.20 (m, 5H), 1.12 (d, *J* = 6.6 Hz, 1H), 0.87 (t, *J* = 6.3 Hz, 3H) .

Linker-payload Cpd6 used in the present invention and the preparation method thereof are as follows:



Step 1: (((9H-fluoren-9-yl)methoxy)carbonyl)-L-valyl-L-alanine (**6-1**, 943 mg, 2.3 mmol), (1S,9S)-1-amino-9-ethyl-5-fluoro-9-hydroxy-4-methyl-1,2,3,9,12,15-hexahydro-10H,13H-benzo[de] pyrano [3',4':6,7]indolizino[1,2-b]quinoline-10,13-dione (1 g, 2.3 mmol) were dissloved in anhydrous N,N-dimethylformamide (20 mL), then N,N'-dicyclohexyl carbodiimide (616 mg, 3 mmol), N-hydroxysuccinimide (264 mg, 2.3 mmol) and N,N-diisopropylethylamine (297 mg, 2.3 mmol) were added under an ice-bath, then the reaction solution was stirred at room temperature for 16 hours. LCMS monitored the reaction was complete, the reaction was extracted with ethyl acetate and water, the organic phase was concentrated, the resiude was purified on silica gel column with methanol and dichloromethane (1: 20) to obtain (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro -1H,12H- benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1-oxopropan-2-yl)amino) -3-methyl-1-oxobutan-2-yl)carbamate (**6-3**, 730 mg). LCMS (ESI) m/z: 828.3 $[M+H]^+$.

Step 2: (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-(((1S,9S)-9-ethyl-5-fluoro-9 -hydroxy-4-methyl-10,13-dioxo- 2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7] indolizino[1,2-b]quinolin-1-yl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamat e (**6-3**, 730 mg, 0.88 mmol) was dissloved in N,N-dimethylformamide (10 mL), then hexahydropyridine (75 mg, 0.88 mmol) was added, the reaction solution was stirred at room temperature for 2 hours. LCMS monitored the reaction of the raw material was complete, the reaction solution was seperated and purified by C18 column chromatography directly to obtain (S)-2-amino-N-((S)-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-he xahydro- 1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1-oxopropan-2-yl)-3-methylbutanamide (**6-4**, 450 mg). LCMS (ESI) m/z: 606.2 $[M+H]^+$.

Step 3: 1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13,16,19,22,25,28-nonaoxa-4-azahentriacontan -31-oic acid (592 mg, 0.89 mmol), HATU (422 mg, 1.11 mmol) and N,N-diisopropylethylamine (143 mg, 1.11 mmol) were dissloved in anhydrous N,N-dimethylformamide (5 mL), then the reaction was stirred at room temperature for 1 h, then

(S)-2-amino-N-((S)-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro- 1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1-oxopropan-2-yl)-3-methylbutanamide (450 mg, 0.74 mmol) was added, then the reaction was stirred at room temperature for 4h. LCMS monitored the reaction was complete, the reaction solution was purified by C18 column chromatography directly to obtain (9H-fluoren-9-yl)methyl (29S,32S)-33-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-29-isopropyl-32-methyl- 27,30,33-trioxo-3,6,9,12,15,18,21,24-octaoxa-28,31-diazatritriacontyl)carbamate (**6-5**, 400 mg). LCMS (ESI) m/z: 1251.6 $[M+H]^+$.

Step 4: (9H-fluoren-9-yl)methyl ((29S,32S)-33-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo- 2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-29-isopropyl- 32-methyl-27,30,33-trioxo-3,6,9,12,15,18,21,24-octaoxa-28,31-diazatritriacontyl)carbamate (**6-5**, 400 mg, 0.32 mmol) was dissloved in N,N-dimethylformamide (5 mL), then hexahydropyridine (27 mg, 0.32 mmol) was added, the reaction solution was stirred at room temperature for 2 hours. LCMS monitored the reaction of the raw material was complete, the reaction solution was seperated and purified by C18 column chromatography directly to obtain 1-amino-N-((S)-1-(((S)-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro- 1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1- oxobutan-2-yl)-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-amide (**6-6**, 210 mg). LCMS (ESI) m/z: 1029.6 $[M+H]^+$.

Step 5: 1-Amino-N-((S)-1-(((S)-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo- 2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-amide (**6-6**, 100 mg, 0.097 mmol), 2-(methylsulfonyl)pyrimidine-5-carboxylic acid (78 mg, 0.39 mmol), 2-chloro-4,6-dimethoxy-1,3,5-triazine (68 mg, 0.39 mmol) and N-methylmorpholine (59 mg, 0.582 mmol) were dissloved in anhydrous N,N-dimethylacetamide (5 mL), the reaction solution was stirred at room temperature for 16 hours. The reaction was separated and purified by C18 column chromatography to obtain N-((29S,32S)-33-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H- benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl) amino)-29-isopropyl-32-methyl-27,30,33-trioxo- 3,6,9,12,15,18,21,24-octaoxa-28,31-diazatritriacontyl)-2-(methylsulfonyl)pyrimidine-5-carboxamide (i.e., **Cpd6**, 11.5 mg). LCMS (ESI) m/z: 1213.6 $[M+H]^+$

^{1}H NMR (400 MHz, DMSO-d6) δ 9.36 (s, 2H), 9.13 (s, 1H), 8.41 (d, *J* = 8.9 Hz, 1H), 8.10 (d, *J* = 6.9 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.79 (d, *J* = 11.0 Hz, 1H), 7.30 (s, 1H), 6.53 (s, 1H), 5.54 (s, 1H), 5.42 (s, 2H), 5.24 (d, *J* = 18.9 Hz, 1H), 5.11 (d, *J* = 19.0 Hz, 1H), 4.32 – 4.21 (m, 1H), 4.17 – 4.06 (m, 1H), 3.58 – 3.52 (m, 8H), 3.48 (s, 25H), 3.44 (s, 6H), 2.40 (s, 3H), 2.29 (dd, *J* = 13.7, 7.1

Hz, 1H), 2.13 (s, 2H), 1.91 (ddd, *J* = 23.2, 22.6, 7.6 Hz, 4H), 1.27 (d, *J* = 7.0 Hz, 3H), 0.87 (t, *J* = 7.4 Hz, 3H), 0.83 – 0.72 (m, 6H) .

Example 3: FGFR2b-ADC preparation

The antibody stock solution (HC18-01, 15mg) was taken from the refrigerator (-80 °C), added to a centrifuge tube after thawing, and then PB (20 mM, pH 7.2, 751 uL) buffer, DTPA-3Na (10 mM, 375 uL), TCEP (10 mM, 94 uL) solution were added, and the mixture was reacted at 25 °C for 2 h. Then, the linker-payload described in Example 2, such as Cpd3 (5 mM, 272 uL) was added, and reacted at 25 °C for 2 h. After the reaction was complete, the reaction was added to an ultrafiltration centrifuge tube (30 KD) and dialyzed with His/His-HCl (10 mM, pH 5.5) to obtain the ADC stock solution (11.4 mg), DAR: 7.5~8, SEC purity≥95%.

As an example, the linker-payload used in the above experiment is Cpd3 described in Example 2. The antibody stock solution can be HC18 or HC18-01; the linker-payload may be a combination of linker and camptothecins that are common in the art, including but not limited to deruxtecan, Cpd3, Cpd5, Cpd6, etc.

Using humanized antibody HC18-01, the above experiments were carried out with other linker-payloads. Each ADC sample was identified, and the test results are shown in Table 2 below.

