ANTIBODY-DRUG CONJUGATE AND PREPARATION METHOD THEREFOR AND USE THEREOF

Technical Field

The present disclosure belongs to the field of medical technology, and relates to various antibodies, antibody-drug conjugates, and preparation methods therefor, as well as uses thereof in the prevention and/or treatment of diseases associated with abnormal cellular activities, including but not limited to uses in the prevention and/or treatment of tumor diseases.

Background Art

Under normal physiological conditions, angiogenesis is in a dynamic regulatory balance, strictly controlled by the complex interactions of a series of factors that play a crucial role in neovascularization during embryonic development. However, tumors can activate the "angiogenic switch", disrupt the dynamic balance of angiogenesis, form tumor vasculature with abnormal structure and function, and accelerate tumor occurrence, growth, and metastasis. Anti-tumor angiogenesis and vascular normalization therapy have become landmark new strategies for tumor treatment.

Bevacizumab (trade name: Avastin) is a humanized monoclonal IgG1 antibody that binds to VEGF-A, blocks the VEGF/VEGFR signaling pathway, thereby inhibiting angiogenesis and inducing tumor vascular regression. In addition, low doses of Bevacizumab can induce a transient normalization phase of tumor vasculature. Currently, Bevacizumab has been approved for use in combination with chemotherapy for the treatment of a wide range of solid tumor types, including colorectal cancer (CRC), non-small cell lung cancer (NSCLC), etc. However, the non-targeted action of chemotherapeutic drugs leads to increased systemic toxicity and drug resistance, increases patient suffering during treatment, and severely limits the clinical application of monoclonal antibody/chemotherapy drug combinations. Therefore, there is an urgent need to find a therapeutic option that can overcome the limitations of drug combinations.

An ADC is a conjugate of an antibody and a small-molecule drug that combines the targeting function of an antibody with the activity of a biologically active

molecule, forming a biological missile with highly promising efficacy and safety advantages. The antibody guides the ADC to bind to the target cell, subsequently the ADC is internalized by the cell, and the small-molecule drug is released by enzymatic cleavage under the action of specific enzymes within the cell, thereby treating the disease. ADC drugs have developed very rapidly in recent years, with 17 ADCs currently on the market. However, existing marketed ADCs generate killing activity by the antibody in the ADC binding tumor cell surface antigens, followed by cellular endocytosis, after which the toxin is cleaved from the ADC. However, ADCs targeting free antigen targets differ from traditional endocytic ADCs, and the development of ADCs targeting free antigen targets is very challenging. For free antigen VEGF, if an ADC targeting free VEGF can be developed, it would provide tumor patients with broader and superior medication options, and would also have broad market prospects.

Although there are also reports in the prior art of a VEGF ADC (Bevacizumab Vedotin) using MMAE as the toxin (Yanchen Li, et al., Bioorg Chem. 2023 Aug:137:106575. doi: 10.1016/j.bioorg.2023.106575.), ADCs using MMAE as the toxin have exhibited characteristic clinical toxicities, e.g., ocular toxicity and peripheral neuropathy.

Therefore, there is currently an urgent need in the art to develop an antibody-drug conjugate targeting VEGF.

Summary of the Invention

The present disclosure provides an anti-VEGF antibody-drug conjugate, as well as uses thereof for treating cancer.

The present disclosure provides a VEGF-ADC with a different mechanism of action, having good anti-tumor activity and/or safety, broadening the range of clinical medication options, and having significant clinical value.

The present disclosure for the first time employs camptothecin-based molecules as the toxin of VEGF-ADC, which is an ADC with a completely new mechanism of action, has good anti-tumor activity, and simultaneously has good systemic circulation stability and/or safety, improving the therapeutic window.

The antibody-drug conjugate of the present disclosure is the world's first ADC molecule targeting VEGF about to enter clinical trials, which is capable of exerting the anti-tumor-angiogenesis effect of the anti-VEGF antibody while simultaneously exerting the tumor cell-killing effect of the toxin (camptothecin-based) carried by the ADC, and both also have a synergistic anti-tumor effect; while it also utilizes the specific release mechanism of the ADC molecule of the present disclosure in the tumor microenvironment. These multiple mechanisms of action can better inhibit tumors, thereby exerting two anti-tumor mechanisms by a single administration, which is also of great convenience to patients.

Antibody-Drug Conjugate

In a first aspect, the present disclosure provides an antibody-drug conjugate, which is a compound of formula (I), or a tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), prodrug, or a pharmaceutically acceptable salt or solvate (for example, hydrate) thereof:

Ab-[L-D]q

Formula (I)

wherein,

Ab is an antibody or an antigen-binding fragment thereof that specifically binds vascular endothelial growth factor (VEGF);

L is a bond or a linking molecule that covalently links Ab and D;

q is an integer between 1 and 16, and represents the number of L-D covalently linked to Ab;

D is a camptothecin-based drug molecule.

Ab

In some embodiments, Ab is an antibody or an antigen-binding fragment thereof that specifically binds VEGF (VEGF antibody or an antigen-binding fragment thereof). In some embodiments, Ab comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises three CDR-Hs, i.e. CDR-H1, CDR-H2, and CDR-H3, and the light chain variable region comprises three CDR-Ls, i.e. CDR-L1, CDR-L2, and CDR-L3.

In some embodiments, the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises: three CDR-Hs of the heavy chain variable region

(VH) sequence of SEQ ID NO: 3 and three CDR-Ls of the light chain variable region (VL) sequence of SEQ ID NO: 4, wherein the CDRs are defined according to the Chothia, AbM, Kabat, IMGT, Contact scheme, or any combination thereof.

In some embodiments, the antibody or the antigen-binding fragment thereof that specifically binds VEGF serving as Ab comprises: three CDR-Hs of the heavy chain variable region (VH) sequence of SEQ ID NO: 3 and three CDR-Ls of the light chain variable region (VL) sequence of SEQ ID NO: 4, wherein the CDRs are defined according to the Chothia scheme.

In some embodiments, the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises a heavy chain variable region or a light chain variable region, wherein the heavy chain variable region comprises CDR-H1, CDR-H2, and CDR-H3, and the light chain variable region comprises CDR-L1, CDR-L2, and CDR-L3; or the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises three CDR-Hs: CDR-H1, CDR-H2, and CDR-H3 and three CDR-Ls: CDR-L1, CDR-L2, and CDR-L3, wherein, defined according to the Chothia scheme,

CDR-H1 comprises an amino acid sequence set forth in SEQ ID NO: 5;

CDR-H2 comprises an amino acid sequence set forth in SEQ ID NO: 10;

CDR-H3 comprises an amino acid sequence set forth in SEQ ID NO: 17;

CDR-L1 comprises an amino acid sequence set forth in SEQ ID NO: 23;

CDR-L2 comprises an amino acid sequence set forth in SEQ ID NO: 19; and

CDR-L3 comprises an amino acid sequence set forth in SEQ ID NO: 22.

In some embodiments, the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises a heavy chain variable region or a light chain variable region, wherein the heavy chain variable region comprises CDR-H1, CDR-H2, and CDR-H3, and the light chain variable region comprises CDR-L1, CDR-L2, and CDR-L3; or the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises three CDR-Hs: CDR-H1, CDR-H2, and CDR-H3 and three CDR-Ls: CDR-L1, CDR-L2, and CDR-L3, wherein, defined according to the Chothia scheme,

CDR-H1 consists of the amino acid sequence set forth in SEQ ID NO: 5;

CDR-H2 consists of the amino acid sequence set forth in SEQ ID NO: 10;

CDR-H3 consists of the amino acid sequence set forth in SEQ ID NO: 17;

CDR-L1 consists of the amino acid sequence set forth in SEQ ID NO: 23;

CDR-L2 consists of the amino acid sequence set forth in SEQ ID NO: 19; and

CDR-L3 consists of the amino acid sequence set forth in SEQ ID NO: 22.

In some embodiments, the antibody or the antigen-binding fragment thereof that specifically binds VEGF serving as Ab comprises: three CDR-Hs of the heavy chain variable region (VH) sequence of SEQ ID NO: 3 and three CDR-Ls of the light chain variable region (VL) sequence of SEQ ID NO: 4, wherein the CDRs are defined according to the AbM scheme.

In some embodiments, the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises a heavy chain variable region or a light chain variable region, wherein the heavy chain variable region comprises CDR-H1, CDR-H2, and CDR-H3, and the light chain variable region comprises CDR-L1, CDR-L2, and CDR-L3; or the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises three CDR-Hs: CDR-H1, CDR-H2, and CDR-H3 and three CDR-Ls: CDR-L1, CDR-L2, and CDR-L3, wherein, defined according to the AbM scheme,

CDR-H1 comprises an amino acid sequence set forth in SEQ ID NO: 6;

CDR-H2 comprises an amino acid sequence set forth in SEQ ID NO: 11;

CDR-H3 comprises an amino acid sequence set forth in SEQ ID NO: 17;

CDR-L1 comprises an amino acid sequence set forth in SEQ ID NO: 23;

CDR-L2 comprises an amino acid sequence set forth in SEQ ID NO: 19; and

CDR-L3 comprises an amino acid sequence set forth in SEQ ID NO: 22.

In some embodiments, the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises a heavy chain variable region or a light chain variable region, wherein the heavy chain variable region comprises CDR-H1, CDR-H2, and CDR-H3, and the light chain variable region comprises CDR-L1, CDR-L2, and CDR-L3; or the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises three CDR-Hs: CDR-H1, CDR-H2, and CDR-H3 and three CDR-Ls: CDR-L1, CDR-L2, and CDR-L3, wherein, defined according to the AbM scheme,

CDR-H1 consists of the amino acid sequence set forth in SEQ ID NO: 6;

CDR-H2 consists of the amino acid sequence set forth in SEQ ID NO: 11;

CDR-H3 consists of the amino acid sequence set forth in SEQ ID NO: 17;

CDR-L1 consists of the amino acid sequence set forth in SEQ ID NO: 23;

CDR-L2 consists of the amino acid sequence set forth in SEQ ID NO: 19; and

CDR-L3 consists of the amino acid sequence set forth in SEQ ID NO: 22.

In some embodiments, the antibody or the antigen-binding fragment thereof that specifically binds VEGF serving as Ab comprises: three CDR-Hs of the heavy chain variable region (VH) sequence of SEQ ID NO: 3 and three CDR-Ls of the light chain variable region (VL) sequence of SEQ ID NO: 4, wherein the CDRs are defined according to the Kabat scheme.

In some embodiments, the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises a heavy chain variable region or a light chain variable region, wherein the heavy chain variable region comprises CDR-H1, CDR-H2, and CDR-H3, and the light chain variable region comprises CDR-L1, CDR-L2, and CDR-L3; or the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises three CDR-Hs: CDR-H1, CDR-H2, and CDR-H3 and three CDR-Ls: CDR-L1, CDR-L2, and CDR-L3, wherein, defined according to the Kabat scheme,

CDR-H1 comprises an amino acid sequence set forth in SEQ ID NO: 7;

CDR-H2 comprises an amino acid sequence set forth in SEQ ID NO: 12;

CDR-H3 comprises an amino acid sequence set forth in SEQ ID NO: 17;

CDR-L1 comprises an amino acid sequence set forth in SEQ ID NO: 23;

CDR-L2 comprises an amino acid sequence set forth in SEQ ID NO: 19; and

CDR-L3 comprises an amino acid sequence set forth in SEQ ID NO: 22.

In some embodiments, the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises a heavy chain variable region or a light chain variable region, wherein the heavy chain variable region comprises CDR-H1, CDR-H2, and CDR-H3, and the light chain variable region comprises CDR-L1, CDR-L2, and CDR-L3; or the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises three CDR-Hs: CDR-H1, CDR-H2, and CDR-H3 and three CDR-Ls: CDR-L1, CDR-L2, and CDR-L3, wherein, defined according to the Kabat scheme,

CDR-H1 consists of the amino acid sequence set forth in SEQ ID NO: 7;

CDR-H2 consists of the amino acid sequence set forth in SEQ ID NO: 12;

CDR-H3 consists of the amino acid sequence set forth in SEQ ID NO: 17;

CDR-L1 consists of the amino acid sequence set forth in SEQ ID NO: 23;

CDR-L2 consists of the amino acid sequence set forth in SEQ ID NO: 19; and

CDR-L3 consists of the amino acid sequence set forth in SEQ ID NO: 22.

In some embodiments, the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises: three CDR-Hs of the heavy chain variable region (VH) sequence of SEQ ID NO: 3 and three CDR-Ls of the light chain variable region (VL) sequence of SEQ ID NO: 4, wherein the CDRs are defined according to the Contact scheme.

In some embodiments, the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises a heavy chain variable region or a light chain variable region, wherein the heavy chain variable region comprises CDR-H1, CDR-H2, and CDR-H3, and the light chain variable region comprises CDR-L1, CDR-L2, and CDR-L3; or the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises three CDR-Hs: CDR-H1, CDR-H2, and CDR-H3 and three CDR-Ls: CDR-L1, CDR-L2, and CDR-L3, wherein, defined according to the Contact scheme,

CDR-H1 comprises an amino acid sequence set forth in SEQ ID NO: 8;

CDR-H2 comprises an amino acid sequence set forth in SEQ ID NO: 13;

CDR-H3 comprises an amino acid sequence set forth in SEQ ID NO: 15;

CDR-L1 comprises an amino acid sequence set forth in SEQ ID NO: 24;

CDR-L2 comprises an amino acid sequence set forth in SEQ ID NO: 25; and

CDR-L3 comprises an amino acid sequence set forth in SEQ ID NO: 21.

In some embodiments, the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises a heavy chain variable region or a light chain variable region, wherein the heavy chain variable region comprises CDR-H1, CDR-H2, and CDR-H3, and the light chain variable region comprises CDR-L1, CDR-L2, and CDR-L3; or the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises three CDR-Hs: CDR-H1, CDR-H2, and CDR-H3 and three CDR-

Ls: CDR-L1, CDR-L2, and CDR-L3, wherein, defined according to the Contact scheme,

CDR-H1 consists of the amino acid sequence set forth in SEQ ID NO: 8;

CDR-H2 consists of the amino acid sequence set forth in SEQ ID NO: 13;

CDR-H3 consists of the amino acid sequence set forth in SEQ ID NO: 15;

CDR-L1 consists of the amino acid sequence set forth in SEQ ID NO: 24;

CDR-L2 consists of the amino acid sequence set forth in SEQ ID NO: 25; and

CDR-L3 consists of the amino acid sequence set forth in SEQ ID NO: 21.

In some embodiments, the antibody or the antigen-binding fragment thereof that specifically binds VEGF serving as Ab comprises: three CDR-Hs of the heavy chain variable region (VH) sequence of SEQ ID NO: 3 and three CDR-Ls of the light chain variable region (VL) sequence of SEQ ID NO: 4, wherein the CDRs are defined according to the IMGT scheme.

In some embodiments, the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises a heavy chain variable region or a light chain variable region, wherein the heavy chain variable region comprises CDR-H1, CDR-H2, and CDR-H3, and the light chain variable region comprises CDR-L1, CDR-L2, and CDR-L3; or the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises three CDR-Hs: CDR-H1, CDR-H2, and CDR-H3 and three CDR-Ls: CDR-L1, CDR-L2, and CDR-L3, wherein, defined according to the IMGT scheme,

CDR-H1 comprises an amino acid sequence set forth in SEQ ID NO: 9;

CDR-H2 comprises an amino acid sequence set forth in SEQ ID NO: 14;

CDR-H3 comprises an amino acid sequence set forth in SEQ ID NO: 16;

CDR-L1 comprises an amino acid sequence set forth in SEQ ID NO: 20;

CDR-L2 comprises an amino acid sequence set forth in SEQ ID NO: 18; and

CDR-L3 comprises an amino acid sequence set forth in SEQ ID NO: 22.

In some embodiments, the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises a heavy chain variable region or a light chain variable region, wherein the heavy chain variable region comprises CDR-H1, CDR-H2, and CDR-H3, and the light chain variable region comprises CDR-L1, CDR-L2, and CDR-L3; or the VEGF antibody or the antigen-binding fragment thereof serving

as Ab comprises three CDR-Hs: CDR-H1, CDR-H2, and CDR-H3 and three CDR-Ls: CDR-L1, CDR-L2, and CDR-L3, wherein, defined according to the IMGT scheme,

CDR-H1 consists of the amino acid sequence set forth in SEQ ID NO: 9;

CDR-H2 consists of the amino acid sequence set forth in SEQ ID NO: 14;

CDR-H3 consists of the amino acid sequence set forth in SEQ ID NO: 16;

CDR-L1 consists of the amino acid sequence set forth in SEQ ID NO: 20;

CDR-L2 consists of the amino acid sequence set forth in SEQ ID NO: 18; and

CDR-L3 consists of the amino acid sequence set forth in SEQ ID NO: 22.

