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(54) Title: A METHOD FOR TREATMENT OF SOLID TUMORS WITH AN ANTIBODY-DRUG CONJUGATE TARGETING CLAUDIN 18.2

(57) Abstract: Provided are a method of treating solid tumors by administering an antibody-drug conjugate targeting Claudin18.2, or a pharmaceutically acceptable salt thereof, to a subject in need thereof, as well as single dose units, kits of parts and uses thereof comprising the same.

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A method for treatment of solid tumors with an antibody-drug conjugate targeting Claudin 18.2

Technical field

The present invention relates to the field of disease treatment. Specifically, the present invention relates to a method for treating a solid tumor using an antibody-drug conjugate targeting Claudin18.2 or a pharmaceutically acceptable salt thereof.

Background of the invention

Gastric cancer is one of the most common malignant tumors in the world, and it is one of the main causes of death due to cancer worldwide. According to GLOBOCAN data, in 2020, the incidence rate and mortality of gastric cancer ranked the fifth and the fourth respectively, and the 5-year survival rate of patients with metastatic gastric cancer was less than 5%. Currently, surgical resection remains the only curative treatment for gastric cancer, and the chemotherapy with a combination of fluoropyrimidine and a platinum chemotherapeutic agent is the standard treatment for patients with advanced metastatic gastric cancer. With the emergence of immunotherapy and targeted therapy such as trastuzumab and ramucirumab, the options for treating gastric cancer have increased. However, the systematic treatments exhibited a limited efficacy for advanced gastric cancer, and the median survival of patients was only about one year. In terms of immunotherapy, based on the research results of ATTRACTION-02, nivolumab has been approved in Japan for use in the patients with advanced gastric and gastro-esophageal junction cancer after receiving at least two systemic therapies but the treatments were failed. In April 2021, based on the investigation results of CheckMate-649, the FDA approved nivolumab in combination with fluoropyrimidine and platinum chemotherapeutic agent as the first-line treatment for advanced or metastatic gastric cancer, gastro-esophageal junction cancer, and esophageal adenocarcinoma. This is also the first First-Line immunotherapy approved by the FDA for gastric cancer. Although these studies have yielded encouraging results, new therapies are still needed to benefit more gastric cancer patients.

Pancreatic cancer is one of the most common malignant digestive system tumors. In 2020, there were 496 thousand new cases of pancreatic cancer, and 466 thousand deaths worldwide, ranking the seventh among all malignant tumors. The prognosis of pancreatic cancer is poor because the diagnosis is often in advanced stage. According to the statistics of the SEER (Surveillance, Epidemiology and End Results) database at the US National Cancer Institute, the 5-year survival rate of pancreatic cancer is only 8%, and only 28.6% of patients survive for more than one year after initial diagnosis. In addition, due to the high degree of malignancy, despite surgical intervention, the patients with pancreatic cancer still often relapse, and the 5-year survival rate of pancreatic cancer patients after radical resection is only 18-27%. Pancreatic ductal adenocarcinoma (PDAC) is the most common pancreatic cancer, and accounts for more than 90% of pancreatic cancer cases. Although traditional therapies such as chemotherapy, surgery, and radiation therapy have been applied, no significant improvement in survival rates has been found. Surgical resection and chemotherapy (gemcitabine combined with albumin-bound paclitaxel and FOLFIRINOX) have been shown to improve the survival of patients with early pancreatic cancer, but these are not enough to treat patients with advanced pancreatic cancer.

Biliary tract cancer is divided into cholangiocarcinoma and gallbladder cancer, and the cholangiocarcinoma can also be divided into extrahepatic cholangiocarcinoma and intrahepatic cholangiocarcinoma based on anatomy. In western countries, the incidence rate of cholangiocarcinoma is extremely low. However, in parts

