

Bispecific antibody-drug conjugates targeting B7H3 and EGFR and the use thereof**Cross-reference to related applications**

This application is based on CN patent application No. 202311786110.0, filed on December 22, 2023, and CN patent application No. 202411857429.2, filed on December 17, 2024, and claims priority to the aforementioned applications, the entire contents of which are hereby incorporated by reference into this application.

Technical Field

The present invention relates to bispecific antibody-drug conjugates (ADCs) targeting B7H3 and EGFR and compositions containing the same. The present invention also relates to therapeutic and diagnostic uses of these antibodies and antibody fragments.

Background

Antibody-drug conjugates (ADCs) are a type of tumor-targeted chemotherapy drug that are generally composed of an antibody and varying numbers of linkers and cytotoxins. The target and cytotoxin-linker are crucial to the effectiveness and safety of ADCs. ADCs selectively deliver cytotoxins to tumor tissues, and induce the death of targeted tumor cells through the interaction of the selected cytotoxins with tubulin or DNA in the target cells. They also further kill cancer cells or paracancerous tissues around the tumor through the bystander effect, thereby improving the effectiveness and therapeutic window of ADC drugs [Chari, et al. *Angew. Chem. Int. Ed.* 2014;53 (15), 3796–3827]. Mylotarg (gemtuzumab ozogamicin) was the first ADC drug approved by the FDA in 2000. In the past two decades, 14 ADCs have been approved for marketing [Fu Z, et al. *Signal Transduct Target Ther.* 2022; 7(1): 93]. Among them, DS8201 (HER2-DXd) is recognized as the most breakthrough blockbuster drug for the treatment of breast cancer to date. In recent years, the clinical development of other ADC drugs has also shown a rapid growth trend.

The receptor tyrosine kinase epidermal growth factor receptor (HER) family, including EGFR (HER1 or ERBB1), HER2 (ERBB2), HER3 (ERBB3) and HER4 (ERBB4), is known to play an important role in cell proliferation, survival, differentiation and migration. Among the four HER family members, only EGFR induces tumor proliferation through homodimerization, while the homodimerization of others such as HER2, HER3 or HER4 is not oncogenic [Cohen, et al., *J. Biol. Chem.* 1996; 271(48):30897–30903]. In tumors with overexpression or abnormal activation of EGFR, including lung cancer, head and neck cancer, breast cancer, kidney cancer, gastric cancer, colon cancer, pancreatic cancer, ovarian cancer, prostate cancer and bladder cancer [Jinfeng Yu et al., *Front. Mol. Biosci.* 2022; 9. <https://doi.org/10.3389/fmolb.2022.847835>], EGFR overexpression or abnormal activation will induce and regulate the activation transduction and regulation of multiple EGFR downstream signals, promote tumor cell proliferation and migration, and mediate and promote tumor angiogenesis through VEGF, playing an important role in the occurrence, development, malignancy and prognosis of tumors. EGFR is a well-validated tumor target, and EGFR-targeted tumor therapy (EGFR monoclonal antibodies and small molecules targeting EGFR-TKI) has been widely used in the treatment of various types of cancers, such as lung cancer, head and neck cancer, colon cancer and pancreatic cancer. Although these targeted

EGFR therapies have shown improvements in progression-free survival, overall survival, and quality of life in cancer patients, EGFR targeted therapies are still limited by toxic side effects and the development of drug resistance mechanisms, most commonly a noticeable rash (similar in appearance to acne, usually limited to the face, upper chest, and back), and other side effects including diarrhea, constipation, stomatitis, fatigue, and electrolyte imbalance [Eli Lilly. Package insert Erbitux® (Cetuximab); 2004. Available from: <http://pi.lilly.com/us/erbitux-uspi.pdf>; OSI. Package insert Tarceva® (Erlotinib), 2004, Available from: https://www.gene.com/download/pdf/tarceva_prescribing.pdf]. At the same time, due to the inclusion of the mutation status of homologous oncogenic genes such as EGFR, K-Ras, B-Raf, PI3K and PTEN, as well as their different roles in the EGFR resistance mechanism, these factors also limit the scope of targeted application of EGFR monoclonal antibodies and EGFR-TKI small molecules [De Luca A, et al., *Curr Drug Targets*. 2010; 11:851–64; Siena S et al., *J Natl Cancer Inst*. 2009; 101:1308–24].

In order to make full use of EGFR as a tumor target and improve the effectiveness of EGFR drugs, many pharmaceutical companies have designed and developed EGFR monoclonal antibody ADC drugs targeting EGFR, such as Depatuxizumab Mafodotin (ABT-414), AVID100, MRG003, and Serclutamab talirine (Ser-T, formerly ABBV-321) that have entered clinical development. These EGFR-ADCs are in clinical development for different tumor indications, such as MRG003 (Clinical Phase I-NCT04868344, dose range 0.1-2.5 mg/kg, toxic side effects: hyponatremia, leukopenia, neutropenia, increased aspartate aminotransferase levels and febrile neutropenia, the results showed manageable safety and promising anti-tumor activity; Clinical Phase II-NCT05126719, two therapeutic doses of 2.0/2.3mg/kg are used to treat recurrent/metastatic nasopharyngeal carcinoma after chemotherapy and immunotherapy, the results showed good anti-tumor activity in advanced r/m NPC patients, with acceptable tolerability and manageable safety. Based on the higher ORR and potentially better efficacy than the 2.0 mg/kg group and the safety of good tolerance, 2.3 mg/kg is recommended as the recommended dose for further key studies) [Miaozhen Qiu et al., *JAMA Oncol*. 2022; 8(7): 1042-1046. doi:10.1001/jamaoncol.2022.0503; F. Han, et al., *Annals of Oncology*. 2023; 2(34). Doi.org/10.1016/j.annonc.2023.09.2006], and low doses showed anti-tumor effects and limiting toxicity such as hematopoiesis and elevated blood biochemistry. In addition to high expression in tumors, EGFR is also expressed in normal tissues and participates in cell growth, proliferation and differentiation. Although EGFR-ADC has improved anti-tumor efficacy and application range compared with EGFR monoclonal antibodies and EGFR-TKI small molecules, EGFR-ADC itself also has limiting toxicity, which limits the EGFR-ADC dosage and effective safety window.

B7H3 protein is a single-pass transmembrane glycoprotein encoded by the CD276 gene and is a member of the B7 protein family. B7H3 is highly expressed in a variety of solid tumors, including lung cancer, esophageal cancer, endometrial cancer, prostate cancer, and breast cancer. Although some physiological functions of B7H3 have been reported, due to the lack of direct evidence for B7H3 receptors, the clear physiological functions and detailed mechanisms of action of B7H3 remain to be further elucidated. Some viewpoints believe that B7H3 is a T cell co-stimulatory molecule [Chapoval AI et al. *Nat Immunol*. 2001; 2: 269–74], and some viewpoints believe that B7H3 is a co-inhibitory molecule that can promote tumorigenesis by regulating tumor-infiltrating immune cells [Kontos F, et al. *Clin Cancer Res*. 2021; 27:1227–35]. Several studies have shown that overexpression of B7H3 is associated with poor prognosis in various types of

cancer [Song J et al., *Onco Targets Ther.* 2016; 9:6257-63; Brunner A et al., *Gynecol Oncol.* 2012; 124:105–11]. Its expression profile and epidemiological data show that B7H3 is a highly attractive potential anti-tumor target.

B7H3 is expressed at low levels in normal tissues such as liver, colon, ovary and prostate, but is not expressed in other normal tissues and is highly expressed in tumors. DS-7300 (B7H3-DXd) has been tested in a Phase I clinical safety and tolerability trial (dose range 0.8-16 mg/kg) and has shown good tolerability and acceptable safety in patients with mCRPC [Manish R. et al, *Journal of Clinical Oncology.* 2022; 6(40). DOI: 10.1200/JCO.2022.40.6 _suppl.087 *Journal of Clinical Oncology* 40, no. 6_suppl (February 20, 2022) 87-87]. There are many treatment methods and development strategies for B7H3-targeted anti-tumor therapy, such as enhanced antibody-dependent cellular cytotoxicity (ADCC) antibodies, CD3 bispecific antibodies, chimeric antigen receptor (CAR)-T cells and antibody-drug conjugates (ADC), but there are no approved B7H3-targeted therapeutic drugs to date. B7H3-ADC is relatively safe, but not as effective as EGFR-ADC.

Therefore, there is still a need to develop new antibody molecules and ADC molecules containing the same, in particular, those antibody molecules and ADC molecules having advantages of high therapeutic effect, high safety (including low side effects), being useful for the treatment of various tumor cancers, being useful for the treatment of drug-resistant tumors, and/or having high product uniformity.

Summary

To further improve the effectiveness of B7H3-targeted ADC and the safety of EGFR-targeted ADC, the inventors selected high-affinity B7H3 antibody targeting B7H3 and low-affinity Zalu antibody targeting EGFR to splice into a bispecific antibody, and then introduced site-specific conjugation technology to prepare B7H3/EGFR bispecific ADC, thereby improving the effectiveness and safety of ADC in various tumor cancers.

The present invention provides an immunoconjugate comprising an antibody targeting B7H3 and EGFR (e.g, a bispecific antibody that specifically binds to B7H3 and EGFR or an antigen-binding fragment thereof of the present invention) and other payloads.

In one embodiment, the immunoconjugate is an antibody drug conjugate (ADC).

The present invention provides a bispecific antibody targeting B7H3 and EGFR and a bispecific antibody-drug conjugate (ADC) targeting B7H3 and EGFR, wherein the bispecific antibody has the following advantages:

- (a) binds specifically to one or two antigens with high affinity;
- (b) can be easily expressed in cultured cells in vitro, and the chains of the antibody molecules can be properly coupled or paired;
- (c) has good physical stability, especially good long-term thermal stability; and can maintain biological activity for a long time;
- (d) upon specifically binding to one or two antigens, exerts biological functions by regulating (for example, inhibiting or activating) the signal transduction pathways involved in each antigen;

- (e) exerts effector functions;
- (f) has better anti-tumor activity.

The antibody-drug conjugate (ADC) has the following advantages:

- (1) binds to, with high affinity, target cells expressing human B7H3 and/or EGFR;
- (2) can enter cells through endocytosis to kill target cells and has high endocytosis efficiency;
- (3) has remarkable bystander killing effect;
- (4) can block the downstream signal effect of the EGFR and inhibit cell growth, and the released Exatecan inhibits topoisomerase I, acts on DNA and induces the death of target cells, so that the ADC has high anti-tumor effect;
- (5) has low toxicity;
- (6) due to the site-specific conjugation of glycosylation, the Fc function of the antibody is silent, and there is no function of Fc segment such as ADCC, ADCP and CDC, thus avoiding the side effects caused by inducing inflammatory factors; and the site-specific conjugation makes the product more uniform and the efficacy more controllable;
- (7) has better stability;
- (8) has better druggability;
- (9) has a wider anti-tumor spectrum, and can be applied to the treatment of various cancers such as lung cancer, colorectal cancer, pancreatic cancer, breast cancer, oral squamous carcinoma, gastric cancer, prostatic cancer, melanoma, cervical cancer, and the like, and expands the scope of ADC indications; in addition, it can be used in combination with IO immune agonists in the later stage to further improve the efficacy or expand clinical indications; and/or
- (10) is effective against drug-resistant cancers.

Description of Figures:

Figure 1 shows the results of proliferation inhibition and endocytosis experiments of B7H3/EGFR bsAb in NCI-H358.

Figure 2 shows the results of proliferation inhibition and endocytosis experiments of B7H3/EGFR bsAb in PC9-B7H3.

Figure 3 shows the results of endocytosis experiment of Hz20G5.26/Zalu bsAb in various tumor cells.

Figure 4 shows the expression levels of EGFR and B7H3 in various tumor cells.

Figure 5 shows the pERK signal blocking effect of Hz20G5.26/Zalu bsAb in NCI-H358.

Figure 6 shows the results of endocytosis experiment of Hz20G5.26/Zalu bsAb in NCI-H358.

Figure 7 shows the results of RP-HPLC analysis of Hz20G5.26/Zalu bsAb-Exatecan.

Figure 8 shows the expression of B7H3 and EGFR in different tumor cells.

Figure 9 shows the in vitro killing activity of Hz20G5.26/Zalu bsAb-Exatecan in different types of tumors and different tumor cells.

Figure 10 shows the in vitro killing activity of Hz20G5.26/Zalu bsAb-Exatecan in different lung cancer cells.

Figure 11 shows the in vitro killing activity of Hz20G5.26/Zalu bsAb-Exatecan in different colorectal cancer cells.

Figure 12 shows the in vitro killing activity of Hz20G5.26/Zalu bsAb-Exatecan in pancreatic cancer cells.

Figure 13 shows the in vitro killing activity of Hz20G5.26/Zalu bsAb-Exatecan in breast cancer cells.

Figure 14 shows the in vitro killing activity of Hz20G5.26/Zalu bsAb-Exatecan in oral squamous cell carcinoma cells.

Figure 15 shows the in vitro killing activity of Hz20G5.26/Zalu bsAb-Exatecan in skin melanoma cells.

Figure 16 shows the in vitro killing activity of Hz20G5.26/Zalu bsAb-Exatecan in gastric cancer cells.

Figure 17 shows the in vitro killing activity of Hz20G5.26/Zalu bsAb-Exatecan in prostate cancer cells.

Figure 18 shows the in vitro activity of Hz20G5.26/Zalu bsAb-Exatecan in tumor cell lines with double low and double high expression of EGFR and B7H3.

Figure 19 shows the results of the bystander cell killing assay of Hz20G5.26/Zalu bsAb-Exatecan.

Figure 20 shows the in vivo efficacy of Hz20G5.26/Zalu bsAb-Exatecan in the H1975 CDX mouse model and the changes in mouse body weight after administration.

Figure 21 shows the in vivo efficacy of Hz20G5.26/Zalu bsAb-Exatecan in the BxPC3 CDX mouse model and the changes in mouse body weight after administration.

Figure 22 shows the in vivo efficacy of Hz20G5.26/Zalu bsAb-Exatecan in the H508 CDX mouse model and the changes in mouse body weight after administration.

Figure 23 shows the in vivo efficacy of Hz20G5.26/Zalu bsAb-Exatecan in the JIMT-1 CDX mouse model and the changes in mouse body weight after administration.

Figure 24 shows the structural representation of the bispecific antibody.

Detailed Description

Before the invention is described in detail below, it should be understood that the invention is not limited to the particular methodology, protocols, and reagents described herein, as these may vary. It should also be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the invention, which will be limited only by the appended claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs.

I. Definition

For the purpose of interpreting this specification, the following definitions will be used, and where appropriate, terms used in the singular may also include the plural, and vice versa. It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

The term "about" used in combination with a numerical value is intended to encompass the numerical values in a range from a lower limit less than the specified numerical value by 5%, 4%, 3%, 2% or 1% to an upper limit greater than the specified numerical value by 5%, 4%, 3%, 2% or 1%.

The term "and/or" as used herein, means any one of the options or two or more of the options.

The term "comprise" or "include" as used herein means including the elements, integers or steps described, but does not exclude any other elements, integers or steps. The term also covers the combination of the elements, integers or steps mentioned herein when the term "comprises" or "include" is used herein, unless otherwise specified. For example, it is also intended to cover the antibody variable region consisting of a specific sequence when referring to the antibody variable region "comprising" the specific sequence.

Unless otherwise indicated, the terms "B7-H3", "B7H3" and "CD276" are used interchangeably herein. B7-H3 is a type I transmembrane glycoprotein belonging to a member of the B7/CD28 superfamily, and is similar in sequence to the extracellular domain of PD-L1. B7-H3 has 316 amino acids and contains one putative signal peptide consisting of 28 amino acids, one extracellular region consisting of 217 amino acids, one transmembrane region and one cytoplasmic domain consisting of 45 amino acids with a molecular weight of about 45-66 kDa. In humans, the extracellular structure of B7-H3 may be an IgV-IgC-like domain (2Ig-B7-H3) or an IgV-IgC-IgV-IgC-like domain (4Ig-B7-H3) due to exon replication. The sequence of cynomolgus monkey B7-H3 has about 90% homology with its human counterpart. In some embodiments of the invention, B7-H3 is human B7-H3. In some embodiments, B7-H3 is a protein under UniProt database accession number Q5ZPR3.

As used herein, the term "EGFR" refers to the epidermal growth factor receptor, a tyrosine kinase receptor, a giant transmembrane glycoprotein with a molecular weight of about 170KDa, which is a member of the ErbB receptor family and is the most common cancer-driving gene of NSCLC. The EGFR activation mutation region occurs mainly in the EGFR exon 18-21 tyrosine kinase domain, and the EGFR antibody binding region is mainly located in the EGFR extracellular ligand domain region, so as to avoid the occurrence of drug-resistant mutations. Meanwhile, the EGFR antibody can inhibit the growth of tumor cells by inhibiting the binding of the EGFR and the ligand, and can also utilize self-specific ADCC (antibody-dependent cell-mediated cytotoxicity) to kill tumors in combination with immune cells, so that the EGFR antibody can play a role in resisting and killing the tumors through multiple action mechanisms. In some embodiments, the EGFR is a protein under Uniprot P00533-1 accession number.

The terms "anti-B7-H3 antibody", "anti-B7-H3", "B7-H3 antibody", or "anti-B7-H3 antibody", as used herein, refer to an antibody that is capable of binding B7-H3 protein with sufficient affinity. The antibodies may be used as diagnostic and/or therapeutic agents in targeting B7-H3, or to construct immunoconjugates, such as antibody drug conjugates.

The term "anti-EGFR antibody", "anti-EGFR", "EGFR antibody" or "anti-EGFR antibody" as used herein refers to an antibody that is capable of binding to EGFR protein with sufficient

affinity. The antibodies may be used as diagnostic and/or therapeutic agents in targeting EGFR, or for the construction of immunoconjugates, such as antibody drug conjugates.

When referring to "first" and "second" herein, it is merely to distinguish the two domains or the two chains but do not indicate the positions of the two domains in any way.

General information on the amino acid sequences or nucleotide sequences of human immunoglobulin light and heavy chains is given in Kabat, E.A. et al, Sequences of Proteins of Immunological Interest, 5th edition, Public Health Service, National Institutes of Health, Bethesda, MD (1991).

As used herein, the amino acid positions of all variable regions of the heavy and light chains are numbered according to the Kabat numbering system described in Kabat et al, Sequences of Proteins of Immunological Interest, 5th edition Public Health Service, National Institutes of Health, Bethesda, MD (1991) and referred to herein as "Kabat numbering".

As used herein, when used in reference to amino acid positions in domains (e.g., constant regions, e.g., Fc regions) of antibodies other than the variable regions, numbering is according to the EU numbering system described in Kabat, E.A. et al, Sequences of Proteins of Immunological Interest, 5th edition, Public Health Service, National Institutes of Health, Bethesda, MD (1991) and referred to herein as "EU numbering". When position numbering and/or amino acid residues are assigned to a particular antibody isotype, it is intended to apply to the corresponding position and/or amino acid residue of any other antibody isotype, as is known to those skilled in the art.

The term "antibody" is used herein in the broadest sense to refer to proteins comprising an antigen-binding site, encompassing natural and artificial antibodies of various structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), single chain antibodies, intact antibodies, and antibody fragments.

The terms "whole antibody", "full-length antibody", "complete antibody" and "intact antibody" are used interchangeably herein to refer to a naturally occurring glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain consists of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region consists of 3 domains CH1, CH2 and CH3 (and optionally CH4). Each light chain consists of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region consists of one domain CL. The VH and VL regions may be further subdivided into hypervariable regions (being Complementarity Determining Regions (CDRs)) interspersed with relatively conserved regions (being Framework Regions (FRs)). Each VH and VL consists of three CDRs and 4 FRs, arranged from N-terminal to C-terminal in the order FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The constant regions are not directly involved in binding of the antibody to the antigen, but exhibit multiple effector functions.

"Half-antibody" or "half-polymer" refers to a monovalent antigen-binding polypeptide. In some embodiments, the half-antibody or half-polymer comprises a VH/VL unit and optionally at least a portion of an immunoglobulin constant domain. In some embodiments, a half-antibody or a half-polymer comprises one immunoglobulin heavy chain, or antigen-binding fragment thereof, associated with one immunoglobulin light chain. In some embodiments, the half-antibody or half-polymer is monospecific, i.e., binds a single antigen or epitope. In some specific embodiments, the half-antibody binds to EGFR and does not bind to B7-H3. In some specific embodiments, the half-antibody binds to B7-H3 and does not bind to EGFR. One skilled in the art will readily

appreciate that a half-antibody may have an antigen-binding domain consisting of a single variable domain, e.g., derived from camelidae.

Herein, antibody constant regions or antibody constant domains, including CH1, CL and Fc domains and the CH2, CH3 and optionally CH4 domains that make up the Fc domains, may be selected according to the intended function of the antibody molecule. For example, the constant region may be an IgA, IgD, IgE, IgG or IgM region, especially an immunoglobulin constant domain of human IgG, e.g. a constant domain of human IgG1, IgG2, IgG3 or IgG4, preferably a constant domain of human IgG1. As another example, a Fab fragment of an antibody may comprise CH1 from IgG1 and CL constant regions. As another example, the Fc region of an antibody may comprise the CH2 and CH3 domains from IgG1. The immunoglobulin constant region may have a native sequence or a variant sequence.

The term "Fc domain" or "Fc region" is used herein to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of a constant region. The term includes native sequence Fc regions and variant Fc regions. A native immunoglobulin "Fc domain" comprises two or three constant domains, namely the CH2 domain, the CH3 domain, and the optional CH4 domain. For example, in natural antibodies, the immunoglobulin Fc domain comprises the second and third constant domains (CH2 and CH3 domains) originated from the two heavy chains of IgG, IgA, and IgD class antibodies; or comprises the second, third and fourth constant domains (CH2 domain, CH3 domain and CH4 domain) originated from the two heavy chains of the IgM and IgE classes antibodies. Unless otherwise indicated herein, the numbering of amino acid residues in the Fc region or heavy chain constant region is according to the EU numbering system (also known as the EU index) as described in Kabat et al, Sequences of Proteins of Immunological Interest, 5th edition, Public Health Service, National Institutes of Health, Bethesda, MD, 1991. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Two Fc regions can be dimerized to form a dimeric Fc, and two different Fc heterodimerizations forms a heterodimeric Fc. Herein, the terms "Fc region", "Fc portion" and "dimeric Fc (e.g., heterodimeric Fc)" do not include the heavy chain variable region VH and the light chain variable region VL and the heavy chain constant region CH1 and the light chain constant region CL of an immunoglobulin, but may in some cases include a hinge region of the heavy chain constant region at the N-terminal. In one embodiment, the human IgG heavy chain Fc region extends from Asp221 or from Cys226 or from Asp231 to the carboxy-terminus of the heavy chain.

In one embodiment, a human IgG1 Fc region polypeptide (comprising a portion of hinge region) comprises or consists of the following amino acid sequence:

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID
NO:47).

In one embodiment, the Fc region is originated from an Fc region of human origin. In one embodiment, the Fc region comprises all or part of a human constant region. The antibody Fc region is directly involved in complement activation, C1q binding, C3 activation, and Fc receptor binding. In one embodiment, the Fc region is a human Fc region. In one embodiment, the Fc region belongs to the subclass human IgG 4. In one embodiment, the Fc region belongs to the subclass human IgG1.

Herein, a "heterodimeric Fc scaffold" refers to a scaffold comprising or formed from two different Fc regions by dimerization, which can be linked at its N-terminus or C-terminus to a domain (e.g., heavy and/or light chain variable regions of an antibody or antigen-binding fragment of an antibody that can bind to a target molecule, or soluble portions of a ligand or receptor that can bind to a target molecule) binding to an antigen for use in constructing multispecific antibodies, e.g., bispecific antibodies.

The term "CH1 region" refers to the portion of an antibody heavy chain polypeptide that extends from EU position 118 to EU position 220 (EU numbering system). In one embodiment, the CH1 domain comprises or consists of the amino acid sequence of

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO: 41).

The term "antibody fragment" includes a portion of an intact antibody. In a preferred embodiment, the antibody fragment is an antigen-binding fragment. The term "antigen-binding fragment" of an antibody is a molecule distinct from a full-length antibody that comprises a portion of the full-length antibody, but is capable of binding to an antigen of the full-length antibody or competes for binding to an antigen with the full-length antibody (i.e., with the full-length antibody from which the antigen-binding fragment is derived). Antigen-binding fragments may be prepared by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact antibodies. Antigen binding fragments include, but are not limited to, Fab, Fab', F(ab')₂, Fv, single chain Fv, diabodies, single domain antibodies (sdAb), or nanobodies.

"Fab fragment" or "Fab" are used interchangeably herein to refer to an immunoglobulin fragment consisting of two polypeptide chains, comprising an immunoglobulin heavy chain variable region VH, a heavy chain constant domain CH1, a light chain variable region VL, and a light chain constant domain CL, wherein one polypeptide chain comprises, from N-terminus to C-terminus, VH and one constant region selected from CH1 and CL, and the other polypeptide chain comprises, from N-terminus to C-terminus, VL and another constant region selected from CL and CH1, wherein the VH and VL domains pair to form an antigen-binding site or an antigen-binding region. Herein, the Fab polypeptide chain comprising the heavy chain constant region CH1 is also referred to as the "Fab heavy chain"; accordingly, the Fab polypeptide chain comprising the light chain constant region CL is also referred to as "Fab light chain".

"Complementarity determining region" or "CDR region" or "CDR" is a region in an antibody variable domain that is highly variable in sequence and forms a structurally defined loop ("hypervariable loop") and/or comprises antigen contact residues ("antigen contact point"). CDRs are primarily responsible for binding to epitopes. The CDRs of the heavy and light chains are generally referred to as CDR1, CDR2, and CDR3, and are numbered sequentially from N-terminus. The CDRs located in the variable domain of the antibody heavy chains are referred to as HCDR1, HCDR2, and HCDR3, while the CDRs located in the variable domain of the antibody light chains are referred to as LCDR1, LCDR2, and LCDR3. In a given amino acid sequence of a light chain variable region or a heavy chain variable region, the exact amino acid sequence boundaries of each CDR can be determined using any one or a combination of many well-known antibody CDR assignment systems including, e.g., Chothia based on the three-dimensional structure of antibodies and the topology of the CDR loops (Chothia et al. (1989) Nature 342: 877-883; Al-Lazikani et al., "Standard conformations for the canonical structures of immunoglobulins", Journal of Molecular Biology, 273, 927-948 (1997)), Kabat based on antibody sequence variability (Kabat et al., Sequences of Proteins of Immunological Interest, 4th edition, U.S. Department of Health and Human Services, National Institutes of Health (1987)), AbM (University of Bath), Contact (University College London), International ImmunoGeneTics database (IMGT) (www.

imgt.cines.fr/), and North CDR definition based on the affinity propagation clustering using a large number of crystal structures. Unless otherwise indicated, in the present invention, the term "CDR" or "CDR sequence" encompasses CDR sequences determined in any of the schemes described above or the combination thereof. CDRs may also be determined based on the same Kabat numbering position as a reference CDR sequence (e.g., any one of the exemplary CDRs of the invention).

In one embodiment, the CDRs of the antibodies of the invention are determined by Kabat schemes for boundaries, or by AbM schemes, or by a combination thereof.

In one embodiment of the invention, in the antigen-binding region that binds to B7H3 of the invention, the HCDR1 of the VH is determined by the AbM scheme, HCDR2 and HCDR3 are determined by the Kabat scheme, and the LCDRs of the VL are determined by the Kabat scheme respectively.

In one embodiment of the present invention, the CDRs of VH and VL in the antigen-binding region that binds to EGFR in the present invention are determined by Kabat scheme respectively.

The term "hinge region" refers to the portion of an antibody heavy chain polypeptide that connects the CH1 and CH2 regions in the wild-type antibody heavy chain, e.g., the IgG1 hinge region, e.g., according to EU numbering, the sequence of D221 to P230. Other hinge region of IgG subclasses can be determined by alignment with cysteine residues in the hinge region of IgG1 subclass sequence. In some embodiments, CH1 may comprise a portion of the hinge region. In some embodiments, Fc region may comprise a portion of the hinge region.

Amino acid mutations are denoted by (original amino acid, amino acid position, mutated amino acid). For example, when the mutation site is located in the Fc region, "T366W" means that T located at EU numbering position 366 is substituted with W. When referring to combinations of mutations, the mutations in a combination are linked by an "and" or "/". " R521K/Y1069C " indicates that both mutations R521K and Y1069C are included. It should be noted that when describing mutations, a particular position encompasses its corresponding amino acid position on other polypeptide chains as well. For example, if C220 is mentioned, it encompasses amino acid 220 under EU numbering of the IgG1 heavy chain, as well as corresponding amino acids in other heavy chains, e.g., amino acid 131 in IgG2, IgG3, or IgG 4. In describing mutations, the original amino acid at a particular position may be the amino acid described, or it may be other amino acid at the corresponding position.

With respect to polypeptide sequences, "conservative alterations" include substitutions, deletions, or additions to the polypeptide sequence that does not substantially alter the desired functional activity of the polypeptide sequence. For example, conservative substitutions often result in the substitution of an amino acid for a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. The followings list 8 groups of amino acids containing conservative substitutions for each other: 1) alanine (A), glycine (G); 2) aspartic acid (D), glutamic acid (E); 3) asparagine (N), glutamine (Q); 4) arginine (R), lysine (K); 5) isoleucine (I), leucine (L), methionine (M), valine (V); 6) phenylalanine (F), tyrosine (Y), tryptophan (W); 7) serine (S), threonine (T); and 8) cysteine (C), methionine (M). In some embodiments, the term "conservative sequence changes" is used to refer to amino acid modifications that do not significantly affect or alter the antigen-binding characteristics of interest of the antibody molecules of the present invention containing the amino acid sequence. For example, conservatively modified variants will retain at least 80%, 85%, 90%, 95%, 98%, 99% or more, e.g., 100-110% or more, binding affinity for the antigen of interest relative to the parent antibody.

The term "target" refers to the substance for binding against which the binding molecule is directed. The target may be an antigen, or may be a ligand or receptor. The term "antigen" refers to a molecule that elicits an immune response. Such an immune response may involve antibody production or activation of specific immune cells, or both. The skilled artisan will appreciate that any macromolecule, including substantially all proteins or peptides, may be used as an antigen. Furthermore, the antigen may be derived from recombinant or genomic DNA. As used herein, the term "epitope" refers to the portion of an antigen that specifically interacts with an antibody molecule. Where the binding molecules of the invention are directed to target binding regions derived from antibodies, "target" and "antigen" may be used interchangeably.

The term "antigen-binding region" as used herein refers to any portion of an antibody or antigen-binding fragment thereof, e.g., a multispecific antibody or bispecific antibody, that binds a particular target or antigen. The antigen-binding region may be, for example, an antibody or immunoglobulin per se or an antibody fragment. Such antigen-binding regions may or may not have tertiary structure independent of the remainder of the multispecific or bispecific antibody, and may or may not bind to their antigen/epitope as separate entities.

As used herein, the term "multispecific" antibody refers to an antibody having at least two antigen-binding regions, each of which binds to a different epitope of the same antigen or to a different epitope of different antigens. Multispecific antibodies are antibodies that have binding specificities for at least two different antigens or epitopes. In one embodiment, provided herein are bispecific antibodies having binding specificity for a first antigen and a second antigen.

Herein, the term "bispecific antibody" comprises antigen binding domains that specifically bind to two antigens or two epitopes. Unless otherwise indicated, the order of antigen binding by the bispecific antibody in the listed bispecific antibody names is arbitrary. That is, in some embodiments, the terms "anti-EGFR/B7H3 bispecific antibody" and "anti-B7H3/EGFR bispecific antibody" are used interchangeably. In some embodiments, the bispecific antibody comprises two half-antibodies, wherein each half antibody comprises a single heavy chain variable region and optionally at least a portion of a heavy chain constant region and a single light chain variable region and optionally at least a portion of a light chain constant region. In some embodiments, the bispecific antibody comprises two half-antibodies, wherein each half-antibody comprises a single heavy chain variable region and a single light chain variable region and does not comprise more than one single heavy chain variable region and does not comprise more than one single light chain variable region. In some embodiments, the bispecific antibody comprises two half-antibodies, wherein each half-antibody comprises a single heavy chain variable region and a single light chain variable region, and wherein the first half-antibody binds to a first antigen/epitope and does not bind to a second antigen and the second half-antibody binds to a second antigen/epitope and does not bind to the first antigen.