TABLE 2. test results of ADC sample

Antibody	Antibody drug conjugates	Linker-Payload	ADC test result		
			DAR	SEC purity	Free drug
HC18-01	HC18-01-ADC1	Cpd3	7.7	98.2%	0.68%
	HC18-01-ADC2	Cpd5	7.6	99.3%	0.004%
	HC18-01-ADC3	Cpd6	7.7	99.4%	Not detectable

Example 4 Proliferation inhibition assay

In this example, the binding ability of HC18-deuxtecan ADC and FPA144 antibodies to human FGFR2b on the surface of tumor cells SNU-5 and SNU-16 (cell line sources are shown in Table 3) was examined by FACS method using Staurosporine as a positive control. Wherein the SNU-5 cell surface does not express FGFR2b.

TABLE 3 sources of cell lines

Cell lines	generation	Cancer species	supplier	Cat. No.	Culture Properties	Culture medium
SNU-5	P22	gastric cancer	ATCC	CRL-5973	suspension	IMDM + 20% FBS
SNU-16	P18	gastric cancer	ATCC	CRL-5974	suspension	RPMI-1640 (GIBCO, #22400-089) + 10% FBS

Experimental method: SNU-5 and SNU-16 were cultured at 37°C and 5% CO_2. Each tumor cell line was routinely subcultured, and cells in the logarithmic growth phase were harvested, counted,

and plated into 96-well plates (Greiner CELLSTAR® 96-well plates, #655090). The cell concentration was adjusted to an appropriate cell density, and 135 μL of cell suspension was added to each well of the 96-well plate, so that the cell density of SNU-5 and SNU-16 was 5000/well, 3000/well, and 2000/well, respectively. 135 μL of culture medium was added to the blank wells, and cultured overnight at 37°C, 5% CO_2, and 100% RH. 15 μL of ADC samples diluted in series (3000, 300, 30, 3 nM) were added to each well, and 15 μL of culture medium was added to the blank wells; the cells were cultured at 37°C, 5% CO_2 for 6 days; the luminescent cell survival rate was determined according to the Promega CellTiter Glo kit (Promega-G7573), and the luminescence was read using a 2104 EnVision multi-label plate reader (PerkinElmer).

The results are shown in FIGs. 1-2: HC18-deuxtecan showed better cell proliferation inhibition than FPA144 in both stomach cancer cells SNU-5 (FIG. 1) and SNU-16 (FIG. 2).

Example 5: affinity of humanized antibodies to antigens of different species

This Example examined the affinity of the humanized antibody HC18-01 for antigenic protein human FGFR2 (IIIb), cynomolgus monkey FGFR2 (IIIb), rat FGFR2 (IIIb), and mouse FGFR2 (IIIb).

The experimental method comprises the following steps:

1. Capture of antibodies

The experiment uses 1× PBS buffer (containing 0.05% Tween 20, pH 7.4) as the test buffer. The antibody was diluted to 1 μg/mL with PBS buffer, the flow rate was set to 10 μL/min, and the antibody was directly captured on the test surface of the Protein A chip. The capture was continued for 20 s, and the capture amount could reach 80-100 RU level.

2. Test conditions for FGFR2b samples

A total of 8 analytical concentrations were set for the antigen. For Human FGFR2b and Cyno FGFR2b, the concentration gradient was 0 nM, 1.5625 nM, 3.125 nM, 6.25 nM, 12.5 nM, 20 nM, 50 nM, 100 nM respectively; For Mouse FGFR2b and Rat FGFR2b, the concentration gradients was 0 nM, 6.25 nM, 12.5 nM, 20 nM, 50 nM, 100 nM, 200 nM, 400 nM, respectively. The flow rate was set at 30 μL/min, the association time was 120 s, the dissociation time was 360 s, and the test temperature was 25 ℃ during sample analysis.

3. Regeneration conditions

Gly-HCl buffer (pH 1.5, 10 mM) was used as regeneration buffer, and the flow rate during regeneration was set at 30 μL/min for 30 s. After regeneration is complete, the chip was stabilized for 60 s before analyzing the next sample.

4. Determination of kinetic parameters

The experiment was run in multiple cycles, analysis time as the abscissa and response signal values as the ordinate. The obtained data are fitted through BIAcore T200 analysis software, the adopted fitting model is a 1:1 Langmuir binding model, and kinetic constants such as an association rate constant, a dissociation rate constant and an association dissociation constant are determined.

The results are shown in Table 4. It shows that the HC18-01 antibody has good affinity for FGFR2b of various species.

Table 4: Binding activity of humanized antibodies against antigens of different species

Antigen	ka (1/Ms)	kd (1/s)	T1/2(s)	KD(M)	$Chi^2(RU^2)$
Human FGFR2b	5.38E+05	1.90E-03	365.7	3.52E-09	0.384
Cynomolgus monkey FGFR2b	5.85E+05	1.96E-03	353.4	3.35E-09	0.564
Mouse FGFR2b	6.57E+04	3.08E-03	224.9	4.69E-08	0.0937
Rat FGFR2b	3.87E+04	9.08E-04	763.1	2.35E-08	0.126

Example 6: Antitumor activity of ADC in SNU-16 mouse subcutaneous transplantation tumor model

In this example, the anti-tumor effect of ADC (HC18-deuxtecan) in animals was evaluated, with FPA144 antibody as the control, and the tumor cells used were gastric cancer cell SNU-16.

Experimental method: female Balb/C nude mice (animal source: Charles River) aged 6-8 weeks and weighing about 20 g were used, 10×10^6 SNU-16 cells were injected into each nude mouse subcutaneously on one side (the SNU-16 cells were cultured in an adherent way, the culture condition was the culture medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin , 37 ºC, 5% CO_2. Routine passage was performed twice a week. When the cells were in an exponential growth period, the cells were collected, counted and inoculated), the inoculation volume was 0.2 mL, and when the average tumor volume reached about 150-200mm^3, the random grouping and cage division was carried out. There were 8 tumor-bearing nude mice in each group, and there were 4 groups in total, including a Vehicle negative control group, a human IgG1-deruxtecan (Dxd) group, a HC18-deruxtecan group, and an FPA144 group (dosage of 10mg/kg, dosing volume of 1 μl/g), and the dosing method was a single dose of 10mg/kg by tail vein injection. Tumor volumes were measured 2 times per week for a total of 11 times/5 weeks. The tumor volume was calculated as: $V = 0.5a \times b^2$, a and b represent the long diameter (mm) and short diameter (mm) of the tumor, respectively. Five weeks after the end of the administration, the mice were euthanized, the tumors were taken and the tumor weight was measured, the tumor volume, tumor weight and mouse weight change data were analyzed to calculate the tumor inhibition rate.

The results of the experiments, as shown in TABLE 5 and FIGs. 3-4, show that there was no significant difference between the weights of mice in each group and there was no significant change in the weight of mice in each group during the treatment, and the specific results, as shown in FIG. 4, indicate that the mice have good tolerance to ADC. HC18-deruxtecan showed significant tumor inhibitory effects, and the specific results are shown in FIG. 3.

TABLE 5 Tumor inhibition by ADC in tumor-bearing mice

	Tumor volume (V) Calculation: $V=a \times b^2/2$ (where a is the longest of the tumor diameters and b is the shortest of the tumor diameters)								
	Measurement (day)								
	8	11	15	18	22	25	29	32	36
PBS	215.5	275	344.625	415.375	518.625	603.75	715.875	816	954.25
HC18-deruxtecan	87	67.25	44.75	44	44	43	69.25	77.25	105.375

10mg/kg									
Human IgG1-Dxd 10mg/kg	160.5	163.625	185.875	234.5	263.5	266.5	314.25	354.75	394.375
FPA144 10mg/kg	165.125	170.25	180.25	196	207.375	229	252	260.25	259.375

Example 7: Antitumor activity of ADC in SNU-16 mouse subcutaneous transplantation tumor model

In this example, the anti-tumor effects of different ADC molecules (HC18-01-derxtecan, HC18-01-ADC1, HC18-01-ADC2 and HC18-01-ADC3) of humanized antibody HC18-01 in animals were evaluated, and the tumor cells used were gastric cancer cells SNU-16.