In some embodiments, the antibody or the antigen-binding fragment thereof that specifically binds VEGF, serving as Ab, comprises CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, CDR-L3 in the following Chothia, AbM, Kabat, IMGT, or Contact definition scheme:

	Definition scheme	Sequence	SEQ ID No
CDR-H1	Chothia	GYTFTNY	5
	AbM	GYTFTNYGMN	6
	Kabat	NYGMN	7
	Contact	TNYGMN	8
	IMGT	GYTFTNYG	9
CDR-H2	Chothia	NTYTGE	10
	AbM	WINTYTGEPT	11
	Kabat	WINTYTGEPTYAADFKR	12
	Contact	WVGWINTYTGEPT	13
	IMGT	INTYTGEPT	14
CDR-H3	Chothia	YPHYYGSSHWYFDV	17
	AbM	YPHYYGSSHWYFDV	17
	Kabat	YPHYYGSSHWYFDV	17
	Contact	AKYPHYYGSSHWYFD	15
	IMGT	AKYPHYYGSSHWYFDV	16

and

	Definition scheme	Sequence	SEQ ID No

CDR-L1	Chothia	SASQDISNYLN	23
	AbM	SASQDISNYLN	23
	Kabat	SASQDISNYLN	23
	Contact	SNYLNWY	24
	IMGT	QDISNY	20
CDR-L2	Chothia	FTSSLHS	19
	AbM	FTSSLHS	19
	Kabat	FTSSLHS	19
	Contact	VLIYFTSSLH	25
	IMGT	FTS	18
CDR-L3	Chothia	QQYSTVPWT	22
	AbM	QQYSTVPWT	22
	Kabat	QQYSTVPWT	22
	Contact	QQYSTVPW	21
	IMGT	QQYSTVPWT	22

In some embodiments, Ab comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises or consists of the amino acid sequence set forth in SEQ ID NO: 3, and/or the light chain variable region comprises or consists of the amino acid sequence set forth in SEQ ID NO: 4.

In some embodiments, Ab comprises a heavy chain variable region and a light chain variable region, wherein

(i) the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 3, and the light chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 4; or

(ii) the heavy chain variable region consists of the amino acid sequence set forth in SEQ ID NO: 3, and the light chain variable region consists of the amino acid sequence set forth in SEQ ID NO: 4.

In some embodiments, Ab comprises a heavy chain variable region and a light chain variable region, wherein

(i) the heavy chain variable region comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 3, and the light chain variable region comprises an amino acid sequence having at least 80%, at least 85%, at least 90%,

at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4; or

(ii) the heavy chain variable region comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 3, and the light chain variable region comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4.

In some embodiments, Ab comprises: the heavy chain variable region (VH) of SEQ ID NO: 3 and the light chain variable region (VL) of SEQ ID NO: 4.

In some embodiments, Ab comprises: a heavy chain variable region (VH) having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 3, and a light chain variable region (VL) having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4.

In some embodiments, Ab comprises: a heavy chain variable region (VH) having at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 3, and a light chain variable region (VL) having at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4.

In some embodiments, Ab comprises a heavy chain and a light chain, wherein the heavy chain comprises or consists of the heavy chain variable region and a heavy chain constant region; and the light chain comprises or consists of the light chain variable region and a light chain constant region.

In some embodiments, the heavy chain constant region is the heavy chain constant region of (human) IgG, for example, the heavy chain constant region of (human) IgG1, IgG2, IgG3, or IgG4. In some embodiments, the light chain constant region is the light chain constant region of (human) Kappa or Lambda.

In some embodiments, Ab comprises a heavy chain and a light chain, wherein the heavy chain comprises or consists of the amino acid sequence set forth in SEQ ID NO: 1, and/or the light chain comprises or consists of the amino acid sequence set forth in SEQ ID NO: 2.

In some embodiments, Ab comprises a heavy chain and a light chain, wherein

(i) the heavy chain comprises an amino acid sequence set forth in SEQ ID NO: 1, and the light chain comprises an amino acid sequence set forth in SEQ ID NO: 2; or

(ii) the heavy chain consists of the amino acid sequence set forth in SEQ ID NO: 1, and the light chain consists of the amino acid sequence set forth in SEQ ID NO: 2.

In some embodiments, Ab comprises a heavy chain and a light chain, wherein

(i) the heavy chain comprises an amino acid sequence having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 1, and the light chain comprises an amino acid sequence having 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 2; or

(ii) the heavy chain comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 1, and the light chain comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 2.

In some embodiments, Ab comprises: the heavy chain of SEQ ID NO: 1 and the light chain of SEQ ID NO: 2.

In some embodiments, Ab comprises: a heavy chain having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 1, and a light chain having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 2.

In some embodiments, Ab comprises: a heavy chain having at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 1, and a light chain having at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 2.

In some embodiments, the Ab comprises two heavy chains and two light chains, for example, two identical heavy chains and two identical light chains.

In some embodiments, Ab is a humanized antibody, a human antibody, or a chimeric antibody.

In some embodiments, Ab is a full-length antibody.

In some embodiments, Ab is an antigen-binding fragment, for example, a single-chain Fv (scFv), Fab, Fab', $F(ab')_2$, disulfide bond-linked Fv (sdFv), Fv, di-scFv, and the like.

In some embodiments, Ab is selected from the antibodies disclosed in WO 9845331 or CN 109053895 B, or antigen-binding fragments thereof, for example, Bevacizumab disclosed therein or a biosimilar thereof, or an antigen-binding fragment thereof.

In some embodiments, Ab is selected from Bevacizumab or a biosimilar thereof, or an antigen-binding fragment thereof.

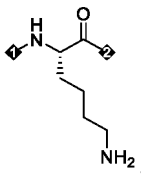
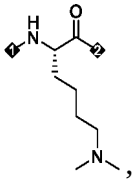
In some embodiments, Ab is selected from any one of the following biosimilars of Bevacizumab, or an antigen-binding fragment thereof, including but not limited to ABP215, BCD-021, FKB238, Mvasi (Bevacizumab-awwb), PF-06439535, SB8, Zirabev (Bevacizumab-bvzr), QL1101, IBI305, SHR-1603, BAT1706, HS008, MIL60, or an antigen-binding fragment thereof.

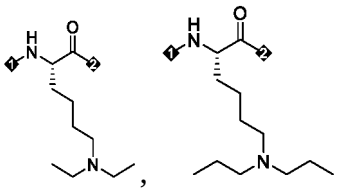
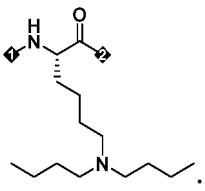
L

In some embodiments, L comprises an amino acid residue or a short peptide consisting of 2-10 amino acid residues.

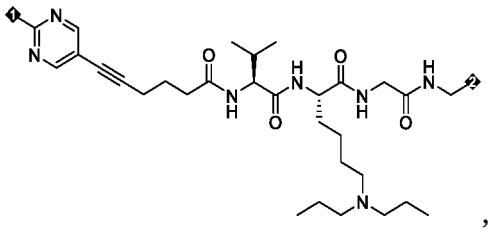
In some embodiments, L comprises an amino acid residue cleavable in a tumor microenvironment or a short peptide consisting of 2-10 amino acid residues.

In some embodiments, the amino acid residue in L is a natural amino acid residue or a modification thereof.

In some embodiments, L comprises an amino acid residue selected from Val, D-Val, Cit, Phe, Lys, Lys(Ac), Leu, Gly, Ala, Asn, Asp, Arg, AA^1, or a short peptide consisting of 2-10 amino acid residues selected from Val, Cit, Phe, Lys, D-Val, Leu, Gly, Ala, Asn, Asp, AA^1, wherein AA^1 is selected from , ,


, and . It is understood that AA^{1}'s ◆ and ◆ represent that the bond is linked to the rest of the molecule.

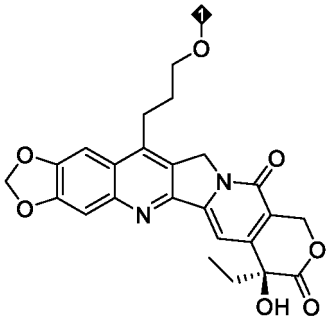
In some embodiments, L comprises Val-AA^{1}-Gly, wherein AA^{1} is as defined herein.

In some embodiments, L is selected from , wherein the 1-position is linked to Ab, and the 2-position is linked to D.

D

In some embodiments, D is selected from camptothecin, SN38, DXD, substituent-modified camptothecin, substituent-modified DXD, or substituent-modified SN38.

In some embodiments, D is selected from:

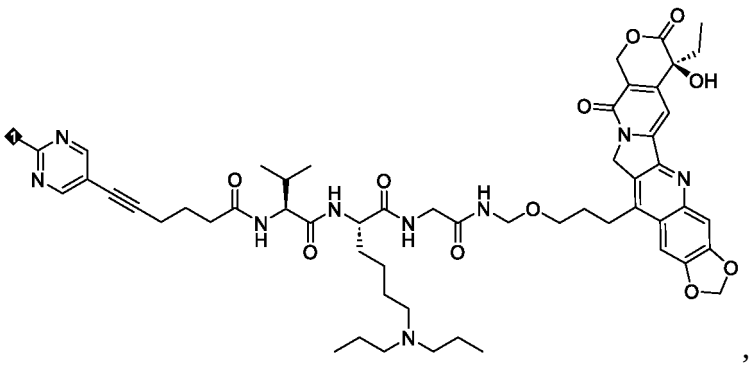
, wherein the 1-position is linked to L.

q

In some embodiments, q is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16. In some embodiments, q is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

L-D

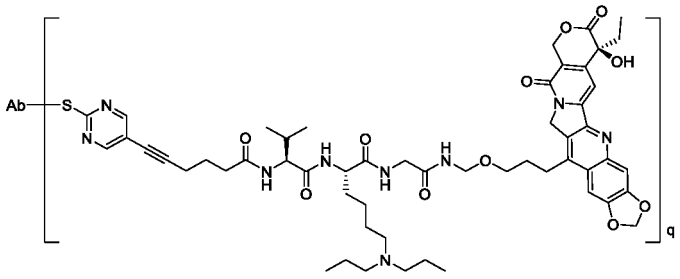
In some embodiments, L-D is selected from:



wherein the 1-position is linked to Ab.

ADC

In some embodiments, the antibody-drug conjugate or compound of formula (I) is selected from



wherein q is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

It is understood that S in the structural formula is derived from Ab, i.e., the -L-D moiety is covalently linked to Ab by the sulfur (S) atom derived from Ab.

In some embodiments, Ab is a VEGF antibody or an antigen-binding fragment thereof, for example, an antibody or an antigen-binding fragment thereof as defined in the "Ab" section herein.

In some embodiments, the VEGF antibody or the antigen-binding fragment thereof serving as Ab comprises: three CDR-Hs of the heavy chain variable region (VH) sequence of SEQ ID NO: 3 and three CDR-Ls of the light chain variable region (VL) sequence of SEQ ID NO: 4, wherein the CDRs are defined according to the Chothia, AbM, Kabat, IMGT, Contact scheme, or any combination thereof.

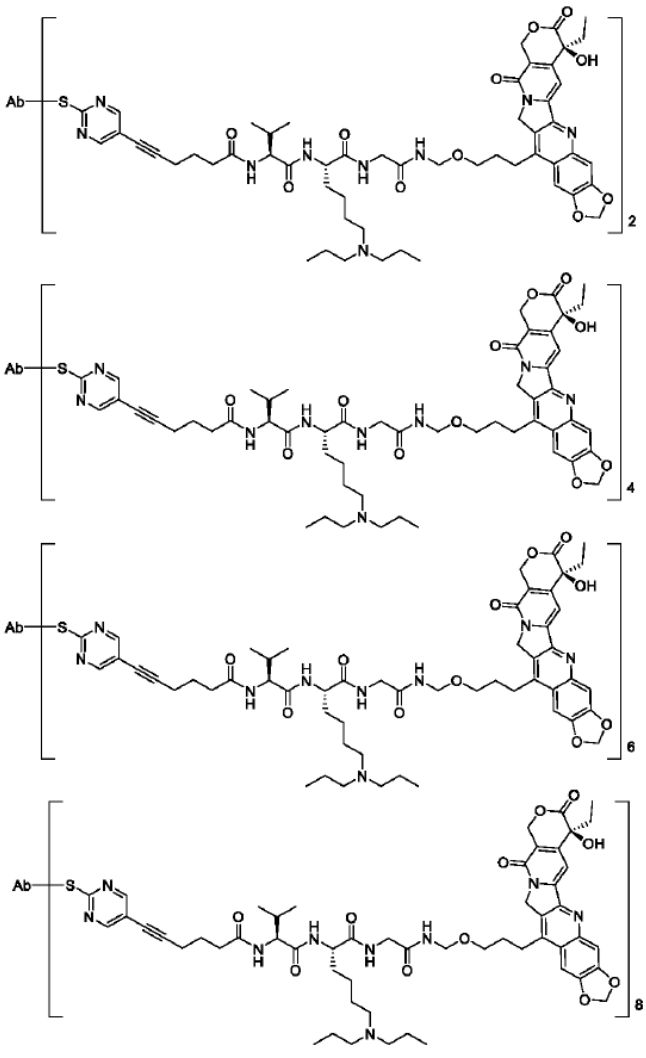
In some embodiments, the antibody or the antigen-binding fragment thereof that specifically binds VEGF serving as Ab comprises: the heavy chain variable region (VH) of SEQ ID NO: 3 and the light chain variable region (VL) of SEQ ID

NO: 4, preferably, Ab is selected from Bevacizumab or a biosimilar thereof, or an antigen-binding fragment thereof.

In some embodiments, q is selected from 2, 4, 6, or 8.

In some embodiments, q is selected from 4 or 8.

In some embodiments, the antibody-drug conjugate or compound of formula (I) is selected from:



,

wherein Ab is selected from Bevacizumab or a biosimilar thereof.

Population of Antibody-Drug Conjugates

In the second aspect of the present disclosure, the present disclosure provides an antibody-drug conjugate population comprising or consisting of the antibody-drug conjugate, or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated

derivative), prodrug, or the pharmaceutically acceptable salt or solvate thereof of the first aspect, wherein the antibody-drug conjugate has one, two, or more q values.

In some embodiments, when one q value of the antibody-drug conjugates in the antibody-drug conjugate population accounts for the majority (e.g., 80%, 85%, 90%, 95%, 95%, 97%, 98%, 99%, or higher), the q value and the average DAR are close.

In some embodiments, when the antibody-drug conjugates in the antibody-drug conjugate population have only one q value, the q value and the average DAR are equal.

In some embodiments, when the antibody-drug conjugates in the antibody-drug conjugate population have two or more q values, the proportion of antibody-drug conjugates having one specific q value among all antibody-drug conjugates in the composition is greater than 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99%.

In some embodiments, the average drug-to-antibody ratio (average DAR) of the antibody-drug conjugates in the antibody-drug conjugate population is selected from an integer or decimal in the range of 1-16, preferably 1-10.

In some embodiments, the average drug-to-antibody ratio (average DAR) of the antibody-drug conjugate population is selected from 1.5-2.5, 3.5-4.5, 5.5-6.5, or 7.5-8.5.

In some embodiments, the average drug-to-antibody ratio (average DAR) of the antibody-drug conjugate population is selected from about 2.0, about 4.0, about 6.0, or about 8.0.

In some embodiments, the average drug-to-antibody ratio (average DAR) of the antibody-drug conjugates in the antibody-drug conjugate population is selected from 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.2, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.7, 8.9, 9, or 9.7.

In some embodiments, the antibody-drug conjugate population contains ADCs with a DAR distribution of 1 to 8, for example, 1.5, 2, 4, 6, and 8 (i.e., drug-loaded populations of 1.5, 2, 4, 6, and 8). It is worth noting that degradation products can be generated, such that the mixture may also contain DARs of 1, 3, 5, and 7. In addition, the antibody-drug conjugate population may also have an average DAR greater than 8. The antibody-drug conjugate is generated by reduction of interchain

disulfides followed by conjugation. In some embodiments, the antibody-drug conjugate comprises both of the following: the antibody-drug conjugate having a DAR of 4 or lower (i.e., a drug-loaded population of 4 or lower) and the antibody-drug conjugate having a DAR of 6 or higher (i.e., a drug-loaded population of 6 or higher).