of Southeast Asia and China, infection with liver fluke and other parasites has led to a significant increase in the incidence rate of cholangiocarcinoma. Meanwhile, the latest epidemiological research shows that the incidence rate and mortality of intrahepatic cholangiocarcinoma are increasing. Cholangiocarcinoma is invasive and is usually diagnosed in the advanced stage. Most patients with cholangiocarcinoma are in an unresectable state at the initially confirmed diagnosis, while in a few patients who can undergo radical surgery, the recurrence rate is high. The 5-year survival rates for stages I, II, III, and IV according to the American Joint Committee on Cancer are 50%, 30%, 10%, and 0%, respectively. For most patients with cholangiocarcinoma, palliative chemotherapy is the only treatment option. The first-line standard treatment for patients with unresectable and metastatic biliary tract cancer is gemcitabine combined with cisplatin. The study on this combination therapy regimen showed an objective response rate of 19.5% to 26.1%, a median PFS of 5.8 to 8.0 months, and a median OS of 11.2 to 11.7 months. At present, there is no approved standard treatment for patients with biliary tract cancer who have progressive disease after first-line systemic treatment. Pembrolizumab has been approved for the treatment of MSI-H/dMMR solid tumors that have progressed after previous treatment, but due to the rarity of MSI-H/dMMR in advanced cholangiocarcinoma, most patients are not suitable for this treatment.

Certain drugs targeting CLDN18.2 have entered into clinical trials and shown good efficacy and safety. Zolbetuximab (IMAB362, claudixmab, Astellas) is the first drug developed against the CLDN18.2 target, and as a chimeric IgG1 monoclonal antibody, it can specifically bind to CLDN18.2 on the surface of tumor cells, causing antibody dependent cell-mediated cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), cell apoptosis, and cell proliferation inhibition. Subsequently, several clinical trials have also been carried out to evaluate its efficacy and safety in patients with gastroesophageal cancer.

A phase IIa trial (NCT01197885, MONO) was used to evaluate the efficacy and safety of zolbetuximab monotherapy in 54 patients with recurrent or refractory advanced gastric or lower esophageal adenocarcinoma, who exhibited moderate or high CLDN18.2 expression in ≥50% of tumor cells. It had been shown that 10 out of 43 evaluable subjects had clinical benefits, in which 4 subjects achieved partial response (PR) (objective response rate (ORR) of 9%), while 6 subjects achieved stable disease (SD) (14%). The median duration of response (DoR) for subjects who responded to zolbetuximab was 24.6 weeks (range: 13.1-156.1 weeks). Among the 10 subjects who had clinical benefits (complete response, CR)+PR+SD), 9 (90%) subjects had moderate or high CLDN18.2 expression in ≥70% of tumor cells. Subgroup analysis of these 9 subjects revealed that 4 (14%) subjects achieved PR, while 5 (17%) subjects achieved SD. Among all subjects, 52 subjects (96%) experienced treatment-emergent adverse events (TEAEs) during the treatment period, most of which were grade 1 or 2. TEAEs that occurred in ≥30% of subjects included nausea (63%), vomiting (57%), fatigue (43%), and decreased appetite (30%). 12 subjects (22%) reported grade 3 vomiting, and 8 subjects (15%) reported grade 3 nausea. Two subjects experienced grade 4 TEAE: dyspnea and diarrhea (one subject for each). 44 subjects (82%) experienced treatment-related adverse events (TRAEs), with nausea, vomiting, fatigue, and decreased appetite occurring in ≥10% of the subjects. Only 5 subjects experienced severe TRAE. For grade 3 and grade 2 TEAEs (nausea and vomiting), each occurred in one subject. Moreover, one subject had grade 2 hematemesis, and two subjects underwent grade 3 vomiting. Except for one subject who experienced grade 3 vomiting, all severe TRAEs occurred in subjects who received a dose of 600 mg/m^2. These TEAEs can be alleviated by pausing or slowing down the infusion of zolbetuximab. Patients who have not undergone prior gastrectomy were more likely to report nausea and vomiting. The incidence of these TEAEs decreased over time as subjects was repeatedly injected with zolbetuximab.