Experimental method: female Balb/C nude mice (animal source: Charles River) aged 6-8 weeks and weighing about 20 g were used, 10×10^6 SNU-16 cells are injected into each nude mouse subcutaneously on one side (the SNU-16 cells were cultured in an adherent way, the culture condition was the culture medium supplemented with10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin, 37 ℃, 5% CO_2. Routine passage was performed twice a week. When the cells were in an exponential growth period, the cells were collected, counted and inoculated), the inoculation volume was 0.2 mL, and when the average tumor volume reached about 150-200mm^3, the random grouping and cage division was carried out. There were 8 tumor-bearing nude mice in each group, and there were 11 groups in total, including a Vehicle negative control group, HC18-deuxtecan group, HC18-01-deuxtecan group, HC18-01-ADC1 group, HC18-01-ADC2 group and HC18-01-ADC3 group (dosage of 1mg/kg, 3mg/kg, dosing volume of 1 μl/g in the experimental groups), and the dosing method was a single dose of 1 or 3mg/kg of single administration by tail vein injection. Tumor volumes were measured 2 times per week for a total of 11 times/5 weeks. The tumor volume was calculated as: $V = 0.5a \times b^2$, a and b represent the long diameter (mm) and short diameter (mm) of the tumor, respectively. Five weeks after the end of the administration, the mice were euthanized, the tumors were taken and the tumor weight was measured, the tumor volume, tumor weight and mouse weight change data were analyzed to calculate the tumor inhibition rate.

The results of the experiments, as shown in FIGs. 5 and 6, show that there was no significant difference between the weights of mice in each group and there was no significant change in the weight of mice in each group during the treatment, and the specific results, as shown in FIG. 6, indicate that the mice have good tolerance to ADC. HC18-01-deruxtecan, HC18-01-ADC1, HC18-01-ADC2, and HC18-01-ADC3 showed significant tumor inhibitory effects, and the specific results are shown in FIG. 5.

Example 8 Anti-tumor effect of antibody drug conjugate HC18-01-ADC1 in the SNU-16 human gastric cancer transplantation tumor model

This Example further explores the inhibitory effect of different single doses of HC18-01-ADC1

on the growth of human gastric cancer SNU-16 transplantation tumors. The experimental method is the same as described in Example 8.

In this Example, a Vehicle negative control group, a 0.5mg/kg HC18-01-ADC1 group, a 1mg/kg HC18-01-ADC1 group, a 3mg/kg HC18-01-ADC1 group, a 10mg/kg HC18-01-ADC1 group, and a 10mg/kg commercially available Bemarituzumab positive control group were set up.

The results of the efficacy of the treatments of the experimental groups are shown in TABLE 6 and FIGs. 7 to 8.

TABLE 6: Inhibitory effect of HC18-01-ADC1 at different doses on human gastric cancer SNU-16 transplantation tumor

Group	**Treatment (i.v., single dose)**	**TGI (%)**	**T/C (%)**	**PRR**	**CRR**
1	Solvent control (Vehicle)	-	-	-	-
2	HC18-01-ADC1, 0.5 mg/kg	35.29	64.71	0/8	0/8
3	HC18-01-ADC1, 1 mg/kg	70.61	29.39	0/8	0/8
4	HC18-01-ADC1, 3 mg/kg	80.00	20.00	0/8	0/8
5	HC18-01-ADC1, 10 mg/kg	97.05	2.95	8/8	0/8
6	Bemarituzumab, 10 mg/kg	22.90	77.10	0/8	0/8

Note:
TGI: Tumor Growth Inhibition Rate
CR: Complete Response, i.e., tumor volume =0 mm^3;
PR: Partial Response, i.e., at least a 30% reduction in tumor volume relative to baseline
CRR: Complete Response Rate, which refers to the proportion of subjects who completely eradicate the tumor, i.e. CR/number of animals used in the experiment
PRR: Partial Response Rate, which refers to the proportion of subjects with partially reduced tumors after treatment, i.e., PR/number of animals used in the experiment
T/C: Relative Tumor Proliferation Rate, T/C (%) = $V_T/V_c \times 100$ (V_T: average tumor volume of a treatment group; V_c: average tumor volume of a solvent control group)

FIG. 7 shows the antitumor efficacy of HC18-01-ADC1 at different doses. A single dose of 0.5-10 mg/kg HC18-01-ADC1 can inhibit the growth of SNU-16 transplantation tumor in a dose-dependent manner, and the anti-tumor effect of 10 mg/kg HC18-01-ADC1 is superior to the anti-tumor effect of the equal dose of Bemarituzumab.

FIG. 8 shows the changes in the body weight of mice at different doses. The experimental results showed that there was no significant difference in the body weight of mice in each group.

Example 9. Anti-tumor effect of the antibody drug conjugate HC18-01-ADC1 on KATO-III human gastric cancer transplantation tumor model

This Example further explores the inhibitory effect of different single doses of HC18-01-ADC1 on the growth of human gastric cancer **KATO-III** transplantation tumors.

In the embodiment, a Vehicle negative control group, a 0.4mg/kg HC18-01-ADC1 group, a 2mg/kg HC18-01-ADC1 group, a 10mg/kg HC18-01-ADC1 group, a 10mg/kg commercially

available Bemarituzumab positive control group and a 10mg/kg HC18-01 naked antibody control group were set up. The experimental protocol was the same as described in Example 11.

Human gastric cancer KATO-III cells were cultured adherently in IMDM medium (supplemented with 20% fetal bovine serum and 1% Antibiotic-Antimycotic) at 37ºC and 5% CO_2. They were routinely subcultured twice a week using trypsin digestion. When the cells were in exponential growth phase and the cell viability was greater than 95%, the cells were harvested, counted and inoculated.

TABLE 7 and FIGs. 9-10 show the results of the experiments.

TABLE 7: Treatment results of HC18-01-ADC1 on KATO-III human gastric cancer transplantation tumor at day 60

Group	Treatment (i.v., single dose)	TGI (%)	T/C (%)	PRR	CRR	IR (%)
1	Vehicle	-	-	-	-	
2	HC18-01-ADC1, 0.4 mg/kg	30.51	69.48	0/8	0/8	30.90
3	HC18-01-ADC1, 2 mg/kg	99.27	0.73	8/8	3/8	99.26
4	HC18-01-ADC1, 10 mg/kg	99.98	0.02	8/8	7/8	99.99
5	Bemarituzumab, 10 mg/kg	80.36	19.64	2/8	0/8	79.80
6	HC18-01 10 mg/kg	66.61	33.38	1/8	0/8	66.30

Note:
TGI: Tumor Growth Inhibition Rate ;
CR: Tumor volume =0 mm^3;
PR: At least a 30% reduction in tumor volume relative to baseline;
CRR: The proportion of subjects who completely eradicate the tumor, i.e. CR/number of animals used in the experiment;
PRR: the proportion of subjects with partially reduced tumors after treatment, i.e., PR/number of animals used in the experiment;
T/C: Relative Tumor Proliferation Rate, T/C (%) = $V_T/V_c \times 100$ (V_T: average tumor volume of a treatment group; V_c: average tumor volume of a solvent control group);
IR: Tumor Weight Inhibition Rate, IR (%) = [1- (average tumor weight of a treatment group/average tumor weight of solvent control group)] ×100.