Pharmaceutical Composition

In the third aspect of the present disclosure, the present disclosure provides a pharmaceutical composition comprising the antibody-drug conjugate or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), prodrug, or the pharmaceutically acceptable salt or solvate thereof of the first aspect, or the population of antibody-drug conjugates of the second aspect, as well as optionally one or more pharmaceutical excipients, e.g., a pharmaceutical carrier or a pharmaceutical vehicle known in the art, including a buffering agent.

In certain embodiments, the pharmaceutical composition comprises an effective amount of the following: the antibody-drug conjugate or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), prodrug, or the pharmaceutically acceptable salt or solvate thereof of the first aspect, or the population of antibody-drug conjugates of the second aspect.

In certain embodiments, the pharmaceutical composition comprises the above-mentioned antibody-drug conjugate, or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), prodrug, or the pharmaceutically acceptable salt or solvate thereof, and a pharmaceutical excipient.

In certain embodiments, the pharmaceutical composition of the present disclosure comprises the population of antibody-drug conjugates of the second aspect, and a pharmaceutical excipient.

In certain embodiments, the pharmaceutical composition of the present disclosure comprises the antibody-drug conjugate or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), prodrug, or the pharmaceutically acceptable salt or solvate thereof of the present disclosure, and a pharmaceutical excipient.

In some embodiments, the drug-to-antibody ratio (average DAR) in the pharmaceutical composition or the antibody-drug conjugate population is selected from an integer or decimal in the range of 1-10.

In some embodiments, the drug-to-antibody ratio (average DAR) in the pharmaceutical composition or the antibody-drug conjugate population is selected from 1.5-2.5, 3.5-4.5, 5.5-6.5, and 7.5-8.5.

In some embodiments, the DAR in the pharmaceutical composition or the antibody-drug conjugate population is selected from: 2±0.5, 4±0.5, 5±0.5, 6±0.5, 7±0.5, 8±0.5.

In some embodiments, the drug-to-antibody ratio (average DAR) in the pharmaceutical composition or the antibody-drug conjugate population is selected from about 2.0, about 4.0, about 6.0, or about 8.0.

In some embodiments, the drug-to-antibody ratio (average DAR) in the pharmaceutical composition or the antibody-drug conjugate population is selected from 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.2, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.7, 8.9, 9, or 9.7.

In some embodiments, the pharmaceutical composition comprises the antibody-drug conjugate or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), prodrug, or the pharmaceutically acceptable salt or solvate thereof of the first aspect, and a buffer.

In some embodiments, the pharmaceutical composition comprises the population of antibody-drug conjugates of the second aspect and a buffer.

In some embodiments, the pharmaceutical composition is for use in therapy. In some embodiments, the pharmaceutical composition is for treating and/or preventing a disease associated with abnormal cellular activities (for example, tumors). In some embodiments, the pharmaceutical composition is for modulating (inhibiting or blocking) the activity of VEGF. In some embodiments, the pharmaceutical composition is for treating or preventing a disease associated with the activity of VEGF or a disease associated with the target of VEGF. In some embodiments, the pharmaceutical composition is for treating or preventing a tumor associated with the activity of VEGF.

Use for Treating Tumors

In the fourth aspect, the present disclosure provides use of the substances of the present disclosure (including the aforementioned antibody-drug conjugate, the population of antibody-drug conjugates, and the aforementioned pharmaceutical composition) as a medicament or for the preparation of a medicament, wherein the medicament is used for therapy, for example, for treating and/or preventing a disease associated with abnormal cellular activities (for example, cancer diseases). In some embodiments, the antibody-drug conjugate, the population of antibody-drug conjugates, and the aforementioned pharmaceutical composition are in an effective amount, for example, prophylactically effective amount or therapeutically effective amount.

In some embodiments, the present disclosure provides use of the antibody-drug conjugate or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), prodrug, or the pharmaceutically acceptable salt or solvate thereof of the first aspect, the population of antibody-drug conjugates of the second aspect, and the pharmaceutical composition of the third aspect, as a medicament or for the preparation of a medicament, wherein the medicament is for modulating (inhibiting or blocking) the activity of VEGF.

In some embodiments, the present disclosure provides use of the antibody-drug conjugate or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), prodrug, or the pharmaceutically acceptable salt or solvate thereof of the first aspect, the population of antibody-drug conjugates of the second aspect, and the pharmaceutical composition of the third aspect, as a medicament or for the preparation of a medicament, wherein the medicament is for treating or preventing a disease associated with the activity of VEGF or a disease associated with the target of VEGF.

In some embodiments, the present disclosure provides use of the antibody-drug conjugate or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), prodrug, or the pharmaceutically acceptable salt or solvate thereof of the first aspect, the population of antibody-drug conjugates of the second aspect, and the pharmaceutical composition of the third aspect, as a medicament or for the preparation of a medicament, wherein the medicament is for treating or preventing a tumor associated with the activity of VEGF.

Method for Treating Tumors

In the fifth aspect, the present disclosure provides a method of using the above-mentioned antibody-drug conjugate or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), prodrug, or the pharmaceutically acceptable salt or solvate thereof of the first aspect, the population of antibody-drug conjugates of the second aspect, and the pharmaceutical composition of the third aspect for treating and/or preventing a disease associated with abnormal cellular activities (for example, tumors).

In some embodiments, the present disclosure provides a method of using the antibody-drug conjugate or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), prodrug, or the pharmaceutically acceptable salt or solvate thereof of the first aspect, the population of antibody-drug conjugates of the second aspect, and the pharmaceutical composition of the third aspect for modulating (inhibiting or blocking) the activity of VEGF.

In some embodiments, the present disclosure provides a method of using the antibody-drug conjugate or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), prodrug, or the pharmaceutically acceptable salt or solvate thereof of the first aspect, the population of antibody-drug conjugates of the second aspect, and the pharmaceutical composition of the third aspect for treating or preventing a disease associated with the activity of VEGF or a disease associated with the target of VEGF. In some embodiments, the disease is a tumor associated with the activity of VEGF.

In some embodiments, the method includes administering the above-mentioned antibody-drug conjugate or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), prodrug, or the pharmaceutically acceptable salt or solvate thereof of the first aspect, the population of antibody-drug conjugates of the second aspect, or the pharmaceutical composition of the third aspect.

In the above-mentioned third, fourth, and fifth aspects, the disease associated with the activity of VEGF, the disease associated with the target of VEGF, or the disease associated with abnormal cellular activities includes tumors, for example, cancer. The cancer may be at an early, intermediate, or advanced stage, or may be

metastatic cancer. In some embodiments, the cancer may be a solid tumor or a hematological tumor.

In one embodiment, the tumor refers to that in tumor tissue or tumor cells of an individual, for example, compared to adjacent normal tissue or normal cells of the individual (for example, normal cells in the tissue) or the same tissue or cells therein of a healthy individual, the protein level (for example, expression) of VEGF is elevated, or the nucleic acid level of VEGF is elevated.

The tumor is selected from, but not limited to: lung cancer (for example, non-small cell lung cancer, lung adenocarcinoma), colon cancer (for example, human colon adenocarcinoma), rectal cancer, gastric cancer, colorectal cancer (e.g., colorectal adenocarcinoma).

It is understood that the various aspects of the present disclosure and their various embodiments and features may be combined with any other aspect, embodiment, and feature to provide further embodiments, and such combined embodiments also fall within the scope of the present application. In addition, the methods, examples, and materials described herein are illustrative only and do not limit the present disclosure in any way. Changes that will readily occur to those skilled in the art will be included within the spirit and scope of the present disclosure. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

Definitions

The terms and symbols used in this application have the meanings typically understood by those skilled in the art, unless otherwise indicated. In particular, the following terms in this application have the meanings indicated below, unless otherwise indicated.

The expressions "a", "an", "the", "said", or the entity itself used herein include both the singular and plural forms thereof, unless otherwise indicated.

The immunoglobulin molecules of the present disclosure may be of any type (for example, IgG, IgE, IgM, IgD, IgA, and IgY), class (for example, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2), or subclass of immunoglobulin. Preferably, the antibody of the present disclosure comprises or consists of a VH region, VH CDR (referred to herein mostly as CDR-H), VL region, or VL CDR (referred to herein

mostly as CDR-L) having any amino acid sequence described in the sequence and specific information table herein, or a fragment or variant thereof.

Preferably, the antibody of the present disclosure comprises or consists of a VH domain, VH CDR (referred to herein mostly as CDR-H), VL domain, or VL CDR (referred to herein mostly as CDR-L) having any amino acid sequence described in the sequence and specific information table herein, or a fragment or variant thereof.

In the present disclosure, the term "monoclonal antibody" refers to an antibody derived from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. The modifier "monoclonal" used herein denotes that the antibody is characterized by being derived from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method.

In some embodiments of the present disclosure, monoclonal antibodies further specifically include chimeric antibodies, i.e., a portion of the heavy chain and/or light chain is identical or homologous to a certain species, class, or subclass of antibody, while the remaining portion is identical or homologous to another species, class, or subclass of antibody, so long as they possess the desired biological activity. Chimeric antibodies useful in the present disclosure include primatized antibodies, which comprise variable region antigen-binding sequences derived from a non-human primate (for example, Old World monkey, ape, etc.) and human constant region sequences.

The term "antigen-binding fragment" refers to a portion of an antibody, preferably the antigen-binding region or variable region. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, Fd, Fv, dAb, and complementarity-determining region fragments, diabodies, linear antibodies, and single-chain antibody molecules. The term "antigen-binding fragment" used herein denotes a partial fragment of an antibody having antigen-binding activity, wherein the fragment has full or partial function of the antibody, and includes but is not limited to single-chain Fv (scFv), Fab, Fab', $F(ab')_2$, disulfide bond-linked Fv (sdFv), Fv, di-scFv, and the like. The term also includes Fab', which is a monovalent fragment of the variable region of an antibody obtained by treating F(ab')2 under reducing conditions. But the term is not

limited to these molecules, as long as the fragment has binding affinity for the antigen. In addition, such functional fragments include not only fragments obtained by treating full-length antibody protein molecules with appropriate enzymes, but also proteins produced in appropriate host cells using genetically modified antibody genes.

The term "Fab'" used herein denotes a monovalent fragment of the variable region of an antibody obtained by treating $F(ab')_2$ under reducing conditions as described above. But Fab' of the present disclosure also includes Fab' produced using genetically modified antibody genes.

As used herein, the term "scFv" refers to a single polypeptide chain comprising VL and VH domains, wherein the VL and VH are connected by a linker or directly connected (see, for example, Bird et al., Science 242:423-426 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Pluckthun, The Pharmacology of Monoclonal Antibodies, Vol. 113, Roseburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994)). Such scFv molecules may have a general structure: NH2-VL-linker-VH-COOH or NH2-VH-linker-VL-COOH. Suitable linkers in the prior art consist of repeated GGGGS amino acid sequences or variants thereof. For example, linkers having an amino acid sequence (GGGGS)4 can be used, but variants thereof can also be used (Holliger et al., (1993), Proc. Natl. Acad. Sci. USA 90: 6444-6448). Other linkers useful in the present disclosure are described by Alfthan et al. (1995), Protein Eng. 8:725-731, Choi et al. (2001), Eur. J. Immunol. 31: 94-106, Hu et al. (1996), Cancer Res. 56:3055-3061, Kipriyanov et al. (1999), J. Mol. Biol. 293: 41-56, and Roovers et al. (2001), Cancer Immunol. In some cases, a disulfide bond may also be present between the VH and the VL of the scFv. As used herein, the term "di-scFv" refers to an antibody fragment formed by linking two scFvs.

The term "variable region", "variable domain", or "variable structural domain" refers to the domain in the heavy chain and/or light chain of an antibody that is involved in binding of the antibody to the antigen. The VH and VL of a native IgG antibody each comprise four conserved framework regions (FRs) and three complementarity-determining regions (CDRs). The term "complementarity-determining region" or "CDR" refers to the region within the variable domain that

primarily contributes to binding to the antigen; "Framework" or "FR" refers to the variable domain residues other than the CDR residues in the variable domain. VH comprises 3 CDR regions: CDR-H1, CDR-H2, and CDR-H3; VL comprises 3 CDR regions: CDR-L1, CDR-L2, and CDR-L3. Each VH and VL is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. A single VH or VL may be sufficient to confer antigen binding specificity.

The boundaries of the amino acid sequences of CDRs can be determined by various well-known definition schemes, for example: the "Kabat" definition scheme rules (see Kabat et al. (1991), "Sequences of Proteins of Immunological Interest", 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD), the "Chothia" definition scheme, the "ABM" definition scheme, the "contact" definition scheme (see Martin, ACR. Protein Sequence and Structure Analysis of Antibody Variable Domains[J]. 2001), and the ImMunoGeneTics (IMGT) definition scheme (Lefranc, M.P. et al., Dev. Comp. Immunol., 27, 55-77 (2003); Front Immunol.2018 Oct 16; 9:2278), etc.; The correspondence among the various definition schemes is well known to those skilled in the art, and is exemplarily shown below.

Relationship among CDR definition schemes

Loop	Kabat	AbM	Chothia	Contact	IMGT
CDR-L1	L24--L34	L24--L34	L24--L34	L30--L36	L27--L32
CDR-L2	L50--L56	L50--L56	L50--L56	L46--L55	L50--L51
CDR-L3	L89--L97	L89--L97	L89--L97	L89--L96	L89--L97
CDR-H1	H31--H35B (Kabat Numbering)	H26--H35B	H26--H32..34	H30--H35B	H26--H35B
CDR-H1	H31--H35 (Chothia Numbering)	H26--H35	H26--H32	H30--H35	H26--H33
CDR-H2	H50--H65	H50--H58	H52--H56	H47--H58	H51--H56
CDR-H3	H95--H102	H95--H102	H95--H102	H93--H101	H93--H102

It should be understood that certain modifications may be made to the antibody or the antigen-binding fragment thereof described herein, for example, amino acid substitutions, deletions, and/or additions (preferably outside the CDR regions, more preferably outside the variable regions), to obtain antibodies that still have the

activity of binding the antigen, and such antibodies are also encompassed within the anti-VEGF antibodies described in the present invention.

The term "intact antibody" or "full-length antibody" refers to an antibody comprising an antigen-binding variable region and a light chain constant region (CL) and heavy chain constant regions (CH1, CH2, and CH3). The constant regions may be native sequences (for example, human native constant region sequences) or amino acid sequence variants thereof. An intact antibody is preferably an intact antibody having one or more effector functions. In the present disclosure, a "humanized" form of a non-human (for example, murine) antibody refers to a chimeric antibody that comprises a minimal amount of non-human immunoglobulin sequence. Most humanized antibodies are human recipient immunoglobulins in which the hypervariable region residues are replaced by non-human (for example, mouse, rat, rabbit, or non-human primate) hypervariable region residues (donor antibody) that possess the desired specificity, affinity, and function. In some embodiments, framework region (FR) residues of the human immunoglobulin are also replaced by non-human residues. Furthermore, humanized antibodies may also comprise residues that are not found in the recipient antibody or the donor antibody. These modifications are made to further optimize the performance of the antibody. A humanized antibody generally comprises at least one, and typically two, variable regions, in which all or substantially all hypervariable loops correspond to those of a non-human immunoglobulin, and the FRs are entirely or substantially entirely sequences of a human immunoglobulin. A humanized antibody may also comprise at least a portion of an immunoglobulin constant region (Fc, typically a human immunoglobulin Fc).

Intact antibodies can be classified into different "classes" according to the amino acid sequence of the heavy chain constant region; the five major classes are IgA, IgD, IgE, IgG, and IgM, and several of these classes can be further divided into different "subclasses" (isotypes), for example, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant regions of the different classes of antibodies are designated α, β, ε, γ, and μ, respectively; the subunit structures and three-dimensional configurations of the different classes of immunoglobulins are well known in the art.

Herein, the CDRs contained in the antibody or the antigen-binding fragment thereof of the present disclosure may be determined according to various definition schemes known in the art. In certain embodiments, the CDRs contained in the antibody or the antigen-binding fragment thereof of the present disclosure are preferably determined by the Kabat, Chothia, AbM, IMGT, or Contact definition schemes, respectively.

As used herein, the term "framework residue region" or "FR residue" refers to those amino acid residues in the antibody variable region other than the CDR residues as defined above.

The twenty conventional amino acids involved in the disclosure are written in accordance with conventional usage. See, for example, Immunology-A Synthesis (2nd Edition, E. S. Golub and D. R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference. Herein, the terms "polypeptide" and "protein" have the same meaning and are used interchangeably. And in the present disclosure, amino acids are typically represented by single-letter and three-letter abbreviations well known in the art. For example, alanine may be represented by A or Ala; arginine may be represented by R or Arg; glycine may be represented by G or Gly; glutamine may be represented by Q or Gln.