For another phase II trial (NCT01630083, FAST) evaluating the efficacy and safety of zolbetuximab combined

with EOX in the first-line treatment of CLDN18.2-positive advanced gastric/gastro-esophageal junction adenocarcinoma (G/GEJ AC), compared to EOX alone, the results indicated an improvement in median progression-free survival (PFS) from 5.3 months to 7.5 months [hazard ratio (HR), 0.44; 95% CI, 0.29-0.67; $P < 0.0005$]; the median overall survival (OS) increased from 8.3 months to 13.0 months (HR, 0.55; 95% CI, 0.39-0.77; $P<0.0005$). The most commonly reported TEAEs in the Zolbetuximab + EOX group were nausea (81.8%) and vomiting (67.5%). The incidence of vomiting was lower in patients undergoing previous gastrectomy, and there was a dose-dependent relationship between the incidence and severity of vomiting and zolbetuximab. The most commonly reported serious adverse events (SAEs) in the Zolbetuximab + EOX group were malignant tumors (3.9%), pneumonia (2.6%), and febrile neutropenia (2.6%). The most commonly reported SAEs in the EOX group were malignant tumors (8.3%), gastric bleeding (3.6%), nausea, renal failure, pulmonary embolism, and febrile neutropenia (2.4% for each). Compared to placebo combined with chemotherapy, two phase III clinical trials (NCT03504397/NCT03653507) are currently underway to evaluate the efficacy of zolbetuximab combined with different chemotherapy regimens (mFOLFOX6 or CAPOX) in the first-line treatment of CLDN18.2-positive and HER2-negative locally advanced and unresectable or metastatic G/GEJ AC, and the obtained results provide safety and efficacy data for drug targeting CLDN18.2.

Gastric cancer, pancreatic cancer and biliary tract cancer are characterized by high malignancy and poor prognosis. The efficacy and benefits of systemic chemotherapy and immune checkpoint inhibitors are very limited for advanced gastric cancer, pancreatic cancer and biliary tract cancer, and thus it is urgent to explore and develop new therapeutic targets and combination therapies. CLDN18.2 is highly expressed in gastric cancer tissues and pancreatic cancer tissues, with the expression rate of 80% in gastric cancer patients; the positive expression in pancreatic cancer accounts for about 60%, in which the medium and high expression is responsible for 54.6%. It has been reported that the positive rate of CLDN18 in extrahepatic cholangiocarcinoma tissues can reach 90%, and the positive rate of CLDN18 in intrahepatic cholangiocarcinoma can also be 43%. Compared to placebo + chemotherapy, two phase III clinical trials (NCT03504397/NCT03653507) are being carried out to evaluate the efficacy of zolbetuximab combined with different chemotherapy regimens (mFOLFOX6 or CAPOX) for first-line treatment of CLDN18.2 positive and HER2 negative, locally advanced, unresectable or metastatic G/GEJAC. Recent data have revealed that both trials have reached the critical endpoints of PFS and OS, confirming the efficacy and safety of the treatment targeting CLDN18.2. However, zolbetuximab is only effective in first-line treatment of gastric/gastro-esophageal junction adenocarcinoma patients with high expression of CLDN18.2, but there is no effective treatment regimen for gastric/gastro-esophageal junction adenocarcinoma with medium/low expression of CLDN18.2 and/or standard treatment failure, as well as other tumors expressing CLDN18.2.

Summary of the invention

The present invention meets the above-described needs by providing a method for treating solid tumors using an antibody-drug conjugate targeting Claudin18.2.

To achieve the above object, the present invention firstly provides a method for preventing or treating a solid tumor in a subject, which comprises administering to a subject in need thereof an effective amount of the antibody-drug conjugate targeting Claudin18.2 or a pharmaceutically acceptable salt thereof.

To achieve the above object, the present invention also provides a single dosage unit comprising an effective amount of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof according to the present invention.

To achieve the above object, the present invention also provides a kit of parts comprising an effective amount of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof according to the present invention.

The present invention also provides use of the single dosage unit or kit of parts according to the invention in the manufacture of a medicament for the prevention or treatment of a solid tumor.

The present invention evaluates the safety and efficacy of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof for treating solid tumors expressing CLDN18.2 and the correlation of the expression level of CLDN18.2 in tumor tissues and the efficacy of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof of the present invention.

The antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof according to the present invention as an ADC with bystander effect has a remarkable tumor inhibition effect in a mouse tumor-bearing model with high expression and low expression of CLDN18.2. In clinical research of the invention, subjects with various tumor types and different expression levels of CLDN18.2 were subjected to a dosing regimen exploration test. The primary proofs show that ORR can reach or even exceed 25%, PFS and OS were improved and good tolerance was achieved, in the case that the antibody-drug conjugate targeting Claudin18.2 of the invention alone is used to treat a gastric/gastroesophageal junction adenocarcinoma with CLDN18.2 high expression, for which a previous second-line systemic therapy has failed.