When referring to a "first antigen-binding region" in a multispecific antibody or bispecific antibody, it is meant a binding region that binds to a first antigen, and is not intended to limit the number of such antigen-binding regions contained in the antibody, e.g., one or more than one first antigen-binding region may be included in a multispecific antibody or bispecific antibody. For example, a bispecific antibody comprises a first antigen-binding region and a second antigen-binding region, but may comprise one or more than one first antigen-binding region and one or more than one second antigen-binding region.

When referring to a "target- or antigen-binding region originated from an antibody", it is meant that the binding domain constituting the target/antigen-binding region is or is derived from a binding domain of the antibody that specifically binds antigen, e.g. a fragment of the antigen-binding region that specifically binds antigen, e.g. Fab, is or is derived from a corresponding

fragment of the antibody, e.g. Fab, or the heavy chain variable region and/or the light chain variable region of the antigen-binding region is or is derived from the heavy chain variable region and/or the light chain variable region of the antibody, or 1, 2, 3, 4, 5 or 6 CDRs of the antigen-binding region are CDRs of the antibody.

The term "derived from" means that the fragment in the antigen-binding region is substantially identical to the fragment of the antibody from which it is derived, but has a mutation, such as a substitution, deletion or addition, at one or more sites. In a specific embodiment, the mutation is not in a CDR of the antibody.

The multispecific or bispecific antibodies of the present invention may comprise a connector. The term "connector" as used herein refers to any molecule that enables direct attachment of different moieties of a multispecific antibody. Examples of connectors to establish covalent linkages between different moieties of the multispecific antibody include peptide connectors and non-proteinaceous polymers, including but not limited to polyethylene glycol (PEG), polypropylene glycol, polyalkylene oxide, or copolymers of polyethylene glycol or polypropylene glycol. In some embodiments, the term "peptide connector" according to the invention refers to a sequence of amino acids, wherein said sequence links together the amino acid sequences of the various moieties of the multispecific antibody. Preferably, the peptide connector has a length sufficient to link the two entities in such a way that they maintain their conformation relative to each other so as not to interfere with the desired activity. The peptide connector may or may not comprise predominantly the following amino acid residues: Gly, Ser, Ala or Thr.

The term "effector functions" refers to those biological activities attributed to the Fc region of an immunoglobulin that vary with the isotype of the immunoglobulin. Examples of immunoglobulin effector functions include: C1q binding and Complement Dependent Cytotoxicity (CDC), Fc receptor binding, antibody dependent cell mediated cytotoxicity (ADCC), Antibody Dependent Cellular Phagocytosis (ADCP), cytokine secretion, immune complex mediated antigen uptake by antigen presenting cells, down regulation of cell surface receptors (e.g., B cell receptors), and B cell activation.

The term "... valent" antibody refers to the number of antigen binding sites present in an antibody molecule. "bivalent," "trivalent," and "tetraivalent" antibodies refer to the presence of 2, 3, and 4 antigen binding sites, respectively, in an antibody molecule.

A "knob-into-hole" mutation or "KIH" mutation is used herein to refer to the introduction of mutations in the first Fc-polypeptide and the second Fc-polypeptide, respectively, using the "KIH" technique to form a bulge ("knob") and a complementary cavity ("hole") at the interface of the first Fc-polypeptide and at the interface of the second Fc-polypeptide. It is known in the art that "knob-into-hole" techniques can engineer the interface between different chains of an antibody molecule to facilitate proper conjugation of the individual chains of the antibody molecule. Generally, this technique involves introducing a "bulge/knob" at the interface of one strand and a corresponding "cavity/hole" at the interface of the other strand to be paired with, so that the bulge can be placed in the cavity. One preferred interface comprises the CH3 domain of the heavy chain constant domain of one chain and the CH3 domain of the heavy chain constant domain of the other chain to be paired with. The bulge may be constructed by replacing the small amino acid side chain from the interface of the CH3 domain of the heavy chain constant domain of one chain with a larger side chain (e.g., tyrosine or tryptophan). Compensatory cavities of the same or similar size to the bulge are constructed at the interface of the CH3 domains of the heavy chain constant domains of the other chain to be paired by replacing large amino acid side chains with smaller side chains (e.g., alanine or threonine). Another alternative interface is the CL domain of the Fab fragment comprising the light chain and the CH1 domain of the heavy chain described above, which

promotes the correct heterodimerization between the two chains of the Fab fragment by constructing a bulge-cavity interaction. In some embodiments, KIH mutations may further comprises cysteine mutations in the two CH3 regions, so as to form non-natural disulfide bond.

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

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

The calculation of sequence identity between sequences is performed as follows. To determine the percent identity of two amino acid sequences or two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., for optimal alignment, gaps can be introduced in the first and second amino acid sequences or in one or both of nucleic acid sequences, or non-homologous sequences can be discarded for comparison purposes). In one preferred embodiment, for comparison purposes, the length of the aligned reference sequence is at least 30%, preferably at least 40%, more preferably at least 50%, 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence. Amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide at the corresponding position in the second sequence, then the molecules are identical at this position.

As used herein, "immunoconjugate" is meant that the payload is attached to the antibody or antigen-binding fragment thereof by a linker so that the antibody or antigen-binding fragment thereof can act as a carrier to transport the payload to a target site in a targeted manner. The term "payload" refers to an active moiety conjugated to an antibody or antibody fragment of the invention, and may include any moiety used to attach an antibody or antibody fragment. In some embodiments, the payload can be a drug, such as a small molecule drug, a radionuclide, DNA, RNA, an enzyme, or a polypeptide, among others. In some embodiments, the immunoconjugate encompasses an Antibody Drug Conjugate (ADC), an antibody immunostimulatory conjugate drug (ISAC), an Antibody Oligonucleotide Conjugate (AOC), an antibody polypeptide conjugate drug (APC), an antibody nuclide conjugate drug (RDC), or an antibody degrading conjugate drug (ADeC), among others. Suitable payloads or active moieties for conjugation to the antibody include, for example, cytotoxic agents, chemotherapeutic agents, innate immune agonists (e.g., Toll-like receptor agonists (TLR) class ISAC drugs SBT6050, SBT6290, BDC-1001; STING agonist ISAC drugs XMT-2056, Treg cell regulatory ISAC drugs ADCT-301, etc.), immune modulators, therapeutic oligonucleotides (siRNA, PMO, etc.), or radionuclides, etc. In some embodiments, the immunoconjugate of the invention is an antibody drug conjugate, i.e., ADC.

As used herein, "antibody-drug conjugate (ADC)" refers to a compound/molecule obtained by linking an antibody to a drug (e.g. small molecule drug) via a linker.

The term "linker" refers to a structural fragment that links a drug (e.g., a small molecule drug) to an antibody moiety. It is understood that the linker, prior to attachment to the antibody or

antigen-binding fragment thereof, has a functional group that can form a bond with a functional group of the antibody or antigen-binding fragment thereof.

The term "linker-payload" refers to a compound formed by linking a payload, such as a drug (e.g., a small molecule drug), to a linker.

The term "spacer" refers to a structural fragment in a linker that can be used to connect other structural fragments in the linker.

The general term "sugar" used herein refers to a monosaccharide, such as glucose (Glc), galactose (Gal), mannose (Man), and fucose (Fuc). The term "sugar derivative" used herein refers to a derivative of a monosaccharide, i.e., a monosaccharide comprising a substituent and/or a functional group. Examples of sugar derivatives include amino sugars and sugar acids, such as glucosamine (GlcN), galactosamine (GalN), N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), N-acetylneuraminic acid (NeuNAc) and N-acetylmuramic acid (MurNAc), glucuronic acid (GlcA), and iduronic acid (IdoA). Examples of sugar derivatives also include compounds represented herein by E(A)_x, wherein E is a sugar or a sugar derivative, and wherein E includes x functional groups A.

The core-N-acetylglucosamine substituent (core-GlcNAc substituent) is defined herein as a GlcNAc bonded to the antibody via C1, preferably via an N-glycosidic bond to the amide nitrogen atom on the side chain of an asparagine amino acid of the antibody. The core-GlcNAc substituent may be present at the native glycosylation site of the antibody, but it may also be introduced at a different site of the antibody. Herein, the core-N-acetylglucosamine substituent is a monosaccharide substituent, or (if the core-GlcNAc substituent is fucosylated) a disaccharide core-(Fuc α 1-6)GlcNAc substituent, also referred to as GlcNAc(Fuc).

"Glycosylation modification" refers to the process of changing the saccharide chain of antibodies by glycosylation engineering. The glycosylation of antibodies may be further modified for various purposes to obtain antibodies with new glycosylation, for example, to deglycosylation in order to eliminate Fc γ R affinity and complement binding/effector function, to reduce fucose and sialic acid groups and increase bisecting N-acetylglucosamine, galactose and mannose in order to enhance Fc-mediated ADCC and CDC effects. Glycosylation modification methods are known in the art, for example, by changing the glycosylation sites of antibodies to increase or decrease the saccharide chains on the surface of antibodies, or by modifying saccharide chains in vitro by chemical or enzymatic methods, or by changing the glycosylation pathway of the expression system (e.g., composed of enzymes such as glycosidases and glycosyltransferases) to catalyze the glycosylation of antibodies, and the glycosylation of antibodies may also be changed by the influence of cell culture conditions. In some embodiments, the "glycosylation modification" of the present invention is carried out by modifying saccharide chains by enzymatic methods in vitro. Preferably, the glycosylation modification of the present invention is performed by modifying the saccharide chain using a glycosidase (eg, endoglycosidase or glycosyltransferase).

The antibody with modified glycosylation of the present invention refers to an antibody whose glycosylation profile is modified compared to an antibody with a native glycosylation profile. Preferably, the antibody with modified glycosylation refers to an antibody obtained after the antibody is expressed in an expression system (e.g., a mammalian cell) and the saccharide chain is modified by an enzymatic method in vitro (e.g., by a glycosidase (e.g., an endoglycosidase or a glycosyltransferase)). More preferably, the antibody with modified

glycosylation of the present invention refers to an antibody comprising a core-GlcNAc and a sugar derivative E(A)_x linked thereto, wherein the GlcNAc is bonded via C1 to the antibody, preferably via an N-glycosidic bond to the amide nitrogen atom in the side chain of an asparagine amino acid of the antibody. If the -GlcNAc substituent in the GlcNAc-E(A)_x substituent is fucosylated, the fucose is usually linked to C6 of the -GlcNAc substituent via α -1,6. The fucosylated-GlcNAc substituent refers to core-GlcNAc(Fuc) and the fucosylated GlcNAc-E(A)_x substituent refers to GlcNAc(Fuc)-E(A)_x.

As used herein, the term "site-specific conjugation" refers to conjugating a drug/active substance specifically to a specific site of an antibody via a linker.

As used herein, the term "alkyl" refers to a fully saturated branched or unbranched hydrocarbon group. The alkyl group preferably contains 1 to 24 carbon atoms, more preferably 1 to 16 carbon atoms, 1 to 12 carbon atoms, 1 to 6 carbon atoms, or 1 to 4 carbon atoms. Representative examples of alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, 3-methylhexyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, n-heptyl, n-octyl, n-nonyl, n-decyl, etc.

The term "alkylene" refers to an alkyl group as defined above, but which is divalent, i.e., has two single bonds linked to two other groups. Non-limiting examples of alkylene groups include -CH₂-, -CH₂CH₂-, -CH₂CH₂CH₂-, -CH₂CH₂CH₂CH₂-, -CH(-CH₂CH₃)- or -CH₂CH(-CH₃)-.

The term "halogen" or "halo" refers to fluoro (-F), chloro (-Cl), bromo (-Br), and iodo (-I).

The term "haloalkyl" refers to an alkyl group as defined herein substituted with one or more halo groups as defined herein. The haloalkyl group may preferably be a monohaloalkyl group, a dihaloalkyl group or a polyhaloalkyl group (including a perhaloalkyl group). The monohaloalkyl group may contain one iodo, bromo, chloro or fluoro in the alkyl group. The dihaloalkyl and polyhaloalkyl groups may contain two or more of the same halogen atoms or a combination of different halo groups in the alkyl group. Preferably, the polyhaloalkyl contains up to 12, 10 or 8 or 6 or 4 or 3 or 2 halo groups. Non-limiting examples of haloalkyl groups include fluoromethyl, difluoromethyl, trifluoromethyl, chloromethyl, dichloromethyl, trichloromethyl, pentafluoroethyl, heptafluoropropyl, difluorochloromethyl, dichlorofluoromethyl, difluoroethyl, difluoropropyl, dichloroethyl, and dichloropropyl. Perhaloalkyl refers to alkyl groups in which all hydrogen atoms are replaced by halogen atoms.

The term "aryl" refers to a monocyclic or bicyclic aromatic hydrocarbon group having 6-20, e.g., 6-12 carbon atoms in the ring portion. Preferably, the aryl group is a (C₆-C₁₀) aryl group. Non-limiting examples include phenyl, biphenyl, naphthyl, or tetrahydronaphthyl, each of which may be optionally substituted with 1-4 substituents, such as alkyl, trifluoromethyl, cycloalkyl, halogen, hydroxy, alkoxy, acyl, alkyl-C(O)-O-, aryl-O-, heteroaryl-O-, amino, thiol, alkyl-S-, aryl-S-, nitro, cyano, carboxyl, alkyl-O-C(O)-, carbamoyl, alkyl-S(O)-, sulfonyl, sulfonamido, heterocyclyl, etc.

As used herein, the term "arylene" refers to a divalent aryl group as defined above, including but not limited to phenylene, such as 1,4-phenylene, 1,3-phenylene, or 1,2-phenylene.

The term "cycloalkyl" is a cyclic alkyl group, that is, a monovalent, saturated or unsaturated hydrocarbon group having a cyclic structure. Cycloalkyl groups include fully saturated or partially saturated (containing 1 or 2 double bonds) hydrocarbon groups having a cyclic structure. Cycloalkyl groups may contain 3 or more, for example 3-18, 3-10, or 3-8 carbon atoms in the ring, and typically, according to the present invention, from 3 to 6 atoms. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl.

As used herein, the term "heteroaryl" refers to a 5-20-membered (e.g., 5-14-membered, 5-8-membered, 5-6-membered) monocyclic, bicyclic or fused polycyclic ring systems containing 1-8 heteroatoms selected from N, O, or S. Preferably, the heteroaryl group is a 5-10 membered ring system. Representative heteroaryl groups include 2- or 3-thienyl, 2- or 3-furyl, 2- or 3-pyrrolyl, 2-, 4- or 5-imidazolyl, 3-, 4- or 5-pyrazolyl, 2-, 4- or 5-thiazolyl, 3-, 4- or 5-isothiazolyl, 2-, 4- or 5-oxazolyl, 3-, 4- or 5-isooxazolyl, 3- or 5-1,2,4-triazolyl, 4- or 5-1,2,3-triazolyl, tetrazolyl, 2-, 3- or 4-pyridyl, 3- or 4-pyridazinyl, 3-, 4- or 5-pyrazinyl, 2-pyrazinyl, 2-, 4- or 5-pyrimidinyl.

The term "optional" or "optionally" means that the subsequently described event or condition occurs or does not occur, and that the description includes instances where said event or condition occurs and instances where it does not. For example, when a group or structure is "optionally substituted", the group or structure may or may not be substituted.

As used herein, when a group is substituted by a "substituent", unless otherwise specified and not contradictory according to the context, it may be substituted by one or more (e.g., 1, 2, 3, or 4) substituents, which may be selected from, for example, hydrogen, halogen, C₁-C₁₂ alkyl-O-, -NO₂, -CN, -S(O)₂R₂, C₁-C₁₂ alkyl, halo C₁-C₁₂ alkyl, cycloalkyl, hydroxyl, acyl, alkyl-C(O)-O-, aryl-O-, heteroaryl-O-, amino, thiol, alkyl-S-, aryl-S-, carboxyl, alkyl-O-C(O)-, carbamoyl, alkyl-S(O)-, sulfonyl, sulfonylamino, and heterocyclyl.

The term "pharmaceutically acceptable salt" refers to a salt that retains the biological effects and properties of the ADC conjugates of the invention, and which is not biologically or otherwise undesirable. The ADC conjugates of the invention may exist in the form of their pharmaceutically acceptable salts, including acid addition salts and base addition salts. In the present invention, pharmaceutically acceptable non-toxic acid addition salts refer to salts formed by the ADC conjugates of the present invention with organic or inorganic acids including, but 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, and the like. Pharmaceutically acceptable non-toxic base addition salts mean salts formed by the ADC conjugates of the invention with organic or inorganic bases, including but not limited to alkali metal salts, such as lithium, sodium or potassium salts; alkaline earth metal salts, such as calcium or magnesium salts; organic base salts, for example ammonium salts formed with organic bases containing N groups.

The term "solvate" refers to an associated complex of one or more solvent molecules with an ADC antibody-drug conjugate of the invention. Solvents that form solvates include, but are not limited to, water, methanol, ethanol, isopropanol, ethyl acetate, tetrahydrofuran, N,N-dimethylformamide, dimethylsulfoxide, and the like.

"Pharmaceutically acceptable" and "pharmaceutically useful" are used interchangeably herein, unless it conflicts with the context,

The term "drug to antibody ratio", "DAR" or similar terms refers to, in an antibody-drug conjugate molecule, a ratio of drug moiety (D) conjugated to the Ab moiety as described herein to the Ab moiety. In some embodiments described herein, the DAR may be determined by r and p in formula (I), or by r, x and y in formula (III). For example, DAR may be from 1 to 16, e.g. 2-16, 4-16, 5-12, 6-10, 2-8, 3-8, 2-6, 4-6, 6-10, e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15. DAR may also be calculated as the average DAR of a population of molecules in a product, i.e., the overall ratio of drug moiety (D) conjugated to the Ab moiety described herein to the Ab moiety as measured by detection methods (e.g., by conventional methods such as mass spectrometry, ELISA

assays, electrophoresis, and/or HPLC) in a product, such DAR being referred to herein as average DAR, the average DAR may be an integer or decimal. In some embodiments, the average DAR value of a conjugate of the invention is 1 to 16, e.g., 2-16, 4-16, 5-12, 6-10, 2-8, 3-8, 2-6, 4-6, 6-10, such as 1.0-8.0, 2.0-6.0, such as 0.5, 0.6, 0.7, 0.8, 0.9, 1, 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 or 10.0, and the ranges with two of these values as endpoints. It should be understood that, in the case that an average DAR value is referred to, the ADCs according to the present invention refer to a population of ADC molecules or a mixture of ADC molecules comprising ADC molecules with the same and/or different DARs.

The term "therapeutic agent" as described herein comprises any substance effective in preventing or treating tumors (such as cancer), including a chemotherapeutic agent, a cytokine, angiogenesis inhibitor, a cytotoxic agent, other antibodies, a small molecule drug or an immunomodulatory agent (such as an immunosuppressant).

As used herein, the term "cytotoxic agent" refers to a substance that inhibits or prevents the cell function and/or causes cell death or destruction.

"Chemotherapeutic agents" include chemical compounds useful in treatment of cancer or immune system disease.

The term "drug" refers to an organic compound capable of modulating a biological process, in particular altering or preventing a pathological process.

The term "prodrug" refers to a chemically modified active or inactive compound that, after administration to an individual, becomes an active drug through physiological actions in the body (e.g., hydrolysis, metabolism, etc.). Techniques for making and using prodrugs are well known to those skilled in the art.

"Angiogenesis inhibitor" refers to a compound that blocks or interferes to some extent with vascular development. The angiogenesis inhibitor may be, for example, a small molecule or an antibody that binds to a growth factor or a growth factor receptor involved in promoting angiogenesis.

The term "small molecule drug" refers to low molecular weight organic compounds that can modulate biological processes, especially change or prevent pathological processes. "Small molecules" are defined as molecules with a molecular weight of less than 10 kD, usually less than 2 kD and preferably less than 1 kD, more preferably less than 500 D. Small molecule drugs include, but are not limited to, organic molecules, organic molecules containing inorganic components, molecules containing radioactive atoms, synthetic molecules, peptide mimetics, and antibody mimetics. As therapeutic agents, small molecules can be more permeable to cells, less susceptible to degradation, and less prone to eliciting immune responses than macromolecules. "Antitumor compounds" are pharmaceutically active compounds that have an effect on tumors, including but not limited to cytotoxic agents or chemotherapeutic agents, such as cytotoxic agents disclosed in WO2021/173773, camptothecins, e.g., exatecan (topoisomerase I inhibitor, Exatecan), Dxd (a novel topoisomerase I inhibitor, a derivative of Exatecan), auristatins, e.g., monomethyl auristatin E (MMAE) or maytansinoids, e.g., small molecule microtubule inhibitor DM1, taxanes such as paclitaxel or docetaxel, anthracyclines, epothilones, mitomycins, combretastatin, vinca alkaloids, calicheamicins, duocarmycin, tubulysins, amatoxin, bleomycin, MEK inhibitors, KSP inhibitors,

etc. It should be understood that the antitumor compound may be substituted by isotopes including but not limited to, for example, deuterium, tritium, etc. For example, after substitution with deuterium, a carbon-deuterium bond replaces a carbon-hydrogen bond, and because the former is more stable than the latter, this substitution can directly affect the absorption, distribution, metabolism and excretion properties of certain drugs, thereby improving the efficacy, safety and tolerability of the drugs. Therefore, the "anti-tumor compound" of the present application encompasses compounds substituted by deuterium or tritium.

"Deuterium substituted" or "substituted with deuterium" means that hydrogen in the molecule is replaced by deuterium, for example, 1 or more hydrogens, for example 1-10 (for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) hydrogens are replaced by deuterium. "Tritium substituted" or "substituted with tritium" has the same or similar interpretation.

The term "immunomodulators" as used herein refers to natural or synthetic active agents or drugs that inhibit or regulate (e.g., activate) immune response. The immune response can be humoral response or cellular response. Immunomodulators include immunosuppressants or immunoagonist. In some embodiments, immunomodulators of the invention include immune checkpoint inhibitors or immune checkpoint agonists.

The term "effective amount" refers to such an amount or dosage of the ADC molecule or composition or combination of the present invention, which produces the desired effect in the patient in need of treatment or prevention after being administered to the patient in a single or multiple doses. Depending on the expected effect, it can be "therapeutically effective amount" and "prophylactically effective amount".

"Therapeutically effective amount" refers to an amount that can effectively achieve the desired therapeutic results at the required dose and for the required period of time. A therapeutically effective amount is also such an amount, where any toxic or deleterious effect of ADC molecule or composition or combination is less than the therapeutic beneficial effect. A "Therapeutically effective amount" preferably inhibits a measurable parameter (such as tumor volume) by at least about 30%, even more preferably by at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or even 100% compared to untreated subjects.

"Prophylactically effective amount" refers to an amount that can effectively achieve the desired prophylactic results at the required dose and for the required period of time. Generally, since the prophylactic dose is used before or at an earlier stage of the disease in a subject, the prophylactically effective amount will be less than the therapeutically effective amount.

The terms "host cell", "host cell line" and "host cell culture" are used interchangeably and refer to the cells in which foreign nucleic acids are introduced, including the descendants of such cells. Host cells include "transformants" and "transformed cells", which include primary transformed cells and offspring derived from them, regardless of the number of passages. The nucleic acid content of the descendants may not be exactly the same as that of the parent cell, but may contain mutations. The mutant descendants with the same function or biological activity screened or selected from the initially transformed cells are included herein.

The term "label" as used herein refers to a compound or composition that is directly or indirectly conjugated or fused to an agent (such as a polynucleotide probe or antibody) and facilitates the detection of the conjugated or fused agent. The label itself can be detectable (for example, radioisotope label or fluorescent label) or can catalyze the chemical changes of detectable substrate compounds or compositions in the case of enzymatic labeling. The term is intended to

cover the direct labeling of probes or antibodies by coupling (i.e., physically connecting) detectable substances to probes or antibodies and the indirect labeling of probes or antibodies by reacting with another directly labeled agent.

"Individuals" or "subjects" include mammals. Mammals include, but are not limited to, domestic animals (such as cattle, sheep, cats, dogs and horses), primates (such as human and non-human primates, such as monkeys), rabbits, and rodents (such as mice and rats). In some embodiments, the individuals or subjects are human.

An "isolated" antibody or other molecule is an antibody or molecule that has been separated from components of its natural environment or the environment in which it is expressed. In some embodiments, the antibody is purified to a purity greater than 95% or 99%, as determined by, for example, electrophoresis (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatography (e.g., ion exchange or reversed phase HPLC).

The term "anti-tumor effect" refers to a biological effect that can be exhibited by a variety of means, including, but not limited to, for example, a reduction in tumor volume, a reduction in tumor cell number, a reduction in tumor cell proliferation, or a reduction in tumor cell survival.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. The cancer may be in an early, intermediate or advanced stage or metastatic cancer. Cancers suitable for treatment by the molecules of the invention are, for example, selected from epithelial cancers or cancers of the digestive tract, such as gastric cancer, pancreatic cancer, intestinal cancer, colon cancer or colorectal cancer, head and neck squamous cell carcinoma, gastric adenocarcinoma, breast cancer, oral squamous cell carcinoma, prostate cancer, melanoma, cervical cancer, lung cancer (e.g. small cell lung cancer and non-small cell lung cancer), esophageal cancer, kidney cancer, bladder cancer, ovarian cancer and head and neck cancer, including metastatic forms of these cancers.

The term "tumor" refers to the growth and proliferation of all neoplastic cells, whether malignant or benign, as well as all pre-cancerous and cancerous cells and tissues. "Tumor" encompasses both solid and hematological tumors and metastatic lesions. The terms "cancer", "cancerous" and "tumor" are not mutually exclusive when referred to herein.

"Tumor immune escape" refers to the process by which tumors escape immune recognition and clearance. As such, as a therapeutic concept, tumor immunity is "treated" when such escape diminishes, and the tumor is recognized and attacked by the immune system. Examples of tumor recognition include tumor binding, tumor shrinkage and tumor clearance.

The term "pharmaceutically acceptable excipient" refers to diluents, adjuvants (e.g., Freund's adjuvant (complete and incomplete)), vehicle, carriers, stabilizers or the like, which are administered together with the active substance.

The term "pharmaceutical composition" refers to a composition that is present in a form that allows for the biological activity of the active ingredients contained therein to be effective, and that does not contain additional ingredients that have unacceptable toxicity to the subject to which the composition is administered.

The term "pharmaceutical combination or combination product" refers to non-fixed combination products or fixed combination products, including but not limited to drug kits and drug compositions. The term "unfixed combination" means that the active ingredients (for example, (i) the ADC of the invention, and (ii) other therapeutic agents) are administered to patients simultaneously, without specific time limits or at the same or different time intervals, in sequence,

in separate entities, where these two or more active agents are administered to provide effective levels of prevention or treatment in patients. In some embodiments, the ADC and other therapeutic agents of the invention used in the pharmaceutical combination are administered at a level not exceeding the level when they are used alone. The term "fixed combination" means that two or more active agents are administered simultaneously to patients in the form of a single entity. It is preferred to select the dose and/or time interval of two or more active agents, so that the combined use of each component can produce greater effect than the single use of any one component in the treatment of disease or disorder. Each component may be in a separate preparation form, and the preparation forms may be the same or different.

The term "combination therapy" refers to the application of two or more therapeutic agents or therapeutic modes (such as radiotherapy or surgery) to treat the diseases described herein. Such application includes the co-administration of these therapeutic agents in a substantially simultaneous manner, such as in a single capsule with a fixed proportion of active ingredients. Alternatively, such application includes the joint administration of each active ingredient in multiple or separate containers (such as tablets, capsules, powders and liquids). The powder and/or liquid can be reconstituted or diluted to the required dose before administration. In addition, this administration also includes the use of each type of therapeutic agent at approximately the same time or at different times in a sequential manner. In either case, the treatment strategy will provide the beneficial effect of pharmaceutical combination in treating the disease or condition described herein.

As used herein, the term "treatment" (or "treat" or "treating") refers to slowing, interrupting, arresting, alleviating, stopping, reducing, or reversing the progression or severity of an existing symptom, disorder, condition, or disease.

As used herein, the term "prevention" (or "prevent" or "preventing") includes the inhibition of the onset or progression of a disease or disorder or a symptom of a particular disease or disorder. In some embodiments, subjects with family history of cancer are candidates for preventive regimens. Generally, in the context of cancer, the term "prevention" refers to the administration of a drug prior to the onset of signs or symptoms of a cancer, particularly in subjects at risk of cancer.

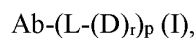
The term "vector" as used herein refers to a nucleic acid molecule capable of proliferating another nucleic acid to which it is linked. The term includes vectors that serve as self-replicating nucleic acid structures as well as vectors binding to the genome of a host cell into which they have been introduced. Some vectors are capable of directing the expression of a nucleic acid to which they are operably linked. Such vectors are called "expression vectors" herein.

"Subject/patient/individual sample" refers to a collection of cells or fluids obtained from a patient or subject. The source of the tissue or cell samples can be solid tissues, e.g., from fresh, frozen and/or preserved organ or tissue samples or biopsy samples or puncture samples; blood or any blood component; body fluids such as cerebrospinal fluids, amniotic fluids, peritoneal fluids(ascites), or interstitial fluids; cells from a subject at any time during pregnancy or development. Tissue samples may comprise compounds which are naturally not mixed with tissues, such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, and the like.

Examples of tumor samples herein include, but are not limited to, tumor biopsies, fine needle aspirates, bronchial lavage, pleural fluid, sputum, urine, surgical specimens, circulating tumor cells, serum, plasma, circulating plasma proteins, ascites, primary cell cultures or cell lines derived from tumors or exhibiting tumor-like properties, and preserved tumor samples, such as formalin-fixed, paraffin-embedded tumor samples or frozen tumor samples.

II. Antibody-drug conjugate

The present invention provides an antibody-drug conjugate of formula (I)



or a pharmaceutically acceptable salt or solvate thereof:

wherein:

Ab is a bispecific antibody or a fragment thereof that specifically binds to B7H3 and EGFR (e.g., human B7H3 and human EGFR);

L is a linker;

D is a drug, preferably an anti-tumor compound; and

p is an integer selected from 1 to 20, e.g., an integer selected from 1-9, 2-8, 3-7, 4-6 or 2-6, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

r is an integer selected from 1-5, such as 1, 2, 3, 4 or 5, preferably 1 or 2.

In some embodiments, the antibody Ab in formula (I) of the present invention is an antibody as defined below, such as a bispecific antibody.

In some embodiments, L and D in formula (I) of the present invention are as defined in the sections below.

II-1. Bispecific antibodies

In one aspect, the present invention provides a multispecific antibody that specifically binds to EGFR and B7H3.

In some embodiments, the multispecific antibodies of the present invention comprise a first binding specificity to EGFR and a second binding specificity to B7H3, and optionally other binding specificities.

In some embodiments, the multispecific antibody is a bispecific antibody.

Accordingly, the present invention relates to a bispecific antibody that specifically binds to EGFR and B7H3.

Accordingly, one aspect of the present invention relates to a multispecific antibody comprising

a first antigen-binding region and a second antigen-binding region, wherein the first antigen-binding region specifically binds to EGFR, and/or the second antigen-binding region specifically binds to B7H3, and optionally other antigen-binding region(s).

Accordingly, one aspect of the present invention relates to a bispecific antibody comprising

a first antigen-binding region and a second antigen-binding region, wherein the first antigen-binding region specifically binds to EGFR and/or the second antigen-binding region specifically binds to B7H3.