The "Vascular Endothelial Growth Factor (VEGF)" described herein is a signaling protein generated by a variety of cells that is capable of stimulating the formation of blood vessels. Specifically, VEGF is a subfamily within the platelet-derived growth factor family and belongs to growth factors with a cysteine knot structure. They are important signaling proteins involved in two processes: vasculogenesis (the de novo formation of the embryonic circulatory system) and angiogenesis (the formation of new blood vessels from the existing vasculature). In mammals, the VEGF family comprises five members: VEGF-A, placental growth factor (PlGF), VEGF-B, VEGF-C, and VEGF-D. Among these members, VEGF-A was the first to be discovered. Therefore, VEGF-A may also be referred to as VEGF. Its activity has been studied primarily on vascular endothelial cells, although it also affects many other cell types (for example, stimulating monocyte/macrophage migration, neurons, cancer cells, and renal epithelial cells). In vitro studies have indicated that VEGF-A can promote the proliferation and migration of endothelial

cells. In addition, VEGF-A also has a vasodilatory effect, and increases the permeability of microvessels, which is why it was originally referred to as vascular permeability factor. Herein, VEGF may be VEGF-A, for example, human VEGF-A. In some embodiments, the VEGF-A is the VEGF165 isoform, for example, human VEGF-165.

The term "antibody that specifically binds VEGF" or "anti-VEGF antibody", "anti-VEGF", "VEGF antibody", or "antibody against VEGF" used herein refers to such an antibody, wherein the antibody or the antigen-binding fragment thereof is capable of binding VEGF protein with sufficient affinity.

As used herein, the term "amino acid" refers to naturally occurring and synthetic amino acids. Amino acids may be L or D isomers. In some embodiments, the amino acid is a natural amino acid, i.e., an amino acid that is naturally synthesized in organisms and participates in protein construction. The names of exemplary natural amino acids and their corresponding abbreviations are listed in the table below:

Amino acid abbreviations		
Name	**Three-letter symbol**	**Single-letter symbol**
Alanine	Ala	A
Arginine	Arg	R
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Histidine	His	H
Isoleucine	Ile	I
Glycine	Gly	G
Asparagine	Asn	N
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F

Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

The amino acids of the present disclosure further include other amino acids, for example, citrulline (Cit; C), which is also a naturally occurring amino acid.

Unless otherwise specified, the amino acids of the present disclosure refer to L-amino acids.

The amino acids described herein may also be modified amino acids, wherein such modifications may be made, for example, by chemically or biologically altering the structure of natural amino acids, thereby imparting new functions or properties to them. Such modifications may occur at the side chain, amino group, or carboxyl group of the amino acid, among other sites.

As used herein, the term "natural amino acid residue or modification thereof" encompasses natural amino acids as well as modified amino acids.

A biosimilar refers to a biological product that is identical in sequence to an approved originator biologic (reference drug, Reference Product, e.g., Bevacizumab or Avastin) and is highly similar in structure, function, quality, safety, and efficacy, but is permitted to have minor differences (owing to differences in the manufacturing process).

As used herein, the term "immunoconjugate" refers to the linkage of an effector molecule to an antibody or an antigen-binding fragment thereof by a linker, such that the antibody or the antigen-binding fragment thereof can serve as a carrier to target-deliver the effector molecule to the target site. The term "effector molecule" refers to the active portion coupled to the antibody or antibody fragment of the present disclosure, and may include any moiety used for attachment to the antibody or antibody fragment. In some embodiments, the effector molecule may be a drug, for example, small-molecule drug, DNA, RNA, enzyme, or polypeptide, etc. In some embodiments, the immunoconjugate encompasses an antibody-drug conjugate

(ADC). Effector molecules or active portions suitable for connecting to antibodies include, for example, anti-tumor agents, immunomodulatory agents, biological response modifiers, lectins, cytotoxic drugs, chromophores, fluorophores, chemiluminescent compounds, enzymes, metal ions, as well as any combination thereof, etc. In some embodiments, the immunoconjugate of the present disclosure is an antibody-drug conjugate, i.e., an ADC.

The term "immunomodulatory agent" used herein refers to a natural or synthetic active agent or drug that inhibits or modulates (for example, activates) an immune response. The immune response may be a humoral response or a cellular response. Immunomodulatory agents comprise immunosuppressants. In some embodiments, the immunomodulatory agent of the present disclosure includes an immune checkpoint inhibitor or an immune checkpoint agonist.

As used herein, the term "prevention" refers to a method implemented to prevent or delay the onset of a disease or condition or symptom (for example, tumors and infectious diseases) in a subject's body. As used herein, the term "treatment" refers to a method implemented in order to obtain a beneficial or desired clinical outcome. For the purposes of the present disclosure, beneficial or desired clinical results include, but are not limited to, alleviating symptoms, diminishing the extent of the disease, stabilizing (i.e., no longer worsening) the state of the disease, delaying or slowing the progression of the disease, ameliorating or palliating the state of the disease, and relieving symptoms (whether partial or total), whether detectable or undetectable. In addition, "treatment" can also refer to an extended survival period compared to the expected survival period (if not treated).

As used herein, the term "subject" refers to a mammal, for example, primate mammal, for example, non-human primate mammal or human. In certain embodiments, the subject (for example, human) has a tumor and an infectious disease, or is at risk of having the above-mentioned diseases.

As used herein, the term "effective amount" refers to an amount sufficient to obtain, or at least partially obtain, the desired effect. For example, a disease-preventing effective amount (for example, tumors and infectious diseases) refers to an amount sufficient to prevent, stop, or delay the occurrence of the disease (for example, tumors and infectious diseases); a disease-treating effective amount refers

to an amount sufficient to cure or at least partially arrest the disease and its complications in a patient who already has the disease. Determining such an effective amount is well within the capabilities of the skilled person. For example, the effective amount for therapeutic use will depend on the severity of the disease to be treated, the general state of the patient's own immune system, the general condition of the patient, for example, age, weight, and sex, the manner in which the drug is administered, as well as other treatments administered at the same time, etc.

The term "pharmaceutical excipient" refers to diluents, adjuvants (for example, Freund's adjuvant (complete and incomplete)), excipients, carriers or stabilizers, and the like administered with an active substance.

The term "pharmaceutical composition" refers to such a composition that is in a form permitting the biological activity of the active ingredient contained therein to be effective, and that does not contain any further ingredient having an unacceptable toxicity to the subject to whom the composition is administered.

The term "label" used herein refers to a compound or composition that is directly or indirectly coupled or fused to a reagent (such as a polynucleotide probe or an antibody), and that facilitates detection of the reagent to which it is coupled or fused. The label itself may be detectable (for example, radioisotope label or fluorescent label) or, in the case of an enzymatic label, may catalyze a chemical alteration of a substrate compound or composition that is detectable. The term is intended to encompass direct labeling of a probe or antibody by conjugation (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reaction with another directly labeled reagent.

An "isolated" antibody or molecule is one that has been separated from a component of its natural environment. In some embodiments, the antibody or molecule is purified to more than 95% or 99% purity, as determined by, for example, electrophoresis (for example, SDS-PAGE, isoelectric focusing (IEF) and capillary electrophoresis) or chromatography (for example, ion exchange or reversed-phase HPLC).

The "percent identity (%)" of an amino acid sequence refers to the percentage of amino acid residues in a candidate sequence that are identical to the amino acid residues in a specific amino acid sequence shown in this specification, after aligning

the candidate sequence with the specific amino acid sequence shown in this specification and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and without considering any conservative substitutions as part of the sequence identity. In some embodiments, the antibodies in the present disclosure also encompass variants of the antibody molecules, wherein the variants have a considerable degree of identity relative to the antibody molecules specifically disclosed herein and their sequences, for example, identity of at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% or higher. The variants may contain conservative changes.

The term "about" when used in connection with a numerical value is meant to encompass numerical values within a range having a lower limit that is 5% smaller than the specified numerical value and an upper limit that is 5% larger than the specified numerical value.

As used herein, the term "and/or" means any one of the optional items or two or more of the optional items.

As used herein, the term "comprise" or "include" means including the described elements, integers, or steps, but not excluding any other elements, integers, or steps. Unless otherwise indicated, the term "comprise" or "include" when used herein also encompasses situations consisting of the described elements, integers, or steps. For example, when reference is made to an antibody variable region that "comprises" a specific sequence, it is also intended to encompass an antibody variable region consisting of that specific sequence.

When referring to "substances of the present disclosure" or "substances of the present invention", it encompasses one or more of the various molecules or compositions defined herein, for example, encompassing one or more of the following: the antibody-drug conjugate defined herein, or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), prodrug, or the pharmaceutically acceptable salt or solvate thereof, the population of antibody-drug conjugates, or the pharmaceutical composition.

The term "pharmaceutically acceptable" means that a molecule itself, a molecular fragment, or a composition does not produce unfavorable, allergic, or other adverse reactions when properly administered to an animal or human. Specific

examples of some substances that may serve as a pharmaceutically acceptable carrier or a component thereof include saccharides (e.g., lactose), starch, cellulose, and derivatives thereof, vegetable oils, gelatin, polyols (e.g., propylene glycol), alginic acid, and the like.

The term "drug-to-antibody ratio" or "DAR" refers to the quantitative ratio of the drug moiety (D) conjugated to the antibody moiety (Ab) described herein to the antibody moiety. The DAR of an ADC can range from 1 to 20, but higher loadings are also possible depending on the number of linking sites on the antibody. The term DAR may be used when referring to the number of drugs loaded onto a single antibody, or alternatively, when referring to the average or mean DAR of a group of ADCs. DAR may also be calculated as the average DAR of the molecular population in the product, i.e., the overall ratio (molar ratio) of the drug moiety (D) conjugated to the Ab moiety described herein to the Ab moiety in the product as measured by a detection method (for example, by conventional methods e.g., mass spectrometry, ELISA assay, electrophoresis, and/or HPLC); this DAR is referred to herein as the average DAR. In some embodiments, the average DAR value of the antibody-drug conjugate of the present disclosure is 1.0-20.0, for example, 1.0-18.0, 1.0-16.0, 2.0-14.0, 3.0-12.0, 4.0-10.0, 5.0-9.0, 6.0-8.0, 1.0-8.0, 2.0-6.0, for example, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 12.0, and 16.0, with ranges having any two of these values as endpoints.

During the determination of DAR values by mass spectrometry, the antibody has been reduced to separated heavy and light chains; DAR1 represents a conjugate comprising a light chain or heavy chain conjugated to 1 toxin molecule; DAR2 represents a conjugate comprising a light chain or heavy chain conjugated to 2 toxin molecules; DAR3 represents a conjugate comprising a light chain or heavy chain conjugated to 3 toxin molecules; and so on.

The term "drug" refers to a chemical substance that can alter or ascertain the physiological functions and pathological states of the body, and can be used for the

prevention, diagnosis, and treatment of diseases; particularly, it includes substances that inhibit or prevent the function of cells and/or cause cell death or destruction. Drugs include cytotoxic agents, particularly small-molecule cytotoxic agents. There is no strict boundary between drugs and poisons; a poison refers to a chemical substance that generates toxic effects on the body and damages human health at relatively small doses, and any drug can generate a toxic reaction if the dose is excessive.

A cytotoxic agent, i.e., a substance that inhibits or prevents the function of cells and/or causes cell death or destruction. In principle, cytotoxic drugs can kill tumor cells at sufficiently high concentrations, but due to their lack of specificity, while killing tumor cells, they also cause apoptosis of normal cells, leading to serious side effects. Cytotoxic agents include toxins, e.g., small-molecule toxins or enzymatically active toxins of bacterial, fungal, plant, or animal origin, radioisotopes (e.g., radioisotopes of At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, and Lu), chemotherapeutic drugs, antibiotics, and nucleolytic enzymes. Additionally, they include but are not limited to DNA topoisomerase inhibitors (for example, camptothecin-based biologically active molecules, for example, camptothecin, DXD, substituent-modified camptothecin or substituent-modified DXD, hydroxycamptothecin, 9-aminocamptothecin, SN-38, irinotecan, topotecan, belotecan, rubitecan) or tubulin inhibitors (for example, MMAF-class tubulin inhibitors, MMAE-class tubulin inhibitors).

For the avoidance of doubt, the "drug" that can be a component of an ADC does not refer only to "medicines" that have been approved by pharmaceutical regulatory authorities, but also includes any compound with potential therapeutic biological activity in clinical settings, or in research and development and academic research. Additionally, it should be understood that this differs from the meaning of "drug" in "use in the preparation of a medicament (medicine)". It should be understood that in order to be linked to a linker, the drug molecule may need to be functionalized or derivatized, and the compounds thus obtained are also included within the scope of drugs of the present disclosure.

As used herein, "substituent-modified camptothecin", "substituent-modified DXD", and "substituent-modified SN38" refer to those that are chemically modified

at any available position on the camptothecin, DXD, or SN38 molecule, respectively, for example, chemically modified by substitution, condensation, oxidation, reduction, linking a protecting group, and the like.

In the present disclosure, the "active drug unit" refers to the portion of an antibody-drug conjugate (also known as ADC) other than the antibody and the linker group of the present disclosure, which is derived from the drug defined above. For convenience, the "active drug unit" in the ADC of the present disclosure may be referred to directly by the name of the above-mentioned drug, which is understood by those skilled in the art.

As used in the present disclosure, the term "pharmaceutically acceptable salt" denotes a salt that retains the biological effectiveness and properties of the antibody-drug conjugate or drug-linker conjugate of the present disclosure, and the salt is not biologically or otherwise undesirable. The conjugates of the present disclosure (including antibody-drug conjugates and drug-linker conjugates) may exist in the form of their pharmaceutically acceptable salts, including acid addition salts and base addition salts. In the present disclosure, pharmaceutically acceptable acid addition salts denote salts formed by the conjugates of the present disclosure with organic or inorganic acids, the organic or inorganic acids include but are not limited to hydrochloric acid, sulfuric acid, hydrobromic acid, hydroiodic acid, phosphoric acid, nitric acid, perchloric acid, acetic acid, oxalic acid, maleic acid, fumaric acid, tartaric acid, benzenesulfonic acid, methanesulfonic acid, salicylic acid, succinic acid, citric acid, lactic acid, propionic acid, benzoic acid, p-toluenesulfonic acid, malic acid, etc. Pharmaceutically acceptable base addition salts denote salts formed by the conjugates of the present disclosure with organic or inorganic bases, including but not limited to alkali metal salts, for example, lithium, sodium, or potassium salts; alkaline earth metal salts, for example, calcium or magnesium salts; organic base salts, for example, ammonium salts formed with organic bases containing N-groups. Pharmaceutically acceptable salts are preferred. But other salts may also be useful, for example, in isolation or purification steps, they may be used during preparation, and are therefore encompassed within the scope of the present application.

Pharmaceutically acceptable salts can be obtained using standard procedures well known in the art, for example, by reacting a sufficient amount of a basic compound with a suitable acid that provides a pharmaceutically acceptable anion.

As used in the present disclosure, the term "stereoisomer" denotes an isomer formed due to at least one asymmetric center. In a compound having one or more (for example, one, two, three, or four) asymmetric centers, it can give rise to a racemic mixture, a single enantiomer, a mixture of diastereomers, and individual diastereomers. Specific individual molecules may also exist as geometric isomers (cis/trans). Similarly, the compounds of the present disclosure may exist as mixtures of two or more structurally different forms in rapid equilibrium (typically referred to as tautomers). Representative examples of tautomers include keto-enol tautomers, phenol-keto tautomers, nitroso-oxime tautomers, imine-enamine tautomers, and the like. It is to be understood that the scope of the present disclosure encompasses all such isomers in any proportion (for example, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%) or mixtures thereof.

In the present disclosure, solid lines (——), solid wedges, or dashed wedges may be used to depict the carbon-carbon bonds of the compounds of the present disclosure. The use of a solid line to depict a bond linked to an asymmetric carbon atom is intended to indicate that all possible stereoisomers at that carbon atom are included (for example, specific enantiomers, racemic mixtures, etc.). The use of a solid or dashed wedge to depict a bond linked to an asymmetric carbon atom is intended to indicate that the stereoisomer shown is present. Unless otherwise indicated, the compounds of the present disclosure are intended to be capable of existing in the form of stereoisomers (which include cis and trans isomers, optical isomers (for example, R and S enantiomers), diastereomers, geometric isomers, rotamers, conformational isomers, atropisomers, and mixtures thereof). The compounds of the present disclosure may exhibit more than one type of isomerism and may consist of mixtures thereof (for example, racemic mixtures and diastereomeric pairs).