FIG. 9 shows the anti-tumor efficacy of HC18-01-ADC1 at different doses. A single dose of 0.4-10 mg/kg HC18-01-ADC1 can dose-dependently and potently inhibit the growth of KATO-III transplantation tumors; 0.4 mg/kg HC18-01-ADC1 has equivalent anti-tumor effects to 10 mg/kg HC18-01; the anti-tumor effects of 10 mg/kg HC18-01-ADC1 is superior to the anti-tumor effects of equal dose of Bemarituzumab.

FIG. 10 shows the changes in the body weight of mice at different doses. The experimental results showed that there was no significant difference in the body weight of mice in each group.

Example 10 Anti-tumor effect of antibody drug conjugate HC18-01-ADC1 on human gastric cancer xKATO-III transplantation tumor model

In the embodiment, a human gastric cancer xKATO-III mouse subcutaneous xenograft tumor model is used for evaluating the in-vivo anti-tumor effect of a test substance HC18-01-ADC1 and the naked antibody HC18-01 thereof on the human gastric cancer, and the efficacy was compared with the test substances Bemarituzumab and Paclitaxel (paclitaxel injection, source: Sichuan Huiyu Pharmaceutical), respectively. At the same time, the efficacy under different dosing regimens were explored to provide a reference for the selection of clinical indications and the design of clinical trial protocols for the test substance HC18-01-ADC1.

Human gastric cancer xKATO-III cells were cultured adherently in IMDM medium (supplemented with 20% fetal bovine serum and 1% Antibiotic-Antimycotic) at 37ºC and 5% CO_2. They were routinely subcultured twice a week using trypsin digestion. When the cells were in exponential growth phase and the cell viability was greater than 95%, the cells were harvested, counted and inoculated (inoculation passage P9).

Cell line	Tumor type	Origin	Supplier	Cat. No.
KATO-III	gastric cancer	Human	American Type Culture Collection	HTB-103
xKATO-III	gastric cancer	Human	Wuxi Apptec, KATO-III was subcutaneously inoculated on CB17 SCID mice, and tumors were taken to prepare cell suspensions	/

Female SCID Beige mice (Shanghai Lingchang Biotechnology Co., Ltd.) aged 6-8 weeks and weighing around 20 g were inoculated with 0.2 mL of a mixed cell suspension containing $10*10^6$ xKATO-III cells and 50% matrix gel. When the average tumor volume reached 202 mm^3, the mice were randomly divided into groups for drug administration. The administration settings are shown in TABLE 8.

TABLE 8: Administration regimen of HC18-01-ADC1

Group	Nunmber	Drug	Dosage (mg/kg)	Volume administered (mL/kg)	Route of administration	Frequency of administration
1	8	Vehicle	-	10	Intravenous Injection (IV)	Single administration
2	8	HC18-01-ADC1	0.4	10		
3	8	HC18-01-ADC1	2	10		
4	8	HC18-01-ADC1	10	10		
5	8	HC18-01	10	10		
6	8	Bemarituzumab	10	10		
7	8	Paclitaxel	10	10		Once every three days *4 weeks

Tumor volumes were measured 2 times per week and were calculated as: $V = 0.5a \times b^2$, a and b represent the long diameter (mm) and short diameter (mm) of the tumor, respectively.

Evaluation of efficacy indicators TGI, T/C, the number of mice with Complete Response (CR)

or Partial Response (PR), Tumor Weight Inhibition Rate (IR), and the like was performed. Wherein each indicator is calculated according to the following formula or mode:

1. TGI (%) = [1-(V_{Ti}/V_{T0})/(V_{ci}/V_{c0})]×100% (V_{Ti}: average tumor volume of the treatment group on the day i of administration, V_{T0}: average tumor volume of the treatment group on the day 0 of administration, V_{ci}: average tumor volume of the solvent control group on the day i of administration, V_{c0}: average tumor volume of the solvent control group on the day 0 of administration);

2. T/C (%) = V_T/V_c× 100 (V_T: average tumor volume of the treatment group; V_c: average tumor volume of the solvent control group).

3. The number of mice with Complete Response CR or Partial Response PR, CR means that the mouse tumor volume is 0 mm^3, PR means that the mouse tumor volume decreases by more than 30% compared with that when grouping.

4. IR (%) = [1- (average tumor weight of the treatment group / average tumor weight of the solvent control group)] ×100%.

Experimental data are expressed as Mean ± standard error (Mean ± SEM). Statistical analyses were performed to assess inter-group differences based on data obtained at the end of the experiment. Differences between groups were statistically analyzed using Graphpad Prism 10.0 software and One-way Analysis of Variance (ANOVA). $p < 0.05$ was considered to be significant..

The results of the experiment are shown in FIGs. 11-12. The test substances HC18-01-ADC1, HC18-01, Bemarituzumab and Paclitaxel had a small effect on the body weight of mice with xKATO-III subcutaneous transplantation tumors. There was no significant difference in the body weight and body weight change rate of animals in each dosing group and the Vehicle group on day 60. There was no significant change in the body weight of animals in each group during the entire experimental period. There was no abnormality in clinical observation. The drug dosage was safe under the dosing regimen of this example.

The average tumor volumes of xKATO-III subcutaneous transplantation model mice were measured on day 60 (D60). The effect of the test substances on the tumor weight of the xKATO-III subcutaneous transplantation tumor model is shown in FIG. 12. Compared with the Vehicle group, except for the 0.4 mg/kg HC18-01-ADC1 group (p>0.05), the average tumor volume of the other dosing groups was significantly reduced (p<0.0001, $^{****}p$<0.0001 means compared with the solvent control Vehicle group); the average tumor volume of 10 mg/kg HC18-01-ADC1 was significantly smaller than that of 0.4 mg/kg HC18-01-ADC1 (p<0.0001, $^{\#\#\#\#}p$<0.0001 means compared with HC18-01-ADC1 0.4 mg/kg).

T/C (%) and TGI (%) of the test substance to xKATO-III subcutaneous transplantation tumor model were calculated based on D60 tumor volume, IR (%) was calculated based on D60 Tumor Weight (TW), and CR rate and PR rate were calculated based on tumor remission, and the results are shown in TABLE 9 below.

TABLE 9: D60, inhibition results of HC18-01-ADC1 on xKATO-III subcutaneous transplantation tumors

Group	Tumor volume (mm^3 Mean±SEM)	T/C （%）	TGI （%）	TW (g, Mean±SEM)	IR (%)	CR	PR
Vehicle	1751.96±349.78	-	-	1.795±0.987	-	-	-
HC18-01-ADC1 0.4 mg/kg	1217.36±240.97	69.48	30.51	1.240±0.665	30.90	0/8	0/8
HC18-01-ADC1 2 mg/kg	12.80±5.66 ****####	0.73	99.27	0.013±0.017 ****####	99.26	3/8	8/8
HC18-01-ADC1 10 mg/kg	0.30±0.30 ****####	0.02	99.98	0.000±0.000 ****####	99.99	7/8	8/8
HC18-01 10 mg/kg	584.94±141.04 ****	33.38	66.61	0.605±0.427 ****	66.30	0/8	1/8
Bemarituzumab 10 mg/kg	344.02±74.42 ****	19.64	80.36	0.363±0.229 ****	79.80	0/8	2/8
Paclitaxel 10 mg/kg	84.36±22.99 ****	4.82	95.18	0.083±0.062 ****	95.38	0/8	5/8

$^{****}p<0.0001$ compared with the solvent control group，$^{####}p<0.0001$ compared with HC18-01-ADC1 0.4 mg/kg group

A single administration of HC18-01-ADC1 can significantly inhibit the growth of human gastric cancer xKATO-III mouse subcutaneous transplantation tumors. The higher the dose in the range of 2-10 mg/kg, the better the tumor inhibitory effect, showing a significant dose-related effect. The tumor growth inhibitory effect of HC18-01-ADC1 is long-lasting, and no rebound effect was observed 60 days after a single administration of 10 mg/kg. At the equal dose, HC18-01-ADC1 has a better tumor inhibitory effect than Bemarituzumab and Paclitaxel.