Other embodiments of the present invention will become apparent by reference to the detailed description that follows.

Detailed description of the invention

Before describing the present invention in detail, it should be understood that the present invention is not limited to the specific methods and experimental conditions described in the specification, because the methods and conditions can be changed. In addition, the terms used herein are only for the purpose of describing specific embodiments and are not intended to be limitative.

Definitions

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. For the purpose of the present invention, the following terms are defined below. It should be understood, whenever appropriate, a term used in a singular form may also cover the plural form, and vice versa.

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% to an upper limit greater than the specified numerical value by 5%.

The term "and/or" when used to connect two or more options should be understood to mean any one of the options or any two or more of the options. As used herein, the term "comprise" or "include" is intended to mean that the described elements, integers or steps are included, but not to the exclusion of any other elements, integers or steps. The term "comprise" or "include" used herein, unless otherwise specified, also encompasses the situation where the entirety consists of the described elements, integers or steps. For example, when

referring to "comprise" an antibody variable region of a particular sequence, it is also intended to encompass an antibody variable region consisting of the specific sequence.

The term "CLAUDIN" or "CLDN" used herein is the most important skeleton protein that determines the structures of tight junctions between cells, and it is involved in adhesion junctions and plays an important role in the metastasis and invasion of tumor cells. Claudin proteins are widely present in mammalian epithelial and endothelial cells, primarily on the lateral surface of epithelial cells and on the plasma membrane of basal cells. Different Claudin proteins have their respective specific expression in different tissues, wherein the Claudin18 (CLDN18) gene is located at 3q22.3, has a molecular weight of 24 kDa, comprises 261 amino acid residues, and is a member of the Claudins superfamily. Its protein structure comprises 2 extracellular loops and 4 transmembrane region. Two subtypes of human CLDN18 or Claudin18 protein are Claudin18.1 or CLDN18.1 (UniProt ID: P56856-1) and Claudin18.2 or CLDN18.2 (UniProt ID: P56856-2), respectively. In the primary structural sequences of the two proteins, only the amino acid residues at some positions from the N-terminus signal peptide to the extracellular loop 1 (Loops) structure are different. Especially at the extracellular loop 1, CLDN18.1 and CLDN18.2 differ by only 8 amino acids. The intergeneric and inerspecies sequence homology of the two subtypes of CLDN18 protein is also very high. The sequences of the extracellular loop 1 of CLDN18.2 are completely identical in different species such as human, mouse and rhesus monkey, while the homology of human and mouse CLDN18.2 proteins reaches 84%, indicating that the sequence of CLDN18.2 protein is extremely conserved (O.Tureci. et al., Gene, 481:83-92, 2011). CLDN18.2 or any variants and isoforms thereof may be isolated from cells or tissues in which they are naturally expressed, or recombinantly produced using techniques well known in the art and/or those described herein. In an embodiment, the CLDN18.2 as described herein is a human CLDN18.2.

The term "antibody targeting CLDN18.2","anti-CLDN18.2 antibody", "anti-CLDN18.2", "CLDN18.2 antibody" or "antibody that binds to CLDN18.2" or "antibody that specifically binds to CLDN18.2", or "antibody targeting to CLDN18.2" used herein refers to an antibody capable of binding to (human) CLDN18.2 with sufficient affinity such that the antibody can be used as a therapeutic agent targeting (human) CLDN18.2. In an embodiment, the (human) CLDN18.2 antibody binds to (human) CLDN18.2 with high affinity in vitro or in vivo. In an embodiment, the (human) CLDN18.2 antibody does not bind to CLDN18.1. In an embodiment, the (human) CLDN18.2 antibody binds to cells expressing CLDN18.2 but does not bind to cells expressing CLDN18.1. In some embodiments, the binding is determined, e.g., by radioimmunoassay (RIA), bio-layer interferometry (BLI), MSD assay, surface plasmon resonance (SPR) or flow cytometry.