The present disclosure further includes all pharmaceutically acceptable isotopic derivatives which are identical to the substance of the present disclosure except that one or more atoms are replaced with an atom having the same atomic number but an

atomic mass or mass number different from the atomic mass or mass number prevalent in nature. Examples of isotopes suitable for inclusion in the substances of the present disclosure include (but are not limited to) isotopes of hydrogen (for example, ^{2}H, ^{3}H); isotopes of carbon (for example, ^{11}C, ^{13}C, and ^{14}C); isotopes of chlorine (for example, ^{36}Cl); isotopes of fluorine (for example, ^{18}F); isotopes of iodine (for example, ^{123}I and ^{125}I); isotopes of nitrogen (for example, ^{13}N and ^{15}N); isotopes of oxygen (for example ^{15}O, ^{17}O and ^{18}O); isotopes of phosphorus (for example ^{32}P); and isotopes of sulfur (for example, ^{35}S). In some embodiments, the isotopic derivative is a deuterated derivative, i.e., one in which one or more hydrogen atoms are deuterium atoms.

The compound of the present disclosure may exist in the form of a solvate (preferably a hydrate), in which the compound of the present disclosure comprises, as a structural element of the crystal lattice of the compound, a polar solvent, in particular, for example, water, methanol, or ethanol. The amount of the polar solvent, in particular water, may be present in a stoichiometric or non-stoichiometric ratio.

Also included within the scope of the present disclosure are metabolites of the compounds of the present disclosure, i.e., substances formed in vivo upon administration of the compounds of the present disclosure. Such products may be generated, for example, by oxidation, reduction, hydrolysis, amidation, deamidation, esterification, defatting, enzymatic hydrolysis, etc. of the administered compound. Therefore, the present disclosure includes metabolites of the compounds of the present disclosure, including compounds generated by a process of contacting the compounds of the present disclosure with a mammal for a period of time sufficient to generate metabolic products thereof.

The present disclosure further includes within its scope prodrugs of the compounds of the present disclosure. Typically such prodrugs will be functional group derivatives of the compounds that are readily converted into the desired therapeutically active compound in vivo. Therefore, in these instances, the term "administration" used in the therapeutic methods of the present disclosure shall include treating various diseases or conditions with a prodrug form of one or more of the claimed compounds, wherein the prodrug form is converted in vivo into the above-mentioned compound after administration to an individual. Conventional

methods for selecting and preparing suitable prodrug derivatives are described, for example, in "Design of Prodrug", ed. H. Bundgaard, Elsevier, 1985.

In the present disclosure, the pharmaceutical excipients refer to vehicles and additives used in the production of pharmaceutical products and in the formulation of prescriptions; they refer to substances other than active ingredients that have been reasonably assessed for safety and are contained in pharmaceutical preparations. In addition to providing shape, serving as a carrier, and improving stability, pharmaceutical excipients or vehicles also have important functions, e.g., solubilization, co-solubilization, and sustained/controlled release, and are important components that may affect the quality, safety, and efficacy of a drug product. They can be classified as natural, semi-synthetic, or fully synthetic according to their origin. According to their function and use, they can be classified as: solvents, propellants, solubilizers, co-solvents, emulsifiers, colorants, binders, disintegrants, fillers, lubricants, wetting agents, osmotic pressure regulators, stabilizers, glidants, flavoring agents, preservatives, suspending agents, coating materials, fragrances, anti-adherents, antioxidants, chelating agents, penetration enhancers, pH adjusting agents, buffering agents, plasticizers, surfactants, foaming agents, defoaming agents, thickening agents, inclusion agents, humectants, absorbents, diluents, flocculating and deflocculating agents, filter aids, release retardants, etc.; according to the route of administration, they can be classified as oral, injectable, mucosal, transdermal or topical, nasal or oral inhalation, and ophthalmic administration, etc. The same pharmaceutical excipient or vehicle may be used in pharmaceutical preparations for different routes of administration, and may have different functions and uses. For the use of pharmaceutical excipients and their applications, see also "Handbook of Pharmaceutical Excipients", 8th edition, R.C. Rowe, P.J. Seskey, and S.C. Owen, Pharmaceutical Press, London, Chicago.

The pharmaceutical composition may be formulated into various suitable dosage forms according to the route of administration. For example, tablets, capsules, granules, oral solutions, oral suspensions, oral emulsions, powders, tinctures, syrups, injections, suppositories, ointments, creams, pastes, ophthalmic preparations, pills, implants, aerosols, powder aerosols, sprays, etc. wherein the pharmaceutical composition or suitable dosage form may contain 0.01 mg to 1000 mg of the

substance of the present disclosure (including the conjugate) or a pharmaceutically acceptable salt thereof, suitably 0.1 mg to 800 mg, preferably 0.5 mg to 500 mg, preferably 0.5 mg to 350 mg, and particularly preferably 1 mg to 250 mg. Amounts outside the above-mentioned range may sometimes also be feasible.

The pharmaceutical composition may be administered in the form of an injection (for example, intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous injection), including injections, sterile powders for injection, and concentrated solutions for injection. Carriers and solvents that may be used include water, Ringer's solution, and isotonic sodium chloride solution. Additionally, sterilized non-volatile oils may also be used as solvents or suspending media, e.g., monoglycerides or diglycerides. The pharmaceutical composition may be administered in the form of an infusion.

In the present disclosure, regarding "L is connected to the antibody by a sulfur atom", those skilled in the art will understand that the sulfur atom is derived from the thiol group (-SH) contained in the antibody itself after opening the disulfide bond (for example, the disulfide bond can be opened by reducing it with the reducing agent TCEP to generate a thiol group -SH); that is, the -S- between L and Ab is not an additionally introduced external sulfur atom.

In the present disclosure, the term "linking group" or "linker" refers to the fragment that links the active drug unit (drug molecule) to the antibody moiety. In this regard, the linker, prior to being linked to the antibody or the antigen-binding fragment thereof (i.e., the linker precursor), has a functional group capable of forming a bond with the functional group of the antibody or the antigen-binding fragment thereof.

In the present disclosure, the term "antibody-drug conjugate" or "ADC" refers to a substance obtained by linking an active drug unit (drug molecule) to an antibody or an antigen-binding fragment moiety thereof. In some embodiments of the present disclosure, the active drug unit is connected to the targeting moiety by a linker. The linker is capable of cleavage in a specific environment (for example, intracellular low-pH environment) or under specific actions (for example, the action of lysosomal proteases), thereby separating the biologically active compound (for example, c-Myc protein degrader) fragment from the targeting moiety or the antibody, or the

antigen-binding fragment thereof. In some embodiments of the present disclosure, the linker comprises a cleavable or non-cleavable unit, for example, peptide or disulfide bond. In some embodiments of the present disclosure, the active drug unit is directly connected to the targeting moiety or the antibody or the antigen-binding fragment thereof by a covalent bond, and the covalent bond is capable of being cleaved in a specific environment or under a specific action, thereby separating the active drug unit from the antibody or the antigen-binding fragment moiety thereof.

The term "population of antibody-drug conjugates" or "antibody-drug conjugate population" refers to a mixture consisting of a group or population of antibody-drug conjugates of the present disclosure, tautomers, stereoisomers, isotopic derivatives (e.g., deuterated derivatives), prodrugs, or pharmaceutically acceptable salts or solvates thereof, wherein the q of the antibody-drug conjugates may be the same or different. Additionally, it may also be referred to as an "antibody-drug conjugate mixture".

Beneficial Effects of the Invention

In some embodiments, the anti-VEGF antibody-drug conjugate of the present disclosure, utilizing the extracellular cleavage capability of its linker in the tumor microenvironment, can bind to VEGF in the tumor microenvironment rather than to tumor cell surface antigens, forming an antibody-drug conjugate composed of such antibodies, and such antibody-drug conjugates still possess high anti-tumor activity. This overcomes the difficulty of traditional ADC technology, which can only employ antibodies that bind to tumor cell surface antigens to make ADCs, and has developed ADCs with high anti-tumor activity targeting free VEGF.

In some embodiments, the antibody-drug conjugate (ADC) of the present disclosure has better solubility and/or excellent chemical stability; experiments have shown that the VEGF-ADC of the present disclosure has excellent plasma stability and systemic circulation stability; the ADC of the present disclosure, when incubated in plasma, almost always maintains the toxin without shedding, which can reduce unintended toxin shedding in non-target cells, thereby having a higher therapeutic index.

By extensive research, the present disclosure has discovered a class of ADCs that can be cleaved in the tumor microenvironment (both within and around tumor cells), capable of achieving release both intracellularly and extracellularly in tumor cells via cathepsins, maximizing ADC delivery to tumor tissues and tumor cells, thereby exerting the efficacy of treating tumors, and thus achieving anti-tumor effects by the free antigen VEGF.

In some embodiments, the antibody-drug conjugate of the present disclosure, by utilizing the extracellular cleavage capability of its linker in the tumor microenvironment as well as its enrichment capability in the tumor microenvironment, can, together with an antibody having no cellular endocytosis capability and an antibody having no capability to bind a tumor extracellular antigen, constitute an antibody-drug conjugate, and such antibody-drug conjugates still have high anti-tumor activity.

The conjugate (ADC) obtained by the above-mentioned method, by adjustment of the physicochemical properties of the linker and the overall ADC molecule, increases the exposure of the entire ADC molecule in the relatively acidic tumor environment, thereby giving the ADC better tumor tissue targeting, i.e., enrichment capability in the tumor microenvironment, increases the ratio of biologically active molecule concentrations in the tumor versus the blood, and reduces the mechanism-related toxicity of the ADC molecule (toxicity generated by ADC binding cell surface antigens in non-tumor tissues, also known as "on-target toxicity"), thus having a higher therapeutic index.

In some embodiments, the conjugate obtained by the above-mentioned method has very high stability in systemic circulation in vivo, reduces shedding of drug molecules in non-target tissues, and reduces "off-target" toxicity caused by toxin shedding in non-target tissues.

In some embodiments, the conjugate is capable of synergistically exerting the anti-tumor activity of the anti-VEGF antibody and the toxin, and its anti-tumor activity is superior to that of the anti-VEGF antibody or the toxin alone or in combination.

In some embodiments, the biologically active molecule of the conjugate has higher anti-tumor-cell activity and thus has an excellent bystander effect, and the

ADC can more effectively kill antigen-high-expressing tumor cells as well as antigen-low-expressing or antigen-non-expressing tumor cells in the tumor tissue.

In summary, the ADC of the present disclosure has significant clinical value.

Brief Description of the Drawings

FIG. 1a. Species cross-reactivity of the anti-VEGF antibody-drug conjugate

FIG. 1b. Species cross-reactivity of the anti-VEGF antibody

FIG. 2. Cross-reactivity of the anti-VEGF antibody-drug conjugate with VEGF family proteins

FIG. 3a. Statistical chart of the effect of the anti-VEGF antibody-drug conjugate on mesh formation by HUVEC cells

FIG. 3b. Statistical chart of the effect of the anti-VEGF antibody-drug conjugate on junction formation by HUVEC cells

FIG. 3c. Fluorescence image of the effect of the anti-VEGF antibody-drug conjugate on tube formation by HUVEC cells

FIG. 4. Efficacy test of anti-VEGF antibody-drug conjugate on the SW480 xenograft tumor model

FIG. 5. Body weight test of anti-VEGF antibody-drug conjugate on the SW480 xenograft tumor model

FIG. 6. Efficacy test of anti-VEGF antibody-drug conjugate on the NCI-H358 xenograft tumor model

FIG. 7. Body weight test of anti-VEGF antibody-drug conjugate on the NCI-H358 xenograft tumor model

FIG. 8. Body weight test of anti-VEGF antibody-drug conjugate on the human colorectal cancer PDX model

FIG. 9. Body weight test of anti-VEGF antibody-drug conjugate on the human colorectal cancer PDX xenograft tumor model

Detailed Description of Embodiments

The present disclosure is further illustrated by the following description of specific embodiments, but this is not intended to limit the present disclosure. Those skilled in the art may make various modifications or improvements according to the

teachings of the present disclosure without departing from the basic spirit and scope of the present disclosure. The reagents or instruments used for which manufacturers are not specified are all conventional products that are commercially available. Unless otherwise stated, solutions mentioned in the examples are solutions in water. Unless otherwise stated, abbreviations used herein typically have meanings well known in the art.

Example 1. Preparation of anti-VEGF antibody

Bevacizumab (trade name **Avastin**) is a VEGF inhibitor that was launched on the market in the United States on February 26, 2004, and is used for the treatment of a variety of tumors e.g., lung cancer and ovarian cancer; WO 9845331 discloses its sequence and preparation method, and meanwhile the sequence and preparation method of this murine antibody and its humanized antibody rhuMab-VEGF (i.e., Bevacizumab) have been disclosed in the U.S. patent with patent No. US 6,054,297 (inventors: Carter, Paul J. and Presta, Leonard G; patent application date: May 9, 1995; patent grant date: April 25, 2000; patent title: Humanized antibodies and methods for making them) and published in the journal Cancer Res (Presta LG et al., Cancer Res, 1997, 57:4593). The sequence and preparation method of Bevacizumab or its biosimilar are also disclosed in CN 109053895 B.

Heavy chain sequence of Bevacizumab or its biosimilar:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWV
GWINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAK
YPHYYGSSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCWVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K(SEQ ID NO:1)

Light chain sequence of Bevacizumab or its biosimilar:

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTS
SLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKV

EIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC(SEQ ID NO:2)

By techniques well known to those skilled in the art, the amino acid sequences of the CDR regions, VH, and VL of Bevacizumab were obtained by analysis, and the results are as follows:

the amino acid sequence of the heavy chain variable region VH is as set forth in SEQ ID NO: 3,

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWV GWINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAK (SEQ ID NO:3)

the amino acid sequence of the light chain variable region VL is as set forth in SEQ ID NO: 4,

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTS SLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVP (SEQ ID NO:4)

the amino acid sequences of the 3 CDR regions of the heavy chain variable region are as follows:

	Definition scheme	Sequence	SEQ ID No
CDR-H1	Chothia	GYTFTNY	5
	AbM	GYTFTNYGMN	6
	Kabat	NYGMN	7
	Contact	TNYGMN	8
	IMGT	GYTFTNYG	9
CDR-H2	Chothia	NTYTGE	10
	AbM	WINTYTGEPT	11
	Kabat	WINTYTGEPTYAADFKR	12
	Contact	WVGWINTYTGEPT	13
	IMGT	INTYTGEPT	14
CDR-H3	Chothia	YPHYYGSSHWYFDV	17
	AbM	YPHYYGSSHWYFDV	17
	Kabat	YPHYYGSSHWYFDV	17
	Contact	AKYPHYYGSSHWYFD	15

	IMGT	AKYPHYYGSSHWYFDV	16

the amino acid sequences of the 3 CDR regions of the light chain variable region are as follows:

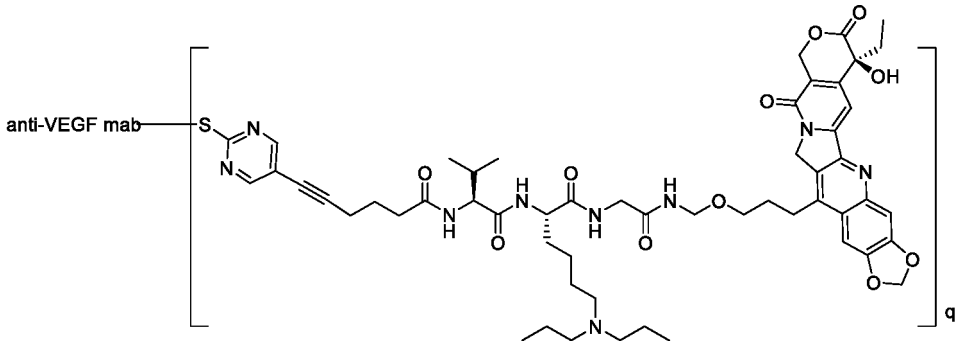
	Definition scheme	Sequence	SEQ ID No
CDR-L1	Chothia	SASQDISNYLN	23
	AbM	SASQDISNYLN	23
	Kabat	SASQDISNYLN	23
	Contact	SNYLNWY	24
	IMGT	QDISNY	20
CDR-L2	Chothia	FTSSLHS	19
	AbM	FTSSLHS	19
	Kabat	FTSSLHS	19
	Contact	VLIYFTSSLH	25
	IMGT	FTS	18
CDR-L3	Chothia	QQYSTVPWT	22
	AbM	QQYSTVPWT	22
	Kabat	QQYSTVPWT	22
	Contact	QQYSTVPW	21
	IMGT	QQYSTVPWT	22

An IgG1 isotype antibody was also prepared, which is a human anti-hen egg lysozyme antibody (human anti-Hen Egg Lysozyme IgG, anti-HEL, e.g., human IgG1, abbreviated as hIgG1), whose sequence is derived from the variable region sequence of Fab F10.6.6 sequence published in the study "Affinity maturation increases the stability and plasticity of the Fv domain of anti-protein antibodies" by Acierno et al. (Acierno et al. J Mol Biol. 2007; 374(1):130-46.). The preparation method is as follows: Nanjing GenScript Biotech was commissioned to perform amino acid codon optimization and gene synthesis for the heavy and light chain genes of the human IgG antibody. Following the standard techniques described in "Molecular Cloning: A Laboratory Manual (3rd Edition)", standard molecular cloning techniques including PCR, enzyme digestion, DNA gel recovery, ligation and transformation, colony PCR, or enzyme digestion identification were employed to subclone the heavy and light chain genes into the antibody heavy chain expression

vector and antibody light chain expression vector of a mammalian expression system, respectively, and the heavy and light chain genes of the recombinant expression vectors were further subjected to sequencing analysis. After sequencing verification was confirmed correct, endotoxin-free grade expression plasmids were prepared in large quantities, and the heavy and light chain expression plasmids were transiently co-transfected into HEK293 cells for recombinant antibody expression. After 7 days of culture, the cell culture medium was collected and subjected to affinity purification using an rProteinA column (GE). The harvested antibody samples were quality-assessed using standard analytical techniques of SDS-PAGE and SEC-HPLC.