Bispecific antibodies of the invention can be prepared using bispecific antibody formats or techniques known in the art. Specific exemplary bispecific formats that may be used in the context

of the present invention are described, for example, in Labrijn, et al., Bispecific antibodies: a mechanical review of the pipeline. *Nature Reviews Drug Discovery*, 2019, 18(8): 1-24. In an embodiment, the bispecific antibody format includes an IgG-like antibody (Fan et al (2015) *Journal of Hematology & Oncology*.8: 130). The most common type of IgG-like antibodies comprises two Fab regions and two Fc regions, the heavy and light chains of each Fab may be derived from a separate monoclonal antibody. In some embodiments, the bispecific antibody of the invention is an IgG-like bispecific antibody comprising as one antigen-binding region a Fab fragment that specifically binds to EGFR and as the other antigen-binding region a Fab fragment that specifically binds to B7H3.

The following provides a detailed description of the components of the multispecific antibodies, such as bispecific antibodies, of the present invention. Those skilled in the art will understand that, unless the context clearly indicates otherwise, any combination of any technical features of these components is within the scope of the present invention. Moreover, those skilled in the art will understand that, unless the context clearly indicates otherwise, the antibodies of the present invention (including antibodies in any form) can comprise any such combination.

II-1-1 An antigen-binding region that specifically binds to EGFR

In some embodiments, the first antigen-binding region applicable for the anti-EGFR/B7H3 bispecific antibodies of the present invention may comprise or consist of an anti-EGFR antibody or antigen binding fragment thereof (e.g. an EGFR antibody disclosed in WO02100348A2(which is incorporated herein as a whole), e.g. Zalutumumab), as long as it is capable of specifically binding to EGFR, including, but not limited to, e.g. a full length antibody, a half antibody, a Fab, a Fab', a Fab'-SH, a Fv, a single chain antibody(e.g. scFv), a (Fab')₂, a single domain antibody such as VHH, dAb (domain antibody), a heavy chain antibody, or a linear antibody that specifically binds to EGFR, and the like.

In some embodiments, the antigen-binding region that specifically binds to EGFR is originated from an antibody that specifically binds to EGFR, e.g., an EGFR antibody disclosed in WO02100348A2, e.g., Zalutumumab.

In some embodiments, the antigen-binding region that specifically binds to EGFR comprises 1, 2, 3, 4, 5, or 6 CDRs of a known antibody that specifically binds to EGFR, e.g., an EGFR antibody disclosed in WO02100348A2, e.g., Zalutumumab.

In some embodiments, the antigen-binding region that specifically binds to EGFR comprises 1, 2, or 3 heavy chain variable region CDRs, i.e., HCDR1, HCDR2, and HCDR3, of a known antibody that specifically binds to EGFR, e.g., an EGFR antibody disclosed in WO02100348A2, e.g., Zalutumumab.

In some embodiments, the antigen-binding region that specifically binds to EGFR comprises 1, 2, or 3 light chain variable region CDRs, i.e., LCDR1, LCDR2, and LCDR3, of a known antibody that specifically binds to EGFR, e.g., an EGFR antibody disclosed in WO02100348A2, e.g., Zalutumumab.

In some embodiments, the antigen-binding region that specifically binds to EGFR comprises 3 heavy chain variable region CDRs and 3 light chain variable region CDRs of a known antibody that specifically binds to EGFR, e.g., an EGFR antibody disclosed in WO02100348A2, e.g., Zalutumumab.

In some embodiments, the antigen-binding region that specifically binds to EGFR comprises the heavy chain variable region and/or the light chain variable region of a known antibody that specifically binds to EGFR, such as an EGFR antibody disclosed in WO02100348A2, e.g., Zalutumumab.

In some embodiments, the antigen-binding region that specifically binds to EGFR comprises a Fab of a known antibody that specifically binds to EGFR such as an EGFR antibody disclosed in WO02100348A2, e.g., Zalutumumab.

In some embodiments, the antigen-binding region that specifically binds to EGFR comprises 3 Complementarity Determining Regions (HCDRs) from a heavy chain variable region, HCDR1, HCDR2, and HCDR3. In some embodiments, the antigen-binding region that specifically binds to EGFR comprises 3 Complementarity Determining Regions (LCDRs) from the light chain variable region, LCDR1, LCDR2, and LCDR3. In some embodiments, the antigen-binding region that specifically binds to EGFR comprises 3 Complementarity Determining Regions (HCDRs) from the heavy chain variable region and 3 Complementarity Determining Regions (LCDRs) from the light chain variable region.

In some aspects, the antigen-binding region that specifically binds to EGFR comprises a heavy chain variable region (VH). In some aspects, the antigen-binding region that specifically binds to EGFR comprises a light chain variable region (VL). In some aspects, the antigen-binding region that specifically binds to EGFR comprises a heavy chain variable region (VH) and a light chain variable region (VL). In some embodiments, the heavy chain variable region comprises 3 Complementarity Determining Regions (CDRs) from a heavy chain variable region, HCDR1, HCDR2 and HCDR3. In some embodiments, the light chain variable region comprises 3 Complementarity Determining Regions (CDRs) from a light chain variable region, LCDR1, LCDR2 and LCDR3.

The HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3, the VH, and/or the VL, comprised by the antigen-binding region that specifically binds to EGFR of the present invention are respectively defined herein.

In some embodiments, the heavy chain variable region of the antigen-binding region that specifically binds to EGFR described in the present invention

(i) comprises or consists of an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 1, or

(ii) comprises or consists of the amino acid sequence of SEQ ID NO: 1; or

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

In some embodiments, the light chain variable region of the antigen-binding region that specifically binds to EGFR described in the present invention

(i) comprises or consists of an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 2, or

(ii) comprises or consists of the amino acid sequence of SEQ ID NO: 2; or

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

In some embodiments, the 3 complementarity determining regions (HCDRs) from the heavy chain variable region of the antigen-binding region that specifically binds to EGFR described in the present invention, HCDR1, HCDR2 and HCDR3 are selected from

(i) the three complementarity determining regions HCDR1, HCDR2 and HCDR3 contained in the VH set forth in SEQ ID NO: 1, or

(ii) sequences which contain at least one and no more than 5, 4, 3, 2 or 1 amino acid change (preferably amino acid substitution, preferably conservative substitution) in total on the three HCDR regions relative to the sequence of (i),

wherein the HCDRs can be determined according to any scheme for determining CDRs, such as determined respectively by the Kabat, AbM, Chothia, Contact, or IMGT schemes or combination thereof;

for example, the HCDRs1-3 are determined by the Kabat scheme respectively.

In some embodiments, the 3 complementarity determining regions (LCDRs) from the light chain variable region of the antigen-binding region that specifically binds to EGFR described in the present invention, LCDR1, LCDR2, and LCDR3 are selected from

(i) the three complementarity determining regions LCDR1, LCDR2 and LCDR3 contained in the VL set forth in SEQ ID NO: 2, or

(ii) sequences which contain at least one and no more than 5, 4, 3, 2 or 1 amino acid change (preferably amino acid substitution, preferably conservative substitution) in total on the three LCDR regions relative to the sequence of (i),

wherein the LCDRs can be determined according to any scheme for determining CDRs, such as determined respectively by the Kabat, AbM, Chothia, Contact, or IMGT schemes or combination thereof;

for example, the LCDRs1-3 are determined by the Kabat scheme respectively.

In some embodiments, the antigen-binding region that specifically binds to EGFR of the present invention comprises 3 complementarity determining regions (HCDRs) comprised by the heavy chain variable region consisting of the amino acid sequence of SEQ ID NO:1 and 3 complementarity determining regions (LCDRs) comprised by the light chain variable region consisting of the amino acid sequence of SEQ ID NO: 2;

wherein the HCDRs and LCDRs can be determined according to any scheme for determining CDRs, such as determined respectively by the Kabat, AbM, Chothia, Contact, or IMGT schemes or combination thereof;

for example, the HCDRs1-3 are determined by the Kabat scheme respectively; and the LCDRs1-3 are determined by the Kabat scheme respectively.

In some embodiments, the HCDR1 comprises or consists of the amino acid sequence of SEQ ID NO:9 or the HCDR1 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) as compared to the

amino acid sequence of SEQ ID NO: 9.

In some embodiments, the HCDR2 comprises or consists of the amino acid sequence of SEQ ID NO:10 or the HCDR2 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) as compared to the amino acid sequence of SEQ ID NO: 10.

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

In some embodiments, the LCDR1 comprises or consists of the amino acid sequence of SEQ ID NO:12 or the LCDR1 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) as compared to the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the LCDR2 comprises or consists of the amino acid sequence of SEQ ID NO:13 or the LCDR2 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) as compared to the amino acid sequence of SEQ ID NO: 13.

In some embodiments, the LCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 14 or the LCDR3 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) as compared to the amino acid sequence of SEQ ID NO: 14.

In some particular embodiments of the invention, the antigen-binding region that specifically binds to EGFR described in the present invention comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and/or LCDR3 as described above.

In some particular embodiments of the invention, the antigen-binding region that specifically binds to EGFR described in the present invention comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 as described above.

In some particular embodiments of the invention, the antigen-binding region that specifically binds to EGFR described in the present invention comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein

the HCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 9;

the HCDR2 comprises or consists of the amino acid sequence of SEQ ID NO: 10;

the HCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 11;

the LCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 12;

the LCDR2 comprises or consists of the amino acid sequence of SEQ ID NO: 13; and

the LCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 14.

In some particular embodiments of the invention, the antigen-binding region that specifically binds to EGFR described in the present invention comprises HCDR1 set forth in SEQ ID NO: 9, HCDR2 set forth in SEQ ID NO: 10, HCDR3 set forth in SEQ ID NO: 11; LCDR1 set forth in SEQ ID NO: 12, LCDR2 set forth in SEQ ID NO: 13 and LCDR3 set forth in SEQ ID NO: 14.

In some particular embodiments of the invention, the antigen-binding region that specifically binds to EGFR described in the present invention comprises a VH and a VL, wherein

the VH comprises or consists of the amino acid sequence set forth in SEQ ID NO: 1 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity thereto, and the VL comprises or consists of the amino acid sequence set forth in SEQ ID NO: 2 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity thereto.

In some particular embodiments of the invention, the antigen-binding region that specifically binds to EGFR described in the present invention comprises a VH and a VL, wherein the VH and VL comprise or consist of, respectively, the amino acid sequences shown below: SEQ ID NO: 1 and 2, respectively.

In some particular embodiments of the invention, the antigen-binding region that specifically binds to EGFR described in the present invention is an anti-EGFR Fab.

II-1-2 An antigen-binding region that specifically bind to B7-H3

In some embodiments, the second antigen-binding region for the anti-EGFR/B7H3 bispecific antibodies of the present invention may comprise or consist of an anti-B7H3 antibody or antigen binding fragment thereof (e.g. the B7-H3 antibody described in PCT/CN2021/140449 (incorporated in its whole), e.g. the mAb (monoclonal antibody) numbered Hz20G5 therein), as long as it is capable of specifically binding to B7H3, including, but not limited to, e.g. a full length antibody, a half antibody, a Fab, a Fab', a Fab'-SH, a Fv, a single chain antibody(e.g. scFv), a (Fab')₂, a single domain antibody such as VHH, dAb (domain antibody), a heavy chain antibody, or a linear antibody that specifically binds to B7-H3, or the like.

In some embodiments, the antigen-binding region that specifically binds to B7H3 is originated from an antibody that specifically binds to B7H3, such as the B7H3 antibody described in PCT/CN2021/140449, for example, the mAb numbered Hz20G5 therein (e.g., Hz20G5.26 mentioned in the examples of the invention).

In some embodiments, the antigen-binding region that specifically binds to B7H3 comprises 1, 2, 3, 4, 5, or 6 CDRs of a known antibody to specifically bind to B7H3, e.g., the B7H3 antibody described in PCT/CN2021/140449, e.g., mAb numbered Hz20G5 therein.

In some embodiments, the antigen-binding region that specifically binds to B7H3 comprises 1, 2, or 3 heavy chain variable region CDRs, i.e., HCDR1, HCDR2, and HCDR3 of a known antibody that specifically binds to B7H3, such as the B7H3 antibody described in PCT/CN2021/140449, e.g., mAb numbered Hz20G5 therein.

In some embodiments, the antigen-binding region that specifically binds to B7H3 comprises 1, 2, or 3 light chain variable region CDRs, i.e., LCDR1, LCDR2, and LCDR3 of a known antibody that specifically binds to B7H3, such as the B7H3 antibody described in PCT/CN2021/140449, e.g., mAb numbered Hz20G5 therein.

In some embodiments, the antigen-binding region that specifically binds to B7H3 comprises 3 heavy chain variable region CDRs and 3 light chain variable region CDRs of a known antibody that specifically binds B7-H3, such as the B7H3 antibody described in PCT/CN2021/140449, e.g., mAb numbered Hz20G5 therein.

In some embodiments, the antigen-binding region that specifically binds to B7H3 comprises

the heavy chain variable region and/or the light chain variable region of a known antibody that specifically bind B7-H3, such as the B7H3 antibody described in PCT/CN2021/140449, e.g., mAb numbered Hz20G5 therein.

In some embodiments, the antigen-binding region that specifically binds to B7H3 comprises a Fab of a known antibody that specifically bind B7-H3, such as the B7H3 antibody described in PCT/CN2021/140449, e.g., mAb numbered Hz20G5 therein.

In some embodiments, the antigen-binding region that specifically binds to B7H3 comprises 3 Complementarity Determining Regions (HCDRs) from a heavy chain variable region, HCDR1, HCDR2, and HCDR3. In some embodiments, the antigen-binding region that specifically binds to B7H3 comprises 3 Complementarity Determining Regions (LCDRs) from the light chain variable region, LCDR1, LCDR2, and LCDR3. In some embodiments, the antigen-binding region that specifically binds B7-H3 comprises 3 Complementarity Determining Regions (HCDRs) from the heavy chain variable region and 3 Complementarity Determining Regions (LCDRs) from the light chain variable region.

In some aspects, the antigen-binding region that specifically binds to B7H3 comprises a heavy chain variable region (VH). In some aspects, the antigen-binding region that specifically binds B7-H3 comprises a light chain variable region (VL). In some aspects, the antigen-binding region that specifically binds B7-H3 comprises a heavy chain variable region (VH) and a light chain variable region (VL). In some embodiments, the heavy chain variable region comprises 3 Complementarity Determining Regions (CDRs) from a heavy chain variable region, HCDR1, HCDR2 and HCDR3. In some embodiments, the light chain variable region comprises 3 Complementarity Determining Regions (CDRs) from a light chain variable region, LCDR1, LCDR2 and LCDR3.

The HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3, the VH, and/or the VL, comprised by the antigen-binding region that specifically binds to B7-H3 of the present invention are respectively defined herein.

In some embodiments, the heavy chain variable region of the antigen-binding region that specifically binds B7-H3 described in the present invention

(i) comprises or consists of an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 3, 5, or 7, or

(ii) comprises or consists of the amino acid sequence of SEQ ID NO: 3, 5, or 7; or

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

In some embodiments, the light chain variable region of the antigen-binding region that specifically binds B7-H3 described in the present invention

(i) comprises or consists of an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 4, 6, or 8, or

(ii) comprises or consists of the amino acid sequence of SEQ ID NO: 4, 6, or 8; or

(iii) comprises or consists of an amino acid sequence having one or more (preferably not more than 10, more preferably not more than 5, 4, 3, 2, 1) amino acid changes (preferably amino acid substitutions, more preferably amino acid conservative substitutions) compared to the amino acid sequence of SEQ ID NO: 4, 6, or 8, preferably said amino acid changes do not occur in the CDR regions.

In some embodiments, the 3 complementarity determining regions (HCDRs) from the heavy chain variable region of the antigen-binding region that specifically binds B7-H3 described in the present invention, HCDR1, HCDR2 and HCDR3 are selected from

(i) the three complementarity determining regions HCDR1, HCDR2 and HCDR3 contained in the VH set forth in SEQ ID NO: 3, 5, or 7, or

(ii) sequences which contain at least one and no more than 5, 4, 3, 2 or 1 amino acid change (preferably amino acid substitution, preferably conservative substitution) in total on the three HCDR regions relative to the anyone sequence of (i),

wherein the HCDRs can be determined according to any scheme for determining CDRs, such as determined respectively by the Kabat, AbM, Chothia, Contact, or IMGT schemes or combination thereof;

for example, the HCDR1 is determined by Abm scheme, the HCDR2 and HCDR3 are determined by the Kabat scheme respectively.

In some embodiments, the 3 complementarity determining regions (LCDRs) from the light chain variable region of the antigen-binding region that specifically binds to B7H3 described in the present invention, LCDR1, LCDR2, and LCDR3 are selected from

(i) the three complementarity determining regions LCDR1, LCDR2 and LCDR3 contained in the VL set forth in SEQ ID NO: 4, 6, or 8, or

(ii) sequences which contain at least one and no more than 5, 4, 3, 2 or 1 amino acid change (preferably amino acid substitution, preferably conservative substitution) in total on the three LCDR regions relative to anyone sequence of (i),

wherein the LCDRs can be determined according to any scheme for determining CDRs, such as determined respectively by the Kabat, AbM, Chothia, Contact, or IMGT schemes or combination thereof;

for example, the LCDRs1-3 are determined by the Kabat scheme respectively.

In some embodiments, the antigen-binding region that specifically binds B7-H3 described in the present invention comprises 3 complementarity determining regions (HCDRs) contained in the heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 3, 5 or 7 and 3 complementarity determining regions (LCDRs) contained in the light chain variable region consisting of the amino acid sequence of SEQ ID NO: 4, 6 or 8;

wherein the HCDRs and LCDRs can be determined according to any scheme for determining CDRs, such as determined respectively by the Kabat, AbM, Chothia, Contact, or IMGT schemes or combination thereof;

for example, the HCDR1 is determined by Abm scheme, the HCDR2 and HCDR3 are determined by the Kabat scheme respectively; and the LCDRs1-3 are determined by the Kabat scheme respectively.

In some embodiments, the HCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 15, 21 or 27, or the HCDR1 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) as compared to the amino acid sequence of SEQ ID NO: 15, 21, or 27.

In some embodiments, the HCDR2 comprises or consists of the amino acid sequence of SEQ ID NO: 16, 22 or 28, or the HCDR2 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) as compared to the amino acid sequence of SEQ ID NO: 16, 22, or 28.

In some embodiments, the HCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 17, 23 or 29, or the HCDR3 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) as compared to the amino acid sequence of SEQ ID NO: 17, 23, or 29.

In some embodiments, the LCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 18, 24 or 30, or the LCDR1 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) as compared to the amino acid sequence of SEQ ID NO: 18, 24, or 30.

In some embodiments, the LCDR2 comprises or consists of the amino acid sequence of SEQ ID NO: 19, 25 or 31, or the LCDR2 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) as compared to the amino acid sequence of SEQ ID NO: 19, 25, or 31.

In some embodiments, the LCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 20, 26 or 32, or the LCDR3 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) as compared to the amino acid sequence of SEQ ID NO: 20, 26, or 32.

In some particular embodiments of the invention, the antigen-binding region that specifically binds to B7H3 described in the present invention comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and/or LCDR3 as described above.

In some particular embodiments of the invention, the antigen-binding region that specifically binds B7-H3 described in the present invention comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 as described above.

In some embodiments of the invention, the antigen-binding region that specifically binds to B7H3 described in the present invention comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein

(i) the HCDR1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 15, the HCDR2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 16, the HCDR3 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 17,

the LCDR1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 18, the LCDR2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 19, the LCDR3 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 20; or the like, or a combination thereof, or

(ii) the HCDR1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 21, the HCDR2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 22, the HCDR3 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 23,

the LCDR1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 24, the LCDR2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 25, the LCDR3 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 26; or the like, or a combination thereof, or

(iii) the HCDR1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 27, the HCDR2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 28, the HCDR3 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 29,

the LCDR1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 30, the LCDR2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 31, the LCDR3 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 32.

In some particular embodiments of the invention, the antigen-binding region that specifically binds to B7H3 described in the present invention comprises

(i) HCDR1 set forth in SEQ ID NO: 15, HCDR2 set forth in SEQ ID NO: 16, HCDR3 set forth in SEQ ID NO: 17; LCDR1 set forth in SEQ ID NO: 18, LCDR2 set forth in SEQ ID NO: 19 and LCDR3 set forth in SEQ ID NO: 20;

(ii) HCDR1 set forth in SEQ ID NO: 21, HCDR2 set forth in SEQ ID NO: 22, HCDR3 set forth in SEQ ID NO: 23; LCDR1 set forth in SEQ ID NO: 24, LCDR2 set forth in SEQ ID NO: 25 and LCDR3 set forth in SEQ ID NO: 26; or

(iii) HCDR1 as set forth in SEQ ID NO: 27, HCDR2 as set forth in SEQ ID NO: 28, HCDR3 as set forth in SEQ ID NO: 29; LCDR1 set forth in SEQ ID NO: 30, LCDR2 set forth in SEQ ID NO: 31 and LCDR3 set forth in SEQ ID NO: 32.

In some embodiments of the invention, the antigen-binding region that specifically binds to B7H3 described in the present invention comprises a VH and a VL, wherein

(i) the VH comprises or consists of the amino acid sequence set forth in SEQ ID NO: 3 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 3 and the VL comprises or consists of the amino acid sequence set forth in SEQ ID NO: 4 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 4;

(ii) the VH comprises or consists of the amino acid sequence set forth in SEQ ID NO: 5 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 5 and the VL comprises or consists of the amino acid sequence set forth in SEQ ID NO: 6 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 6; or

(iii) the VH comprises or consists of the amino acid sequence set forth in SEQ ID NO: 7 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 7, and the VL comprises or consists of the amino acid sequence set forth in SEQ ID NO: 8 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 8.

In some particular embodiments of the invention, the antigen-binding region that specifically binds to B7H3 described in the present invention comprises a VH and a VL, wherein VH and VL

comprise or consist of the amino acid sequences shown below, respectively:

SEQ ID NO: 3 and SEQ ID NO: 4;

SEQ ID NO: 5 and SEQ ID NO: 6, or

SEQ ID NO: 7 and SEQ ID NO: 8.

In some embodiments of the invention, the antigen-binding region that specifically binds to B7H3 described in the present invention is a Fab.

II-1-3 Fab fragment

In some embodiments, the first antigen-binding region and/or the second antigen-binding region of the invention is a Fab fragment. A Fab fragment suitable for use as an antigen-binding region of the multispecific antibody such as bispecific antibody as described herein consists of two polypeptide chains comprising the antibody VH, CH1, VL and CL domains, wherein the VH is paired with VL and the CH1 is paired with CL to form the antigen-binding region.

In some embodiments, in a Fab, one chain comprises or consists of VH and CH1 (i.e., VH-CH1) from N-terminus to C-terminus, and the other chain comprises or consists of VL and CL (i.e., VL-CL) from N-terminus to C-terminus. In some embodiments, in the multispecific or bispecific antibodies of the present invention, the Fab may be linked to the N-terminus of the Fc domain of the antibody via the C-terminus of the chain comprising the VH. Preferably, the Fab comprises a VH-CH1 chain and a VL-CL chain and may be linked to an antibody Fc domain via the C-terminus of CH1 of the VH-CH1 chain. In some embodiments, the linkage is a direct linkage, or through a connector. Herein, Fab chain comprising VH-CH1 is also referred to as a Fab heavy chain, while Fab chain comprising VL-CL is also referred to as a Fab light chain.

In some embodiments, the CH1 is a CH1 from IgG1, IgG2, IgG3, or IgG4, preferably CH1 from IgG1. In some embodiments, the CH1

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 41, or

(ii) comprises or consists of the amino acid sequence of SEQ ID NO: 41; or

(iii) comprises or consists of an amino acid sequence having one or more (preferably not more than 10, more preferably not more than 5, 4, 3, 2, 1) amino acid changes (preferably amino acid substitutions, more preferably amino acid conservative substitutions) as compared to the amino acid sequence of SEQ ID NO: 41.

In some embodiments, the CL is a Kappa light chain constant region or a Lambda light chain constant region. In some embodiments, CL is a Kappa light chain constant region. In some embodiments, the CL

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 54;

(ii) comprises or consists of the amino acid sequence of SEQ ID NO: 54; or

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

acid substitutions, more preferably amino acid conservative substitutions) as compared to the amino acid sequence of SEQ ID NO: 54.

In some embodiments, the first antigen-binding region is a Fab that specifically binds to EGFR, wherein the Fab fragment is from an anti-EGFR antibody, and comprises the heavy chain variable region VH and the light chain variable region VL of the anti-EGFR antibody. In some embodiments, the Fab that specifically binds to EGFR as the first antigen-binding region comprises the 6 CDRs of the antigen-binding regions that specifically bind to EGFR described herein. In some embodiments, the Fab that specifically binds to EGFR as the first antigen-binding region comprises a VH or a VL of the antigen-binding region that specifically binds to EGFR as described herein, or comprises a VH and a VL of the antigen-binding region that specifically binds to EGFR as described herein.

In some embodiments, the second antigen-binding region is a Fab that specifically binds to B7H3, wherein the Fab fragment is from an anti-B7-H3 antibody, and comprises the heavy chain variable region VH and the light chain variable region VL of the anti-B7H3 antibody. In some embodiments, the Fab that specifically binds to B7H3 as the second antigen-binding region comprises the 6 CDRs of the antigen-binding regions that specifically bind B7-H3 as described herein. In some embodiments, the Fab that specifically binds B7-H3 as the second antigen-binding region comprises a VH or a VL of the antigen-binding region that specifically binds to B7H3 as described herein, or comprises a VH and a VL of the antigen-binding region that specifically binds to B7H3 as described herein.

II-1-4 Fc region

In some embodiments, the bispecific antibody of the present invention further comprises an Fc region, wherein the Fc regions comprised may be the same or different.

In some embodiments, the antibody molecular of the present invention comprises the first Fc region and the second Fc region, wherein the first Fc region and the second Fc region are the same or different.

In some embodiments, the first Fc region and the second Fc region are different and are capable of dimerizing to form a heterodimeric Fc scaffold.

Herein, the Fc region refers to the C-terminal region of an immunoglobulin heavy chain containing at least a portion of a constant region, and may include native sequence Fc regions and variant Fc regions. The native sequence Fc region encompasses the naturally occurring Fc sequences of various immunoglobulins, such as the Fc regions of various Ig subclass and allotypes thereof (Gestur Vidarsson et al, IgG subclasses and allotypes: from structure to effector functions, 20 October 2014, doi: 10.3389/fimmu.2014.00520). In some embodiments, the Fc region of the present invention comprises the antibody CH2 and CH3. In some embodiments, the antibody Fc region may also have an IgG hinge region or a partial IgG hinge region at the N-terminus, e.g., an IgG1 hinge region or a partial IgG1 hinge region, e.g., according to EU numbering, the sequence of D221 to P230. A mutation may be comprised in the hinge region.

Unless otherwise indicated herein, the numbering of amino acid residues in the Fc region is according to the EU numbering system, also known as the EU index, as described in Kabat, E.A. et al, Sequences of Proteins of Immunological Interest, 5th edition, Public Health Service, National Institutes of Health, Bethesda, MD (1991), NIH Publication 91-3242.

In some embodiments, the Fc region is a human IgG Fc, e.g., human IgG1 Fc, human IgG2

Fc, human IgG3 Fc or human IgG4 Fc. In one embodiment, the Fc region comprises or consists of the amino acid sequence SEQ ID NO: 46 or 47, or an amino acid sequence having at least 90% identity, e.g., 95%, 96%, 97%, 98%, 99% or more identity thereto.

As understood by those skilled in the art, to facilitate formation of the multispecific antibodies of the present invention as heterodimers, the Fc region comprised by the multispecific antibodies of the present invention may comprise mutations that facilitate heterodimerization of a first Fc region with a second Fc region. In one embodiment, mutations are introduced in the CH3 regions of both Fc regions.

Methods for promoting heterodimerization of Fc regions are known in the art. For example, the CH3 region of the first Fc region and the CH3 region of the second Fc region are engineered in a complementary manner such that each CH3 region (or the heavy chain comprising it) can no longer homodimerize with itself but is forced to heterodimerize with other CH3 regions that are complementarily engineered (such that the CH3 regions of the first and the second Fc regions heterodimerize and no homodimers are formed between the two first CH3 regions or the two second CH3 regions).

Preferably, based on the Knob-in-Hole technology, the respective Knob mutations and Hole mutations are introduced in the first Fc region and the second Fc region. See, e.g., US 5, 731, 168; US 7, 695, 936; Ridgway et al, Prot Eng 9, 617-621(1996) and Carter, J Immunol Meth 248, 7-15 (2001) for the technique. In some embodiments, the Knob mutations and Hole mutations may further comprise mutations to cysteine residue and thus to introduce disulfide bond. Disulfide bonds can be used to stabilize the final antibody product or the ADC of the antibody.

In a particular embodiment, in the CH3 region of an Fc region, the threonine residue at position 366 is replaced with a tryptophan residue (T366W) (knob mutation); in the CH3 region of the other Fc region, the tyrosine residue at position 407 was replaced with a valine residue (Y407V) (hole mutation), optionally the threonine residue at position 366 was replaced with a serine residue (T366S) and the tyrosine residue at position 407 was replaced with a valine residue (Y407V) (numbering according to the EU index).

In some embodiments, the Fc regions may further comprise cysteine residue substitution, so that to obtain non-natural disulfide bond linkage. In some embodiments, in one Fc region, the serine residue at position 354 is replaced with a cysteine residue (S354C) or the glutamic acid residue at position 356 is replaced with a cysteine residue (E356C) (in particular, the serine residue at position 354 is replaced with a cysteine residue), while in the other Fc region, the tyrosine residue at position 349 is replaced with a cysteine residue (Y349C) (numbering according to the EU index). Accordingly, in yet another embodiment, in the CH3 region of one Fc region, the threonine residue at position 366 is replaced with a tryptophan residue (T366W) and the serine residue at position 354 is replaced with a cysteine residue (S354C) or the glutamic acid residue at position 356 is replaced with a cysteine residue (E356C) (in particular, the serine residue at position 354 is replaced with a cysteine residue); whereas in the CH3 region of the other Fc region, the tyrosine residue at position 407 is replaced with a valine residue (Y407V) (hole mutation), optionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A) (numbering according to the EU index), optionally the tyrosine residue at position 349 is replaced with a cysteine residue (Y349C) (numbering according to the EU index).

In a particular embodiment, one Fc region comprises the amino acid substitutions T366W and the other Fc region comprises the amino acid substitutions T366S, L368A and Y407V (numbering according to the EU index).

In a particular embodiment, one Fc region comprises the amino acid substitutions S354C and T366W and the other Fc region comprises the amino acid substitutions Y349C, T366S, L368A and Y407V (numbering according to the EU index).

Mutations may also be introduced in the first Fc region and the second Fc region based on the Innobody technique. See, e.g., PCT/CN2021/143141 for this technique, which is incorporated herein as its entirety.

In a particular embodiment,

the first CH3 region comprises the S364R/K mutation (preferably S364R), and optionally one or more additional mutations. In some embodiments, the second CH3 region comprises a K370S/T/A/V mutation (preferably K370S), and optionally one or more additional mutations. In some embodiments, the first CH3 region includes the S364R/K mutation, and the second CH3 region includes the K370S/T/A/V mutation. In some embodiments, the first CH3 region comprises the S364R mutation, and the second CH3 region comprises the K370S mutation.

In some embodiments, the first CH3 region comprises S364R/K (preferably S364R) and D399K/R (preferably D399K) mutations. In some embodiments, the second CH3 region comprises a K370S/T/A/V (preferably K370S) mutation and a K409D/E (preferably K409D) mutation. In some embodiments, the first CH3 region comprises S364R/K + D399K/R and the second CH3 region comprises K370S/T/A/V + Y349T/S/A/V. In some embodiments, the first CH3 region comprises S364R + D399K and the second CH3 region comprises K370S + Y349T. In some embodiments, the first CH3 region further comprises E375N/Q (preferably E375N) and/or T350V/A (preferably T350V). In some embodiments, the second CH3 region further comprises K409D/E (preferably K409D), Q347D/E (preferably Q347D) and/or T350V/A (preferably T350V).