Example 11 Anti-tumor effect of antibody drug conjugate HC18-01-ADC1 on lung cancer tumor xenograft (PDX) model

The purpose of the experiment is to evaluate the antitumor effect of the test substance HC18-01-ADC1 on a human lung cancer tumor xenograft (PDX) model, and to compare the drug effect with the test substances Docetaxel and Bemarituzumab.

In the embodiment, a human lung cancer tumor tissue sample (NO. LD1-0025-200783) is selected for carrying out an efficacy experiment, and the sample has positive FGFR2 expression through verification.

A tumor tissue (NO. LD1-0025-200783, FP2+1 generation) was obtained from the biobank, cleaned and trimmed aseptically, non-tumor tissues such as blood clots, fat, and internal necrotic foci were removed, and the tissue was trimmed into a suitable 3×3×3 mm^3 tissue block, washed again, and an appropriate amount of Matrigel matrix gel was added according to the volume of the tumor

block, and placed on an ice box for standby. After Nu/Nu mice were treated according to standard operating procedures, 3×3×3 mm^3 tumor tissue blocks were taken and inoculated into the subcutaneous part of the limbs of Nu/Nu mice, and 1-2 sites were inoculated per animal. The tumor volume and body weight of the mice were observed twice a week. When the tumor volume reached about 100-200 mm^3, it indicated that the tumor tissue was successfully revived. After the tumor tissue was successfully revived, when the average tumor volume grew to 620 mm^3, the tumor-bearing animals were euthanized and the tumor was removed in a biosafety cabinet. After removing non-tumor tissue and necrotic tissue, the tumor tissue blocks for inoculation (FP2+2 generation) were made. BALB/c Nude mice were used for inoculation, and the tumor tissue blocks (FP2+2 generation) were divided into several 3×3×3 mm^3 tissue blocks and inoculated subcutaneously into the mice (FP2+3 generation). After the mice were inoculated, the tumor volume and body weight changes were monitored twice a week. When the average tumor volume reached 118 mm^3 (tumor latency was 14 days), it meant that the PDX modeling was successful and met the grouping criteria.

The PDX model mice were administrated according to the scheme disclosed in Table 10.

TABLE 10. Administration regimen of HC18-01-ADC1 for treatment of human lung cancer tumor xenografts

Group	**Number**	**Drug**	**Dosage (mg/kg)**	**Administration mode**	**Frequency of administration**
1	6	Solvent control (Vehicle)	-	Intravenous Injection *i.v.*	Single administration
2	6	HC18-01-ADC1	3		
3	6	HC18-01-ADC1	10		
4	6	Bemarituzumab	10		
5	6	Docetaxel	10		Once a week *4 weeks

Body weights were recorded twice a week at fixed times, tumor diameters were measured twice a week with a vernier caliper to calculate tumor volume.

The tumor volume was calculated as: $V = 0.5a \times b^2$, a and b represent the long diameter (mm) and short diameter (mm) of the tumor, respectively.

At the end of the experiment, after euthanasia of the animals, tumors were dissected and weighed.

1. The rate of change of body weight of the mice over the experimental period was calculated, RCBW（%）=(BW_{Di}-BW_{D0})/BW_{D0}×100% (BW_{Di}: body weight on day i of administration, BW_{D0}: body weight on day 0 of administration).

2. The anti-tumor efficacy of the drug on mice was evaluated by TGI (Tumor Growth

Inhibition, %) or relative tumor proliferation rate T/C (%); where TGI (%) =[1-(V_{Ti}/V_{T0})/(V_{ci}/V_{c0})]×100% (V_{Ti}: average tumor volume of the treatment group on the day i of administration, V_{T0}: average tumor volume of the treatment group on the day 0 of administration, V_{ci}: average tumor volume of the solvent control group on the day i of administration, V_{c0}: average tumor volume of the solvent control group on the day 0 of administration); T/C (%) = V_T/V_c× 100% (V_T: average tumor volume of the treatment group; V_c: average tumor volume of the solvent control group).

3. Counting number of mice with Complete Response CR or Partial Response PR, wherein, CR means that the mouse tumor volume is 0 mm^3, PR means that the mouse tumor volume decreases by more than 30% compared with that when grouping.

4. Calculating the tumor regression rate TRR (%), TRR（%）=（1-V_{Dn}/V_{D0}）×100 (V_{Dn}: tumor volume measured on day n; V_{D0}: tumor volume at the time of grouping on day 0).

The test substances HC18-01-ADC1, Bemarituzumab and Docetaxel had a small effect on the body weight of human lung cancer PDX mice. There was no significant change in the body weight of the animals in each group during the entire experimental period, and there was no abnormality in clinical observation, indicating that the drug dosage was safe under the dosing regimen of this Example.

The results of this Example are shown in FIGs 13-14 and TABLEs 11-12. On day 31, compared with the solvent control group, it was found that the 10 mg/kg Bemarituzumab group had almost no inhibitory effect on tumor volume, so the experiment of this group was terminated. Compared with the solvent control group, the average tumor volume of the dosing groups of HC18-01-ADC1 was significantly reduced ($p<0.001$), and the experiment was continued until the end of day 38. At the end of the experiment, the average tumor volume of the 10 mg/kg HC18-01-ADC1 group was significantly reduced compared to the 10 mg/kg Docetaxel group ($p<0.05$); the average tumor volume of the 10 mg/kg HC18-01-ADC1 group was significantly smaller than that of the 3 mg/kg HC18-01-ADC1 group ($p<0.05$). Among them, $^{*}p$ represents the comparison with the solvent control group, and $^{\#}p$ represents the comparison with the 10 mg/kg HC18-01-ADC1 group. (Note: On the 31st day of the experiment, due to the large tumor volume of the animals, the experimental process of the solvent control group and the Bemarituzumab group was terminated to meet the ethical standards of experimental animals; the experimental process of the remaining groups continued until the end of the 38th day).

The tumor weights of the remaining groups were measured at the experimental endpoint D38. The results are shown in FIG. 14 and TABLE 12. Compared with the 10 mg/kg Docetaxel group, the average tumor weight of the 10 mg/kg HC18-01-ADC1 group was significantly reduced ($p<0.01$); the average tumor weight of the 10 mg/kg HC18-01-ADC1 group was significantly less than that of the 3 mg/kg HC18-01-ADC1 group ($p<0.01$). Among them, $^{\&\&}p$, $^{\&}p$ represent the comparison with the 10 mg/kg HC18-01-ADC1 group.