Example 2. Preparation of ADC

Preparation of VEGF-ADC-01



q is selected from any integer of 1-10, and anti-VEGF mab is a monoclonal antibody that specifically binds VEGF.

2.1 Preparation of VEGF-ADC-01 (DAR8) sample

The anti-VEGF antibody prepared in Example 1 (1000 mg) was diluted with diluent (20 mM phosphate (PB) buffer, pH 7.2), and sodium edetate solution was added to a final concentration of 5 mM, and the mixture was mixed evenly; a 20 mmol/L TCEP solution at 6.25 molar equivalents relative to the antibody was added, mixed evenly, and allowed to stand at room temperature for 90 minutes; DL-037 (obtained as described in Example 2.37 of WO 2022170971, CAS No.: 2964513-44-6) dissolved in dimethyl sulfoxide at 10 mmol/L was added to the above-mentioned solution system at 11 molar equivalents relative to the antibody, mixed evenly, and allowed to stand at room temperature for 2 hours to obtain the conjugated sample. After the reaction was complete, the sample was transferred into

20 mM histidine buffer at pH 6.0 using a 30 KDa ultrafiltration tube to remove low-molecular-weight substances, and the sample was finally concentrated to obtain the solution VEGF-ADC-01 (DAR8) containing the anti-VEGF antibody ADC composition. The average DAR value was determined to be 8.1 by mass spectrometry according to Example 3.

As determined by mass spectrometry, the antibody light chain of VEGF-ADC-01 (DAR8) was conjugated to 0-1 toxin molecule (obtained as described in Example A1.9 of WO 2022170971, CAS No. 2821768-98-1) (proportions of LC0 and LC1 were 0% and 100%, respectively), and the heavy chain was conjugated to 0-4 toxin molecules (proportions of HC0, HC1, HC2, HC3, and HC4 were 0%, 0%, 0%, 97%, and 3%, respectively), from which it can be inferred that the q value may be 8, 9, or 10.

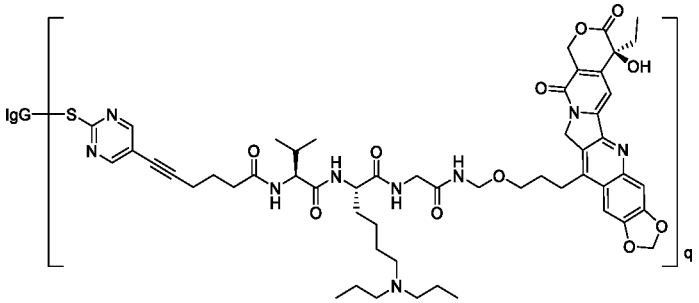
2.2 Preparation of VEGF-ADC-01 (DAR4) sample

The anti-VEGF antibody prepared in Example 1 (1000 mg) was diluted with diluent (20 mM PB, pH 7.2), and sodium edetate solution was added to a final concentration of 5 mM, and the mixture was mixed evenly; a 20 mmol/L TCEP solution at 2.8 molar equivalents relative to the antibody was added, mixed evenly, and allowed to stand at room temperature for 90 minutes; DL-037 (obtained as described in Example 2.37 of WO 2022170971) dissolved in dimethyl sulfoxide at 10 mmol/L was added to the above-mentioned solution system at 4.8 molar equivalents relative to the antibody, mixed evenly, and allowed to stand at room temperature for 2 hours to obtain the conjugated sample. After the reaction was complete, the sample was transferred into 20 mM histidine buffer at pH 6.0 using a 30 KDa ultrafiltration tube to remove low-molecular-weight substances, and the sample was finally concentrated to obtain the solution VEGF-ADC-01 (DAR4) containing the anti-VEGF antibody ADC composition. The average DAR value was determined to be 4.5 by mass spectrometry.

As determined by mass spectrometry, the antibody light chain of VEGF-ADC-01 (DAR4) was conjugated to 0-1 toxin molecule (proportions of LC0 and LC1 were 48% and 52%, respectively), and the heavy chain was conjugated to 0-4 toxin molecules (proportions of HC0, HC1, HC2, HC3, and HC4 were 22%, 16%, 30%,

32%, and 0%, respectively), from which it can be inferred that the q value may be 1, 2, 3, 4, 5, 6, 7, or 8.

2.3 Preparation of IgG1-ADC



2.3.1 Preparation of IgG1-ADC (DAR8)

The antibody in the preparation method of Example 2.1 was replaced with the IgG1 isotype antibody (anti-hen egg lysozyme antibody, prepared in Example 1) to obtain the conjugation product of DL-037 and IgG1 antibody, IgG1-ADC. The DAR value was determined to be 8.0 by mass spectrometry according to Example 3.

2.3.2 Preparation of IgG1-ADC (DAR4)

The antibody in the preparation method of Example 2.2 was replaced with the IgG1 isotype antibody (anti-hen egg lysozyme antibody, prepared in Example 1) to obtain the conjugation product of DL-037 and IgG1 antibody, IgG1-ADC. The DAR value was determined to be 4.4 by mass spectrometry according to Example 3.

Example 3. Identification of the preparation of anti-VEGF ADC

Detection: LC-MS molecular weight and DAR value analysis was performed on VEGF-ADC-01 (DAR4) and VEGF-ADC-01 (DAR8). The chromatographic conditions are as follows:

Chromatographic column: PLRP-S, 2.1*50 mm, 5 μm;

Mobile phase A: 0.1%FA/H_2O; Mobile phase B: 0.1%FA/ACN

Column temperature: 70°C

Flow rate: 0.3 ml/min

Injection volume: 1 μl

Time (min)	0.0	1.0	6.0	6.5	8.0	8.1	10.0
Mobile phase A	80	80	30	5	5	80	80

Mobile phase B	20	20	70	95	95	20	20

Sample treatment: 200 μg of each sample was taken, diluted with ultrapure water to 0.2 mg/ml, 10 μl of 1 mol/L DTT was added, and the mixture was reacted at 37°C for 30 minutes before direct injection.

LC/MS model: AB SCIEX X500B

Mass spectrometry conditions are as follows:

Ion Source Gas 1 (Gas1): 45	Ion Source Gas 2 (Gas2): 45
Curtain Gas (CUR): 30	Temperature (TEM): 450
IonSpray Voltage Floating (ISVF): 5000	Declustering Potential (DP): 120
Collision Energy (CE): 12	Time bins to sum: 80
Accumulation time: 1 secs	TOF Mass: 600-4000

The results are shown below:

Table 1. Theoretical molecular weight and measured molecular weight

Peptide chain	LC0	LC1	HC0	HC1	HC2	HC3	HC4
Theoretical value (Da)	23451.03	24469.03	51164.52	52182.52	53200.52	54218.52	55236.52
Measured value (Da)	23446.64	24464.93	51156.52	52174.73	53192.88	54211.27	55229.68

In Table 1, LC0 represents the antibody light chain not conjugated to any toxin; LC1 represents the antibody light chain conjugated to 1 toxin; HC0 represents the antibody heavy chain not conjugated to any toxin; HC1, HC2, HC3, and HC4 represent the antibody heavy chains conjugated to 1, 2, 3, and 4 toxins, respectively. The theoretical molecular weights of LC0 and HC0 are calculated based on amino acid sequences, wherein the theoretical molecular weight of HC is calculated based on the G0F glycoform with K removed.

Table 2. Proportions of each component and DAR values

Sample	Proportion (%)							Determined DAR value
	LC0	LC1	HC0	HC1	HC2	HC3	HC4	
VEGF-ADC-01 (DAR4)	48	52	22	16	30	32	Not detected	4.5

VEGF-ADC-01 (DAR8)	Not detected	100	Not detected	Not detected	Not detected	97	3	8.1

The detection results showed that the drug-to-antibody ratio (DAR value) of the VEGF-ADC-01 (DAR4) sample was 4.5; the drug-to-antibody ratio (DAR value) of the VEGF-ADC-01 (DAR8) sample was 8.1.

Example 4. Protein affinity detection of VEGF ADC

The affinity of the anti-VEGF antibody prepared as described in Example 1 and the VEGF-ADC-01 (DAR4) prepared as described in Example 2 for VEGF proteins of various species was detected by ELISA assay. The specific experimental procedure is as follows: human/monkey VEGF protein (VEGF165, P15692, from Swiftbio), rat VEGF protein (VEGF 164, purchased from Novoprotein, Cat. No. CJ96), and mouse VEGF protein (VEGF164, purchased from Novoprotein, Cat. No. CX73) were diluted to a final concentration of 1 μg/mL with carbonate buffer at pH 9.6, added to 96-well ELISA plates at 100 μL/well, and coated overnight at 4°C; the coating solution was discarded, the plates were washed 3 times with PBST, 300 μL PBS (containing 2% BSA) was added per well, and the plates were blocked at 37°C for 1 hour; then 100 μL PBS (containing 2% BSA)-diluted anti-VEGF antibody and VEGF-ADC-01 (DAR4) (starting at 1000 ng/mL, 4-fold dilution, 2 replicates) were added per well and incubated at 37°C for 1.5 h; the plates were washed 3 times with PBST, and horseradish peroxidase-labeled anti-human Fab antibody (Invitrogen, A56868, diluted 1:2000 in PBS containing 2% BSA) was added at 100 μL/well and incubated at 37°C for 1 hour; the plates were washed 5 times with PBST. TMB chromogenic solution was added for color development (100 μL/well, 37°C, 7 min), and stop solution was added to terminate the reaction (50 μL/well), and absorbance was measured at 450 nm. EC50 values were calculated from the raw data. The results are shown in Table 3 and FIGs. 1a and 1b. The anti-VEGF antibody and VEGF-ADC-01 (DAR4) showed strong binding to human/monkey VEGF protein and did not bind to rat and mouse VEGF proteins, and the binding ability of VEGF ADC to VEGF antigens of different species was similar to that of the anti-VEGF antibody.

Table 3. Species cross-reactivity of anti-VEGF antibody and VEGF ADC

Sample	Human/monkey VEGF EC50 (nM)	Rat VEGF EC50 (nM)	Mouse VEGF EC50 (nM)
Anti-VEGF antibody	0.069	NA	NA
VEGF-ADC-01 (DAR4)	0.046	NA	NA

Example 5. Kinetic affinity detection of VEGF ADC

The kinetic affinity of the anti-VEGF antibody prepared as described in Example 1 and the VEGF-ADC-01 (DAR4) prepared as described in Example 2 for human VEGF protein was detected by surface plasmon resonance. A brief description of the method is as follows: Human VEGF protein (Acrobiosystems, VE5-H4210: isoform VEGF165) was desalted and buffer-exchanged with HEPES buffer; the concentration was determined using SPECTROstarNano (purchased from BMGLABTECH), and serial dilutions were performed with HEPES buffer (starting concentration of 62.5 nM, 2-fold dilution, 7 concentrations in total). The anti-VEGF antibody and VEGF-ADC-01 (DAR4) were diluted to 6 μg/mL with HEPES buffer and injected into the Protein A capture chip experimental channel (Fc2). Antigen dilutions were sequentially injected into the experimental channel (Fc2) and the reference channel (Fc1), with association for 90 s and dissociation for 210 s in HEPES buffer. After one analysis cycle, the sensor was regenerated with 10 mM Gly (pH 1.5) for 30 s. Data analysis was performed using Biacore Insight Evaluation Software with a 1:1 model to determine the association (Ka) and dissociation (Kd) rate constants, which were used to calculate the dissociation equilibrium constant (KD). The results are shown in Table 4. The anti-VEGF antibody and VEGF-ADC-01 (DAR4) exhibited high kinetic affinity for human VEGF.

Table 4. Kinetic affinity analysis of anti-VEGF antibody and VEGF ADC with VEGF protein

Ligand	Capture Level (RU)	Analyte	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi^2 (RU^2)
VEGF-ADC-01 (DAR4)	172.0	Human VEGF	9.66E+05	1.52E-04	1.57E-10	58.3	0.50

Anti-VEGF antibody	179.7	Human VEGF	1.11E+06	6.85E-05	6.16E-11	62.4	0.64

Example 6. Specificity detection of VEGF ADC

The binding characteristics of the anti-VEGF antibody (Bevacizumab) prepared as described in Example 1 and the VEGF-ADC-01 (DAR4) prepared as described in Example 2 with other VEGF family proteins were detected by ELISA assay. The specific experimental procedure is as follows: human VEGFA protein (VEGF165, from Swiftbio), human VEGF-B (purchased from Novoprotein, CS78), human VEGF-C (purchased from Novoprotein, C546), human VEGF-D (purchased from Novoprotein, C498), and human PLGF protein (purchased from Novoprotein, CW90) were diluted to a final concentration of 1 μg/mL with carbonate buffer at pH 9.6, added to 96-well ELISA plates at 100 μL/well, and coated overnight at 4°C; the next day, the coating solution was discarded, the plates were washed 3 times with PBST, 300 μL PBS (containing 2% BSA) was added per well, and the plates were blocked at 37°C for 1.5 h; after washing the plates 3 times with 1×PBST, the test samples (anti-VEGF antibody or VEGF-ADC-01 (DAR4)) at a concentration of 1000 ng/mL were added and incubated at 37°C for 1 hour; after the plates were washed 3 times with PBST, horseradish peroxidase-labeled anti-human Fab antibody (Invitrogen, A56868, diluted 1:2000 in PBS containing 2% BSA) was added at 100 μL/well and incubated at 37°C for 1 hour; the plates were washed 5 times with PBST. TMB chromogenic solution (Huzhou InnoReagents, TMB-S-001) was added for color development, and stop solution was added to terminate the reaction, and absorbance was read at 450 nm using a microplate reader. The results are shown in Table 5 and FIG. 2. The anti-VEGF antibody and VEGF-ADC-01 (DAR4) showed significant binding to VEGFA and did not bind to VEGF-B, VEGF-C, VEGF-D, or PLGF.

Table 5. Specificity detection results of anti-VEGF antibody and VEGF ADC

Sample	Human VEGF-A OD value	Human VEGF-B OD value	Human VEGF-C OD value	Human VEGF-D OD value	Human PLGF OD value
Anti-VEGF antibody	1.360	0.067	0.071	0.058	0.071

VEGF-ADC-01 (DAR4)	1.115	0.070	0.068	0.056	0.077

Example 7. In vitro anti-angiogenesis detection of VEGF ADC

The effect of the anti-VEGF antibody prepared in Example 1 and the VEGF-ADC-01 (DAR4) prepared in Example 2 on tube formation of vascular endothelial cells HUVEC (ATCC, Cat. No. CRL-1730) was detected by a tube formation assay.

The specific experimental procedure is as follows: Extracellular Matrix Gel (BD, 354248) was thawed at 4°C in advance, and the 96-well plate and pipette tips were pre-cooled; 50 μL of the matrix gel was added to each well, and the plate was placed in a 37°C incubator for 30-45 minutes until the matrix gel solidified uniformly and coated the bottom of the 96 wells. HUVEC cells in the logarithmic growth phase were added to the 96-well plate containing the matrix gel at 10,000 cells per well, 50 μL/well; subsequently, 3 μM/L anti-VEGF antibody (prepared in Example 1), 3 μM/L VEGF-ADC-01 (DAR4), 12 μM/L toxin (obtained with reference to Example A1.9 of WO 2022170971), and 3.35 μM/L Avastin (purchased from MCE, HY-P9906) were added to each experimental well to treat the HUVEC cells, and wells to which only VEGF was added served as the control. After 6 hours, the culture medium was washed away, and the cells were washed twice with 100 μL of washing solution; 100 μL of staining solution was added to each well for staining at 37°C for 0.5 h, fluorescence photographs were taken under a 4× objective, the relevant indicators of tube formation were calculated using Image J software, and graphs were plotted using GraphPad Prism. The results are shown in Table 6 and FIGs. 3a to 3c. Compared with the control group (VEGF), Avastin, toxin, anti-VEGF antibody, and VEGF-ADC-01 (DAR4) could significantly inhibit the formation of HUVEC cell meshes, junctions, and other structures, and tube formation was reduced. In summary, the toxin, anti-VEGF antibody, and VEGF-ADC-01 (DAR4) can significantly inhibit the ability of HUVEC cells to form tubes.