In some embodiments, the first CH3 region comprises S364R + D399K and the second CH3 region comprises K370S + Y349T + K409D. In some embodiments, the first CH3 region further comprises E357N. In some embodiments, the second CH3 region further comprises Q347D. In some embodiments, the first CH3 region also includes E357N, and the second CH3 region also includes Q347D. In some embodiments, the first CH3 region and the second CH3 region further comprise T350V, respectively, or both comprises T350V.

Thus, in some embodiments, the first CH3 region comprises S364R + D399K and the second CH3 region comprises K370S + Y349T + K409D + Q347D. In some embodiments, the first CH3 region comprises S364R + D399K + E357N and the second CH3 region comprises K370S + Y349T + K409D + Q347D. In some embodiments, the first CH3 region comprises S364R + D399K + E357N + T350V, and the second CH3 region comprises K370S + Y349T + K409D + Q347D + T350V.

In some embodiments, the first CH3 region comprises K409E/D (preferably K409E). In some embodiments, the second CH3 region comprises D399K/R (preferably D399K) or K370T/S/A/V (preferably K370T). In some embodiments, the first CH3 region comprises K409E/D (preferably K409E) and the second CH3 region comprises D399K/R (preferably D399K). In some embodiments, the first CH3 region further comprises T411R/K (preferably T411R). In some embodiments, the second CH3 region further comprises K370T/S/A/V (preferably K370T). In some embodiments, the CH3 region comprises K409E/D + T411R/K and the second CH3 region comprises D399K/R + K370T/S/A/V. In some embodiments, the CH3 region comprises K409E + T411R and the second CH3 region comprises D399K + K370T.

In some particular embodiments, the first and the second CH3 regions have the following combination of mutations:

The first CH3 region	the second CH3 region
S364R, D399K, E357N, T350V	K370S, Y349T, Q347D, K409D, T350V
K409E, T411R	D399K, K370T
S364R, D399K, E357N	K370S, Y349T, Q347D, K409D
S364R, D399K	K370S, Y349T, Q347D, K409D
S364R, D399K	K370S, Y349T, K409D

In one embodiment, the CH3 of one Fc region comprises the S364R and D399K mutations and the CH3 mutation of the other Fc region comprises the Y349T, K370S and K409D mutations.

Thus, in a specific embodiment, the two Fc regions comprised by the multispecific antibodies of the present invention heterodimerize, wherein

a) one Fc region polypeptide comprises the mutation T366W, while the other Fc region polypeptide comprises T366S, L368A and Y407V, or

b) one Fc-region polypeptide comprises the mutations S354C and T366W, while the other Fc-region polypeptide comprises the mutations Y349C, T366S, L368A and Y407V, or

c) one Fc-region polypeptide comprises mutations S364R and D399K, while the other Fc-region polypeptide comprises mutations Y349T, K370S and K409D.

Thus, in a particular embodiment, the two Fc regions comprised by the multispecific antibodies of the present invention heterodimerize, wherein one Fc region polypeptide comprises or consists of the amino acid sequence set forth in SEQ ID NO: 49 or 50 and the other Fc region polypeptide comprises or consists of the amino acid sequence set forth in SEQ ID NO: 52 or 53.

Thus, in a particular embodiment, the two Fc regions comprised by the multispecific antibodies of the present invention heterodimerize, wherein one Fc region polypeptide comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to the amino acid sequence set forth in SEQ ID NO: 49 or 50, and the other Fc region polypeptide comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to the amino acid sequence set forth in SEQ ID NO: 52 or 53.

Thus, in a particular embodiment, the two Fc regions comprised by the multispecific antibodies of the present invention heterodimerize, wherein one Fc region polypeptide comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to the amino acid sequence set forth in SEQ ID NO: 49 or 50 and comprises the mutations Y349T, K370S and K409D, and the other Fc region comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to the amino acid sequence set forth in SEQ ID NO: 52 or 53 and comprises the mutations S364R and D399K.

In some embodiments, the Fc region further comprises additional mutations that facilitate purification of the heterodimer.

In one embodiment, the Fc region is modified in characteristics of an effector function of the Fc region (e.g., complement activation function of the Fc region). In one embodiment, the effector function has been reduced or eliminated relative to a wild-type isotype Fc region. In one

embodiment, effector function is reduced or eliminated by a method selected from the group consisting of: Fc isoforms, which naturally have reduced or eliminated effector function, and Fc region modifications are used.

In a preferred embodiment, the Fc region has reduced effector function mediated by the Fc region, such as reduced or eliminated ADCC or ADCP or CDC effector function, e.g. comprising mutations to achieve the above. As understood by those skilled in the art, binding molecules, e.g., antibody molecules, of the present invention may also comprise modifications in the Fc domain that alter binding affinity to one or more Fc receptors, depending on the intended use of the binding molecule, e.g., antibody molecule, of the present invention. In one embodiment, the Fc receptor is the Fc γ receptor, particularly the human Fc γ receptor. In some embodiments, the Fc region comprises a mutation that reduces binding to the Fc γ receptor. For example, in some embodiments, the Fc region used in the invention has a mutation that reduces binding to the Fc γ receptor, e.g., the L234A/L235A mutation. In yet another preferred embodiment, the Fc fragment may have a mutation that results in increased serum half-life, such as a mutation that improves binding of the Fc fragment to FcRn.

II-1-5 Exemplary Bispecific antibody molecules

In some embodiments, the anti-B7H3/EGFR bispecific antibody of the invention has one or more of the following properties:

(1) on one hand, the bispecific antibody of the invention can block the binding of EGFR ligand and EGFR, inhibit biological signal transmission and block the corresponding biological activity of tumor; on the other hand, it can stimulate EGFR endocytosis and eventually be degraded by intracellular lysosomes and the like;

(2) the bispecific antibody of the invention adopts an EGFR antibody parent sequence with low affinity to EGFR, thereby greatly reducing the toxic and side effects of EGFR monoclonal antibodies on normal epithelial tissues such as skin;

(3) the bispecific antibody of the invention introduces the antibody parent sequence with high affinity to B7H3 on the basis of low affinity to EGFR, greatly improves the EGFR signal blocking activity, and improves the pharmacodynamic bioactivity and pharmacodynamic safety window of the bispecific antibody of the invention;

(4) the bispecific antibody of the invention has higher pharmacodynamic bioactivity and safety;

(5) the bispecific antibody of the invention has excellent tumor killing and inhibiting effects; and/or

(6) The ADC constructed by the bispecific antibody of the invention has reduced toxic and side effects and high effectiveness and safety.

In some embodiments, in the antibody molecule of the invention, the heavy chain variable region of the antigen-binding region that specifically binds to EGFR as described herein is linked to the heavy chain constant region CH, e.g., wherein the C-terminus of the heavy chain variable region is linked to the N-terminus of the heavy chain constant region CH. In some embodiments, in the antibody molecule of the invention, the light chain variable region of the antigen-binding region that specifically binds to EGFR as described herein is linked to a light chain constant region CL, e.g., wherein the C-terminus of the light chain variable region is linked to the N-terminus of

the light chain constant region CL. In some embodiments, in the antibody molecules of the invention, the heavy chain variable region of the antigen-binding region that specifically binds to EGFR as described herein is linked to a heavy chain constant region CH and the light chain variable region thereof is linked to a light chain constant region CL.

In some embodiments, in the antibody molecules of the invention, the heavy chain variable region of the antigen-binding region that specifically binds to B7H3 as described herein is linked to the heavy chain constant region CH, e.g., wherein the C-terminus of the heavy chain variable region is linked to the N-terminus of the heavy chain constant region CH. In some embodiments, in the antibody molecules of the invention, the light chain variable region of the antigen-binding region that specifically binds to B7H3 as described herein is linked to a light chain constant region CL, e.g., wherein the C-terminus of the light chain variable region is linked to the N-terminus of the light chain constant region CL. In some embodiments, in the antibody molecules of the invention, the heavy chain variable region of the antigen-binding region that specifically binds B7-H3 as described herein, is linked to the heavy chain constant region CH and the light chain variable region thereof is linked to the light chain constant region CL.

In some embodiments, the heavy chain constant region comprises CH1 and an Fc region as described herein, connected via or not via a hinge region.

In some embodiments, the heavy chain constant region is a heavy chain constant region of IgG1, IgG2, IgG3, or IgG4, for example, of human IgG1, human IgG2, human IgG3, or human IgG 4.

In some embodiments, the CH1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 41 or 42 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 41 or 42.

In some embodiments, the hinge region comprises or consists of the amino acid sequence set forth in SEQ ID NO: 43 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 43.

In some embodiments, the CH1 comprises a portion of a hinge region. In some embodiments, the Fc region comprises a portion of a hinge region. Thus, when CH1 is linked to an Fc region to constitute a heavy chain constant region, if CH1 and the Fc region include a portion of a hinge region, the heavy chain constant region after the linkage of the two comprises a hinge region, and thus there is no need to add a hinge region.

In some embodiments, the light chain constant region is a Kappa light chain constant region or a Lambda light chain constant region, such as a human Kappa or human Lambda light chain constant region. In some embodiments, the light chain constant region comprises or consists of the amino acid sequence set forth in SEQ ID NO: 54 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 54.

In some preferred embodiments, the present invention provides a bispecific antibody molecule comprising one Fab fragment that specifically binds to EGFR as describe herein, one Fab fragment that specifically binds to B7H3 as describe herein, and an Fc dimer as described herein, wherein the Fab fragment that specifically binds to EGFR forms a half-antibody that specifically binds to EGFR with one Fc and the Fab fragment that specifically binds to B7-H3 forms a half-antibody that specifically binds to B7H3 with one Fc.

In some embodiments, the bispecific antibody is an IgG-like antibody having the

configuration set forth in Figure 24.

In a particular embodiment, the first Fc region and the second Fc region comprise an Innobody mutation.

In some embodiments, the bispecific antibody comprises a heavy chain 1 and a light chain 1, and a heavy chain 2 and a light chain 2, wherein heavy chain 1 and light chain 1 constitute the first half antibody, and heavy chain 2 and light chain 2 constitute the second half antibody; wherein heavy chain 1 comprises a heavy chain variable region of the first antigen-binding region and a first heavy chain constant region; light chain 1 comprises a light chain variable region of the first antigen-binding region and a first light chain constant region; and heavy chain 2 comprises a heavy chain variable region of the second antigen-binding region and a second heavy chain constant region; light chain 2 comprises a light chain variable region of the second antigen-binding region and a second light chain constant region.

In a specific embodiment, the bispecific antibody of the invention specifically binds to EGFR and B7H3 and comprises or consists of the followings:

heavy chain 1: from N-terminus to C-terminus comprises or consists of: a heavy chain variable region of a Fab that specifically binds to EGFR-heavy chain constant region CH1-a first Fc region, wherein the heavy chain constant region CH1 is linked at its C-terminus to the N-terminus of the first Fc region, with or without a connector (e.g., hinge region);

light chain 1: from N-terminus to C-terminus comprises or consists of: a light chain variable region of a Fab that specifically binds to EGFR -light chain constant region;

heavy chain 2: from N-terminus to C-terminus comprises or consists of: a heavy chain variable region of a Fab that specifically binds to B7H3-heavy chain constant region CH1-a second Fc region, wherein heavy chain constant region CH1 is linked at its C-terminus to the N-terminus of the second Fc region, with or without a connector (e.g., hinge region);

light chain 2: from N-terminus to C-terminus comprises or consists of: a light chain variable region of a Fab which specifically binds to B7-H3 light chain constant region,

preferably, each domain is linked directly;

optionally, the first Fc region comprises mutations K370S, Y349T and K409D and the second Fc region comprises S364R and D399K. Each domain of the bispecific antibody of the present invention is described herein.

In some embodiments, in the bispecific antibody, heavy chain 1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 33 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 33.

In some embodiments, in the bispecific antibody, light chain 1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 34 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 34.

In some embodiments, in the bispecific antibody, heavy chain 1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 33 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 33, and light chain 1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 34 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98% or 99% identity to the amino acid sequence of SEQ ID NO: 34.

In some embodiments, in the bispecific antibody, heavy chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 35, 37, or 39 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence set forth in SEQ ID NO: 35, 37, or 39.

In some embodiments, in the bispecific antibody, light chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 36, 38 or 40 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence set forth in SEQ ID NO: 36, 38, or 40.

In some embodiments, in the bispecific antibody,

(1) heavy chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 35 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence set forth in SEQ ID NO: 35; and light chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 36 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence set forth in SEQ ID NO: 36;

(2) heavy chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 37 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence set forth in SEQ ID NO: 37; and light chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 38 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence set forth in SEQ ID NO: 38;

(3) heavy chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 39 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence set forth in SEQ ID NO: 39; and light chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 40 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence set forth in SEQ ID NO: 40.

In some embodiments, in the bispecific antibody,

heavy chain 1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 33 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 33, and light chain 1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 34 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 34; and heavy chain 2 and light chain 2 respectively comprise the amino acid sequences set forth in SEQ ID NOs as follows or an amino acid sequences having at least 85%, 90%, 95%, 97%, 98%, 99% identity thereto, or consist of the amino acid sequences set forth in SEQ ID NOs:

- i) SEQ ID NO: 35 and SEQ ID NO: 36;
- ii) SEQ ID NO: 37 and SEQ ID NO: 38;
- iii) SEQ ID NO: 39 and SEQ ID NO: 40.

In some embodiments, in the bispecific antibody,

(i) heavy chain 1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 33, and light chain 1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 34,

heavy chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 35 and light chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 36; or,

(ii) heavy chain 1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 33, and light chain 1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 34,

heavy chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 37 and light chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 38; or,

(iii) heavy chain 1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 33, and light chain 1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 34,

heavy chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 39 and light chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 40.

In some embodiments, the bispecific antibody comprises or consists of a heavy chain 1, a heavy chain 2, a light chain 1, and a light chain 2 as described herein.

II-2 engineered glycosylated antibodies

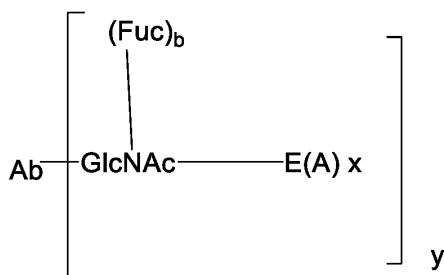
The antibody Ab in formula (I) of the present invention may be an antibody with engineered glycosylation. In some embodiments, the antibody is obtained after enzymatic modification of the sugar chain in vitro, for example, modification of the sugar chain by a glycosidase (e.g., an endoglycosidase or a glycosyltransferase). In some embodiments, the antibody with engineered glycosylation refers to an antibody in which the sugar chains at the glycosylation site of the antibody are engineered from non-uniform structure N-sugar chains into single structure N-sugar chains with reactive groups (e.g., any reactive groups capable of reacting with a linker moiety, such as azide, keto, and alkyne). In a preferred embodiment, the N-glycosylation site of the antibody is a conserved N-glycosylation site, e.g., Asn297, on the Fc domain of the antibody.

Suitable methods for engineering antibody glycosylation of the present invention are described, for example, in PCT/NL2013/050744, PCT/EP2016/059194, or PCT/EP2017/052792, which are incorporated herein in their entirety.

In a preferred embodiment, the antibody of the invention having engineered glycosylation is an antibody comprising a substituent GlcNAc-E(A)_x, wherein GlcNAc is N-acetylglucosamine, wherein E(A)_x is a saccharide derivative comprising x functional groups A, wherein A is independently selected from the group consisting of azido, keto, and alkynyl and x is 1, 2, 3, or 4; wherein the substituent GlcNAc-E(A)_x is bonded to the antibody by C1 of the N-acetylglucosamine of the substituent GlcNAc-E(A)_x, wherein the N-acetylglucosamine is optionally fucosylated. In the case where N-acetylglucosamine is fucosylated, it is bonded to fucose (Fuc) at C6 through a glycosidic bond.

In one embodiment, the antibody with engineered glycosylation of the invention is the antibody of formula (II), wherein Ab represents an antibody, GlcNAc is N-acetylglucosamine, Fuc is fucose, b is 0 or 1, wherein E(A)_x is a saccharide derivative comprising x functional groups A, wherein A is azido, keto, and alkynyl, and x is 1, 2, 3, or 4. In a preferred embodiment, y is 1 to 10, more preferably, y is 1, 2, 3, 4, 5, 6, 7 or 8, even more preferably y is 1, 2, 3 or 4, and most

preferably y is 1 or 2.



Formula (II)

The saccharide derivative E(A)x in the substituent GlcNAc-E(A)x in the antibody with engineered glycosylation may be bonded to C4 of said GlcNAc, for example by a $\beta(1,4)$ - or $\beta(1,3)$ -glycosidic bond or to C3 of said GlcNAc by an $\alpha(1,4)$ - or $\alpha(1,3)$ - glycosidic bond, preferably to C4 of said GlcNAc by a $\beta(1,4)$ - glycosidic bond. The N-acetylglucosamine of the substituent GlcNAc-E(A)x is bonded to the antibody by C1, preferably to the amide nitrogen atom (GlcNAc β 1-Asn) in the side chain of the asparagine of the antibody by an N-glycosidic bond. The GlcNAc on the substituent GlcNAc-E(A)x is optionally fucosylated. Accordingly, GlcNAc-E in an antibody-drug conjugate, when present, may also be attached as described above.

In a preferred embodiment, the functional group a is an azido group. When A is an azido group, preferably A is bonded to C2, C3, C4, or C6. As mentioned above, one or more azide substituents in E(A)x may be bonded to C2, C3, C4 or C6 of the saccharide or saccharide derivative E, replacing the hydroxyl group (OH) at said position. It is understood that the bonding position of functional group A corresponds to the position where the A-containing saccharide is attached to the linker.

In a preferred embodiment, the saccharide derivative E(A)x is derived from a saccharide or a saccharide derivative E. In a preferred embodiment, E is a saccharide or saccharide derivative selected from the group consisting of galactose (Gal), mannose (Man), N-acetylglucosamine (GlcNAc), glucose (Glc), N-acetylgalactosamine (GalNAc), glucuronic acid (Gcu), fucose (Fuc) and N-acetylneuraminic acid (sialic acid), preferably Gal, GlcNAc, glucose and GalNAc, most preferably GalNAc.

In another preferred embodiment, said E(A)x is GalNAc-N₃, preferably E(A)x is 6-azido-6-deoxy-2-acetamidogalactose.

It is to be understood that, without being contradictory, the description and illustration of the saccharides of formula (II) (including but not limited to the glycosidic linkage of GlcNAc-E(A)x) may be used to explain or define the corresponding saccharides in the conjugate of formula (III), for example, the glycosidic linkage thereof. It will be appreciated by those skilled in the art that although in some embodiments, a reactive group (e.g., functional group A) is attached to the saccharide chain of an antibody in the antibody glycosylation engineering, and thus "an antibody with engineered glycosylation" is defined as an antibody comprising the reactive group, upon formation of an antibody-drug conjugate (formula (I) or formula (III)), the reactive group may react with the linker moiety, thereby forming a new group with the linker moiety, such that in the antibody-drug conjugate, the new group is considered to be part of the linker.

When the modified antibody is the bispecific antibody of the invention, the antibody preferably comprises two or more, more preferably two, substituents GlcNAc-E(A)x, wherein the

substituents GlcNAc-E(A)_x are optionally fucosylated. However, if the modified antibody is an antibody fragment, such as a Fab or Fc fragment, the antibody may have only one substituent GlcNAc-E(A)_x, which is optionally fucosylated. The substituent GlcNAc-E(A)_x may be located at any position of an antibody, provided that the substituent does not interfere with the binding of an antigen to the antigen binding site of the antibody. In one embodiment, the substituent GlcNAc-E(A)_x is located in the Fc domain of an antibody, more preferably in the CH2 domain.

As noted above, antibodies of the invention with engineered glycosylation comprise more than one substituent GlcNAc-E(A)_x, for example two substituents GlcNAc-E(A)_x.

In a preferred embodiment, the substituent GlcNAc-E(A)_x is present on a native N-glycosylation site of the antibody (e.g., a native conserved N-glycosylation site), such as a glycosylation site of the Fc region (more preferably the CH2 domain). In yet another preferred embodiment, the antibody is an IgG antibody and the substituent GlcNAc-E(A)_x is present on a native N-glycosylation site (a native conserved N-glycosylation site) of the IgG antibody. In yet another preferred embodiment, the native site is an Asn 297-glycosylation site of an IgG antibody. The Asn297-glycosylation site is present in the Fc region of the heavy chain of an IgG antibody. In a preferred embodiment, the substituent GlcNAc-E(A)_x is present at an Asn297- glycosylation site of the two heavy chains of the antibody.

II-3. Linker and drug

In some embodiments, L in formula (I) of the present invention is a linker. Any linker known in the art can be used to connect to the anti-human B7H3 and EGFR antibodies of the present invention, preferably, the linker can achieve site-specific conjugation of ADC.

In some embodiments, the linker suitable for the present invention can be any linker that can achieve coupling of antibodies to drugs. In some embodiments, the linker can be a linker used in a technology that capable of achieving site-specific conjugation.

In a preferred embodiment, the linker of the present invention is a linker connected to an antibody oligosaccharide. The "linker connected to an antibody oligosaccharide" defined herein refers to any linker that is connected to a reactive group on a saccharide chain at an antibody glycosylation site to couple an antibody to a drug. The saccharide chain on the antibody glycosylation site is generally an N-saccharide chain, and is usually modified to transform the heterogeneous N-saccharide chain into a single structure N-saccharide chain with a reactive group, and then the reactive group carried by the saccharide chain is connected to the "linker" to achieve site-specific conjugation of the drug to the antibody to obtain an antibody-drug conjugate. In a preferred embodiment, the N-glycosylation site of the antibody is an antibody Fc domain, preferably a conserved N-glycosylation site on the CH2 domain, such as Asn297. Therefore, in one embodiment, the "linker connected to an antibody oligosaccharide" of the present invention is particularly a linker obtained by reacting with a reactive group on an N-saccharide chain at a conserved N-glycosylation site (e.g., Asn297) on an antibody Fc domain to achieve site-specific conjugation, such as the linker described in PCT/NL2013/050744 or the linker described in PCT/EP2021/075401, which are incorporated herein in their entirety. In one embodiment, the reactive group of the present invention is an azido group, a keto group, or an alkynyl group. In one embodiment, the linker of the present invention is a linker comprising an alkynyl group. In one embodiment, the reactive group of the present invention is an azido group, a keto group, or an alkynyl group, preferably an azido group, and the linker of the present invention is a linker

comprising an alkynyl group. When referring to such linkers in the present invention, since a new group is formed after the reactive group reacts with the group of the linker, the group formed after the reactive group in the antibody-drug conjugate reacts can also be defined as a part of the "linker", such as shown in formula (III) of the present invention.

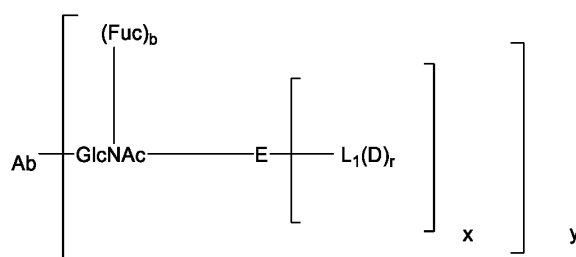
Linkers suitable for use in the present invention also include, for example, cathepsin-degradable linkers, such as Val-Cit linkers (e.g., vc-PAB), cBu-Cit linkers, and CX linkers; non-cleavable linkers such as SMCC linkers or MD linkers; acid-cleavable linkers, silicone-structured linkers, disulfide-carbamate linkers, MC-GGFG linkers, TRX linkers, galactoside-containing linkers, pyrophosphate linkers, near-infrared-light cleavable linkers, and ultraviolet cleavable linkers such as PC4AP (Antibody-drug conjugates: Recent advances in linker chemistry, Su, Z., Xiao, D., Xie, F., Liu, L., Wang, Y., Fan, S., ... Li, S. (2021). Antibody-drug conjugates: Recent advances in linker chemistry. Acta Pharmaceutica Sinica B.). The linker applicable to the present invention can also be a combination of one or more linkers, for example, a cathepsin-degradable linker can be combined with other types of linkers to form a new linker. Therefore, the "linker" described in the present invention covers a single type of linker, or a combination of different types of linkers, as long as it can couple the antibody of the present invention to the drug. Therefore, in one embodiment, the linker applicable to the present invention is MC-VC-PAB, vc-PAB, SMCC or MC-GGFG.

D in formula (I) of the present invention can be any antitumor compound, and is not particularly limited, as long as it has an antitumor effect and has a substituent or a structural moiety that can be connected to the linker structure. For example, the antitumor compound can be a pharmaceutically active compound that has an effect on tumors. For antitumor compounds, it is preferable that a part or whole of the linker is cleaved in a tumor cell to release the antitumor compound, thereby exhibiting an antitumor effect. When the linker is cleaved at the part connected to the drug, the antitumor compound is released in an unmodified structure and exhibits its original antitumor effect.

In some embodiments, the anti-tumor compound can be, for example, a cytotoxic or chemotherapeutic agent, e.g., camptothecins, e.g., exatecan (topoisomerase I inhibitor, Exatecan), Dxd (a novel topoisomerase I inhibitor, a derivative of Exatecan), auristatins, e.g., monomethyl auristatin E (MMAE), maytansinoids, e.g., small molecule microtubule inhibitor, DM1, taxanes, e.g., paclitaxel or docetaxel, anthracyclines, epothilones, mitomycins, combretastatin, vinca alkaloids, calicheamicins, duocarmycin, Tubulysins, amatoxin, bleomycin, MEK inhibitors, KSP inhibitors, and the like.

II-4. Antibody-drug conjugates linked by carbohydrates

The present invention relates to an antibody-drug conjugate of formula (III) or a pharmaceutically acceptable salt or solvate thereof



Formula (III)

Ab is a bispecific antibody or fragment thereof of the present invention that specifically binds to B7H3 and EGFR (e.g., human B7H3 and EGFR), as defined herein,

L_1 is a linker;

E is a sugar or a sugar derivative; for example, a sugar or a sugar derivative as defined above;

and GlcNAc is N-acetylglucosamine, Fuc is fucose;

D is as defined in Formula I above;

r is 1-5, for example, 1, 2, 3, 4 or 5, preferably 1 or 2;

b is 0 or 1, for example, 0;

x is 1, 2, 3 or 4; preferably 1 or 2;

y is an integer selected from 1 to 20, for example, an integer selected from 1 to 10, more preferably, y is 1, 2, 3, 4, 5, 6, 7 or 8, even more preferably, y is 1, 2, 3 or 4.

In some embodiments, x is 1 or 2, more preferably 1, and/or y is 1 or 2, more preferably 2.

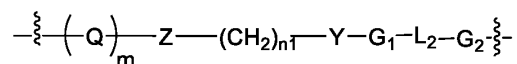
In some embodiments, in formula (III), E is selected from galactose (Gal), mannose (Man), N-acetylglucosamine (GlcNAc), glucose (Glc), N-acetylgalactosamine (GalNAc), glucuronic acid (Gcu), fucose (Fuc) and N-acetylneuraminic acid (sialic acid), preferably Gal, GlcNAc, glucose and GalNAc, most preferably GalNAc, such as 6-deoxy-2-acetamidogalactose, which is connected to L_1 via C2, C3, C4 and/or C6, preferably connected to L_1 via the C atom at C6.

In some embodiments, in formula (III), the monosaccharide or sugar derivative E may be bonded to C4 of the GlcNAc via a $\beta(1,4)$ - or $\beta(1,3)$ -glycosidic bond or bonded to C3 of said GlcNAc via an $\alpha(1,4)$ - or $\alpha(1,3)$ -glycosidic bond, preferably bonded to C4 of the GlcNAc via a $\beta(1,4)$ -glycosidic bond, and the GlcNAc linked to Ab is bonded via C1 to the antibody, preferably via an N-glycosidic bond to the amide nitrogen atom in the side chain of an asparagine amino acid of the antibody (GlcNAc β 1-Asn), and said GlcNAc is optionally fucosylated.

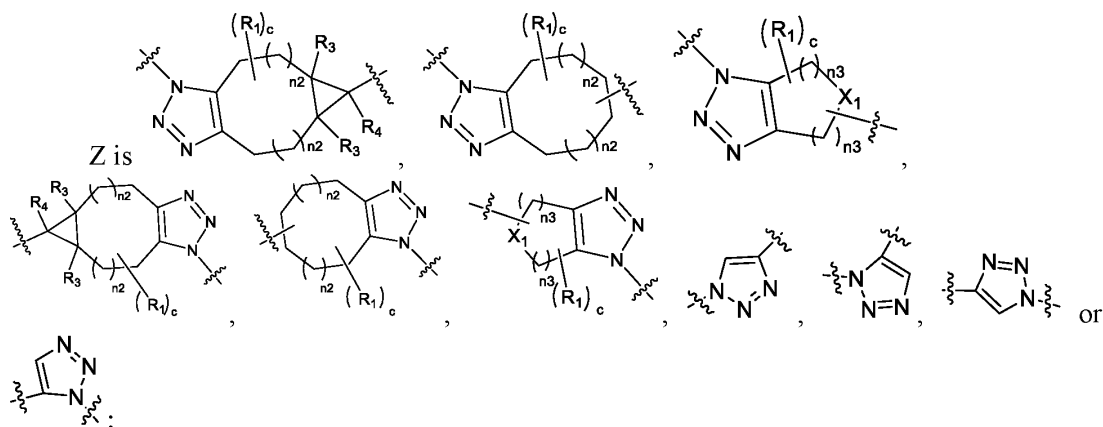
In a preferred embodiment, the GlcNAc linked to Ab is present at a native N-glycosylation site (e.g., a native conserved N-glycosylation site) of the antibody, such as a glycosylation site in the Fc region. In another preferred embodiment, the antibody is IgG and the GlcNAc is present at a native N-glycosylation site (e.g., a native conserved N-glycosylation site)

of IgG, such as a glycosylation site in the Fc region. In another preferred embodiment, the native site is the Asn297-glycosylation site of IgG. The Asn297-glycosylation site is present in the Fc region of the heavy chain of the IgG antibody. In a preferred embodiment, the GlcNAc group is present at the Asn297-glycosylation site of the two heavy chains of the antibody.