Table 11: Anti-tumor efficacy and statistical results on day 31

Group	Tumor volume (mm^3) (Mean±SEM)	T/C (%)	TGI (%)	PR	CR
Solvent control (Vehicle)	1760.89 ± 282.97	/	/	/	/
HC18-01-ADC1 3 mg/kg	344.39 ± 79.72***	19.65	80.35	1/6	/
HC18-01-ADC1 10 mg/kg	21.67 ± 7.72****	1.23	98.77	6/6	2/6
Bemarituzumab 10 mg/kg	1623.30 ± 423.16	92.41	7.59	/	/
Docetaxel 10 mg/kg	376.69 ± 78.97***	21.40	78.60	/	/

* means compared with solvent control group

Table 12: Antitumor efficacy and statistical results on day 38 (end point)

Group	Tumor volume (mm^3) (Mean±SEM)	Tumor weight (mg) (Mean±SEM)	PR	CR
Vehicle	/	/	/	/
HC18-01-ADC1 3 mg/kg	508.54 ± 118.60#	0.509 ± 0.120&&	1/6	/
HC18-01-ADC1 10 mg/kg	37.57 ± 16.24	0.035 ± 0.152	5/6	2/6
Docetaxel 10 mg/kg	477.21 ± 124.63#	0.472 ± 0.119&	/	/

#p、&p compared with HC18-01-ADC1 10 mg/kg group

A single administration of HC18-01-ADC1 can significantly inhibit the tumor growth of mouse subcutaneous human lung cancer PDX (NO. LD1-0025-200783) model. The higher the dose, the better the tumor inhibitory effect, showing a significant dose-dependency. No rebound effect was observed 38 days after a single administration of 10 mg/kg HC18-01-ADC1, and the tumor inhibitory effect was long-lasting. At the equal dose, the tumor inhibition effect of HC18-01-ADC1 was significantly better than that of antibody drug Bemarituzumab and chemotherapy drug Docetaxel.

Example 12: linker-toxin Cpd3 plasma stability validation

The test ADC molecule HC18-01-ADC1 (concentration of 0.2 mg/mL) was incubated in plasma of different species (human/monkey/rat/mouse) for 21 days at 37 °C, during which the concentration of analyte in plasma at different time points was detected by LC-MS/Ms. The control sample of this Example was mc-Exd (commercially available). Table 13 below sets forth the experimental parameters of HC18-01-ADC1 (analyte Cpd3) and a control HC18-01-mc-Exd (analyte mc-Exd, i.e., Cpd3 methanesulfonamide linker replaced by maleic acid linker, the rest of the linker parts are the same, the toxin part is Exd). Table 13 describes the plasma stability of HC18-01-ADC1.

The release rate (%) refers to the falling-off rate of the free drug in the plasma obtained by measuring the content of the free drug in the plasma after the ADC and the plasma have been incubated for a certain time and calculating according to the ratio of the content of the free drug in the plasma to the total amount of the drug in the ADC.

	Release rate%					
Time (day)	Mouse (cpd3)	Rat (cpd3)	PBS (cpd3)	Monkey (cpd3)	Human (cpd3)	Human (MCEXD)
0	0.022579	0.007904	0.010831	0.005913	0.00524	0.004414
1	0.225079	0.021867	0.008157	0.012929	0.014422	0.018863
2	0.379084	0.03584	0.007962	0.018247	0.024872	0.038196
3	0.459457	0.050437	0.009504	0.026307	0.034688	0.055581
5	0.518569	0.069552	0.016012	0.034396	0.048173	0.0729
7	0.919939	0.118262	0.032815	0.04912	0.082101	0.123981
14	1.29914	0.171207	0.063883	0.066713	0.12079	0.18601
21	1.223323	0.281205	0.18117	0.055209	0.1574	0.241082

The experimental results show that the humanized antibody HC18-01 conjugated with Cpd3 (DAR8) has good plasma stability and are stable in the plasma of different species. Cpd3 showed extremely high stability in human, monkey, and rats, and is superior to the control mc-EXD.

Sequence listing

HC18	HCDR1	SEQ ID NO:1	SYWMH
	HCDR2	SEQ ID NO:2	SIYPENSDTSYNQKFKG
	HCDR3	SEQ ID NO:3	YHGYDGDY
	LCDR1	SEQ ID NO:4	SASSSVSYMY
	LCDR2	SEQ ID NO:5	STSNLAS
	LCDR3	SEQ ID NO:6	QQRSSYPYT
	VH	SEQ ID NO:7	EVQLQQSGTVLARPGASVKMSCKTSGYTFTSYWMHWV KQRPGQGLEWIGSIYPENSDTSYNQKFKGKAKLTAVTSA STAYMDLSSLTNEDSAVYYCSIYHGYDGDYWGQGTTLT VSS
	VL	SEQ ID NO:8	QIVLTQSPAIMSASPGEKVTITCSASSSVSYMYWFQQKP GTSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISRMEA EDAATYYCQQRSSYPYTFGGGTKLEIK
	Heavy chain	SEQ ID NO:9	EVQLQQSGTVLARPGASVKMSCKTSGYTFTSYWMHWV KQRPGQGLEWIGSIYPENSDTSYNQKFKGKAKLTAVTSA STAYMDLSSLTNEDSAVYYCSIYHGYDGDYWGQGTTLT VSSASTKPGSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLPGK
	Light chains	SEQ ID NO:10	QIVLTQSPAIMSASPGEKVTITCSASSSVSYMYWFQQKP GTSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISRMEA EDAATYYCQQRSSYPYTFGGGTKLEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV THQGLSSPVTKSFNRGEC
HC18 -01	HCDR1	SEQ ID NO:1	SYWMH
	HCDR2	SEQ ID NO:2	SIYPENSDTSYNQKFKG
	HCDR3	SEQ ID NO:11	YHGYDADY
	LCDR1	SEQ ID NO:4	SASSSVSYMY
	LCDR2	SEQ ID NO:5	STSNLAS
	LCDR3	SEQ ID NO:6	QQRSSYPYT
	VH	SEQ ID NO:12	EVQLVQSGAEVKKPGASVKVSCKTSGYTFTSYWMHWV RQAPGQGLEWMGSIYPENSDTSYNQKFKGRVTLTADTSI STAYMELSRLRSDDTVVYYCSIYHGYDADYWGQGTLVT VSS
	VL	SEQ ID NO:13	EIVLTQSPATLSLSPGERATLSCSASSSVSYMYWFQQKPG QAPKLWIYSTSNLASGIPARFSGSGSGTDYTLTISSLEPED FAVYYCQQRSSYPYTFGQGTKLEIK

	Heavy chain	SEQ ID NO:14	EVQLVQSGAEVKKPGASVKVSCKTSGYTFTSYWMHWV RQAPGQGLEWMGSIYPENSDTSYNQKFKGRVTLTADTSI STAYMELSRLRSDDTVVYYCSIYHGYDADYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
	Light chains	SEQ ID NO:15	EIVLTQSPATLSLSPGERATLSCSASSSVSYMYWFQQKPG QAPKLWIYSTSNLASGIPARFSGSGSGTDYTLTISSLEPED FAVYYCQQRSSYPYTFGQGTKLEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC
Human IgG1 heavy chain constant region		SEQ ID NO:16	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
human light chain constant region, Kappa-type		SEQ ID NO:17	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC

Claims

1. An anti-FGFR2b antibody or the antigen-binding fragment thereof that specifically binds FGFR2b, comprising:

(1) the 3 CDRs (HCDR1, HCDR2, HCDR3) comprised in the VH as shown in SEQ ID NO:7 or variants thereof; and/or the 3 CDRs (LCDR1, LCDR2, LCDR3) comprised in the VL as shown in SEQ ID NO:8 or variants thereof; or,

(2) the 3 CDRs (HCDR1, HCDR2, HCDR3) comprised in the VH as shown in SEQ ID NO:12 or variants thereof; and/or the 3 CDRs (LCDR1, LCDR2, LCDR3) comprised in the VL as shown in SEQ ID NO:13 or variants thereof.