Table 6. Statistical table of results for HUVEC cell tube formation

Group	Number of meshes (Mean)	Number of junctions (Mean)
VEGF, 100 ng/mL	34	72
VEGF + Avastin, 100 ng/mL + 3.35 μM	12	34
VEGF + toxin, 100 ng/mL + 12 μM	3	14

VEGF + anti-VEGF antibody, 100 ng/mL + 3 μM	16	37
VEGF + VEGF-ADC-01 (DAR4), 100 ng/mL + 3 μM	12	33

Example 8. Cellular functional activity of VEGF ADC

VEGF ADC does not rely on cell-specific endocytosis; instead, it is cleaved by enzymes in the tumor microenvironment to release the toxin and exert its cytotoxic effect. Therefore, this experiment used an in vitro model to simulate the cleavage of VEGF ADC in the tumor microenvironment and assess its cytotoxic effect on cells.

The specific experimental procedure is as follows: 200 μg/mL VEGF-ADC-01 (DAR4) was incubated with 50 μg/mL Cathepsin L (Acrobiosystems, Cat. No. CAL-H5253) at 37°C for 72 h to obtain the VEGF ADC enzyme-incubated solution. NCI-H358 (human non-small cell lung cancer cells, from ECACC, Cat. No. 95111733), SW480 (human colon cancer cells, from ECACC, Cat. No. 87092801), SNU-16 (human gastric cancer cells, from ATCC, Cat. No. CRL-5974), and NCI-H441 (human lung adenocarcinoma cells, from ATCC, Cat. No. HTB-174) cells were cultured at 37°C under 5% CO_2; cells in the logarithmic growth phase were harvested and counted, and cell viability was assessed by trypan blue staining method to ensure that cell viability was above 90%. Cell density was adjusted, and cells were then seeded in 96-well cell culture plates at 120 μL per well; the seeding numbers for NCI-H358, SW480, SNU-16, and NCI-H441 were 4,000, 6,000, 6,000, and 2,000 cells per well, respectively. The culture plates were placed in the incubator and cultured overnight. The next day, sample solutions were prepared; the maximum working concentration for each cell line was 260 nM, with 4-fold serial dilutions for a total of 9 concentrations, with 3 replicate wells per concentration; 30 μL/well of sample working solution was added to the 96-well plates. The 96-well plates with added samples were placed in the incubator and cultured for an additional 6 days, after which cell viability analysis was performed. CellTiter-Glo reagent (from Promega, Cat. No. G7573) was thawed and the cell plates were equilibrated to room temperature for 30 minutes; 75 μL of CellTiter-Glo reagent was added per well, the plates were shaken on an orbital shaker for 2 minutes to lyse the cells, the cell plates were then placed at room temperature for 10 minutes to stabilize the luminescent signal, and finally the luminescence values were read and data collected. The specific results are shown in Table 7, indicating that the active components released

from the VEGF ADC of the present disclosure after incubation with Cathepsin L have significant cytotoxic activity against tumor cells.

Table 7. Cell-killing activity detection results of VEGF ADC enzyme-incubated solution

Sample	NCI-H358 IC50 (nM)	SW480 IC50 (nM)	SNU-16 IC50 (nM)	NCI-H441 IC50 (nM)
VEGF-ADC-01 (DAR4) + enzyme-incubated solution	1.78	2.82	1.85	0.21
VEGF-ADC-01 (DAR4)	NA	NA	NA	NA
Toxin	4.08	4.96	7.68	0.13

NA indicates that the IC50 value was too high to be obtained (Not Available).

Example 9. Determination of in vivo activity of VEGF ADC

9.1. Efficacy test of anti-VEGF antibody-drug conjugate on SW480 xenograft tumor

The present disclosure demonstrates in vivo efficacy by evaluating the anti-tumor effects of the anti-VEGF antibody and ADC drug on a subcutaneous human colon cancer cell line SW480 cell (ATCC origin) xenograft tumor model in Balb/c nude mice. The specific steps were as follows: 35 Balb/c nu nude mice, female, 5-6 weeks of age, were purchased from GemPharmatech (Chengdu) Co., Ltd. SW480 cells were cultured in 1640 medium containing 10% FBS in 15 cm diameter dishes; when confluence reached approximately 80-90%, cells were digested with trypsin-EDTA, washed twice with PBS, then centrifuged and resuspended in pre-cooled PBS; cells were counted using a cell counter and diluted with PBS to a cell concentration of 5×10^7/mL. Balb/c nu mice were acclimatized to the laboratory environment for 3-5 days, then SW480 cells were inoculated subcutaneously in the right flank at 5×10^6 cells/mouse in an inoculation volume of 0.2 ml (containing 50% Matrigel); when tumors grew to approximately 180 mm^3, mice were randomly grouped according to tumor size, with 5 mice per group: normal saline (vehicle (saline)) group, anti-VEGF antibody group, anti-hen egg lysozyme human IgG1-ADC (DAR8) (prepared in Example 2) group, VEGF-ADC-01 (DAR4) dose group, as well as VEGF-ADC-01 (DAR8) dose group. All samples were administered by

tail vein injection, once per week, for a total of 3 weeks. After administration, the tumor volume and body weight of the mice were observed and measured periodically.

1) Relative tumor proliferation rate, T/C (%), i.e., at a certain time point, the percentage value of relative tumor volume or tumor weight of the treatment group and the control group.

2) Relative tumor inhibition rate, TGI (%), is calculated by the formula: TGI% = (1 - T/C) × 100% (T and C are the relative tumor volumes (RTV) of the treatment group and the control group, respectively, at a specific time point).

The specific results are shown in Table 8 and FIG. 4. The experimental results showed that all dose groups inhibited tumor growth in the SW480 human colon cancer xenograft tumor model, and VEGF-ADC-01 (DAR4) and VEGF-ADC-01 (DAR8) demonstrated good tumor growth inhibition at each dose group, showing a dose-gradient trend. Body weight results are shown in FIG. 5, suggesting good safety.

Table 8. Efficacy results of the SW480 + Balb/c nude mouse model

Group	Average tumor volume (mm^3)	Average tumor volume (mm^3, Mean ± SD)	T/C (%)	Tumor inhibition rate TGI (%)	*P* value D29**
	D1	D29	D29	D29	
Normal saline	146.16±11.93	2884.96±1282.34*	-	-	-
IgG1-ADC (DAR8), 10 mg/kg	144.66±14.70	1261.82±931.35	44.19	55.8	0.113
Anti-VEGF antibody, 5 mg/kg	145.96±11.63	2291.48±363.40	79.54	20.5	1.000
VEGF-ADC-01 (DAR8) 5 mg/kg	144.77±12.69	796.22±997.50	27.86	72.1	0.014
VEGF-ADC-01 (DAR8) 10 mg/kg	145.43±12.39	94.67±40.45###	3.30	96.7	0.000
VEGF-ADC-01 (DAR4) 10 mg/kg	146.00±12.11	536.66±269.85	18.62	81.4	0.012
VEGF-ADC-01 (DAR4) 20 mg/kg	144.75±14.68	194.57±175.16##	6.81	93.2	0.000

*: Due to excessively rapid tumor growth in the control group, the animals were necropsied early according to animal ethics and welfare requirements; the volume is the tumor volume on the day of necropsy (D19).

**: Statistical analysis was performed between each treatment group and the normal saline control group employing Dunnett's test.

Compared with the IgG1-ADC (DAR8) group, ## indicates $p < 0.01$, ### indicates $p < 0.001$; $p < 0.05$ is considered statistically significant.

9.2. Efficacy test of anti-VEGF antibody-drug conjugate on NCI-H358 xenograft tumor

The present disclosure demonstrates in vivo efficacy by evaluating the anti-tumor effects of the anti-VEGF antibody and ADC drug on a subcutaneous human non-small cell lung cancer cell line NCI-H358 cell (Nanjing Cobioer Biosciences) xenograft tumor model in Balb/c nude mice. The specific steps were as follows: 56 Balb/c nu nude mice, female, 5-6 weeks of age, were purchased from GemPharmatech (Chengdu) Co., Ltd. NCI-H358 cells were cultured in 1640 medium containing 10% FBS in 15 cm diameter dishes; when confluence reached approximately 80-90%, cells were digested with trypsin-EDTA, washed twice with PBS, then centrifuged and resuspended in pre-cooled PBS; cells were counted using a cell counter and diluted with PBS to a cell concentration of 5×10^7/mL. Balb/c nu mice were acclimatized to the laboratory environment for 3-5 days, then NCI-H358 cells were inoculated subcutaneously in the right flank at 5×10^6 cell/mouse in an inoculation volume of 0.2 ml (containing 50% Matrigel); when tumors grew to approximately 180 mm^3, mice were randomly grouped according to tumor size, with 5 mice per group: normal saline group, anti-VEGF antibody group, toxin (obtained as described in Example A1.9 of WO 2022170971) group, anti-hen egg lysozyme human IgG1-ADC (DAR4) (prepared in Example 2) group, and VEGF-ADC-01 (DAR4) 0.5 mg/kg, 1 mg/kg, and 3 mg/kg dose groups. All samples were administered by tail vein injection, once per week, for a total of 3 weeks. After administration, the tumor volume and body weight of the mice were observed and measured periodically.

1) Relative tumor proliferation rate, T/C (%), i.e., at a certain time point, the percentage value of relative tumor volume or tumor weight of the treatment group and the control group.

2) Relative tumor inhibition rate, TGI (%), is calculated by the formula: TGI% = (1 - T/C) × 100% (T and C are the relative tumor volumes (RTV) of the treatment group and the control group, respectively, at a specific time point).

The specific results are shown in Table 9 and FIG. 6. The experimental results showed that all dose groups of VEGF-ADC-01 (DAR4) significantly inhibited tumor growth in the NCI-H358 human non-small cell lung cancer xenograft tumor model, and showed a dose-gradient trend. Body weight results are shown in FIG. 7, suggesting good safety.

Table 9. Efficacy results of the NCI-H358 Balb/c nude mouse model

Group	Average tumor volume (mm^3 Mean ± SD)	Average tumor volume (mm^3, Mean ± SD)	T/C (%)	Tumor inhibition rate TGI (%)	*P* value D22*
	D1	D22	D22	D22	
Normal saline	183.39±7.12	1031.45±214.47	-	-	-
Anti-VEGF antibody, 3 mg/kg	182.75±15.21	902.57±375.27	87.8	12.2	0.930
Toxin, 0.07 mg/kg	182.81±14.28	742.74±245.36	72.2	27.8	0.456
IgG1-ADC (DAR4), 3 mg/kg	183.43±10.73	684.25±299.58	66.3	33.7	0.163
VEGF-ADC-01 (DAR4) 0.5 mg/kg	183.10±7.83	710.67±660.22	69.0	31.0	0.049
VEGF-ADC-01 (DAR4) 1 mg/kg	183.13±7.57	506.40±199.31	49.2	50.8	0.015
VEGF-ADC-01 (DAR4) 3 mg/kg	182.87±16.40	256.52±124.04##	24.9	75.1	0.000

*: Statistical analysis was performed between each treatment group and the normal saline control group employing Dunnett's test.

Compared with the IgG1-ADC (DAR4) group, ## indicates $p < 0.01$; $p < 0.05$ is considered statistically significant.

9.3. Efficacy test of anti-VEGF antibody-drug conjugate on LD1-2012-200671 PDX xenograft tumor

The present disclosure demonstrates in vivo efficacy by evaluating the anti-tumor effects of the anti-VEGF antibody and ADC drug on a subcutaneous patient-derived xenograft (PDX) model of human colorectal cancer LD1-2012-200671 (Shanghai LIDE Biotech Co., Ltd.) in mice. The specific steps were as follows: 16 NU/NU mice, female, were purchased from Zhejiang Vital River Laboratory Animal Technology Co., Ltd. Human colorectal cancer LD1-2012-200671 tumor tissue was uniformly cut into tumor pieces of about 3 mm × 3 mm × 3 mm in size and about 30-60 mg in weight, and inoculated subcutaneously on the right side of NU/NU mice; when tumors grew to approximately 157 mm^3, mice were randomly grouped according to tumor size, with 4 mice per group: normal saline group (Vehicle), anti-hen egg lysozyme human IgG1-ADC (DAR4) (prepared in Example 2) group, anti-VEGF antibody group, as well as VEGF-ADC-01 (DAR4) dose group. All samples were administered by tail vein injection, once per week, for a total of 3 weeks. After administration, the tumor volume and body weight of the mice were observed and measured periodically.

1) Relative tumor proliferation rate, T/C (%), i.e., at a certain time point, the percentage value of relative tumor volume or tumor weight of the treatment group and the control group.

2) Relative tumor inhibition rate, TGI (%), is calculated by the formula: TGI% = (1 - T/C) × 100% (T and C are the relative tumor volumes (RTV) of the treatment group and the control group, respectively, at a specific time point).

The specific results are shown in Table 10 and FIG. 8. The experimental results showed that all ADC dose groups were able to inhibit tumor growth in the LD1-2012-200671 subcutaneous colon cancer PDX model in mice, and VEGF-ADC-01 (DAR4) demonstrated superior inhibitory effects compared to IgG1-ADC (DAR4) and the anti-VEGF antibody. Body weight results are shown in FIG. 9, suggesting good safety.

Table 10. Efficacy results of the LD1-2012-200671 PDX mouse model

Group	Average tumor volume (mm^3 Mean ± SEM)	Average tumor volume (mm^3 Mean ± SEM)	T/C (%)	Tumor inhibition rate TGI (%)	*P* value D21*
	D0	D21	D21	D21	
Normal saline (Vehicle)	156.12±2.95	1262.74±117.63	-	-	-
IgG1-ADC (DAR4), 5 mg/kg	156.45±3.68	735.18±164.23	58.10	41.90	0.040
Anti-VEGF antibody, 5 mg/kg	157.85±5.21	665.61±127.23	52.13	47.87	0.014
VEGF-ADC-01 (DAR4), 5 mg/kg	157.12±4.64	41.85±7.16##	3.29	96.71	<0.0001

**: Statistical analysis was performed between each treatment group and the normal saline control group employing One-Way ANOVA Uncorrected Fisher's LSD test. Compared with the IgG1-ADC (DAR4) group, ## indicates $p < 0.01$; $p < 0.05$ is considered statistically significant.

Example 10. ADC plasma stability test

Solution preparation: The ADC stock solution to be tested was diluted with PBS to a working solution at a concentration of 2 mg/mL.

Sample incubation: The bacteriostatic agent ProClin was added to both the VEGF-ADC-01 working solution and human plasma, respectively, to a final concentration of 0.1% ProClin, under aseptic conditions. The ADC solution to which the bacteriostatic agent had been added was added to the above-mentioned sterile plasma at a final ADC concentration of 200 μg/mL, placed in a 37°C cell culture

incubator for incubation, with a shaking speed of 80 times/minute. After incubation for 0, 7, and 14 days, the ADC samples were removed; 100 μl of Protein A was added to each tube, adsorbed for 2 h, and then eluted to obtain the incubated ADC. DAR value detection was performed on the incubated ADC (detection and calculation methods as in Example 3) to determine the plasma stability of the samples.

The test results indicated that the DAR values of VEGF-ADC-01 (DAR4) and VEGF-ADC-01 (DAR8) of the present disclosure remained essentially stable during plasma incubation (i.e., almost no toxin-linker was shed from the antibody), demonstrating good plasma stability.

Example 11. ADC toxicity test

In the present disclosure, different doses of VEGF-ADC-01 (DAR4) were administered to cynomolgus monkeys by intravenous infusion, with repeated dosing for 4 weeks followed by a 4-week recovery period after discontinuation, to observe the toxic reactions and the degree of reversibility thereof, and to determine the toxic target organs or target tissues. The specific steps were as follows: Cynomolgus monkeys, half male and half female, were selected and administered vehicle (glucose solution) control or different doses of VEGF-ADC-01 (DAR4) by intravenous infusion, once every 2 weeks for a total of 3 doses, followed by a 4-week recovery period after discontinuation. All surviving animals were euthanized and necropsied at the end of the dosing period and at the end of the recovery period, respectively. Evaluation indicators included clinical observations, body weight, food consumption, body temperature, ophthalmological examination, clinical pathology, gross necropsy findings, organ weights, and histopathological examination, etc.