In some embodiments, in formula (III), L_1 has the following structure



Q is -NHC(O)CH₂- or -CH₂-;

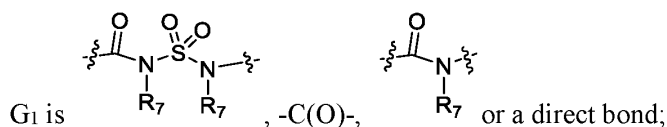


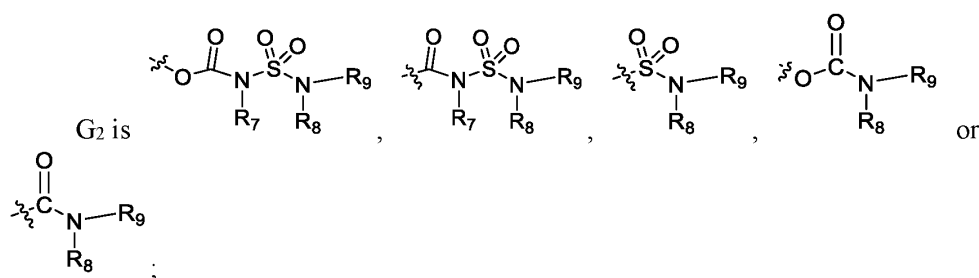
R₁ is independently selected from hydrogen, halogen, -OR₂, -NO₂, -CN, -S(O)₂R₂, C₁-C₁₂ alkyl, C₆-C₂₀ aryl, 5-20 membered heteroaryl, C₁-C₁₂ alkyl-C₆-C₂₀ aryl, C₁-C₁₂ alkyl-5-20 membered heteroaryl, C₆-C₂₀ aryl-C₁-C₁₂ alkyl and 5-20 membered heteroaryl-C₁-C₁₂ alkyl, and wherein the alkyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, arylalkyl and heteroarylalkyl are optionally substituted, wherein two substituents R₁ may be linked together to form a fused, bridged or spiro-connected C₃-C₁₈ cycloalkyl or a fused or spiro-connected aromatic or heteroaromatic substituent, and wherein R₂ is independently selected from hydrogen, C₁-C₂₄ alkyl, C₆-C₂₀ aryl, 5-20 membered heteroaryl, C₁-C₁₂ alkyl-C₆-C₂₀ aryl, C₁-C₁₂ alkyl-5-20 membered heteroaryl, C₆-C₂₀ aryl-C₁-C₁₂ alkyl and 5-20 membered heteroaryl-C₁-C₁₂ alkyl;

R₃ and R₄ are each independently selected from hydrogen, halogen, C₁-C₂₄ alkyl, C₆-C₂₀ aryl, 5-20 membered heteroaryl, C₁-C₁₂ alkyl-C₆-C₂₀ aryl, C₁-C₁₂ alkyl-5-20 membered heteroaryl, C₆-C₂₀ aryl-C₁-C₁₂ alkyl and 5-20 membered heteroaryl-C₁-C₁₂ alkyl;

Y is -O-, -S-, -NR₂- or absent;

X₁ is -O-, -S- or -NR₂-:





R₇ is independently selected from hydrogen and C₁-C₁₂ alkyl;

R₈ is hydrogen, C₁-C₁₂ alkyl or $\text{---L}_3\text{---L}_4\text{---}$;

R₉ is $\text{---L}_3\text{---L}_4\text{---}$;

L₂ and L₃ are independently C₁-C₁₂ alkylene, and one or more (e.g. 1, 2, 3 or 4) carbon atoms in the alkylene are optionally replaced by heteroatoms selected from O, N and S, provided that the O, N and/or S are not directly connected to each other;

L₄ is independently a cleavable spacer;

n₁ is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

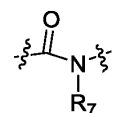
n₂ is independently 0, 1 or 2;

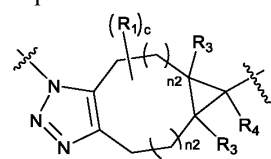
n₃ is independently 0, 1, 2, 3, 4 or 5;

c is 0, 1, 2, 3 or 4;

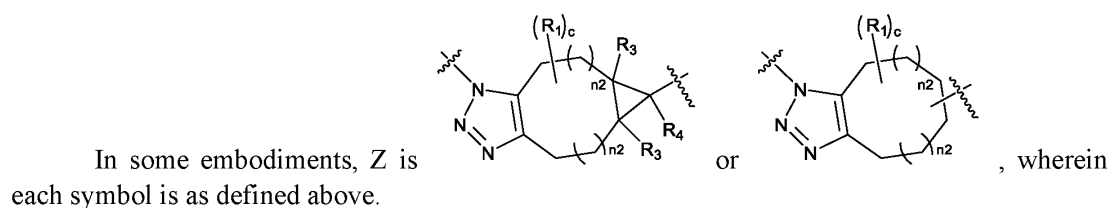
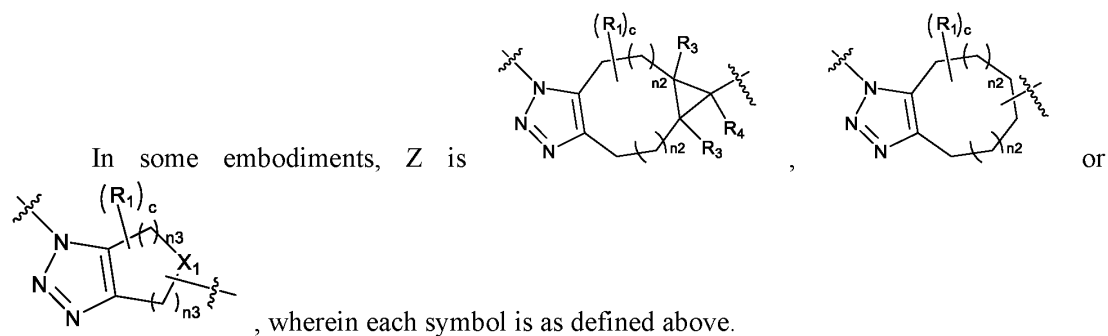
m is 0 or 1.

It should be understood that, unless otherwise specified and not contradictory according to the context, for the ADC of the present invention, the bond on the left side of the divalent group shown herein is connected to Ab or a group near the Ab end, and the bond on the right side of said

divalent group is connected to D or a group near the D end. For example, when G₁ is , wherein the carbonyl group is connected to the Y group, and N is connected to L₂; the bond on the

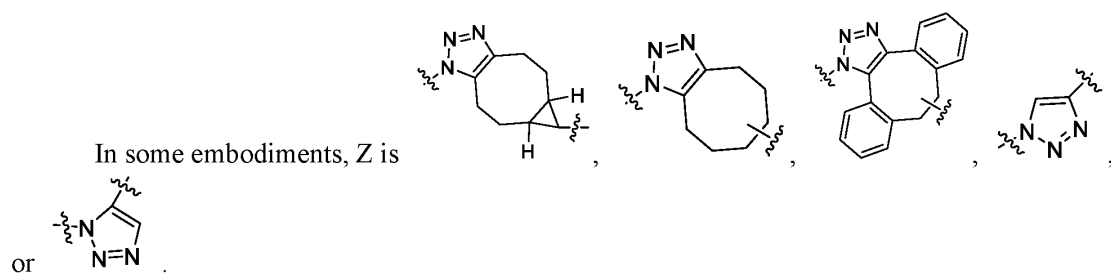
triazole of the Z group  is connected to the sugar E on the left side of Z, and the bond on the cyclopropyl group is connected to CH₂ on the right side of Z.

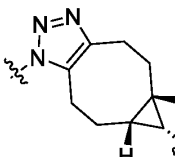
It should be understood that when m is 0, it means that Q does not exist, so that the relevant nitrogen atom of the triazole is directly connected to the E moiety through a covalent single bond.

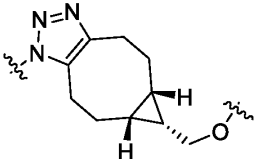


In some embodiments, n_2 is 1.

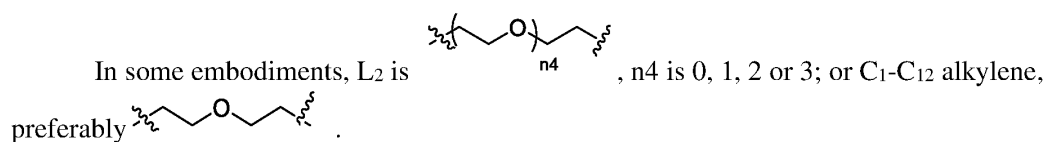
In some embodiments, R_3 and R_4 are H.

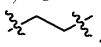
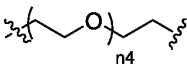


In some embodiments, Z is .

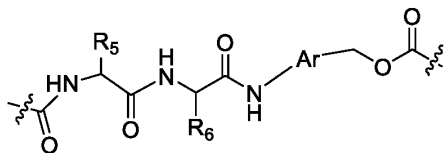
In some embodiments, $\text{---Z---(CH}_2\text{)}_{n_1}\text{---Y---}$ is .

In some embodiments, m is 0.



In some embodiments, L_3 is each independently , preferably , n_4 is 0, 1, 2 or 3; or

In some embodiments, each L_4 is independently selected from

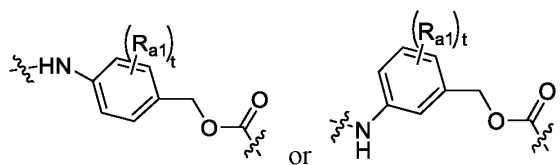


wherein R_5 and R_6 are each independently selected from H, C_1 - C_{12} alkyl or $-(CH_2)_3NHC(O)NH_2$;

or

$-\text{CO}-L_5-L_6-$,

wherein L_5 is a peptide residue consisting of 2, 3, 4, or 5 amino acids;



L_6 is selected from absent, $-\text{NH}-\text{CH}_2-$,

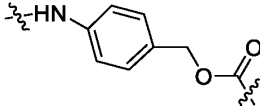
wherein each R_{a1} is independently selected from C_{1-6} alkyl-, C_{1-6} alkoxy-, halo C_{1-6} alkyl-, halo, nitro and cyano; and

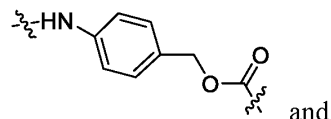
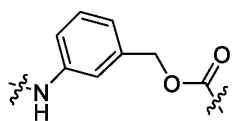
t is 0, 1, 2, 3 or 4.

In some embodiments, the amino acid is selected from, for example, glycine, alanine, valine, glutamine, glutamic acid, phenylalanine, leucine, tyrosine, lysine, citrulline, serine, tryptophan, aspartic acid, asparagine, isoleucine, arginine and proline.

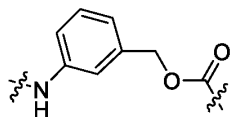
In some embodiments, the amino acid is selected from glycine, alanine, valine, glutamine, glutamic acid, phenylalanine, and leucine.

In some embodiments, L_5 is selected from: $-\text{Val}-\text{Ala}-$, $-\text{Gln}-\text{Val}-\text{Ala}-$, $-\text{Gly}-\text{Val}-\text{Ala}-$, $-\text{Gln}-\text{Phe}-\text{Ala}-$, $-\text{Gly}-\text{Phe}-\text{Ala}-$, $-\text{Gly}-\text{Gly}-\text{Phe}-\text{Gly}-$, $-\text{Val}-\text{Cit}-$, $-\text{Ala}-\text{Ala}-$, $-\text{Ala}-\text{Cit}-$, $-\text{Ala}-\text{Lys}-$, $-\text{Ala}-\text{Val}-$, $-\text{Asn}-\text{Cit}-$, $-\text{Asp}-\text{Cit}-$, $-\text{Asn}-\text{Lys}-$, $-\text{Asp}-\text{Val}-$, $-\text{Cit}-\text{Ala}-$, $-\text{Cit}-\text{Asn}-$, $-\text{Cit}-\text{Asp}-$, $-\text{Cit}-\text{Cit}-$, $-\text{Cit}-\text{Lys}-$, $-\text{Cit}-\text{Ser}-$, $-\text{Cit}-\text{Val}-$, $-\text{Glu}-\text{Val}-$, $-\text{Glu}-\text{Gly}-$, $-\text{Ile}-\text{Cit}-$, $-\text{Ile}-\text{Pro}-$, $-\text{Ile}-\text{Val}-$, $-\text{Leu}-\text{Cit}-$, $-\text{Lys}-\text{Cit}-$, $-\text{Phe}-\text{Arg}-$, $-\text{Phe}-\text{Cit}-$, $-\text{Phe}-\text{Lys}-$, $-\text{Pro}-\text{Lys}-$, $-\text{Ser}-\text{Cit}-$, $-\text{Trp}-\text{Cit}-$, $-\text{Ala}-\text{Val}-$, $-\text{Val}-\text{Asp}-$, $-\text{Cit}-\text{Val}-$, $-\text{Val}-\text{Glu}-$, $-\text{Val}-\text{Lys}-$, $-\text{Gly}-\text{Gly}-\text{Gly}-$, $-\text{Gly}-\text{Gly}-\text{Arg}-$, $-\text{Phe}-\text{Lys}-\text{Gly}-$, $-\text{Leu}-\text{Lys}-\text{Gly}-$, $-\text{Leu}-\text{Leu}-\text{Gly}-$, $-\text{Glu}-\text{Val}-\text{Cit}-$, $-\text{Cit}-\text{Ala}-\text{Glu}-$, $-\text{Val}-\text{Lys}-\text{Gly}-$, $-\text{Val}-\text{Lys}-\text{Ala}-$, $-\text{Val}-\text{Gly}-\text{Gly}-$, $-\text{Val}-\text{Cit}-\text{Gly}-$, $-\text{Val}-\text{Gln}-\text{Gly}-$, $-\text{Val}-\text{Glu}-\text{Gly}-$, $-\text{Val}-\text{Lys}-\text{Gly}-$, $-\text{Val}-\text{Lys}-\text{Leu}-$, $-\text{Ala}-\text{Ala}-\text{Ala}-$, $-\text{Asn}-\text{Ala}-\text{Ala}-$, $-\text{Gly}-\text{Gly}-\text{Gly}-\text{Gly}-$, $-\text{Gly}-\text{Gly}-\text{Leu}-\text{Gly}-$, $-\text{Gly}-\text{Phe}-\text{Leu}-\text{Gly}-$, $-\text{Gly}-\text{Val}-\text{Lys}-\text{Gly}-$, $-\text{Ala}-\text{Leu}-\text{Ala}-\text{Leu}-$, $-\text{Gly}-\text{Phe}-\text{Leu}-\text{Gly}-$, $-\text{Ala}-\text{Leu}-\text{Ala}-\text{Leu}-$, $-\text{Gly}-\text{Phe}-\text{Gly}-\text{Gly}-$ and $-\text{Val}-\text{Lys}-\text{Gly}-\text{Gly}-$.

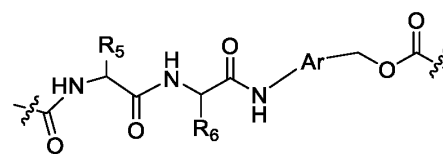
In some embodiments, L_6 is selected from $-\text{NH}-\text{CH}_2-$, , and



In some embodiments, L_6 is selected from

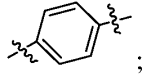


In some embodiments, each L_4 is independently $-\text{CO}-L_5-L_6-$, wherein each symbol (L_5 , L_6) is as defined above.

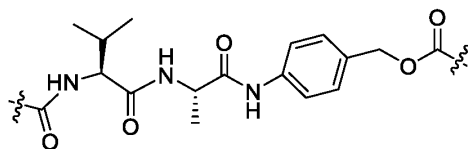


In some embodiments, L_4 is each independently

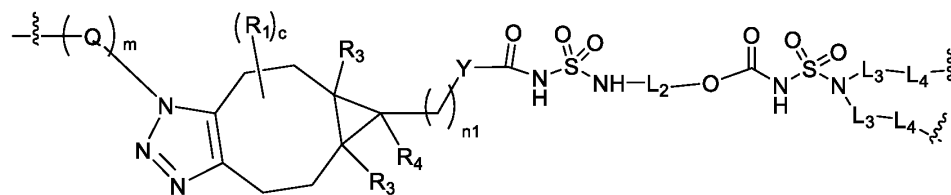
wherein R_5 and R_6 are each independently selected from hydrogen, C_1 - C_{12} alkyl or $-(\text{CH}_2)_3\text{NHC}(\text{O})\text{NH}_2$; preferably, R_5 is isopropyl and R_6 is methyl;

Ar is selected from arylene, preferably phenylene, more preferably ;

In some embodiments, L_4 is each independently

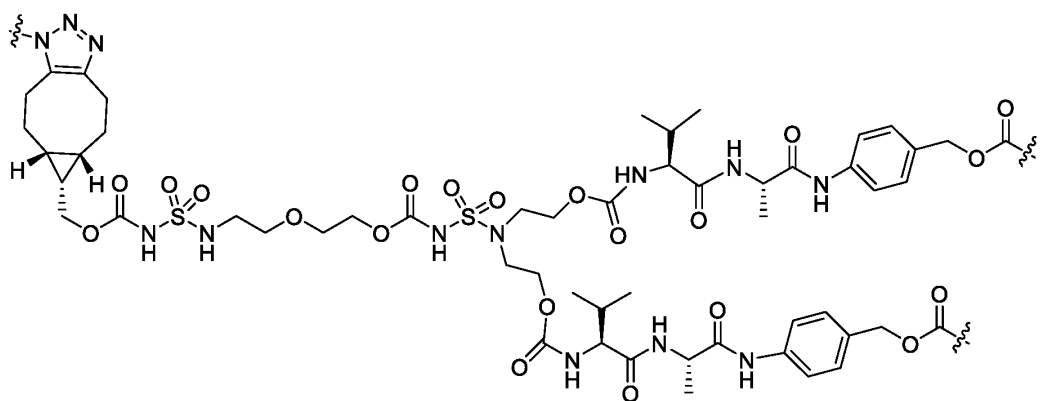


In some embodiments, in formula (III), $-\text{L}_1-$ is represented by the following structure

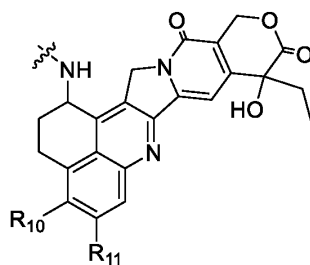


wherein Q , R_1 , R_2 , R_3 , R_4 , c , m , n_1 , Y , L_2 , L_3 and L_4 are as defined above.

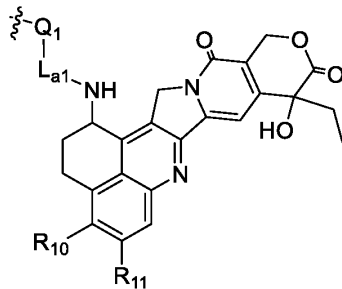
In some embodiments, in formula (III), $-\text{L}_1-$ is represented by the following structure



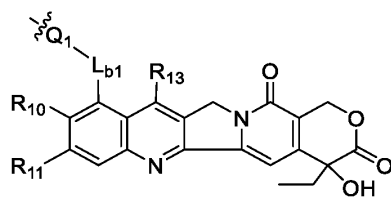
In some embodiments, D is represented by formula (D-1a), formula (D-1b), formula (D-1c), or formula (D-1d):



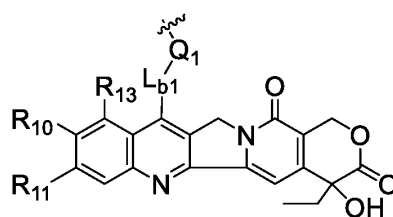
(D-1a)



(D-1b)



(D-1c)



(D-1d)

each R_{10} is independently selected from H, halo, C_1 - C_6 alkyl, C_1 - C_6 haloalkyl, $-OR_{12}$ and $-SR_{12}$; R_{11} is selected from H, halo, CN, C_1 - C_6 alkyl, C_1 - C_6 haloalkyl and $-OR_{12}$; or R_{10} and R_{11} together form $-O(CH_2)_{n5}O-$ or $-O(CF_2)_{n5}O-$, wherein $n5$ is 1 or 2;

each R_{12} is independently selected from H or C_1 - C_4 alkyl;

wherein each R_{13} is independently selected from H, C_1 - C_6 alkyl, C_1 - C_6 alkoxy, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_1 - C_6 haloalkyl, C_2 - C_6 haloalkenyl and C_2 - C_6 haloalkynyl;

each Q_1 is independently $-O-$ or $-S-$;

L_{a1} is $-(C_1-C_{10}alkylene)C(O)-$;

each L_{b1} is independently $-(C_1-C_{10}alkylene)-$, $-(C_1-C_{10}alkylene)-C(O)N(R_{14})-$ or $-(C_1-C_{10}alkylene)-N(R_{14})C(O)-$, wherein, R_{14} is H or C_1 - C_6 alkyl.

It is understood that, consistent with the description above, divalent groups such as L_{a1} and L_{b1} (e.g., $-(C_1-C_{10}alkylene)C(O)-$) shown herein are covalently attached to Q_1 at their left terminal ends. The wavy line on the structure/fragment herein has the generally understood meaning, it indicates the position of attachment of the structure/fragment.

In some embodiments, L_{a1} is $-(C_1-C_4alkylene)C(O)-$.

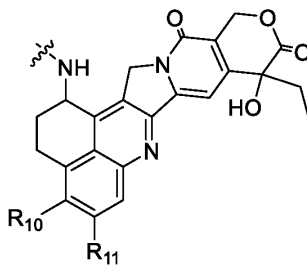
In some embodiments, L_{a1} is $-CH_2-C(O)-$.

In some embodiments, L_{b1} is $-(C_1-C_6alkylene)-$.

In some embodiments, L_{b1} is $-CH_2-CH_2-CH_2-CH_2-$ or $-CH_2-CH_2-CH_2-$.

In some embodiments, Q_1 is $-O-$.

In some embodiments, D is represented by formula (D-1a):



wherein

R_{10} is selected from H, halogen, C_1 - C_6 alkyl, C_1 - C_6 haloalkyl, $-OR_{12}$ and $-SR_{12}$; R_{11} is selected from H, halogen, CN, C_1 - C_6 alkyl, C_1 - C_6 haloalkyl and $-OR_{12}$; or R_{10} and R_{11} together form $-O(CH_2)_{n5}O-$ or $-O(CF_2)_{n5}O-$, wherein $n5$ is 1 or 2;

R_{12} is selected from H or C_1 - C_4 alkyl.

In some embodiments, R_{10} is C_1 - C_6 alkyl or C_1 - C_4 alkoxy, preferably methyl or methoxy, and R_{11} is halo, preferably F;

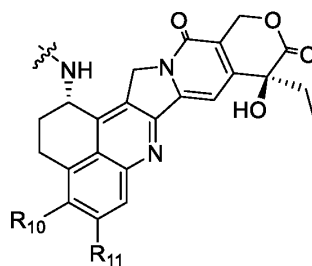
or R_{10} and R_{11} together form $-OCH_2O-$.

In some embodiments, R_{10} and R_{11} together form $-OCH_2O-$.

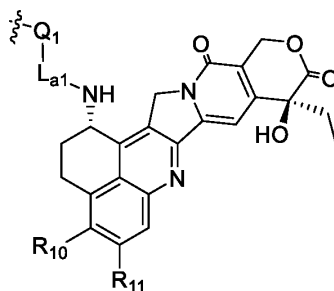
In some embodiments, R_{10} is C_1 - C_6 alkyl, preferably methyl, and R_{11} is halogen, preferably F.

In some embodiments, each R_{13} is independently selected from H and C_1 - C_6 alkyl, preferably is H.

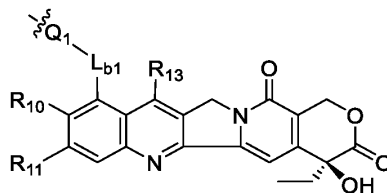
In some embodiments, D is represented by formula (D-2a), (D-2b), (D-2c) or (D-2d):



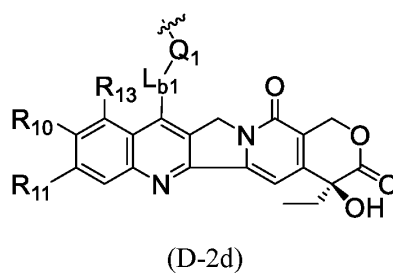
(D-2a)



(D-2b)

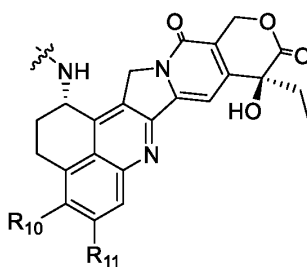


(D-2c)



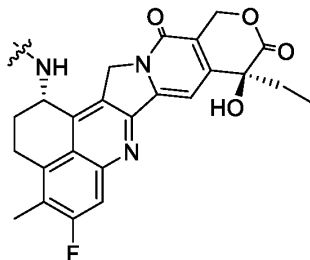
wherein each symbol (R_{10} , R_{11} , R_{13} , Q_1 , L_{a1} and L_{b1}) is as defined above.

In some embodiments, D is represented by formula (D-2a):



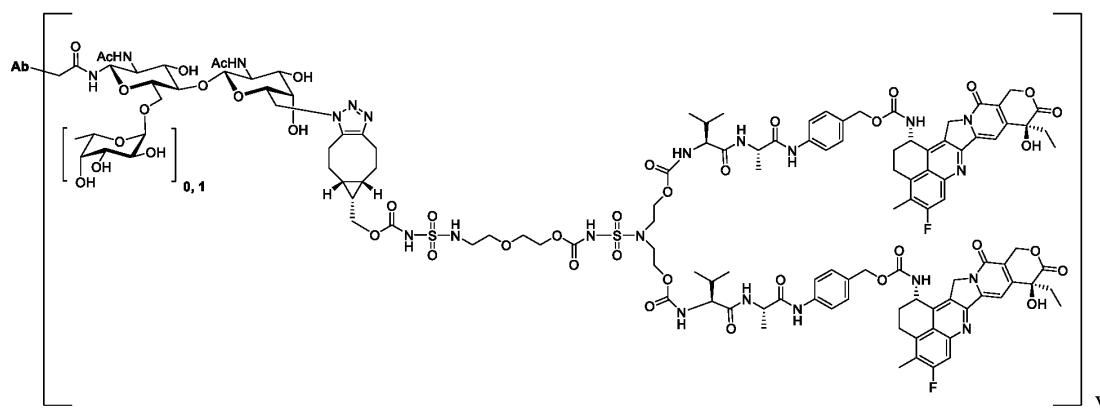
wherein R_{10} and R_{11} are as defined above.

In some embodiments, D is represented by formula (D-3a):



In some embodiments, the antibody-drug conjugate has an average DAR of 1-10, such as 2-8, 2-6 or 3-5.

In some embodiments, the antibody-drug conjugate of the present invention is selected from:



wherein Ab is a bispecific antibody of the present invention, preferably Hz20G5.26/Zalu bsAb;
y is 1 or 2, preferably 2.

Preferably, the antibody-drug conjugate has an average DAR of, for example, 2-6, 3-5, or 3-4.

In another aspect, the present invention provides a modified glycosylated antibody shown in formula (II).

III. Preparation of ADC molecules of the present invention

In one aspect, the invention provides a method of preparing an antibody having engineered glycosylation comprising

(1) Preparation of glycosylated antibody: culturing a host cell comprising a nucleic acid encoding the antibody (e.g., any one and/or more polypeptide chains) or comprising an expression vector of the nucleic acid, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium) to obtain an antibody comprising a core N-acetylglucosamine substituent (core-GlcNAc substituent), wherein the core N-acetylglucosamine and the core N-acetylglucosamine substituent are optionally fucosylated;

(2) preparation of the pruned antibody: deglycosylating the antibody prepared in step (1) in the presence of an endoglycosidase to obtain an antibody comprising a core N-acetylglucosamine substituent, wherein the core N-acetylglucosamine and the core N-acetylglucosamine substituent are optionally fucosylated;

(3) contacting the pruned antibody obtained in (2) with a compound of formula E(A)_x-P in the presence of a suitable catalyst to obtain an antibody comprising a Substituent GlcNAc-E(A)_x bonded to the antibody by the C1 of the N-acetylglucosamine of the Substituent GlcNAc-E(A)_x; wherein the catalyst is a glycosyltransferase, wherein P is selected from the group consisting of Uridine Diphosphate (UDP), Guanosine Diphosphate (GDP), and Cytidine Diphosphate (CDP).

To perform step (1), nucleic acid encoding an antibody (e.g., an antibody described above, e.g., any one polypeptide chain and/or multiple polypeptide chains) is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acids are readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of an

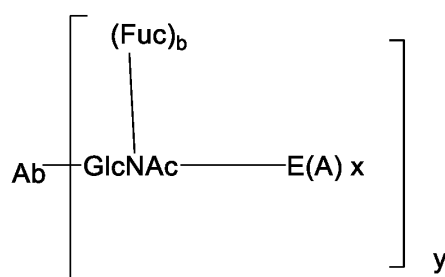
antibody).

The endoglycosidase in step (2) may be selected according to the nature of the glycosylated antibody, for example, selected from Endo S, Endo S2, Endo A, Endo F, Endo M, Endo D and Endo H enzymes and/or combinations thereof, further for example, Endo S, Endo S2, Endo S49, Endo F or combinations thereof. In a preferred embodiment, the endoglycosidase is an endoglycosidase described in PCT/EP2017/052792, most preferably an Endo SH in PCT/EP 2017/052792.

The glycosyltransferase in step (3) is preferably a glycosyltransferase that is or is derived from a β - (1,4) -N-acetylgalactosamine transferase, more preferably any of the β - (1,4) -GalNAcT enzymes described in PCT/EP 2016/059194. In some embodiments, the β - (1,4) -GalNAcT enzyme is or is derived from a β - (1,4)-GalNAcT that originates from invertebrate animal species. The β - (1,4) -GalNAcT enzyme may be or may be derived from any invertebrate β - (1,4) -GalNAcT enzyme known to the skilled person. Preferably, the β - (1,4) -GalNAcT enzyme is or is derived from a β - (1,4) -GalNAcT enzyme that originates from the phylum of Nematoda, preferably of the class of Chromadorea or Secernentea, or from the phylum of Arthropoda, preferably of the class of Insecta. More preferably, the β - (1,4) -GalNAcT enzyme is or is derived from *Caenorhabditis elegans*, *Ascaris suum*, *Trichoplusia ni*, *Drosophila melanogaster*, *Saproscit fruit nematode*, *Caenorhabditis briggsae*, *Wuchereria*, *Loa Loa*, *Cerapachys biroi*, *Zootermopsis nevadensis*, *Camponotus floridanus*, *Crassostrea gigas*, and *Danaus plexippus*. Preferably, the glycosyltransferase suitable for use in step (3) is a *Trichoplusia ni* β - (1,4) -GalNAcT enzyme designated TnGalNAcT as disclosed in PCT/EP2016/059194 (e.g., His-TnGalNAcT).

In another aspect, the present invention provides a method for preparing ADC using the antibody of the present invention. "ADC" in the present invention is defined as an antibody conjugated to an active substance (D) having biological and/or pharmaceutical activity through a linker (L). The method comprises coupling the antibody of the present invention to one or more active substances D through one or more linkers (L) defined in the present invention. Preferably, the linker-active substance is site-specifically coupled to the antibody.

In some embodiments, a method for preparing an ADC of the present invention is provided, comprising reacting a glycosylated antibody of formula (II)

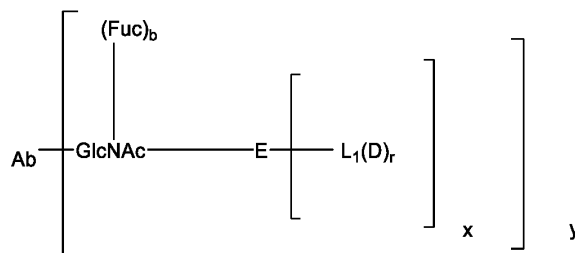


Formula (II)

wherein the meaning of each variable or symbol is as defined above;

with a linker-drug compound (linker-Drug) containing an alkynyl group and one or more (e.g., 1, 2, 3 or 4) drug molecules,

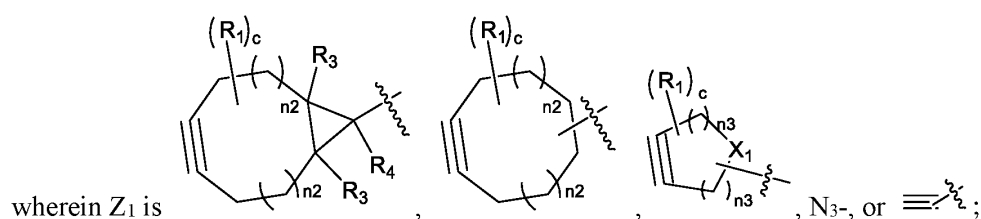
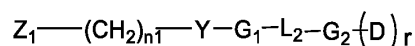
to produce an antibody-drug conjugate of formula (III)



Formula (III)

wherein the meaning of each variable or symbol is as defined above.

In some embodiments, the linker-drug compound is the following structure:



the variables in each linker-drug compound (including the variables in Z_1) are as defined above.

IV. Pharmaceutical composition

In some embodiments, the present invention provides a composition comprising any of the ADC molecules described herein, or a pharmaceutically acceptable salt thereof, preferably the composition is a pharmaceutical composition or pharmaceutical formulation. In some embodiments, the composition further comprises a pharmaceutically acceptable excipient. In some embodiments, a composition, e.g., a pharmaceutical composition, comprises a combination of an ADC molecule of the invention, and one or more other therapeutic agents.

The invention also includes compositions (including pharmaceutical compositions) comprising the ADC molecules of the invention, or pharmaceutically acceptable salts thereof. These compositions may also contain suitable pharmaceutically acceptable excipients, such as pharmaceutically acceptable carriers and pharmaceutically acceptable vehicles known in the art, including buffers.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, isotonic and absorption delaying agents, and the like that are physiologically compatible.