2. The anti-FGFR2b antibody or the antigen-binding fragment thereof of claim 1, comprising:

1) HCDR1, comprising or consisting of the sequence as shown in SEQ ID NO:1;
HCDR2, comprising or consisting of the sequence as shown in SEQ ID NO:2;
HCDR3, comprising or consisting of the sequence as shown in SEQ ID NO:3;
LCDR1, comprising or consisting of the sequence as shown in SEQ ID NO:4;
LCDR2, comprising or consisting of the sequence as shown in SEQ ID NO:5; and
LCDR3, comprising or consisting of the sequence as shown in SEQ ID NO 6; or

2) HCDR1, comprising or consisting of the sequence as shown in SEQ ID NO:1;
HCDR2, comprising or consisting of the sequence as shown in SEQ ID NO:2;
HCDR3, comprising or consisting of the sequence as shown in SEQ ID NO:11;
LCDR1, comprising or consisting of the sequence as shown in SEQ ID NO:4;
LCDR2, comprising or consisting of the sequence as shown in SEQ ID NO:5; and
LCDR3, comprising or consisting of the sequence as shown in SEQ ID NO 6.

3. The anti-FGFR2b antibody and the antigen-binding fragment thereof of claim 1 or 2, comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein:

(1) The heavy chain variable region comprises the amino acid sequence as shown in SEQ ID NO:7, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:7, or consists of SEQ ID NO:7; the light chain variable region comprises the amino acid sequence as shown in SEQ ID NO:8, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:8, or consists of SEQ ID NO:8; or

(2) The heavy chain variable region comprises the amino acid sequence as shown in SEQ ID NO:12, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:12, or consists of SEQ ID NO:12; the light chain variable region comprises the amino acid sequence as shown in SEQ ID NO:13, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:13, or consists of SEQ ID NO:13.

4. The anti-FGFR2b antibody and the antigen-binding fragment thereof of any one of claims 1-3,

comprising a heavy chain and a light chain, wherein

1) The heavy chain comprises the amino acid sequence as shown in SEQ ID NO:9, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:9, or consists of SEQ ID NO:9; the light chain comprises an amino acid sequence as shown in SEQ ID NO:10, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:10, or consists of SEQ ID NO:10; or

2) The heavy chain comprises the amino acid sequence as shown in SEQ ID NO:14, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:14, or consists of SEQ ID NO:14; the light chain comprises an amino acid sequence as shown in SEQ ID NO:15, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the amino acid sequence of SEQ ID NO:15, or consists of SEQ ID NO:15.

5. An anti-FGFR2b antibody drug conjugate according to formula (I), an isomer thereof, a pharmaceutically acceptable salt thereof, or a mixture thereof:

Ab- (L-D)m [formula (I)]

Wherein,

L is a linker unit;

D is a cytotoxic small molecule drug;

m is an average number of L-D units conjugated to Ab, and m is selected from 1 to 10, preferably 2 to 8;

Ab is the anti-FGFR2b antibody or antigen-binding fragment thereof of any one of claims 1-4.

6. The antibody drug conjugate, an isomer thereof, a pharmaceutically acceptable salt thereof, or the mixture thereof according to claim 5, wherein the anti-FGFR2b antibody or fragment thereof comprises the following CDRs: HCDR1 comprising or consisting of the sequence as shown in SEQ ID NO:1, HCDR2 comprising or consisting of the sequence as shown in SEQ ID NO:2, HCDR3 comprising or consisting of the sequence as shown in SEQ ID NO:11, LCDR1 comprising or consisting of the sequence as shown in SEQ ID NO:4, LCDR2 comprising or consisting of the sequence as shown in SEQ ID NO:5, and LCDR3 comprising or consisting of the sequence as shown in SEQ ID NO:6.

7. The antibody drug conjugate, isomers thereof, pharmaceutically acceptable salts thereof, or the mixtures thereof according to claim 5 or 6, wherein the anti-FGFR2b antibody or fragment thereof comprises a light chain variable region and a heavy chain variable region:

the light chain variable region comprising or consisting of the amino acid sequence as shown in SEQ ID NO:13;

the heavy chain variable region comprising or consisting of the amino acid sequence as shown in SEQ ID NO:12.

8. The antibody drug conjugate, isomer thereof, pharmaceutically acceptable salt thereof, or the

mixture thereof according to any one of claims 5-7, wherein,

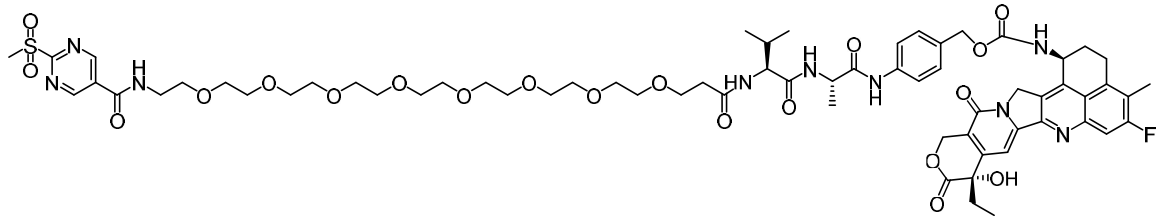
L is a linker unit, which is a combination of one or more L'; the L' is selected from carbonyl, amino, amido, aminoacyl, -$(PEG)_n$-, -$(CH_2)_n$-, -$(CH_2)_n$- comprising a hetreoatom, -(C ≡ C)-, -(CH=CH)-, -O-, -S-, maleimido (mc), maleimide propionyl (MP), methylsulfonyl pyrimidinyl, valine-citrulline (val-cit), valine-alanine (val-ala), N-succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), alanine-phenylalanine (ala-phe), phenylalanine-lysine (phe-lys), p-aminobenzyloxycarbonyl (PAB), dimethyl ethanediamine (DMED), N-succinimidyl 4-(2-pyridinylthio)pentanoate (SPP), N-succinimidyl 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC), N-succinimidyl (4-iodo-acetyl)aminobenzoate (SIAB), N-succinimidyl 4-(2-pyridinyldithio)butanoate (SPDB), N-succinimidyl 3-(pyridin-2-yldithio)-propionate (SPDP), glycine-glycine-phenylalanine-glycine (GGFG), acetyl-lysine-valine-citrulline-p-aminobenzyloxycarbonyl (AcLys-vc-PABC); wherein n is independently selected from an integer of 1-20;

D is a cytotoxic small molecule drug, which is selected from one or more of monomethyl auristatins, camptothecins or mertansine alkaloids;

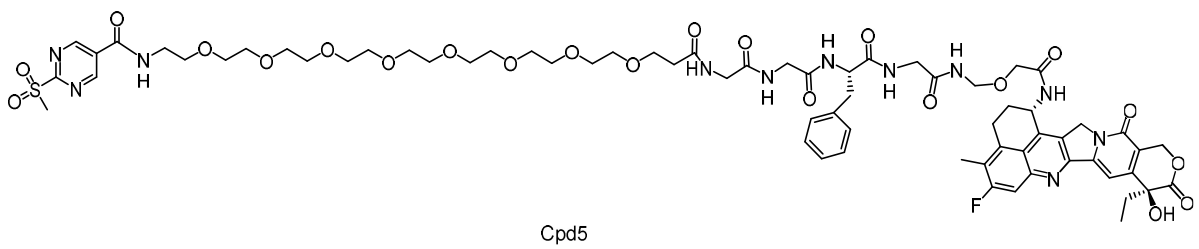
m is an average number of L-D units conjugated to Ab, wherein m is selected from 1-10.