The results indicated that under the conditions of this study, the highest non-severely toxic dose (HNSTD) of VEGF-ADC-01 (DAR4) in cynomolgus monkeys was 30 mg/kg; no severe toxic reactions were observed in this study, only mild toxic reactions were observed, all of which were reversible after discontinuation of dosing.

Although specific embodiments of the present disclosure have been described in detail, those skilled in the art will understand that: according to all the teachings disclosed, various modifications and changes can be made to the details, and these changes all fall within the scope of protection of the present disclosure. The scope

of protection of the present disclosure is given by the appended claims and any equivalents thereof.

CLAIMS

1. An antibody-drug conjugate, characterized in that the antibody-drug conjugate is a compound of formula (I), or a tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), or a pharmaceutically acceptable salt or solvate thereof:

Ab-[L-D]q

Formula (I)

wherein,

Ab is an antibody or an antigen-binding fragment thereof that specifically binds vascular endothelial growth factor (VEGF);

L is a bond or a linking molecule that covalently links Ab and D;

q is an integer between 1 and 16, and represents the number of L-D covalently linked to Ab; preferably, q is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

D is a camptothecin-based drug molecule.

2. The antibody-drug conjugate of claim 1, or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), or the pharmaceutically acceptable salt or solvate thereof, characterized in that the antibody or the antigen-binding fragment thereof that specifically binds VEGF comprises: three CDR-Hs of the heavy chain variable region (VH) sequence of SEQ ID NO: 3 and three CDR-Ls of the light chain variable region (VL) sequence of SEQ ID NO: 4, wherein the CDRs are defined according to the Chothia, AbM, Kabat, IMGT, Contact definition scheme, or any combination thereof;

optionally, the antibody or the antigen-binding fragment thereof that specifically binds VEGF comprises:

three CDR-Hs of the heavy chain variable region (VH) sequence of SEQ ID NO: 3 and three CDR-Ls of the light chain variable region (VL) sequence of SEQ ID NO: 4, wherein the CDRs are defined according to the Chothia scheme,

three CDR-Hs of the heavy chain variable region (VH) sequence of SEQ ID NO: 3 and three CDR-Ls of the light chain variable region (VL) sequence of SEQ ID NO: 4, wherein the CDRs are defined according to the AbM scheme,

three CDR-Hs of the heavy chain variable region (VH) sequence of SEQ ID NO: 3 and three CDR-Ls of the light chain variable region (VL) sequence of SEQ ID NO: 4, wherein the CDRs are defined according to the Kabat scheme,

three CDR-Hs of the heavy chain variable region (VH) sequence of SEQ ID NO: 3 and three CDR-Ls of the light chain variable region (VL) sequence of SEQ ID NO: 4, wherein the CDRs are defined according to the IMGT scheme, or

three CDR-Hs of the heavy chain variable region (VH) sequence of SEQ ID NO: 3 and three CDR-Ls of the light chain variable region (VL) sequence of SEQ ID NO: 4, wherein the CDRs are defined according to the Contact scheme;

preferably, the antibody or the antigen-binding fragment thereof that specifically binds VEGF comprises CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, CDR-L3 in the Chothia, AbM, Kabat, IMGT, or Contact definition scheme, wherein

(i) defined according to the Chothia scheme,

CDR-H1 comprises an amino acid sequence set forth in SEQ ID NO: 5;

CDR-H2 comprises an amino acid sequence set forth in SEQ ID NO: 10;

CDR-H3 comprises an amino acid sequence set forth in SEQ ID NO: 17;

CDR-L1 comprises an amino acid sequence set forth in SEQ ID NO: 23;

CDR-L2 comprises an amino acid sequence set forth in SEQ ID NO: 19; and

CDR-L3 comprises an amino acid sequence set forth in SEQ ID NO: 22;

(ii) defined according to the AbM scheme,

CDR-H1 comprises an amino acid sequence set forth in SEQ ID NO: 6;

CDR-H2 comprises an amino acid sequence set forth in SEQ ID NO: 11;

CDR-H3 comprises an amino acid sequence set forth in SEQ ID NO: 17;

CDR-L1 comprises an amino acid sequence set forth in SEQ ID NO: 23;

CDR-L2 comprises an amino acid sequence set forth in SEQ ID NO: 19; and

CDR-L3 comprises an amino acid sequence set forth in SEQ ID NO: 22

(iii) defined according to the Kabat scheme,

CDR-H1 comprises an amino acid sequence set forth in SEQ ID NO: 7;

CDR-H2 comprises an amino acid sequence set forth in SEQ ID NO: 12;

CDR-H3 comprises an amino acid sequence set forth in SEQ ID NO: 17;

CDR-L1 comprises an amino acid sequence set forth in SEQ ID NO: 23;

CDR-L2 comprises an amino acid sequence set forth in SEQ ID NO: 19; and
CDR-L3 comprises an amino acid sequence set forth in SEQ ID NO: 22;
(iv) defined according to the Contact scheme,
CDR-H1 comprises an amino acid sequence set forth in SEQ ID NO: 8;
CDR-H2 comprises an amino acid sequence set forth in SEQ ID NO: 13;
CDR-H3 comprises an amino acid sequence set forth in SEQ ID NO: 15;
CDR-L1 comprises an amino acid sequence set forth in SEQ ID NO: 24;
CDR-L2 comprises an amino acid sequence set forth in SEQ ID NO: 25; and
CDR-L3 comprises an amino acid sequence set forth in SEQ ID NO: 21;
(v). defined according to the IMGT scheme,
CDR-H1 comprises an amino acid sequence set forth in SEQ ID NO: 9;
CDR-H2 comprises an amino acid sequence set forth in SEQ ID NO: 14;
CDR-H3 comprises an amino acid sequence set forth in SEQ ID NO: 16;
CDR-L1 comprises an amino acid sequence set forth in SEQ ID NO: 20;
CDR-L2 comprises an amino acid sequence set forth in SEQ ID NO: 18; and
CDR-L3 comprises an amino acid sequence set forth in SEQ ID NO: 22;
(vi) defined according to the Chothia scheme,
CDR-H1 consists of the amino acid sequence set forth in SEQ ID NO: 5;
CDR-H2 consists of the amino acid sequence set forth in SEQ ID NO: 10;
CDR-H3 consists of the amino acid sequence set forth in SEQ ID NO: 17;
CDR-L1 consists of the amino acid sequence set forth in SEQ ID NO: 23;
CDR-L2 consists of the amino acid sequence set forth in SEQ ID NO: 19; and
CDR-L3 consists of the amino acid sequence set forth in SEQ ID NO: 22;
(vii). defined according to the AbM scheme,
CDR-H1 consists of the amino acid sequence set forth in SEQ ID NO: 6;
CDR-H2 consists of the amino acid sequence set forth in SEQ ID NO: 11;
CDR-H3 consists of the amino acid sequence set forth in SEQ ID NO: 17;
CDR-L1 consists of the amino acid sequence set forth in SEQ ID NO: 23;
CDR-L2 consists of the amino acid sequence set forth in SEQ ID NO: 19; and
CDR-L3 consists of the amino acid sequence set forth in SEQ ID NO: 22
(viii). defined according to the Kabat scheme,
CDR-H1 consists of the amino acid sequence set forth in SEQ ID NO: 7;

CDR-H2 consists of the amino acid sequence set forth in SEQ ID NO: 12;
CDR-H3 consists of the amino acid sequence set forth in SEQ ID NO: 17;
CDR-L1 consists of the amino acid sequence set forth in SEQ ID NO: 23;
CDR-L2 consists of the amino acid sequence set forth in SEQ ID NO: 19; and
CDR-L3 consists of the amino acid sequence set forth in SEQ ID NO: 22;

(ix). defined according to the Contact scheme,

CDR-H1 consists of the amino acid sequence set forth in SEQ ID NO: 8;
CDR-H2 consists of the amino acid sequence set forth in SEQ ID NO: 13;
CDR-H3 consists of the amino acid sequence set forth in SEQ ID NO: 15;
CDR-L1 consists of the amino acid sequence set forth in SEQ ID NO: 24;
CDR-L2 consists of the amino acid sequence set forth in SEQ ID NO: 25; and
CDR-L3 consists of the amino acid sequence set forth in SEQ ID NO: 21;

(x). defined according to the IMGT scheme,

CDR-H1 consists of the amino acid sequence set forth in SEQ ID NO: 9;
CDR-H2 consists of the amino acid sequence set forth in SEQ ID NO: 14;
CDR-H3 consists of the amino acid sequence set forth in SEQ ID NO: 16;
CDR-L1 consists of the amino acid sequence set forth in SEQ ID NO: 20;
CDR-L2 consists of the amino acid sequence set forth in SEQ ID NO: 18; and
CDR-L3 consists of the amino acid sequence set forth in SEQ ID NO: 22.

3. The antibody-drug conjugate of claim 1 or 2, or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), or the pharmaceutically acceptable salt or solvate thereof, characterized in that Ab comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein

(i) the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 3, and the light chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 4; or

(ii) the heavy chain variable region consists of the amino acid sequence set forth in SEQ ID NO: 3, and the light chain variable region consists of the amino acid sequence set forth in SEQ ID NO: 4;

(iii) the heavy chain variable region comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 3, and the light chain variable region comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4; or

(iv) the heavy chain variable region comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 3, and the light chain variable region comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4.

4. The antibody-drug conjugate of any one of claims 1-3, or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), or the pharmaceutically acceptable salt or solvate thereof, characterized in that Ab comprises a heavy chain and a light chain, wherein

(i) the heavy chain comprises an amino acid sequence set forth in SEQ ID NO: 1, and the light chain comprises an amino acid sequence set forth in SEQ ID NO: 2;

(ii) the heavy chain consists of the amino acid sequence set forth in SEQ ID NO: 1, and the light chain consists of the amino acid sequence set forth in SEQ ID NO: 2;

(iii) the heavy chain comprises an amino acid sequence having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 1, and the light chain comprises an amino acid sequence having 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 2; or

(iv) the heavy chain comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 1, and the light chain comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 2;

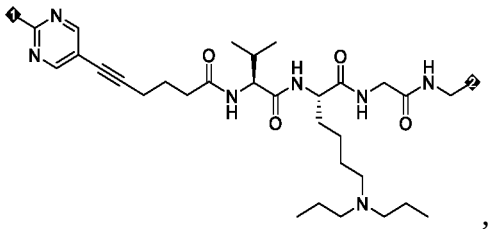
preferably, Ab is selected from Bevacizumab or a biosimilar thereof, or an antigen-binding fragment thereof;

preferably, Ab is selected from any one of the following biosimilars of Bevacizumab, or an antigen-binding fragment thereof: ABP215, BCD-021, FKB238, Mvasi (Bevacizumab-awwb), PF-06439535, SB8, Zirabev (Bevacizumab-bvzr), QL1101, IBI305, SHR-1603, BAT 1706, HS008, MIL60, or an antigen-binding fragment thereof.

5. The antibody-drug conjugate of any one of claims 1-4, or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), or the pharmaceutically acceptable salt or solvate thereof, characterized in that L comprises an amino acid residue or a short peptide consisting of 2-10 amino acid residues;

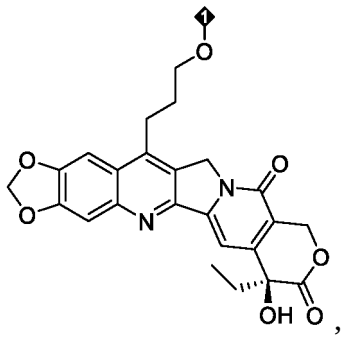
preferably, L comprises an amino acid residue cleavable in the tumor microenvironment or a short peptide consisting of 2-10 amino acid residues;

optionally, the amino acid residue in L is a natural amino acid residue or a modification thereof;

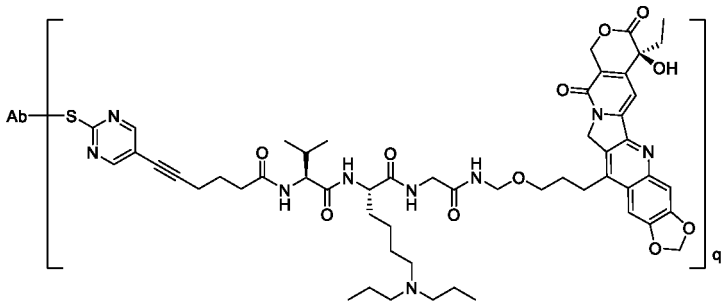
preferably, L is selected from , wherein the 1-position is linked to Ab, and the 2-position is linked to D.

6. The antibody-drug conjugate of any one of claims 1-5, or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), or the pharmaceutically acceptable salt or solvate thereof, characterized in that D is selected from camptothecin, SN38, DXD, substituent-modified camptothecin, substituent-modified DXD, or substituent-modified SN38;

or, D is selected from:


, wherein the 1-position is linked to L.

7. The antibody-drug conjugate of any one of claims 1-6, or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), or the pharmaceutically acceptable salt or solvate thereof, characterized in that the compound of formula (I) is selected from:



wherein q is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

preferably, the antibody or the antigen-binding fragment thereof that specifically binds VEGF comprises: three CDR-Hs of the heavy chain variable region (VH) sequence of SEQ ID NO: 3 and three CDR-Ls of the light chain variable region (VL) sequence of SEQ ID NO: 4, wherein the CDRs are defined according to the Chothia, AbM, Kabat, IMGT, Contact scheme, or any combination thereof;

more preferably, the antibody or the antigen-binding fragment thereof that specifically binds VEGF comprises: the heavy chain variable region (VH) of SEQ ID NO: 3 and the light chain variable region (VL) of SEQ ID NO: 4, further preferably, Ab is selected from Bevacizumab or an antigen-binding fragment thereof, or a biosimilar thereof.

8. A population of antibody-drug conjugates, characterized in that the population of antibody-drug conjugates comprises or consists of the antibody-drug

conjugate of any one of claims 1-7, or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), or the pharmaceutically acceptable salt or solvate thereof, wherein the antibody-drug conjugates have one, two, or more q values;

optionally, when the antibody-drug conjugates in the antibody-drug conjugate population have two or more q values, the proportion of antibody-drug conjugates having one specific q value among all antibody-drug conjugates in the composition is greater than 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99%;

optionally, an average drug-to-antibody ratio (average DAR) of the antibody-drug conjugate population is selected from 1.5-2.5, 3.5-4.5, 5.5-6.5, or 7.5-8.5;

preferably, the average drug-to-antibody ratio (average DAR) of the antibody-drug conjugate population is selected from about 2.0, 4.0, 6.0, or 8.0.

9. A pharmaceutical composition, characterized in that the pharmaceutical composition comprises the antibody-drug conjugate of any one of claims 1-7, or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), or the pharmaceutically acceptable salt or solvate thereof, or the population of antibody-drug conjugates of claim 8, as well as optionally one or more pharmaceutical excipients, e.g., a pharmaceutical carrier or a pharmaceutical vehicle, including a buffering agent;

optionally, the drug-to-antibody ratio (average DAR) in the pharmaceutical composition or the antibody-drug conjugate population is selected from an integer or decimal in the range of 1-10;

preferably, the drug-to-antibody ratio (average DAR) in the pharmaceutical composition or the antibody-drug conjugate population is selected from 1.5-2.5, 3.5-4.5, 5.5-6.5, and 7.5-8.5;

preferably, the DAR in the pharmaceutical composition or the antibody-drug conjugate population is selected from: 2±0.5, 4±0.5, 5±0.5, 6±0.5, 7±0.5, and 8±0.5;

preferably, the drug-to-antibody ratio (average DAR) in the pharmaceutical composition or the antibody-drug conjugate population is selected from about 2.0, 4.0, 6.0, and 8.0.

10. The antibody-drug conjugate of any one of claims 1-7, or the tautomer, stereoisomer, isotopic derivative (e.g., deuterated derivative), or the pharmaceutically acceptable salt or solvate thereof, the population of antibody-drug conjugates of claim 8, or the pharmaceutical composition of claim 9 for use as a medicament or for use in the preparation of a medicament, wherein the medicament is for treating or preventing a disease associated with the activity of VEGF or a disease associated with a target of VEGF, including tumors, for example, cancer, for example, solid tumors or hematological tumors;

optionally, the tumor is selected from lung cancer (for example, non-small cell lung cancer, lung adenocarcinoma), colon cancer (for example, human colon adenocarcinoma), rectal cancer, gastric cancer, colorectal cancer (e.g., colorectal adenocarcinoma).