For the application of pharmaceutically acceptable excipients and their use, see also "Handbook of Pharmaceutical Excipients", eighth edition, R.C. Rowe, P.J. Seskey and S.C. Owen, Pharmaceutical Press, London, Chicago.

The compositions of the present invention may be in a variety of forms. Such forms include, for example, liquid, semi-solid, and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), powders or suspensions, liposomal formulations, and suppositories. The preferred form depends on the intended mode of administration and therapeutic use.

Medicaments comprising the ADCs described herein may be prepared by mixing the ADC molecules of the invention of the desired purity with one or more optional pharmaceutically acceptable excipients, preferably in the form of lyophilized formulations or aqueous solutions.

The pharmaceutical compositions or formulations of the present invention may also contain more than one active ingredient as required for the particular indication being treated, preferably those having complementary activities that do not adversely affect each other. For example, it may be desirable to further provide other therapeutic agents, including chemotherapeutic agents, angiogenesis inhibitors, cytokines, cytotoxic agents, other antibodies, small molecule drugs, or immune modulators (e.g., immune checkpoint inhibitors or agonists), and the like. The active ingredients are suitably present in combination in an amount effective for the intended use.

Sustained release formulations can be prepared. Appropriate examples of sustained release formulations include semipermeable matrices of solid hydrophobic polymers containing the ADC molecule, which matrices are in the form of shaped articles, e.g., films, or microcapsules.

VII. Pharmaceutical combination and Kit

In some embodiments, the present invention further provides a pharmaceutical combination or a pharmaceutical combination product comprising the ADC of the present invention, and one or more other therapeutic agents (such as chemotherapeutic agents, angiogenesis inhibitors, cytokines, cytotoxic agents, other antibodies, small molecule drugs, or immune modulators (e.g., immune checkpoint inhibitors or agonists) etc.).

Another object of the invention is to provide a kit comprising the pharmaceutical combination of the invention, preferably in the form of drug dose unit. Therefore, the dose unit can be provided according to the regimen or interval of the administration.

In some embodiments, the kit of the invention comprises:

- a first container comprising a pharmaceutical composition containing the ADC molecule of the invention;
- a second container comprising a pharmaceutical composition containing other therapeutic agent(s).

VII. Use and Methods

In one aspect, the present invention provides a method of preventing or treating a disease in a subject, comprising administering to the subject an effective amount of the ADC molecule, pharmaceutical composition, pharmaceutical combination or kit of the present invention.

In some embodiment, the disease is B7H3 and/or EGFR related disease. In some embodiments, the disease is related to aberrant expression or aberrant activity of B7H3 and/or EGFR.

In some embodiments, the disease is a tumor, e.g., cancer.

In some embodiments, the tumor, e.g., cancer, is B7H3 positive, e.g., it comprises tumor cells that express B7H3. In some embodiments, the patient's tumor comprises tumor cells that express B7H3. In some embodiments, the patient's tumor cells express B7H3, e.g., moderately express B7H3, preferably highly express B7H3. In some embodiments, the tumor (e.g., cancer) patient's tumor tissue has, e.g., elevated levels, such as nucleic acid or protein levels or activity of B7H3, such as compared to the level of B7H3 in the same tissue of a healthy individual or in a healthy tissue adjacent to the patient's tumor tissue. In some embodiments, the patient's tumor cells have, e.g., elevated levels, such as nucleic acid or protein levels or activity of B7H3, such as compared to the level of B7H3 in the same cell of a healthy individual or in a healthy cell adjacent to the patient's tumor cell.

In some embodiments, the tumor, e.g., cancer, is EGFR positive, e.g., it comprises tumor cells that express EGFR. In some embodiments, the patient's tumor comprises tumor cells that express EGFR. In some embodiments, the patient's tumor cells express EGFR, e.g., moderately express EGFR, preferably highly express EGFR. In some embodiments, the tumor (e.g., cancer) patient's tumor tissue has, e.g., elevated levels, such as nucleic acid or protein levels or activity of EGFR, such as compared to the level of EGFR in the same tissue of a healthy individual or in a healthy tissue adjacent to the patient's tumor tissue. In some embodiments, the patient's tumor cells have, e.g., elevated levels, such as nucleic acid or protein levels or activity of EGFR, such as compared to the level of EGFR in the same cell of a healthy individual or in a healthy cell adjacent to the patient's tumor cell.

In some embodiments, the tumor, e.g., cancer, is B7H3 positive and EGFR positive, e.g., it comprises tumor cells that express EGFR. In some embodiments, the patient's tumor comprises tumor cells that express B7H3 and EGFR. In some embodiments, the patient's tumor cells express B7H3 and EGFR, e.g., moderately or highly express B7H3 and EGFR. In some embodiments, the tumor (e.g., cancer) patient's tumor tissue has, e.g., elevated levels, such as nucleic acid or protein levels or activity of B7H3 and EGFR, such as compared to the level of B7H3 and EGFR in the same tissue of a healthy individual or in a healthy tissue adjacent to the patient's tumor tissue. In some embodiments, the patient's tumor cells have, e.g., elevated levels, such as nucleic acid or protein levels or activity of B7H3 and EGFR, such as compared to the level of B7H3 and EGFR in the same cell of a healthy individual or in a healthy cell adjacent to the patient's tumor cell.

In some embodiments, the tumors, such as cancers, include solid tumors and hematological tumors as well as metastatic lesions. In some embodiments, examples of solid tumors include malignant tumors. The cancer may be in an early, intermediate or advanced stage or metastatic cancer.

In a specific embodiment, the ADC molecule of the present invention is capable of killing tumor cells and/or inhibiting the proliferation of tumor cells, such as tumor cells expressing B7H3 and/or EGFR, such as epithelial tumor cells, digestive tract tumor cells, such as gastric cancer cells or pancreatic cancer cells or intestinal cancer cell or colon cancer cells or colorectal cancer cells, head and neck squamous cell carcinoma cell, gastric adenocarcinoma cells, breast cancer cells, oral squamous cell carcinoma cells, prostate cancer cells, melanoma cells, cervical cancer cells, lung cancer (such as small cell lung cancer and non-small cell lung cancer) cells, head and neck cancer cells.

In some embodiments, the tumor is tumor immune escape.

In some embodiments, the tumor is a cancer, such as an epithelial cancer or a digestive tract cancer, wherein the cancer is selected from, for example, gastric cancer, pancreatic cancer, intestinal cancer, colon cancer, colorectal cancer, head and neck squamous cell carcinoma, gastric adenocarcinoma, breast cancer, oral squamous cell carcinoma, prostate cancer, melanoma, cervical cancer, lung cancer (such as small cell lung cancer and non-small cell lung cancer) and head and neck cancer.

In some embodiments, the tumor is a tumor resistant to TKI small molecules.

In some embodiments, the tumor is a mutated tumor, such as a tumor having the mutations described in Figures 11 and 12, such as a tumor with EGFR and/or KRAS mutations, and the mutations can be the following mutations: EGFR^{L858R/T790M}, KRAS^{G12C}, EGFR (L858R/T790M/C797S) and/or Exon20ins^{S768_D770dup}.

In some embodiments, the tumor cells of the tumor have one or more of the following characteristics, compared to normal cells of the same tissue or adjacent normal tissue in the same subject, or compared to normal cells of the same tissue in a healthy subject:

(i) overexpresses wild-type EGFR (e.g., wild-type EGFR with increased nucleic acid or protein levels) and/or expresses a mutant EGFR, e.g., mutant EGFR comprising a mutation listed in Passaro A, et al. Nat Cancer. 2021, preferably the mutant EGFR comprises one or more mutations selected from R521K, L858R, T790M, G719X, C797S, Y1069C, Exon19 deletion (Del 19), Exon20ins (e.g., S768_D770 dup), preferably the mutant EGFR comprises R521K/Y1069C, R521K, L858R/T790M/C797S, Del19/T790M/C797S or S768_D770dup, compared to normal cells of an adjacent tissue or compared to normal cells of the same tissue in a healthy subject;

(ii) overexpresses a wild-type KRAS (e.g., wild-type KRAS with an increased level of nucleic acid or protein) or expresses a mutant KRAS, preferably the mutant KRAS comprises a mutation at position G12 or G13, e.g., G12D or G12C, compared to normal cells of an adjacent tissue or compared to normal cells of the same tissue in a healthy subject;

(iii) having elevated nucleic acid levels or protein levels of B7H3 compared to normal cells of an adjacent tissue or compared to normal cells of the same tissue in a healthy subject;

(iv) the tumor cells are resistant to tyrosine kinase inhibitors, such as to the first generation (Erlotinib) and the third generation (Osimertinib), such as resistant to Osimertinib.

The subject can be a mammal, e.g., a primate, preferably a higher primate, e.g., a human (e.g., a subject having or at risk of having a disease as described herein). In some embodiments, the subject has or is at risk of having a disease described herein (e.g., cancer). In some embodiments, the subject receives or has received other treatment, such as chemotherapy and/or radiation therapy. In some embodiments, the subject has previously received or is receiving immunotherapy.

In another aspect, the present invention provides the use of an ADC molecule or pharmaceutical composition or pharmaceutical combination or kit as described above in the manufacture or preparation of a medicament for use as described herein, for example for the prevention or treatment of a related disease or condition mentioned herein.

In another aspect, the present invention provides the above ADC molecules or pharmaceutical compositions or pharmaceutical combinations or kits for use in therapy, e.g. for use as described herein, e.g. for the prevention or treatment of a related disease or disorder mentioned herein.

In some embodiments, the ADC molecule or pharmaceutical composition or pharmaceutical combination or kit of the present invention delays the onset of a disease or condition and/or

symptoms associated with a disease or condition.

In some embodiments, the ADC molecules or pharmaceutical compositions of the present invention can also be administered in combination with one or more other therapies, e.g., therapeutic modalities and/or other therapeutic agents, for uses described herein, e.g., for the prevention and/or treatment of related diseases or conditions mentioned herein.

In some embodiments, the treatment modality includes surgery (e.g., tumor resection); radiation therapy, localized or focused irradiation, or the like.

In some embodiments, the therapeutic agent is selected from a chemotherapeutic agent, angiogenesis inhibitor, cytokine, cytotoxic agent, another antibody, small molecule drug, or immunomodulatory agent (e.g., an immune checkpoint inhibitor or agonist).

Exemplary other antibodies include antibodies that specifically bind to immune checkpoints.

In some embodiments, the small molecule drug is selected from a KRAS small molecule inhibitor, such as a KRAS G12C inhibitor (such as AMG510 (Sotorasib) or GFH925), a KRAS G12D inhibitor (such as MRTX1133) or a KRAS G12S inhibitor.

Immunomodulators include immune checkpoint molecule inhibitors and co-stimulatory molecule activators.

In some further embodiments, the ADC molecules of the present invention are used in combination with KRAS small molecule inhibitors, such as KRAS G12C inhibitors (such as AMG510 (Sotorasib) or GFH925), KRAS G12D inhibitors (such as MRTX1133) or KRAS G12S inhibitors.

In some embodiments, the ADC molecules of the present invention can be administered in combination with a therapy comprising adoptively transferring T cells (such as cytotoxic T cells or CTLs) expressing a chimeric antigen receptor (CAR).

In some embodiments, the ADC molecules of the present invention can be administered in combination with an anti-tumor agent.

In some embodiments, the ADC molecules of the present invention can be administered in combination with a cytokine. Cytokines can be administered as fusion molecules with antibody molecules of the invention, or as separate compositions. In some embodiments, the ADC molecules of the invention are administered in combination with one, two, three or more cytokines (e.g., as fusion molecules or as separate compositions).

In some embodiments, the ADC molecules of the invention can be combined with conventional cancer therapies in the art, including but not limited to: (i) radiation therapy; (ii) chemotherapy, or the use of cytotoxic drugs, which generally affect rapidly dividing cells; (iii) targeted therapy, or agents that specifically affect the deregulation of cancer cell proteins; (iv) immunotherapy, or enhancing the host immune response (e.g., vaccines); (v) hormone therapy, or blocking hormones (e.g., when the tumor is hormone-sensitive), (vi) angiogenesis inhibitors, or blocking blood vessel formation and growth, and (vii) palliative care.

In some embodiments, the ADC molecules of the invention can be combined with conventional methods for enhancing host immune function.

The various combination therapies described above can be further combined for treatment.

The combination therapy of the present invention encompasses combined administration (e.g.,

two or more therapeutic agents are included in the same formulation or in separate formulations), and separate administration, in which case the administration of the ADC molecules of the present invention may occur before, simultaneously with, and/or after the administration of the other therapy, e.g., treatment modality and/or therapeutic agent. ADC molecules and/or other therapies, such as therapeutic agents or treatment modalities, can be administered during active disease or during remission or less active disease. ADC molecules can be administered before, simultaneously with or after other treatments, or during disease remission.

The combination therapy of the present invention encompasses combined administration (e.g., two or more therapeutic agents are included in the same formulation or in separate formulations), and separate administration, in which case the administration of the ADC molecules of the present invention may occur before, simultaneously with, and/or after the administration of the therapeutic agent and/or active agent.

The route of administration of the pharmaceutical composition is according to known methods, for example, orally, by intravenous injection, intraperitoneally, intracerebral (intraparenchymal), intracerebroventricular, intramuscular, intraocular, intraarterial, intraportal or intralesional routes; by sustained release systems or by implant devices. In certain embodiments, the composition may be administered by bolus injection or by continuous infusion or by implant devices.

The composition may also be administered topically via an implant membrane, sponge, or another suitable material onto which the desired molecule is absorbed or encapsulated. In certain embodiments, when an implant device is used, the device may be implanted into any suitable tissue or organ and the desired molecule may be delivered via diffusion, timed-release bolus, or continuous administration.

These and other aspects and embodiments of the present invention are described in the accompanying drawings (a brief description of the drawings follows immediately thereafter) and in the following detailed description of the invention and are exemplified in the following examples. Any or all features discussed above and throughout this application may be combined in various embodiments of the present invention. The following examples further illustrate the present invention. However, it should be understood that the examples are described in an illustrative rather than a limiting manner, and that various modifications may be made by those skilled in the art.

Examples

Related information of some cell lines used in the experiments is shown below:

NCI-H1975: ATCC, CRL-5908;

NCI-H358: CoBioer, CBP60136;

H508: CoBioer, CBP60795;

SW620: CoBioer, CBP60036;

HT55: CoBioer, CBP60012;

BxPC3: CoBioer, CBP60542;

PE/PJ-41: CoBioer, CBP60606;

SW601: CoBioer, CBP60507;

LNCaP: CoBioer, CBP60346;

PC-9: Ybio, Shanghai, YB-H3210;

NCI-H1703: ATCC, CRL-5889;

NCI-H2030: ATCC, CRL-5914.

H322-EGFR20ins (Exon20ins^{S768_D770dup}): Lentviruses + EGFR^{S768_D770dup} were constructed and packaged, and NCI-H322 (Cobioer, CBP60134) was infected with Lentviruses + EGFR^{S768_D770dup} virus, and subjected to pressure screening to obtain an H322-EGFR^{S768_D770dup} stable cell line.

MDA-MB-453^{OX}: c Lentvirus + EGFR, Lentvirus + B7-H3(4Ig) were constructed and packaged respectively and MAD-MB-453 (Cobioer, CBP60386) was infected with Lentvirus + EGFR and Lentvirus + B7-H3(4Ig) viruses, and subjected to pressure screening to obtain an MDA-MB-453+ EGFR + B7H3 stable cell line, wherein B7H3 is the protein under UniProt database Accession No. Q5ZPR3, and EGFR is the protein under Uniprot Accession No. P00533-1.

Example 1: Design, preparation and validation of Effect of B7-H3/EGFR bsAb bispecific antibody

Example 1.1 B7-H3/EGFR bsAb design

The anti-B7-H3/EGFR bispecific antibody molecules of the present invention are prepared by assembling an anti-B7H3 antibody parent and an anti-EGFR antibody parent into an antibody format of IgG1 through Innobody technology platform (Application Number: PCT/CN2021/143141(WO2022143912A1), Invention Name: Protein containing heterodimer antibody Fc, and preparation method therefor) of the Innovent Biologics (Suzhou) Co., Ltd. The bispecific antibody format contains four polypeptide chains and can bind to two antigens, antigen A is B7H3 and antigen B is EGFR.

Wherein, the parent antibodies used for constructing the bispecific antibody are anti-EGFR monoclonal antibody Zalutumumab (hereinafter referred to as Zalu, Publication No: WO02100348A2, Invention Name: Human monoclonal antibodies to Epidermal Growth Factor Receptor (EGFR)) and anti-B7-H3 monoclonal antibodies, which are derived from Hybridoma Screening Platform of Innovent and has been humanized to obtain antigen-binding region sequences of anti-B7H3 monoclonal antibodies Hz20G5.26, Hz19A2.25 and Hz5C2.9 (Application No: PCT/CN2021/140449, Invention Name: anti-B7-H3 antibody and the uses thereof). Meanwhile, the Innobody technology is adopted to mutate in the Fc region to improve the formation of specific antibody heterodimer (Application Number: PCT/CN2021/143141, Invention Name: Protein containing heterodimer antibody Fc and preparation method thereof). Three bispecific antibodies were obtained by screening, which were numbered as bispecific antibody molecules Hz5C2.9/Zalu bsAb, Hz19A2.25/Zalu bsAb, and Hz20G5.26/Zalu bsAb, and which simultaneously bound to B7H3 and EGFR.

Sequence information is shown below:

TABLE 1 Details of the B7H3/EGFR bsAb molecule sequence

Bispecific antibody molecules	B7H3 antibody parent	Heavy and light chains involved	EGFR antibody parent	Heavy and light chains involved
Hz20G5.26/Zalu	Hz20G5.26	Heavy chain 2: SEQ ID NO: 35 Light chain 2: SQ ID NO: 36	Zalu	Heavy chain 1: SEQ ID NO:33, Light chain 1: SEQ ID NO:34;
Hz19A2.25/Zalu	Hz19A2.25	Heavy chain 2: SEQ ID NO:37 Light chain 2: SQ ID NO:38	Zalu	Heavy chain 1: SEQ ID NO:33, Light chain 1: SEQ ID NO:34;
Hz5C2.9/Zalu	Hz5C2.9	Heavy chain 2: SEQ ID NO:39 Light chain 2: SQ ID NO:40	Zalu	Heavy chain 1: SEQ ID NO:33, Light chain 1: SEQ ID NO:34;

Example 1.2 B7H3/EGFR bsAb bispecific antibody preparation

The heavy chain sequence of the EGFR antibody, the light chain sequence of the EGFR antibody, the heavy chain sequence of the B7H3 antibody and the light chain sequence of the B7H3 antibody are inserted into a vector pcDNA3.1(Invitrogen, V790-20) to respectively obtain a heavy chain plasmid and a light chain plasmid of an anti-EGFR end and a heavy chain plasmid and a light chain plasmid of an anti-B7H3 end.

The heavy chain plasmid and the light chain plasmid of the EGFR parent antibody, and the heavy chain plasmid and the light chain plasmid of the B7H3 parent antibody were transiently transfected into ExpicCHO (Invitrogen, A29133) cells, respectively, after 7 days, cell fermentation broth is harvested, filtered and clarified, and captured by a Hitrap Mabselect Sure chromatography column (GE Healthcare, 11-0034-95), respectively, to obtain the EGFR antibody parent and the B7H3 antibody parent.

After the concentration was detected by the A280 method, the antibody parents were mixed at a molar ratio of 1:1, and an appropriate amount of reducing agent GSH was added. The reaction was allowed to proceed overnight at room temperature, and the reducing agent was removed by ultrafiltration to terminate the reaction. Fine purification was performed using MonoS cation exchange chromatography column (GE Healthcare, 17-5168-01). The B7-H3 antibody parent solution was 20 mM sodium phosphate buffer (pH 6.6), and the EGFR antibody parent solution was 20 mM sodium phosphate buffer (pH 6.6) containing 1M sodium chloride. The elution gradient was 0-50% (30 column volumes). The eluted protein solution was ultrafiltered and replaced with PBS (Gibco, 70011-044), and the purity was tested by SEC-HPLC.

Example 1.3 B7-H3/EGFR bsAb bispecific antibody screening and mechanism

The B7-H3/EGFR bsAb-ADC is designed to improve the effectiveness and safety of the bsAb-ADC. Molecules with strong effects on B7-H3/EGFR bsAb endocytosis by tumor cells are screened as far as possible while the effect of B7-H3 on enhancing the effect of blocking downstream signals of EGFR is retained. Hz20G5.26/Zalu bsAb which has strong blocking to EGFR signal and good promotion for tumor cell endocytosis is finally screened as a preferred bsAb sequence through a proliferation inhibition experiment for blocking EGFR signals, a Fab-ZAP endocytosis assay and affinity KD detection, and the Hz20G5.26/Zalu bsAb is subjected to verification on promotion to tumor cell endocytosis activity in various tumor cells. Meanwhile, an in vitro activity experiment is designed, to evaluate and explain the mechanism that Hz20G5.26/Zalu bsAb is suitable for preparing bsAb-ADC.

Experimental method

1. Proliferation inhibition assay

(1) 1000 cells/100 μ l were plated in 96-well white plates (NUNC, 136101) for 2D cell culture.

(2) The antibody molecules prepared by dilution in advance (maximum concentration 10nM, 4-fold dilution) were added to the corresponding cell well plate, mixed well, and cultured at 37 °C with 5% CO₂ for 4 days.

(3) After culturing for 4 days in the proliferation inhibition assay, Cell-Titer reagent (Promega, G7572) prepared in advance was added to the corresponding cell wells, and the cells were left standing in the dark at room temperature for 30 minutes, and then subjected to detection by a multifunctional microplate reader (Molecular Devices, Spectra MAXi3).

2. Fab-ZAP endocytosis assay

(1) 1000 cells/100 μ l were plated in 96-well white plates (NUNC, 136101) and cells were cultured overnight.

(2) The antibody molecules diluted in advance (maximum concentration 10nM, 4-fold dilution or maximum concentration 12.5nM, 4-fold dilution) and Fab-ZAP (ATSBIO, IT-51) were mixed at a molar ratio of 1:4 uniformly, and reacted for binding for 0.5 hour at room temperature.

(3) The Ab-ZAP in above (2) was added to the corresponding cell well plate, mixed well, and cultured at 37 °C with 5% CO₂ for 4 days.

(4) After 4 days of cell culture and killing, Cell-Titer reagent (Promega, G7572) prepared in advance was added to the corresponding cell well, and the cells were left standing in the dark at room temperature for 30 minutes, and then subjected to detection by a multifunctional microplate reader (Molecular Devices, Spectra MAXi3).

3. KD assay

The affinity (K_D) of the bispecific antibodies of the invention for binding to B7H3 and EGFR was determined using Bio-Layer Interferometry (BLI).

Half an hour before the start of the experiment, an appropriate number of AHC sensors (18-5060, Sartorius) were soaked in SD buffer (1 x PBS, 0.1% BSA, 0.05% Tween-20) depending on the number of samples. The antibody prepared above and human B7H3 (B73-H52E2, Acro Biosystem) and human EGFR (EGR-H5222, Acro Biosystem) were respectively diluted to 100 nM. SD buffer, the antibody solution, human B7H3, and human EGFR were added to 96-well black polystyrene microwell plates (Greiner, 655209), respectively. Detection was performed

using ForteBio Octet Red96e, and arranging the plate and selecting the sensor position according to the sample position. The instrument set-up parameters were as follows: the operation steps are as follows: 120 s of baseline equilibrium, 100 s of loading and immobilizing of antibodies, 120 s of baseline equilibrium, 100 s of antigen binding and 120 s of dissociation, at a speed of 1000 rpm and a temperature of 30°C. After the experiment was completed, K_D values were analyzed using ForteBio Octet analysis software.

4. Cisbio pERK (Cisbio, 64AERPEG) Activity assay

(1) 25000 cells/100 μ l were plated in 96-well white plates (Nest, 701001), and cultured for 6 hours.

(2) The antibody molecules prepared by dilution in advance (maximum concentration 300nM, 3.5-fold dilution) were added to the corresponding cell well plate, mixed well, and cultured at 37 °C with 5% CO₂ for 16 hours.

(3) The medium was removed, 50 μ l of lysine buffer was added, and the mixture was lysed by shaking for 0.5 hour.

(4) The lysate was mixed uniformly and 16 μ l were applied to HTRF 96 well cell culture plates.

(5) A mixed solution of 4 μ l Eu Cryptate antibody and d2 antibody which are prepared in advance was added into (4), and placed for 4 hours at room temperature in a dark.

(6) After 4 hours, the detection was performed with a multifunctional microplate reader (Molecular Devices, Spectra MAXi3).

Results of the experiment:

1. B7H3/EGFR bsAb sequence screening

As analyzed from the results of Figure 1 and Figure 2, the endocytosis of Hz20G5.26/Zalu bsAb and Hz5C2.9/Zalu bsAb was stronger in different tumor cells than that of Hz19A2.25/Zalu bsAb. There is little difference between the endocytosis of Hz20G5.26/Zalu bsAb and Hz5C2.9/Zalu bsAb. From the results of the proliferation inhibition assay on blocking EGFR signaling in Figures 1 and 2, among the three B7-H3/EGFR bsAb candidate molecules, Hz20G5.26/Zalu bsAb has a stronger inhibitory effect on proliferation by blocking EGFR signaling than that of Hz5C2.9/Zalu bsAb and Hz19A2.25/Zalu bsAb, and the affinity detection data in Table 1 shows that the Hz20G5.26 antibody parent has the highest affinity for B7H3 among the three B7-H3/EGFR bsAb candidate molecules.

Therefore, according to the proliferation inhibition of blocking EGFR signal and the strength of inducing tumor cell endocytosis activity as well as the affinity detection results, the Hz20G5.26/Zalu bsAb sequence is preferably used as a B7H3/EGFR bsAb-ADC preferred target sequence.

2. Action mechanism of Hz20G5.26/Zalu bsAb as B7H3/EGFR bsAb-ADC

The main action mechanism of ADC is that tumor cells transfer the payload toxin to the tumor target cells by using the strong endocytosis of a targeting antibody, thereby exerting an anti-tumor effect. We conjugated Hz20G5.26/Zalu bsAb to ZAP toxin (Fab-ZAP human (Ats-Bio, IT-51)), in a variety of tumor cell lines. According to the product instructions, the potent potential of Hz20G5.26/Zalu bsAb as B7H3/EGFR bsAb-ADC was rapidly validated. The results in Figures 3 and 4 show that Hz20G5.26/Zalu bsAb-ADC has good anti-tumor killing effect positively correlated with the expression levels of EGFR and B7H3 in various tumor cells, and meanwhile,

the results in Figures 3 and 4 show that the endocytosis effect for EGFR is stronger than that for B7H3.

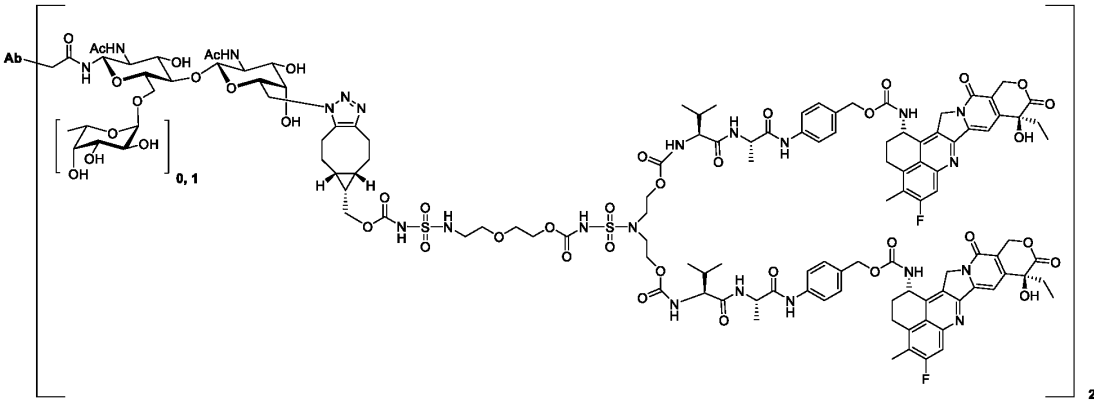
The Hz20G5.26/Zalu bsAb-ADC is designed to improve the effectiveness and safety of the B7-H3/EGFR bsAb-ADC, and meanwhile, to maintain the activity of the B7H3 antibody parent to enhance the effect of blocking EGFR signals. The results in Figure 5 shows that Hz20G5.26/Zalu bsAb significantly enhanced the effect of blocking downstream signals of EGFR, and the results in Figure 6 show that the endocytosis of Hz20G5.26/Zalu bsAb is significantly stronger than that of the parent monoclonal antibody. As shown in Figure 3 and Figure 4, the endocytosis of Zalu mAb is stronger than that of Hz20G5.26 mAb. By combining the Hz20G5.26 antibody parent with strong affinity with the Zalu antibody parent with low affinity, the toxic and side effects brought by the EGFR mAb-ADC can be reduced and the effectiveness of the B7H3 mAb-ADC can be improved, and the effectiveness and safety of the B7H3/Zalu bsAb-ADC can be comprehensively improved.

TABLE 1 B7H3/EGFR bsAb affinity assays

Antibody Name	Parent	KD(M)	Kon(1/Ms)	Kdis(1/s)
Hz20G5.26/Zalu	EGFR	4.59E-09	2.70E+05	1.24E-03
	B7H3	2.48E-09	6.55E+05	1.63E-03
Hz19A2.25/Zalu	EGFR	4.19E-09	2.89E+05	1.21E-03
	B7H3	6.73E-09	3.26E+05	2.19E-03
Hz5C2.9/Zalu	EGFR	4.69E-09	2.64E+05	1.24E-03
	B7H3	8.25E-09	4.27E+05	3.52E-03

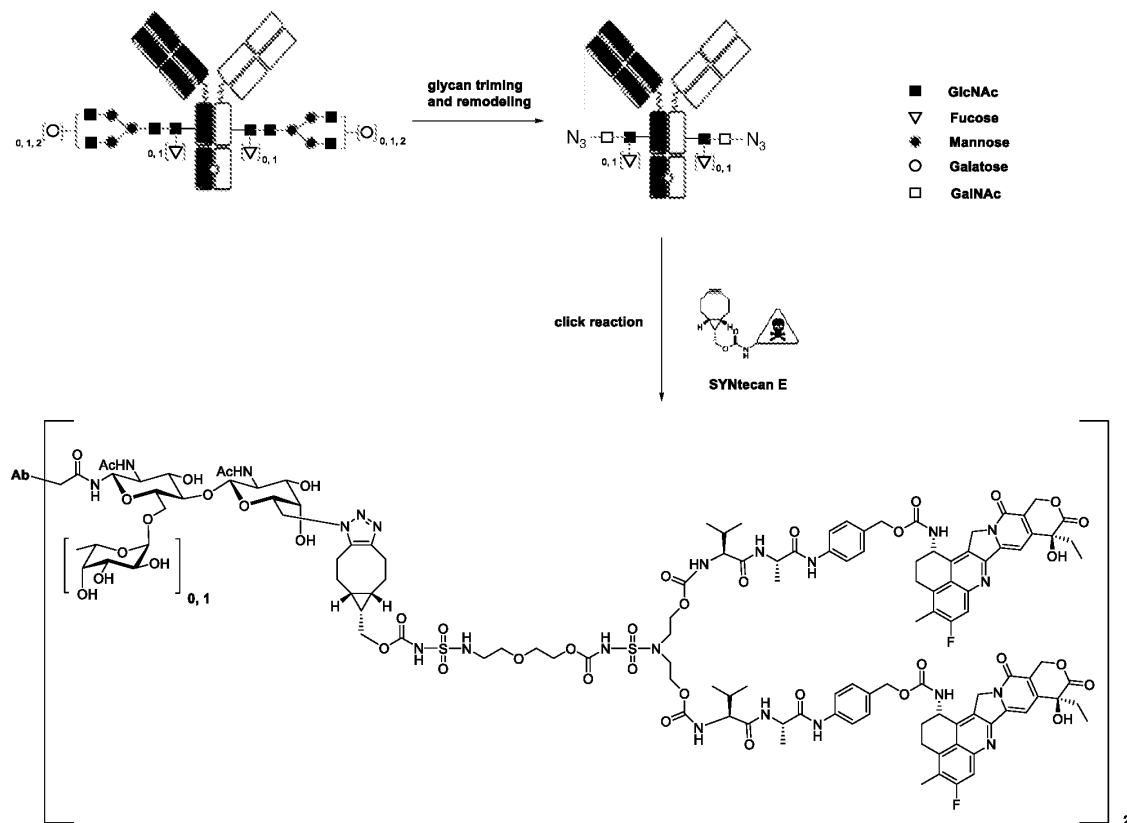
Example 2 Preparation of Hz20G5.26/Zalu bsAb-Exatecan

The Hz20G5.26/Zalu bsAb was subjected to glycosylation site-specific conjugation using glycosylation site-specific conjugation technology (see, for example, PCT/EP2017/052792 and PCT/CN2022/139637) to prepare Hz20G5.26/Zalu bsAb-Exatecan (DAR=4).