9. The antibody drug conjugate, the isomer thereof, the pharmaceutically acceptable salt thereof or the mixture thereof according to any one of claims 5-8, wherein the conjugate comprises multiple D components, the multiple D components may be a combination of different therapeutic active substances or pharmaceutical active ingredients, or a combination of the same therapeutic active substances or pharmaceutical active ingredients, the D is preferably a camptothecin compound, more preferably Exatecan, Dxd (1), Dxd (2) or SN-38.

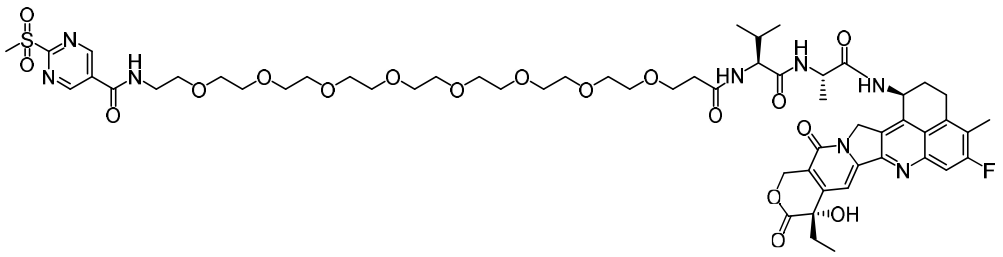
10. The antibody drug conjugate, the isomer thereof, the pharmaceutically acceptable salt thereof, or the mixture thereof according to claim 9, wherein the (L-D) moiety is derived from the structures as shown in formula Cpd3, formula Cpd5, and formula Cpd6:



Cpd3 ,

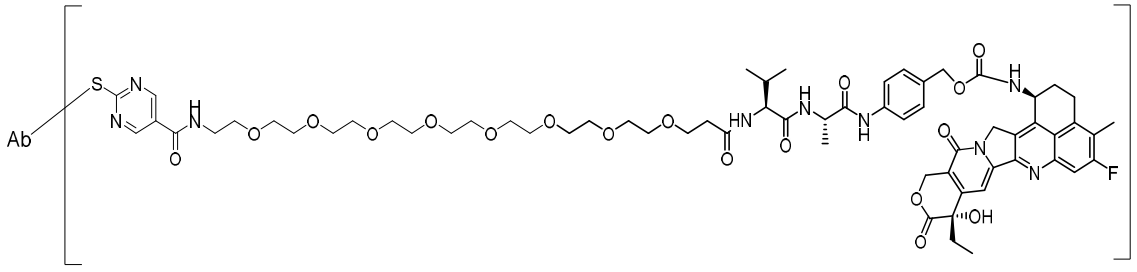


Cpd5 ,

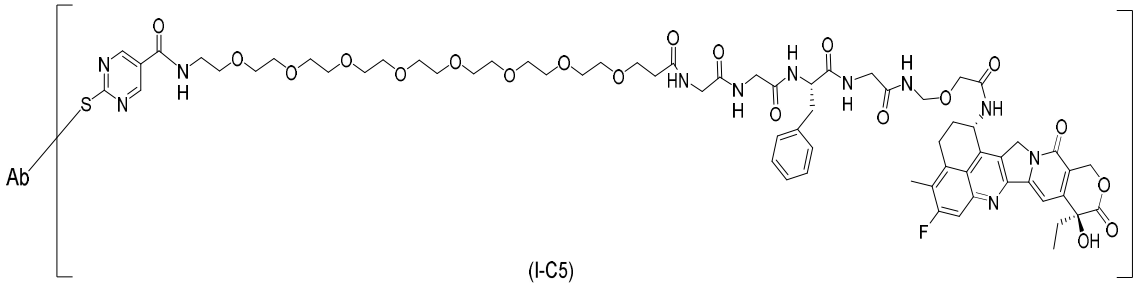


Cpd6 。

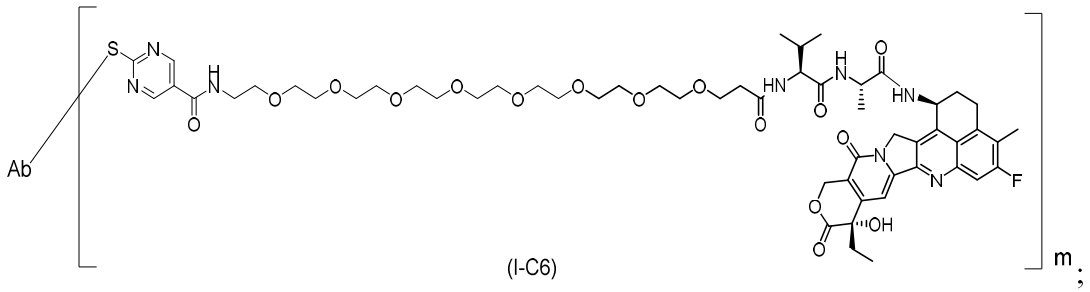
11. The antibody drug conjugate, the isomer thereof, the pharmaceutically acceptable salt thereof, or the mixture thereof according to any one of claims 5 to 10, wherein the structures of the antibody drug conjugates are shown in formula (I-C3), formula (I-C5), and formula (I-C6):



(I-C3) ,



(I-C5) ,



(I-C6) ;

wherein,

m is an average conjugation number, and m is independently selected from 1 to 10;

Ab is the anti-FGFR2b antibody or the antigen-binding fragment thereof according to any one of claims 1-4.

12. An isolated nucleotide molecule encoding the anti-FGFR2b antibody or the antigen-binding fragment thereof according to any one of claims 1-4, or the heavy chain variable region and/or the light chain variable region thereof.

13. An expression vector comprising the nucleotide molecule according to claim 12.

14. A host cell comprising the nucleotide molecule according to claim 12 or the expression vector according to claim 13.

15. A pharmaceutical composition comprising the antibody or the antigen-binding fragment thereof according to any one of claims 1-4, the antibody drug conjugate, the isomer thereof, the pharmaceutically acceptable salt thereof, or the mixture thereof according to any one of claims 5-11, and a pharmaceutically acceptable excipient.

16. Use of the antibody or the antigen-binding fragment thereof according to any one of claims 1-4, the antibody drug conjugate, the isomer thereof, the pharmaceutically acceptable salt thereof, or the mixture thereof according to any one of claims 5-11, or the pharmaceutical composition according to claim 15 in the manufacture of a medicament for the treatment and/or prevention of a FGFR2b-mediated disease or disorder.

17. A method of treating and/or preventing a FGFR2b-mediated disease or disorder, comprising administering to a subject in need thereof the antibody or the antigen-binding fragment thereof according to any one of claims 1-4, the antibody drug conjugate, the isomer thereof, the pharmaceutically acceptable salt thereof, or the mixture thereof according to any one of claims 5-11, or the pharmaceutical composition of claim 15.

18. The use according to claim 16 or the method according to claim 17, wherein the disease or disorder is a tumor and/or cancer; the tumor and/or cancer is selected from the group consisting of lung cancer, lung squamous carcinoma, lung adenocarcinoma, ovarian cancer, endometrial cancer, breast cancer, triple-negative breast cancer, intrahepatic cholangiocarcinoma, bladder cancer, colon cancer, prostate cancer, cervical cancer, colorectal cancer, pancreatic cancer, gastric cancer, esophageal cancer, hepatocellular carcinoma, renal cell carcinoma, head and neck cancer, mesothelioma, melanoma, sarcoma, brain tumor, gastroesophageal adenocarcinoma, malignant uterine tumor, gastroesophageal junction adenocarcinoma, bile duct cancer, gallbladder cancer, intrahepatic bile duct cancer, oral mucosal cancer, and urothelial cancer.