Hz20G5.26/Zalu bsAb-Exatecan (DAR=4)

The preparation process is as follows:



The Hz20G5.26/Zalu bsAb (20.5 mg) prepared in the above example was dissolved in 25 mM Tris (pH = 8.0) to a final concentration of 10 mg/mL, and the enzyme Endo-SH (the mass ratio of enzyme to antibody was 0.1% w/w), glycosyltransferase His-TnGalNAcT (to a final concentration of 0.25 mg/mL), and glycosyl donor 6-N₃-GalNAc-UDP (the molar ratio of glycosyl donor to antibody was 20:1) (see PCT/EP2017/052792) were added in sequence, and 1 M MnCl₂ solution was added (to a final concentration of 10 mM Mn²⁺) for adjustment, the reaction solution was mixed evenly and reacted at 30 °C for 20 hours.

The above reaction product (glycosylated modified antibody Hz20G5.26/Zalu bsAb-(GlcNAc(Fuc)_{0,1}-6-N₃-GalNAc)₂) was dissolved in 50 mM PB/DMF (3:1, v/v) mixed solvent (pH = 7.0), and a solution of SYNtecan E (commercially available from synaffix) in DMF was slowly added, with the molar ratio of SYNtecan E to antibody being 4:1~6:1. After mixing evenly, the mixture was reacted at 25 °C for 20 hours. Protein A affinity chromatography was used for purification and removal of small molecule impurities, and the buffer was replaced with 20 mM histidine (pH = 6.5) to obtain the target product Hz20G5.26/Zalu bsAb-Exatecan. The observed molecular weight was 150730, which was consistent with the theoretical value.

The obtained Hz20G5.26/Zalu bsAb-Exatecan was subjected to RP-HPLC analysis. The result was shown in FIG.7, and the actual DAR was measured to be 3.28.

Example 3 In vitro efficacy study of Hz20G5.26/Zalu bsAb-Exatecan

B7H3 and EGFR are highly expressed in many tumors. The in vitro efficacy of Hz20G5.26/Zalu bsAb-Exatecan was verified in different types of tumors and different tumor cells.

Example 3.1 Detection of EGFR and B7H3 expression in tumor cells

The cells were resuspended in FACS buffer, 200,000 cells were plated in 96-well plates (Corning, CLS3799-50EA), 10 µg/ml EGFR antibody (Cetuximab), 10 µg/ml B7-H3 antibody (Hz20G5.26mAb) and 10 µg/ml IgG were added to the cell plates and incubated at 4 °C for 1 hour. Then, after washing twice with PBS, the prepared APC-anti human Fc antibody (Biolegend, 410712) was added to the corresponding cell plate, and incubated at 4 °C for 30 minutes. Then, the cells were washed twice with PBS and detected by FACS (BD, Celesta).

From the experimental results shown in Figure 8, significant expression of both B7H3 and EGFR was observed in different types of tumors and different tumor cells, indicating that Hz20G5.26/Zalu bsAb-Exatecan can be used for the treatment of various types of tumors.

Example 3.2 In vitro pharmacodynamic activity of Hz20G5.26/Zalu bsAb-Exatecan in various tumor cells**2D cell killing assay**

(1) Cells were plated in the middle 60 wells of a 96-well white plate (NUNC, 136101) at 1000 cells/100 µl and incubated overnight.

(2) The pre-diluted drug (400nM, 4-fold dilution) was added to the corresponding cell plate, for 6 days cell killing and incubating.

(3) The cell killing assay continued for 6 days, and Cell-Titer reagent (Promega, G7572) prepared in advance was added to the corresponding cell wells, and the mixture was left standing at room temperature in the dark for 30 minutes, followed by detection with a multifunctional microplate reader (Molecular Devices, Spectra MAXi3).

3D cell killing assay

Some cell line related information used in the experiments is shown below:

Calu-6: ATCC, HTB-56; JIMT-1: CoBioer, CBP60378; AsPC-1: CoBioer, CBP60546;

A375: ATCC, CRL-1619TM.

NCI-H1975^{LTC}: Constructing and packaging Lentivirus + EGFR^{L858R/T790M/C797S}, the NCI-H1975 (ATCC, CRL-5908) was infected with Lentivirus + EGFR^{L858R/T790M/C797S} virus, and H1975-EGFR^{L858R/T790M/C797S} stable cell line was obtained by pressurized screening.

(1) Cells were plated at 1500 cells/100 µL in the middle 60 wells of a 96-well low adsorption plate (Corning, CLS7007-24 EA).

(2) The pre-diluted drug (400nM, 4-fold dilution) was added to the corresponding cell plate, for 6 days cell killing and incubating.

(3) The cell killing assay continued for 6 days, Cell-Titer reagent (Promega, G7572) prepared in advance was added to the corresponding cell wells and left standing at room temperature in the dark for 30 minutes.

(4) The mixture of Cell-Titer and the cells was transferred into a 96-well white bottom plate (NUNC, 136101), and detected with a multifunctional microplate reader (Molecular Devices, Spectra MAXi3).

Results of the experiment

Tumor cell killing was performed in different types of tumors and different tumor cells using Hz20G5.26/Zalu bsAb and Hz20G5.26/Zalu bsAb-Exatecan. As shown in Figure 9, the results of the experiment showed that Hz20G5.26/Zalu bsAb-Exatecan possessed both the proliferation inhibitory activity by blocking EGFR signaling and the ADC-induced target tumor cell death effect.

As shown in Figure 9 and Figure 10, Hz20G5.26/Zalu bsAb-Exatecan not only has antitumor activity in different types of lung cancer, but also has in vitro killing antitumor effect on EGFR-TKI small molecule drug-resistant cell lines caused by EGFR self mutation such as H1975 (NSCLC, EGFR^{L858R/T790M}) and EGFR downstream signal mutation such as H358 (NSCLC, EGFR^{WT}, KRAS^{G12C}), particularly has good antitumor effect on some rare EGFR mutation tumor cell lines such as H1975-EGFR (L858R/T790M/C797S) and H322-EGFR20ins (Exon20ins^{S768_D770 dup}).

The experimental result shows that Hz20G5.26/Zalu bsAb-Exatecan can be used not only for tumor cell lines expressing B7H3 and EGFR, but also for TKI small molecule drug-resistant tumor cell lines.

From the analysis of the in vitro efficacy shown in Figures 9 to 17, Hz20G5.26/Zalu bsAb-Exatecan can be used not only for the treatment of various lung cancers, but also for colorectal cancer, pancreatic cancer, breast cancer, oral squamous cell carcinoma, gastric cancer, prostate cancer, melanoma, cervical cancer, and the like.

Hz20G5.26/Zalu bsAb-Exatecan has the proliferation inhibitory activity by blocking EGFR signaling and target cell death effect induced by ADC, and in EGFR insensitive tumor cells, the overall killing result of the tumor cells only shows the cell killing effect mediated by ADC; in EGFR sensitive cells, the overall killing result shows not only the target cell death activity induced by ADC, but also the proliferation inhibition activity by blocking EGFR signals, and in the two action mechanisms, ADC plays a leading role in inducing the death of target tumor cells.

Example 4 Bystander Effect of Hz20G5.26/Zalu bsAb-Exatecan

Hz20G5.26/Zalu bsAb-Exatecan shows EGFR signal blocking activity and ADC inducing target cell death activity in EGFR sensitive tumor cells; to further verify whether Hz20G5.26/Zalu bsAb-Exatecan has ADC bystander effect, the MDA-MB-453+ EGFR + B7H3 stable cell line MDA-MB-453^{OX} with high expression of both EGFR and B7H3 was constructed on the basis of MDA-MB-453 (EGFR and B7H3 double low expression) tumor cell line.

The method of MDA-MB-453 and MDA-MB-453^{OX} killing assay is as the 2D cell killing assay in example 3.1, as analyzed from the results of the assay in Figure 18, MDA-MB-453^{WT} was not sensitive to Hz20G5.26/Zalu bsAb and Hz20G5.26/Zalu bsAb-Exatecan and was sensitive to Exatecan; MDA-MB-453^{OX} was insensitive to Hz20G5.26/Zalu bsAb, and sensitive

to Hz20G5.26/Zalu bsAb-Exatecan and Exatecan. We chose 10nM Hz20G5.26/Zalu bsAb-Exatecan, 10nM Hz20G5.26/Zalu bsAb, 10nM Exatecan for the bystander effect experiments, while setting 10nM IgG1-Exatecan and IgG1 as negative controls.

Experimental method for bystander effect

70,000 cells/MDA-MB-453^{WT} and 90,000 cells/MDA-MB-453^{OX} were plated in six-well plates and incubated overnight. The drugs or control diluted in advance was added into the corresponding cell well plate with both final concentration of 10nM, mixed evenly, for 5 days killing and culturing at 37 °C with 5% CO₂. On day 5, the cells were digested and total counts N of cells were counted and the percentage relationship between MDA-MB-453^{OX} and MDA-MB-453^{WT} was marked up by FACS (BD, Celesta) based on B7H3 expression.

Method for calculating absolute number of cells:

$$\text{MDA-MB-453}^{\text{OX}} = N * \text{FACS (B7H3}^+ \text{ - MDA-MB-453}^{\text{OX}}) * 100$$

$$\text{MDA-MB-453} = N * \text{FACS (B7H3}^- \text{ - MDA-MB-453)} * 100$$

Results of the experiment

From the results of the experiment shown in Figure 19, Hz20G5.26/Zalu bsAb-Exatecan was found to have a strong bystander effect, and the antitumor activity was further improved.

Example 5 In vivo efficacy assay of Hz20G5.26/Zalu bsAb-Exatecan

In vitro activity demonstrated that Hz20G5.26/Zalu bsAb-Exatecan had proliferation inhibitory activity by blocking EGFR signals and ADC inducing target tumor cell death activity and ADC bystander effect.

We designed NCI-H508 (CRC), JIMT-1 (BCAR), BxPC3 (PAAD) and NCI-H1975 (NSCLC) CDX mouse tumor models to validate the pharmacodynamic activity of Hz20G5.26/Zalu bsAb-Exatecan in vivo.

Example 5.1 In vivo efficacy assay of Hz20G5.26/Zalu bsAb-Exatecan in NCI-H1975 (NSCLC) CDX mouse tumor model

Experimental method

NCI-H1975 cells were subcultured, resuspended in PBS and Matrigel matrix at equal ratio, and inoculated into the right abdomen of CB17/SCID mice (purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.) at 8E6/200ul, performing grouping and tail vein dosing on day 7, with a dosing cycle of once a week, twice in total, and tumor size and body weight were measured twice a week.

Tumor growth inhibition rate (TGI%) was calculated by the following equation: $\text{TGI\%} = 100\% * (\text{control group tumor volume} - \text{treatment group tumor volume}) / (\text{control tumor group volume} - \text{control group initial tumor volume})$.

Tumor volume measurement: the maximal long axis (L) and maximal wide axis (W) of the tumor were measured using a vernier caliper and the tumor volume was calculated by the following equation: $V=L*W^2/2$

Results of the experiment

Mouse tumor growth inhibition rate calculation was performed on day 36 from NCI-H1975 CDX modeling to evaluate the in vivo efficacy of Hz20G5.26/Zalu bsAb-Exatecan.

As shown from the experimental results of Figure 20, after two administrations, once a week, it was found that 1mg/kg and 3mg/kg of Hz20G5.26/Zalu bsAb-Exatecan had significant in vivo antitumor activities, and the antitumor activity at 3mg/kg was better than 1 mg/kg.

On day 36, the tumor growth inhibition rate of the mice was calculated, and the average tumor size was 112 mm³ at a dose of 1mg/kg, the tumor growth inhibition rate was 104%; and the average tumor size was 31 mm³ at a dose of 3mg/kg, and the tumor growth inhibition rate was 109%.

Analysis of changes in body weight in the 1mg/kg and 3mg/kg groups showed that Hz20G5.26/Zalu bsAb- Exatecan was safe and effective.

Example 5.2 In vivo efficacy assay of Hz20G5.26/Zalu bsAb-Exatecan in BxPC3 (PAAD)

CDX mouse tumor model

Experimental method

BxPC3 cells were subcultured, resuspended in PBS and Matrigel matrix at equal ratio, and inoculated into the right abdomen of CB17/SCID mice at 3E6/200ul, performing grouping and tail vein dosing on day 8, single administration, tumor size and body weight were measured twice a week for the first 46 days and once a week after 46 days.

Tumor growth inhibition rate (TGI%) was calculated by the following equation: $TGI\% = 100\% * (\text{control group tumor volume} - \text{treatment group tumor volume}) / (\text{control group tumor volume} - \text{control group initial tumor volume})$.

Tumor volume determination: the maximal long axis (L) and maximal wide axis (W) of the tumor were measured using a vernier caliper and the tumor volume was calculated by the following equation: $V=L*W^2/2$

Results of the experiment

Mouse tumor growth inhibition rate calculation was performed on day 53 from BxPC3 CDX modeling to evaluate the in vivo efficacy of Hz20G5.26/Zalu bsAb-Exatecan.

As shown in Figure 21, Hz20G5.26/Zalu bsAb showed an antitumor effect by blocking EGFR signaling with a significant inhibition of BxPC3 tumor growth by a single administration, but Hz20G5.26/Zalu bsAb-Exatecan tumor-inhibiting effect was much better than that of Hz20G5.26/Zalu bsAb blocking EGFR signaling at the same dose, indicating that Hz20G5.26/Zalu bsAb-Exatecan having a multiple tumor killing mechanism had a better antitumor effect than that of Hz20g5.26/Zalu bsAb.

On day 53, the tumor growth inhibition rate of the mice was calculated, and for a dose of 1mg/kg of Hz20G5.26/Zalu bsAb-Exatecan, the average tumor size was 468 mm³, the tumor growth inhibition rate was 79%; for a dose of 3mg/kg of Hz20G5.26/Zalu bsAb, the average tumor size was 1050 mm³, the tumor growth inhibition rate was 44%; for a dose of 3mg/kg of Hz20G5.26/Zalu bsAb-Exatecan, the average tumor size was 410 mm³, the tumor growth inhibition rate was 83%; for a dose of 10mg/kg of Hz20G5.26/Zalu bsAb-Exatecan, the average tumor size was 84 mm³, the tumor growth inhibition rate was 103%.

Analysis of changes in body weight in BxPC3 CDX mouse model showed that the high dose Hz20G5.26/Zalu bsAb-Exatecan had good safety.

Example 5.3 In vivo efficacy assay of Hz20G5.26/Zalu bsAb-Exatecan in H508 (CRC) CDX mouse tumor model

Experimental method

H508 cells were subcultured, resuspended in PBS and Matrigel matrix at equal ratio, and inoculated into the right abdomen of NOG mice (purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.) at 6E6/200ul, performing grouping and tail vein dosing on day 7, the mice were administrated twice a week, for a total of 8 low-dose administrations, and tumor size and body weight were measured twice a week.

Tumor growth inhibition rate (TGI%) was calculated by the following equation: $TGI\% = 100\% * (\text{control group tumor volume} - \text{treatment group tumor volume}) / (\text{control group tumor volume} - \text{control group initial tumor volume})$.

Tumor volume measurement: the maximal long axis (L) and maximal wide axis (W) of the tumor were measured using a vernier caliper and the tumor volume was calculated by the following equation: $V = L * W^2 / 2$

Results of the experiment

Cetuximab is an EGFR monoclonal antibody used for first-line therapy for colorectal cancer, and in the H508 tumor model, we designed low-dose multiple administration regimens, and compared Hz20G5.26/Zalu bsAb and Hz20G5.26/Zalu bsAb-Exatecan with Cetuximab in term of efficacy.

As analyzed from the results in Figure 22, the antitumor activity of low-dose Cetuximab was stronger than that of Hz20G5.26/Zalu bsAb, but the antitumor activity of Hz20G5.26/Zalu bsAb-Exatecan was far superior to that of Cetuximab.

Mouse tumor growth inhibition rate calculation was performed on day 53 from H508 modeling, for a dose of 1mg/kg Cetuximab, the average tumor size was 486 mm³, and the tumor growth inhibition rate was 64%; for a dose of 1mg/kg Hz20G5.26/Zalu bsAb, the average tumor size was 687mm³, and the tumor growth inhibition rate was 40%; for a dose of 1mg/kg Hz20G5.26/Zalu bsAb-Exatecan, the average tumor size was 11 mm³, and the tumor growth inhibition rate was 121%, and complete tumor elimination occurred in 3 of 6 mice.

Analysis of the change in body weight in the mouse model showed that Hz20G5.26/Zalu bsAb-Exatecan was safe and effective with multiple low dose administrations.

Example 5.4 In vivo efficacy assay of Hz20G5.26/Zalu bsAb-Exatecan in JIMT-1 (BCAR)**CDX mouse tumor model****Experimental method**

JIMT-1 cells were subcultured, resuspended in PBS and Matrigel matrix at equal ratio, and inoculated into the right abdomen of CB17/SCID mice at 8E6/200ul, and performing grouping and tail vein dosing on day 8, single administration, and tumor size and body weight were measured twice a week.

Tumor growth inhibition rate (TGI%) was calculated by the following equation: $TGI\% = 100\% * (\text{control group tumor volume} - \text{treatment group tumor volume}) / (\text{control group tumor volume} - \text{control group initial tumor volume})$.

Tumor volume measurement: the maximal long axis (L) and maximal wide axis (W) of the tumor were measured using a vernier caliper and the tumor volume was calculated by the following equation: $V = L * W^2 / 2$

Results of the experiment

From the results in Figure 23, it was analyzed that Hz20G5.26/Zalu bsAb-Exatecan had antitumor activity at various doses in the JIMT-1 CDX mouse tumor model.

On day 28, the tumor growth inhibition rate of the mice was calculated, and for 1mg/kg dose, the average tumor size was 583 mm³, the tumor growth inhibition rate was 59%; for 3mg/kg dose, the average tumor size was 31mm³, the tumor growth inhibition rate was 107%; for 10mg/kg dose, the average tumor size was 21 mm³, the tumor growth inhibition rate was 108%, and complete tumor elimination occurred in one of five mice.

Analysis of changes in body weight in the mouse model showed that the high dose of Hz20G5.26/Zalu bsAb-Exatecan was safe.

Sequence information:

Name	SEQ ID NO	Sequence
VH of Zalutumu mab antibody	SEQ ID NO:1	QVQLVESGGGVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPG KGLEWVAVIWDDGSYKYYGDSVKGRFTISRDN SKNTLYLQM NSLRAEDTAVYYCAR <u>DGITMVRGVMKDY</u> FDYWGGQGLVTVS S
VL of Zalutumu mab antibody	SEQ ID NO:2	AIQLTQSPSSLSASVGDRVITITCRASQDISSALVWYQQKPGKAP KLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QFNSYPLTFGGGGTKVEIK
VH of Hz20G5. 26 antibody	SEQ ID NO:3	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEYIMHWVRQAP GQRLEWMGGINPGTGTTYNQKFKDRVTITVDTSASTAYME LSSLRSED TAVYYCTRTPPW HFAVWGQGLVTVSS
VL of Hz20G5. 26 antibody	SEQ ID NO:4	DIQLTQSPSFLSASVGDRVITITCSASSSVSYIHWYQQKPGKAPK RWIYDTSRLASGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQ WSSAPLTFGGGGTKVEIK
VH of Hz19A2. 25 antibody	SEQ ID NO:5	QVQLVQSGAEVKKPGASVKVSCKASGYIFTSYWIHWVRQAPG QGLEWMGRIYPGTESTFYNEKFKGRVTMTRDTSTSTVYMELS SLRSED TAVYYCHFITASDWYFDVWGQGLVTVSS
VL of Hz19A2. 25 antibody	SEQ ID NO:6	EIVLTQSPATLSLSPGERATLSCSVSSSVQSNLYWYQQKPGQA PRLLIYGTSNLASGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCY QWSSYPFTFGGQGTKLEIK
VH of Hz5C2.9 antibody	SEQ ID NO:7	QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDYVISWVRQAPG QGLEWMGEIYPRGGIIHYNEKFKARVTITADKSTSTAYMELSS LRSED TAVYYCARLWDWYFDVWGQGLVTVSS
VL of Hz5C2.9 antibody	SEQ ID NO:8	EIVLTQSPGTLTLSPGERATLSCRSSQSLVHSQGITYLDWYQQK PGQAPRLLIYKVSNRFSGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCSQGTHVPPWTFGGGGTKVEIK
HCDR1 of	SEQ ID NO:9	TYGMH (Kabat scheme)

Zalutumu mab antibody		
HCDR2 of Zalutum umab antibody	SEQ ID NO:10	VIWDDGSYKYYGDSVKG (Kabat scheme)
HCDR3 of Zalutumu mab antibody	SEQ ID NO:11	DGITMVRGVMKDY (Kabat scheme)
LCDR1 of Zalutumu mab antibody	SEQ ID NO:12	RASQDISSALV (Kabat scheme)
LCDR2 of Zalutumu mab antibody	SEQ ID NO:13	DASSLES (Kabat scheme)
LCDR3 of Zalutumu mab antibody	SEQ ID NO:14	QQFNSYPLT (Kabat scheme)
HCDR1 of Hz20G5. 26 antibody	SEQ ID NO:15	GYTFTEYIMH (AbM scheme)
HCDR2 of Hz20G5. 26 antibody	SEQ ID NO:16	GINPGTGGTTYNQKFKD (Kabat scheme)
HCDR3 of	SEQ ID NO:17	RTPPWFAV (Kabat scheme)

Hz20G5. 26 antibody		
LCDR1 of Hz20G5. 26 antibody	SEQ ID NO:18	SASSSVSYIH (Kabat scheme)
LCDR2 of Hz20G5. 26 antibody	SEQ ID NO:19	DTSRLAS (Kabat scheme)
LCDR3 of Hz20G5. 26 antibody	SEQ ID NO:20	QQWSSAPLT (Kabat scheme)
HCDR1 of Hz19A2. 25 antibody	SEQ ID NO:21	GYIFTSYWIH (AbM scheme)
HCDR2 of Hz19A2. 25 antibody	SEQ ID NO:22	RIYPGTESTFYNEKFKG (Kabat scheme)
HCDR3 of Hz19A2. 25 antibody	SEQ ID NO:23	ITASDWYFDV (Kabat scheme)
LCDR1 of Hz19A2. 25 antibody	SEQ ID NO:24	SVSSSVQSNYLY (Kabat scheme)
LCDR2 of	SEQ ID NO:25	GTSNLAS (Kabat scheme)

Hz19A2. 25 antibody		
LCDR3 of Hz19A2. 25 antibody	SEQ ID NO:26	YQWSSYPFT (Kabat scheme)
HCDR1 of Hz5C2.9 antibody	SEQ ID NO:27	GYTFSDYVIS (AbM scheme)
HCDR2 of Hz5C2.9 antibody	SEQ ID NO:28	EIYPRGGIIHYNEKFKA (Kabat scheme)
HCDR3 of Hz5C2.9 antibody	SEQ ID NO:29	ARLWDWYFDV (Kabat scheme)
LCDR1 of Hz5C2.9 antibody	SEQ ID NO:30	RSSQSLVHSQGITYLD (Kabat scheme)
LCDR2 of Hz5C2.9 antibody	SEQ ID NO:31	KVSNRFS (Kabat scheme)
LCDR3 of Hz5C2.9 antibody	SEQ ID NO:32	SQGTHVPPWT (Kabat scheme)
HC1 of HZ205.26 /Zalu bispecific antibody	SEQ ID NO:33	QVQLVESGGGVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPG KGLEWVAVIWDDGSYKYYGDSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARDGITMVRGVMKDYFDYWGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE

		KTISKAKGQPREPQVTTLPSPRDELTKNQVSLTCLVSGFYPSDIA VEWESNGQPENNYKTTTPVLDSGDSFFLYSDLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK
LC1 of HZ205.26 /Zalu bispecific antibody	SEQ ID NO:34	AIQLTQSPSSLSASVGDRVTITCRASQDISSALVWYQQKPGKAP KLLIYDASSLESGVPSRFGSGESGTDFTLTISLQPEDFATYYCQQ FNSYPLTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSST LTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
HC2 of HZ205.26 /Zalu bispecific antibody	SEQ ID NO:35	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEYIMHWVRQAPG QRLEWMGGINPGTGGTTYNQKFKDRVTITVDTSASTAYMELSS LRSEDTAVYYCTRTPPWFAVWGQGTLLTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVELTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSRDELTKNQVRLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
LC2 of HZ205.26 /Zalu bispecific antibody	SEQ ID NO:36	DIQLTQSPSFLSASVGDRVTITCSASSSVSYIHWHYQQKPGKAPKR WIYDTSRLASGVPSRFGSGSGTEFTLTISLQPEDFATYYCQQW SSAPLTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
HC1 of Hz19A2. 25/Zalu bispecific antibody	SEQ ID NO:33	QVQLVESGGGVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPG KGLEWVAVIWDDGSYKYYGDSVKGRFTISRDNKNTLYLQMN SLRAEDTAVYYCARDGITMVRGVMKDYFDYWGQGTLLTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVTTLPSPRDELTKNQVSLTCLVSGFYPSDIA VEWESNGQPENNYKTTTPVLDSGDSFFLYSDLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK
LC1 of Hz19A2. 25/Zalu bispecific antibody	SEQ ID NO:34	AIQLTQSPSSLSASVGDRVTITCRASQDISSALVWYQQKPGKAP KLLIYDASSLESGVPSRFGSGESGTDFTLTISLQPEDFATYYCQQ FNSYPLTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSST LTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
HC2 of	SEQ ID	QVQLVQSGAEVKKPGASVKVSCKASGYIFTSYWIHWVRQAPG

Hz19A2.25/Zalu bispecific antibody	NO:37	QGLEWMGRIYPGTESTFYNEKFKGRVTMTRDTSTSTVYMELSS LRSEDTAVYYCHFITASDWYFDVWGQGLTVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSKVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVELTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSRDELTKNQVRLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
LC2 of Hz19A2.25/Zalu bispecific antibody	SEQ ID NO:38	EIVLTQSPATLSLSPGERATLSCSVSSSVQSNLYWYQQKPGQA PRLLIYGTSNLAGSIPARFSGSGGTDFTLTISSLEPEDFAVYYCY QWSSYPFTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSLSS TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
HC1 of Hz5C2.9/Zalu bispecific antibody	SEQ ID NO:33	QVQLVESGGGVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPG KGLEWVAVIWDDGSYKYYGDSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARDGITMVRGVMKDYFDYWGQGLTVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSKVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVTTLPSPSRDELTKNQVSLTCLVSGFYPSDIA VEWESNGQPENNYKTTTPVLDSGDSFFLYSDLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK
LC1 of Hz5C2.9/Zalu bispecific antibody	SEQ ID NO:34	AIQLTQSPSSLSASVGDRVITTCRASQDISSALVWYQQKPGKAP KLLIYDASSLESGVPSRFSGSESGTDFTLTISSLQPEDFATYYCQQ FNSYPLTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSLSS TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
HC2 of Hz5C2.9/Zalu bispecific antibody	SEQ ID NO:39	QVQLVQSGAEVKKPGSSVKVCKASGYTFSDYVISWVRQAPG QGLEWMGEIYPRGGIIHYNEKFKARVTITADKSTSTAYMELSSL RSEDTAVYYCARLWDWYFDVWGQGLTVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSKVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVELTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSRDELTKNQVRLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA

		LHNHYTQKSLSLSPGK
LC2 of Hz5C2.9/ Zalu bispecific antibody	SEQ ID NO:40	EIVLTQSPGTLSPGERATLSCRSSQSLVHSQGITYLDWYQQKP GQAPRLLIYKVSNRFSGIPDRFSGSGSGTDFTLTISRLEPEDFAVY YCSQGTHVPPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
Wild-type IgG1- CH1 (with partial hinge region)	SEQ ID NO:41	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSC
Wild-type IgG1- CH1	SEQ ID NO:42	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKV
Hinge region	SEQ ID NO:43	EPKSCDKTHTCPPCP
Wild-type IgG1- CH2	SEQ ID NO:44	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAK
Wild-type IgG1- CH3	SEQ ID NO:45	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
Wild-type IgG1-Fc CH2+CH 3 (with no hinge region)	SEQ ID NO:46	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSG DSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSP GK
Wild-type IgG1-Fc CH2+CH 3 (with	SEQ ID NO:47	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYT

hinge region)		QKSLSLSPGK
CH3 mutation (CH3 of Fc portion linked to EGFR antigen-binding region) K370S, Y349T, K409D	SEQ ID NO:48	GQPREPQVTTLPSPRDELTKNQVSLTCLVSGFYPSDIAVEWESN GQPENNYKTTPVLDSGDSFFLYSDLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
Fc region with K370S, Y349T, K409D (with no hinge region)	SEQ ID NO:49	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVTTLPSPRDELTKNQVSLTCLVSGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSDLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPG K
Fc region with K370S, Y349T, K409D (with hinge region)	SEQ ID NO:50	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVTT LPPSRDELTKNQVSLTCLVSGFYPSDIAVEWESNGQPENNYKTT PPVLDSGDSFFLYSDLTVDKSRWQQGNVFSCSV MHEALHNHYT QKSLSLSPGK
CH3 , comprising S364R, D399K	SEQ ID NO:51	GQPREPQVYTLPPSRDELTKNQVRLTCLVKGFYPSDIAVEWESN GQPENNYKTTPVLKSDGDSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
Fc region	SEQ ID	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK

with S364R, D399K (with no hinge region)	NO:52	FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL KNQVRLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
Fc region with S364R, D399K (with hinge region)	SEQ ID NO:53	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVRLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
Light chain constant region	SEQ ID NO:54	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC

Claims

1. An antibody-drug conjugate of formula (I)



or a pharmaceutically acceptable salt or solvate thereof:

wherein:

Ab is a bispecific antibody or a fragment thereof that specifically binds to B7H3 and EGFR (e.g., human B7H3 and human EGFR);

L is a linker;

D is a drug, preferably an anti-tumor compound; and

p is an integer selected from 1 to 20, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

r is an integer selected from 1-5, preferably 1 or 2.

2. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of claim 1,

wherein Ab in formula (I) is a bispecific antibody that specifically binds to B7H3 and EGFR comprising a first antigen-binding region that specifically binds to EGFR and a second antigen-binding region that specifically binds to B7H3, wherein the second antigen-binding region that specifically binds to B7H3 comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein

(i) HCDR1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 15, HCDR2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 16, HCDR3 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 17,

LCDR1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 18, LCDR2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 19, LCDR3 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 20; or,

(ii) HCDR1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 21, HCDR2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 22, HCDR3 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 23,

LCDR1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 24, LCDR2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 25, LCDR3 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 26; or,

(iii) HCDR1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 27, HCDR2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 28, HCDR3 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 29,

LCDR1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 30, LCDR2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 31, and LCDR3 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 32.

3. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of claim 2, wherein the second antigen-binding region that specifically binds to B7H3 comprises VH and

VL, wherein

(i) the VH comprises or consists of the amino acid sequence set forth in SEQ ID NO: 3 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 3 and the VL comprises or consists of the amino acid sequence set forth in SEQ ID NO: 4 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 4;

(ii) The VH comprises or consists of the amino acid sequence set forth in SEQ ID NO: 5 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 5 and the VL comprises or consists of the amino acid sequence set forth in SEQ ID NO: 6 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 6; or

(iii) The VH comprises or consists of the amino acid sequence set forth in SEQ ID NO: 7 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 7 and the VL comprises or consists of the amino acid sequence set forth in SEQ ID NO: 8 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 8.

4. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of any one of claims 1-3, wherein the second antigen-binding region that specifically binds to B7H3 comprises a VH and a VL, wherein VH and VL comprise or consist of, respectively, the amino acid sequences shown as follows:

SEQ ID NO: 3 and SEQ ID NO: 4;

SEQ ID NO: 5 and SEQ ID NO: 6; or

SEQ ID NO: 7 and SEQ ID NO: 8.

5. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of any one of claims 1-4, wherein the first antigen-binding region that specifically binds to EGFR comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein

HCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 9; HCDR2 comprises or consists of the amino acid sequence of SEQ ID NO: 10; HCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 11; and the LCDR1 of the first antigen-binding region comprises or consists of the amino acid sequence of SEQ ID NO: 12; LCDR2 comprises or consists of the amino acid sequence of SEQ ID NO: 13; and LCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 14.

6. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of anyone of claims 1-5, wherein the first antigen-binding region that specifically binds to EGFR comprises a VH comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 1 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 1, and a VL comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 2 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 2.

7. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of anyone of claims 1-6, wherein the first antigen-binding region that specifically binds to EGFR comprises a VH and a VL, wherein VH and VL comprise or consist of, respectively, the amino acid sequences shown as follows: SEQ ID NO: 1 and SEQ ID NO: 2.

8. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of any one of claims 1-7, wherein the bispecific antibody comprises a first Fc region and a second Fc region, wherein the first Fc region and the second Fc region are the same or different.

9. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of claim 8, wherein the first Fc region and the second Fc region are, respectively, human IgG Fc, e.g., human IgG1 Fc, human IgG2 Fc, human IgG3 Fc, or human IgG4 Fc, e.g., comprise or consist of amino acid sequence SEQ ID NO: 46 or 47 or an amino acid sequence having at least 90% identity, e.g., 95%, 96%, 97%, 99% or more identity thereto.

10. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of claim 8 or 9, wherein mutations that promote heterodimerization of the first Fc region and the second Fc region are introduced into the first Fc region and the second Fc region of the bispecific antibody.

11. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of claim 10, wherein the mutation(s) of the Fc region are introduced based on the Innobody technology.

12. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of claim 11, wherein the CH3 of one Fc region comprises the S364R and D399K mutations and the CH3 of the other Fc region comprises the Y349T, K370S, and K409D mutations.

13. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of claim 11, wherein

a) One Fc region polypeptide comprises or consists of the amino acid sequence set forth in SEQ ID NO: 49 or 50 and the other Fc region polypeptide comprises or consists of the amino acid sequence set forth in SEQ ID NO: 52 or 53;

b) One Fc region polypeptide comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to the amino acid sequence set forth in SEQ ID NO: 49 or 50, and the other Fc region polypeptide comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to the amino acid sequence set forth in SEQ ID NO: 52 or 53.

14. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of claim 10, wherein the mutation(s) of the Fc region are introduced based on the Knob-into-Hole technology, wherein the corresponding Knob mutation(s) and Hole mutation(s) are introduced in the first Fc region and the second Fc region, respectively.

15. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of any one of claims 8-14, wherein the first antigen-binding region of the bispecific antibody is a Fab fragment, and/or the second antigen-binding region is a Fab.

16. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of claim 15, wherein the Fab, which is the first antigen-binding region or the second antigen-binding region, comprises CH1, wherein the CH1 is CH1 from IgG1, IgG2, IgG3, or IgG4, preferably CH1 from IgG1.

17. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of

claim 16, wherein the CH1

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence of SEQ ID NO: 41; or

(ii) comprises or consists of an amino acid sequence of SEQ ID NO: 41.

18. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of any one of claims 15-17, wherein the Fab, which is the first antigen-binding region or the second antigen-binding region, comprises a light chain constant region, wherein the light chain constant region is a Kappa light chain constant region or a Lambda light chain constant region.

19. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of claim 18, wherein the Kappa light chain constant region

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence of SEQ ID NO: 54; or

(ii) comprises or consists of an amino acid sequence of SEQ ID NO: 54.

20. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of any one of claims 15-19, wherein the first antigen-binding region that specifically binds to EGFR comprises a first Fab linked at its C-terminus of CH1 to the N-terminus of the first Fc region (with or without a connector, e.g., a hinge region) and the second antigen-binding region that specifically binds B7-H3 comprises a second Fab linked at its C-terminus of CH1 to the N-terminus of the second Fc region (with or without a connector, e.g., a hinge region).

21. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of claim 20, wherein the bispecific antibody comprises a first Fab as a first antigen-binding region that specifically binds to EGFR and a second Fab as a second antigen-binding region that specifically binds to B7H3, wherein the bispecific antibody comprises or consists of the follows:

Heavy chain 1: from N-terminus to C-terminus comprises or consists of: a heavy chain variable region of a Fab that specifically binds to EGFR-heavy chain constant region CH1-a first Fc region, wherein heavy chain constant region CH1 is linked at its C-terminus to the N-terminus of the first Fc region, with or without a connector (e.g., hinge region);

Light chain 1: from N-terminus to C-terminus comprises or consists of: a light chain variable region of a Fab that specifically binds to EGFR -light chain constant region;

Heavy chain 2: from N-terminus to C-terminus comprises or consists of: a heavy chain variable region of a Fab that specifically binds to B7H3-heavy chain constant region CH1-a second Fc region, wherein heavy chain constant region CH1 is linked at its C-terminus to the N-terminus of the second Fc region, with or without a connector (e.g., hinge region);

Light chain 2: from N-terminus to C-terminus comprises or consists of: the light chain variable region of a Fab which specifically binds to B7-H3-light chain constant region,

preferably, each domain is linked directly;

optionally, the first Fc region comprises mutations K370S, Y349T and K409D and the second Fc region comprises S364R and D399K.

22. The antibody-drug conjugate, or a pharmaceutically acceptable salt or solvate thereof of

claim 21, wherein

Heavy chain 1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 33 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 33, and light chain 1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 34 or an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence of SEQ ID NO: 34; and heavy chain 2 and light chain 2 comprise the amino acid sequences set forth in SEQ ID NOs as follows or an amino acid sequences having at least 85%, 90%, 95%, 97%, 98%, 99% identity thereto, or consist of the amino acid sequences set forth in SEQ ID Nos as follows:

- i) SEQ ID NO: 35 and SEQ ID NO: 36;
- ii) SEQ ID NO: 37 and SEQ ID NO: 38;
- iii) SEQ ID NO: 39 and SEQ ID NO: 40.

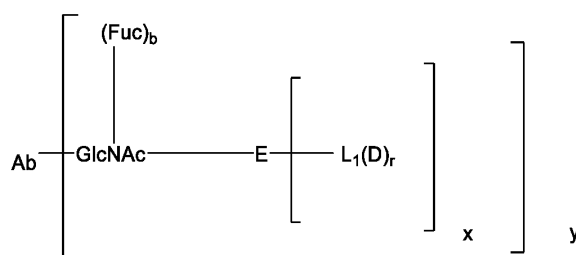
23. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of claims 1 to 22, wherein the linker is MC-VC-PAB, vc-PAB, SMCC or MC-GGFG.

24. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of claims 1 to 23, wherein the linker is a linker capable of achieving site-specific conjugation, preferably a linker connected to an antibody oligosaccharide, preferably, the oligosaccharide linker is a linker obtained by reacting with a reactive group (e.g., an azide group) on an N-saccharide chain at a conserved N-glycosylation site (e.g., Asn297) on an antibody Fc domain to achieve site-specific conjugation.

25. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof of any one of claims 1 to 24, wherein the anti-tumor compound is a cytotoxic agent or a chemotherapeutic agent, such as camptothecin compounds, auristatins, maytansinoids, taxanes, anthracyclines, vinca alkaloids, MEK inhibitors, KSP inhibitors.

26. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to claim 25 wherein the anti-tumor compound is select from Exatecan, DXd, MMAE or DM1, paclitaxel, docetaxel, epothilone, mitomycin, combretastatin, calicheamicin, duocarmycin, tubulysin, amatoxin, and bleomycin.

27. An antibody-drug conjugate of formula (III) or a pharmaceutically acceptable salt or solvate thereof



Formula (III)

wherein

Ab is as defined in any one of claims 1 to 22,

L_1 is a linker;

E is a sugar or a sugar derivative, preferably selected from galactose (Gal), mannose (Man), N-acetylglucosamine (GlcNAc), glucose (Glc), N-acetylgalactosamine (GalNAc), glucuronic acid (Gcu), fucose (Fuc) and N-acetylneuraminic acid (sialic acid);

and GlcNAc is N-acetylglucosamine, Fuc is fucose;

D and r are as defined in Formula I according to any one of claims 1 to 26;

b is 0 or 1;

x is 1, 2, 3 or 4;

y is an integer selected from 1 to 10.

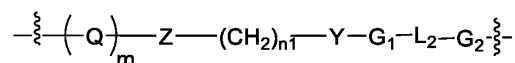
28. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to claim 27, wherein x is 1 or 2; and y is 1, 2, 3 or 4; preferably, x is 1 and y is 2.

29. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to claim 27, wherein E is GalNAc.

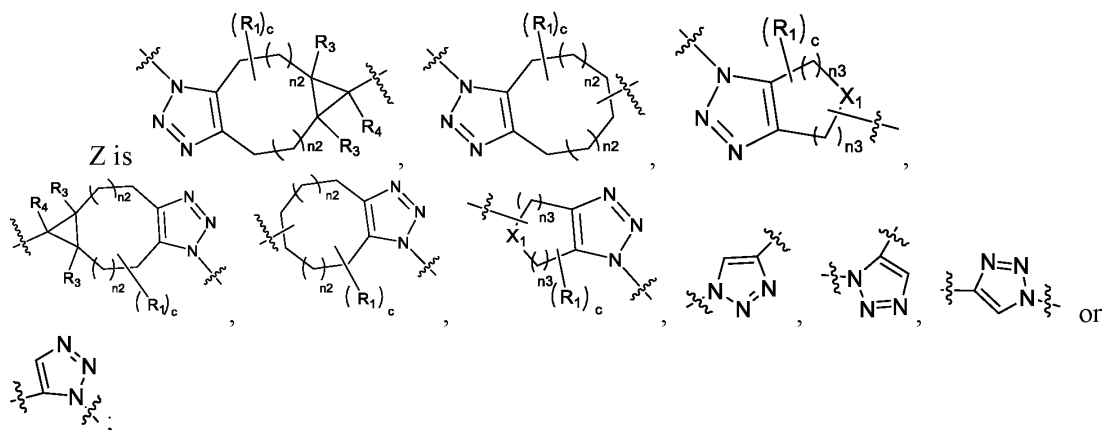
30. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of claims 27-29, wherein E is 6-deoxy-2-acetamidogalactose and is connected to L₁ via the C atom at position C6.

31. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of claims 27-29, wherein the GlcNAc linked to Ab is present at the Asn297-glycosylation site of the two heavy chains of the antibody.

32. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of claims 27 to 31, wherein L₁ is represented by the following structure



Q is -NHC(O)CH₂- or -CH₂-;



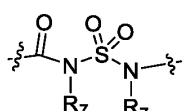
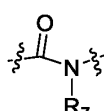
R₁ is independently selected from hydrogen, halogen, -OR₂, -NO₂, -CN, -S(O)₂R₂, C₁-C₁₂ alkyl, C₆-C₂₀ aryl, 5-20 membered heteroaryl, C₁-C₁₂ alkyl-C₆-C₂₀ aryl, C₁-C₁₂ alkyl-5-20

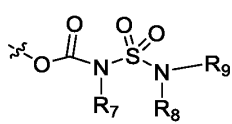
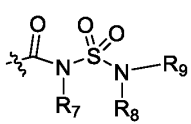
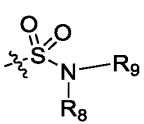
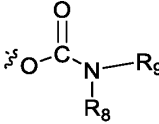
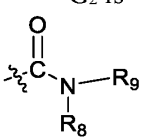
membered heteroaryl, C₆-C₂₀ aryl-C₁-C₁₂ alkyl and 5-20 membered heteroaryl-C₁-C₁₂ alkyl, and wherein the alkyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, arylalkyl and heteroarylalkyl are optionally substituted, wherein two substituents R₁ may be linked together to form a fused, bridged or spiro-connected C₃-C₁₈ cycloalkyl or a fused or spiro-connected aromatic or heteroaromatic substituent, and wherein R₂ is independently selected from hydrogen, C₁-C₂₄ alkyl, C₆-C₂₀ aryl, 5-20 membered heteroaryl, C₁-C₁₂ alkyl-C₆-C₂₀ aryl, C₁-C₁₂ alkyl-5-20 membered heteroaryl, C₆-C₂₀ aryl-C₁-C₁₂ alkyl and 5-20 membered heteroaryl-C₁-C₁₂ alkyl;

R₃ and R₄ are each independently selected from hydrogen, halogen, C₁-C₂₄ alkyl, C₆-C₂₀ aryl, 5-20 membered heteroaryl, C₁-C₁₂ alkyl-C₆-C₂₀ aryl, C₁-C₁₂ alkyl-5-20 membered heteroaryl, C₆-C₂₀ aryl-C₁-C₁₂ alkyl and 5-20 membered heteroaryl-C₁-C₁₂ alkyl;

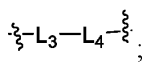
Y is -O-, -S-, -NR₂- or absent;

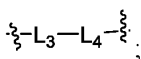
X₁ is -O-, -S- or -NR₂-;

G₁ is , -C(O)-,  or a direct bond;

G₂ is , , ,  or ;

R₇ is independently selected from hydrogen and C₁-C₁₂ alkyl;

R₈ is hydrogen, C₁-C₁₂ alkyl or ;

R₉ is .

L₂ and L₃ are independently C₁-C₁₂ alkylene, and one or more (e.g. 1, 2, 3 or 4) carbon atoms in the alkylene are optionally replaced by heteroatoms selected from O, N and S, provided that the O, N and/or S are not directly connected to each other;

L₄ is independently a cleavable spacer;

n₁ is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

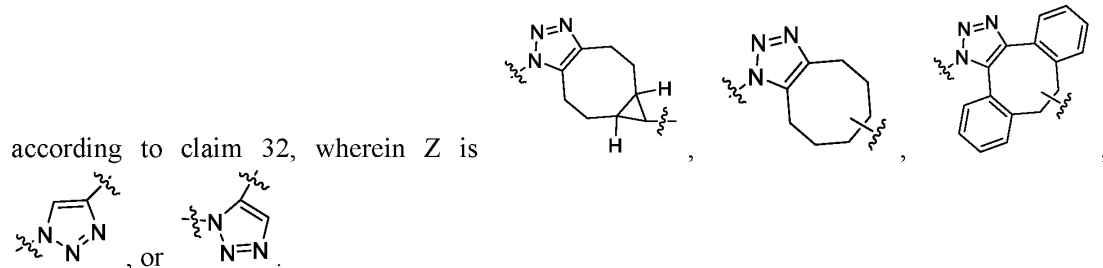
n₂ is independently 0, 1 or 2;

n₃ is independently 0, 1, 2, 3, 4 or 5;

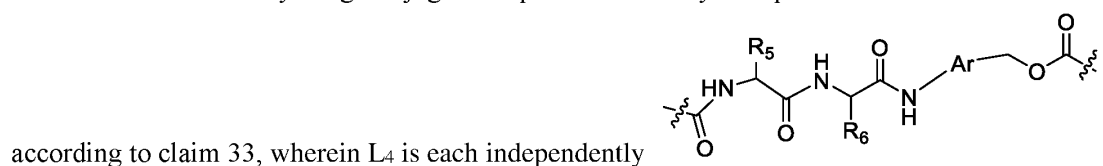
c is 0, 1, 2, 3 or 4;

m is 0 or 1.

33. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof



34. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof

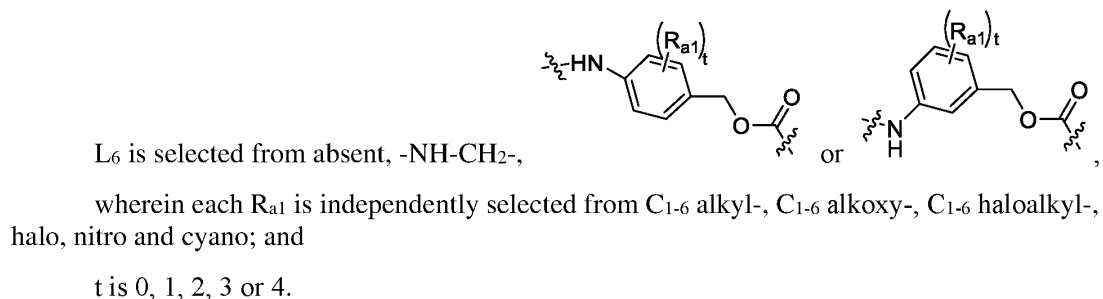


wherein R₅ and R₆ are each independently selected from hydrogen, C₁-C₁₂ alkyl or - (CH₂)₃NHC(O)NH₂; and

Ar is selected from arylene; or

L₄ is each independently -CO-L₅-L₆-,

wherein L₅ is a peptide residue consisting of 2, 3, 4, or 5 amino acids; wherein the amino acid is selected from glycine, alanine, valine, glutamine, glutamic acid, phenylalanine, leucine, tyrosine, lysine, citrulline, serine, tryptophan, aspartic acid, asparagine, isoleucine, arginine and proline;



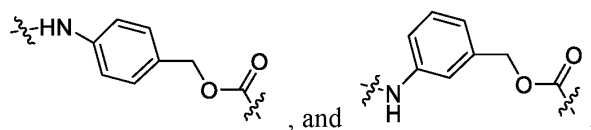
35. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to claim 34,

wherein L₄ is independently -CO-L₅-L₆-,

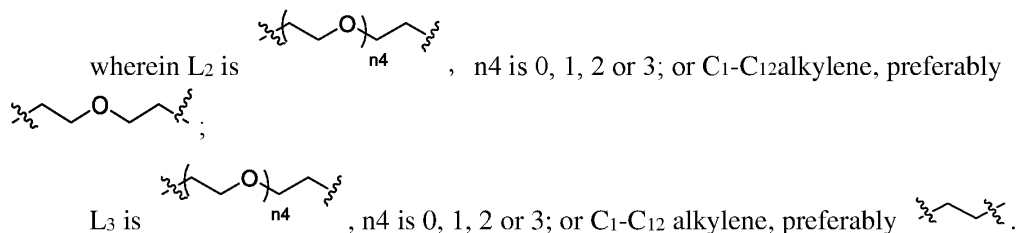
L₅ is selected from: -Val-Ala-, -Gln-Val-Ala-, -Gly-Val-Ala-, -Gln-Phe-Ala-, -Gly-Phe-Ala-, -Gly-Gly-Phe-Gly-, -Val-Cit-, -Ala-Ala-, -Ala-Cit-, -Ala-Lys-, -Ala-Val-, -Asn-Cit-, -Asp-Cit-, -Asn-Lys-, -Asp-Val-, -Cit-Ala-, -Cit-Asn-, -Cit-Asp-, -Cit-Cit-, -Cit-Lys-, -Cit-Ser-, -Cit-Val-, -Glu-Val-, -Glu-Gly-, -Ile-Cit-, -Ile-Pro-, -Ile-Val-, -Leu-Cit-, -Lys-Cit-, -Phe-Arg-, -Phe-Cit-, -Phe-Lys-, -Pro-Lys-, -Ser-Cit-, -Trp-Cit-, -Ala-Val-, -Val-Asp-, -Cit-Val-, -Val-Glu-, -Val-Lys-, -Gly-Gly-Gly-, -Gly-Gly-Arg-, -Phe-Lys-Gly-, -Leu-Lys-Gly-, -Leu-Leu-Gly-, -Glu-Val-Cit-, -Cit-Ala-Glu-, -Val-Lys-Gly-, -Val-Lys-Ala-, -Val-Gly-Gly-, -Val-Cit-Gly-, -Val-Gln-Gly-, -Val-Glu-Gly-, -Val-Lys-Gly-, -Val-Lys-Leu-, -Ala-Ala-Ala-, -Asn-Ala-Ala-, -Gly-Gly-Gly-Gly-, -Gly-Gly-

Leu-Gly-, -Gly-Phe-Leu-Gly-, -Gly-Val-Lys-Gly-, -Ala-Leu-Ala-Leu-, -Gly-Phe-Leu-Gly-, -Ala-Leu-Ala-Leu-, -Gly-Phe-Gly-Gly- and -Val-Lys-Gly-Gly; and

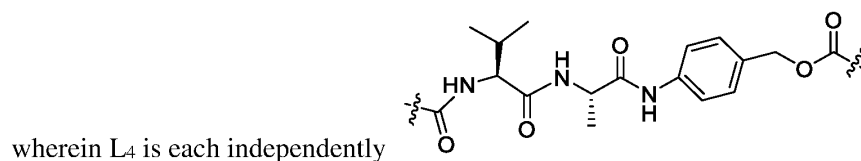
L_6 is selected from $-NH-CH_2-$,



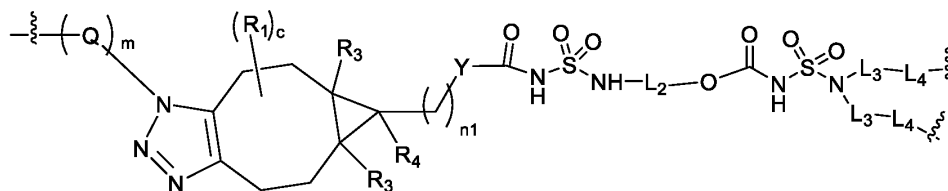
36. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of claims 32 to 35,



37. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of claims 32 to 36,

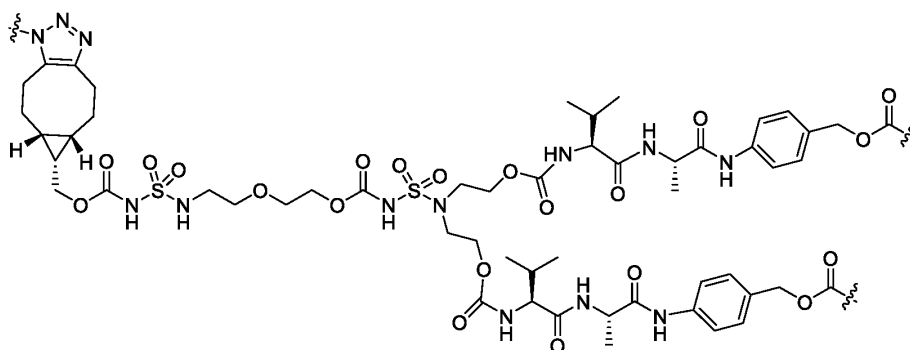


38. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of claims 32 to 37, wherein $-L_1-$ is represented by the following structure

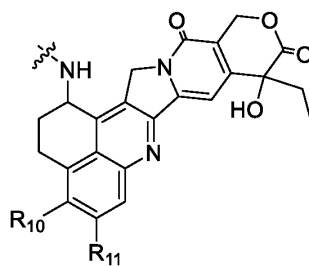


wherein Q , R_1 , R_2 , R_3 , R_4 , c , m , n_1 , Y , L_2 , L_3 and L_4 are as defined in any one of claims 32 to 37.

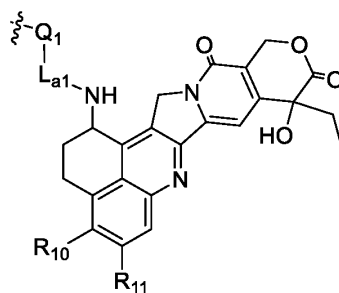
39. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to claim 32, wherein $-L_1-$ is represented by the following structure



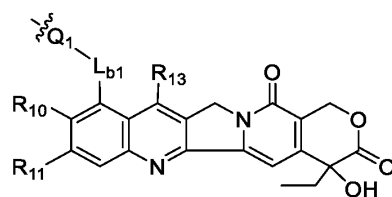
40. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of claims 1 to 39, wherein D is represented by formula (D-1a), formula (D-1b), formula (D-1c), or formula (D-1d):



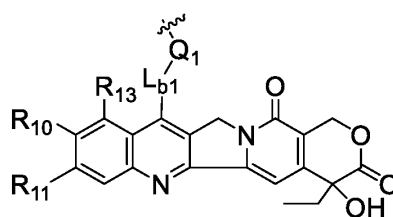
(D-1a)



(D-1b)



(D-1c)



(D-1d)

each R_{10} is independently selected from H, halo, C_1 - C_6 alkyl, C_1 - C_6 haloalkyl, $-OR_{12}$ and $-SR_{12}$; R_{11} is selected from H, halo, CN, C_1 - C_6 alkyl, C_1 - C_6 haloalkyl and $-OR_{12}$; or R_{10} and R_{11} together form $-O(CH_2)_{n5}O-$ or $-O(CF_2)_{n5}O-$, wherein $n5$ is 1 or 2;

each R_{12} is independently selected from H or C_1 - C_4 alkyl;

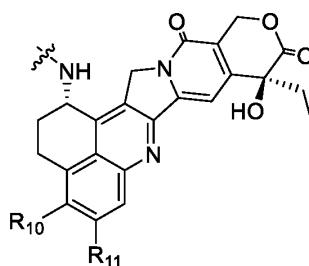
wherein each R_{13} is independently selected from H, C_1 - C_6 alkyl, C_1 - C_6 alkoxy, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_1 - C_6 haloalkyl, C_2 - C_6 haloalkenyl and C_2 - C_6 haloalkynyl;

each Q_1 is independently $-O-$ or $-S-$;

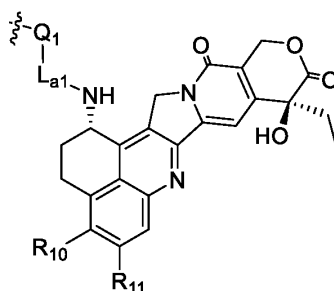
L_{a1} is $-(C_1-C_{10}alkylene)C(O)-$;

each L_{b1} is independently $-(C_1-C_{10}alkylene)-$, $-(C_1-C_{10}alkylene)-C(O)N(R_{14})-$ or $-(C_1-C_{10}alkylene)-N(R_{14})C(O)-$, wherein, R_{14} is H or C_1 - C_6 alkyl;

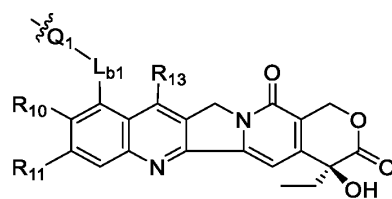
preferably, D is represented by formula (D-2a), (D-2b), (D-2c) or (D-2d):



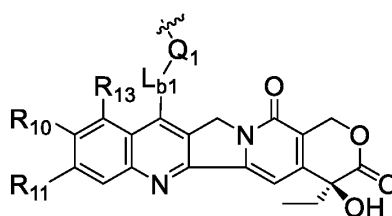
(D-2a)



(D-2b)



(D-2c)



(D-2d)

R₁₀ is C₁-C₆alkyl or C₁-C₄alkoxy, preferably methyl or methoxy, and R₁₁ is halo, preferably F;

or R₁₀ and R₁₁ together form -OCH₂O-;

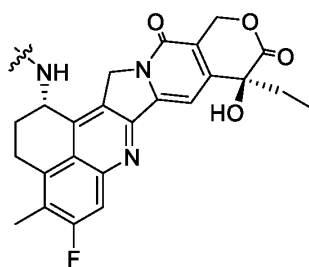
R₁₃ is H;

each Q₁ is independently -O- or -S-;

L_{a1} is -(C₁-C₄alkylene)C(O)-; and

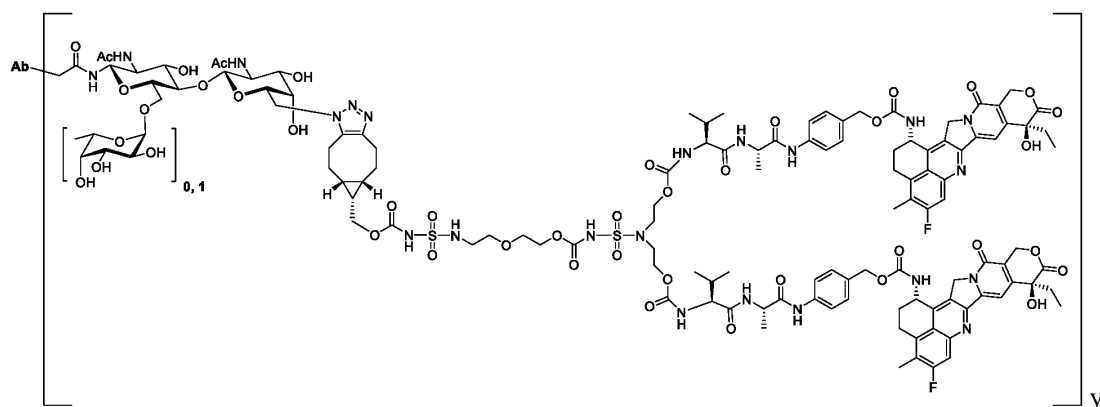
L_{b1} is -(C₁-C₆alkylene)-;

more preferably, D is represented by formula (D-3a):



41. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of claims 1 to 40, which has an average DAR of 1-10, such as 3-5.

42. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to claim 27, wherein the antibody-drug conjugate is selected from:



wherein Ab is a bispecific antibody that specifically binds to EGFR and B7H3, comprising heavy chain 1, light chain 1, heavy chain 2 and light chain 2, or consisting thereof,

wherein, heavy chain 1 comprises an amino acid sequence as shown in SEQ ID NO:33, and light chain 1 comprises an amino acid sequence as shown in SEQ ID NO:34,

heavy chain 2 comprises an amino acid sequence as shown in SEQ ID NO:35, and light chain 2 comprises an amino acid sequence as shown in SEQ ID NO:36;

y is 1 or 2, preferably 2;

preferably, the antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof has an average DAR of 2-6 or 3-4.

43. A pharmaceutical composition comprising the antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of claims 1 to 42, and optionally one or more other therapeutic agents, such as chemotherapeutic agents, angiogenesis inhibitors, cytokines, cytotoxic agents, other antibodies, small molecule drugs or immunomodulators, and optionally pharmaceutically acceptable excipients.

44. A pharmaceutical combination comprising the antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of claims 1 to 42, and one or more other therapeutic agents, such as chemotherapeutic agents, angiogenesis inhibitors, cytokines, cytotoxic agents, other antibodies, small molecule drugs or immunomodulators.

45. A method for preventing or treating a tumor in a subject, comprising administering to the subject an effective amount of the antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of claims 1 to 42, or the pharmaceutical composition according to claim 43, or the pharmaceutical combination according to claim 44.

46. The method according to claim 45, wherein the tumor is a cancer, preferably, the cancer has an elevated level (e.g., nucleic acid or protein level) of B7H3 and/or EGFR, preferably, the tumor cells of the subject moderately express or highly express B7H3 and/or EGFR, and the cancer is, for example, an epithelial cancer or a digestive tract cancer, such as gastric cancer, pancreatic cancer, intestinal cancer, colon cancer, colorectal cancer, head and neck squamous cell carcinoma, gastric adenocarcinoma, breast cancer, oral squamous cell carcinoma, prostate cancer, melanoma, cervical cancer, lung cancer (e.g., small cell lung cancer and non-small cell lung cancer), esophageal cancer, kidney cancer, bladder cancer, ovarian cancer, and head and neck cancer.

47. The method according to any one of claims 45-46, wherein the method further comprises administering to the patient one or more therapies, such as treatment modalities and/or other therapeutic agents, preferably, the treatment modality includes radiotherapy or surgery, or the therapeutic agent includes a chemotherapeutic agent, angiogenesis inhibitor, cytokine, cytotoxic agent, other antibody, small molecule drug, or immunomodulator.