The expression of CLDN18.2 in cell may be determined by multiple method, for example anti-CLDN18.2 antibody. For example, the binding strength of cell with "high expression of CLDN18.2" and anti-CLDN18.2 antibody (e.g., determined by FACS) may be over 500, 600, 700, 800, 900 or preferably over 1000 times, such as over 1100 times, over 1200, 1300, 1400 times or higher times than that of anti-CLDN18.2 antibody and cell without CLDN18.2 expression. For example, the binding strength of cell with "moderate expression of CLDN18.2" and anti-CLDN18.2 antibody (e.g., determined by FACS) may be 5-500 times than that of anti-CLDN18.2 antibody and cell without CLDN18.2 expression, such as 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100 times or higher, but no more than 500 times.

For the conventional four-chain IgG antibody, the full-length antibody heavy chain generally consists of heavy chain variable regions (abbreviated herein as VH) and heavy chain constant regions, wherein the heavy chain constant region comprises at least 3 domains CH1, CH2, and CH3. The full-length antibody light chain consists of light chain variable regions (abbreviated herein as VL) and light chains constant region, wherein the light chain constant region consists of one domain CL. Each heavy chain variable region VH or each light chain

variable region consists of three CDRs and 4 FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4.

The term “antibody fragment” includes a portion of a whole antibody. In preferred embodiments, the antibody fragment is antigen-binding fragment.

"Antigen-binding fragment" refers to a molecule different from a whole antibody, which comprises a portion of the whole antibody and binds to an antigen to which the whole antibody binds. Examples of the antibody fragment include, but are not limited to, Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; a dAb (domain antibody); a linear antibody; a single-chain antibody (e.g., scFv); a single-domain antibody, for example VHH; a bivalent or bispecific antibody or a fragment thereof; a Camelidae antibody.

The term "antigen" refers to the molecule that elicits an immune response. This immune response may involve the production of antibody or the activation of specific immune cells, or both. Technicans will understand that any large molecule, including almost all proteins or peptides, can be used as antigens. In addition, antigens can be derived from recombinant or genomic DNA. As used herein, the term "epitope" refers to a moiety of an antigen (e.g., CLDN18.2) that specifically interacts with an antibody molecule.

"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-contacting residues ("antigen contact site"). CDRs are primarily responsible for binding to antigen epitopes. The CDRs of heavy and light chains are generally referred to as CDR1, CDR2, and CDR3, which are numbered sequentially from N-terminus. The CDRs located in heavy chains variable domain of an antibody are referred to as HCDR1, HCDR2, and HCDR3, whereas the CDRs located in light chains variable domain of an antibody are referred to as LCDR1, LCDR2, and LCDR3. In a given amino acid sequence of light chain variable regions or heavy chain variable regions, the exact amino acid sequence boundary of each CDR can be determined using any one or a combination of many well-known antibody CDR numbering 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) (imgt.cines.fr/ on the World Wide Web), and North CDR definition based on the affinity propagation clustering using a large number of crystal structures (North et al, “A New Clustering of Antibody CDR Loop Conformations”, Journal of Molecular Biology, 406, 228-256 (2011)).

The followings are the range of CDRs defined by kabat, AbM, Chothia, Contact and IMGT protocols.

CDR	Kabat protocol	AbM protocol	Chothia protocol	Contact protocol	IMGT protocol
LCDR1 (Kabat and Chothia numbering system)	L24-L34	L24-L34	L26-L32	L30-L36	L27-L32
LCDR2 (Kabat and Chothia numbering system)	L50-L56	L50-L56	L50-L52	L46-L55	L50-L52

LCDR3 (Kabat and Chothia numbering system)	L89-L97	L89-L97	L91-L96	L89-L96	L89-L96
HCDR1 (Kabat numbering system)	H31-H35B	H26-H35B	H26-H32	H30-H35B	H26-H35B
HCDR1 (Chothia numbering system)	H31-H35	H26-H35	H26-H32	H30-H35	H26-H35
HCDR2 (Kabat and Chothia numbering system)	H50-H65	H50-H58	H53-H55	H47-H58	H51-H57
HCDR3 (Kabat and Chothia numbering system)	H95-H102	H95-H102	H96-H101	H93-H101	H93-H102

CDRs may also be determined based on having the same Kabat numbering positions as the reference CDR sequences (e.g., any of the exemplary CDRs of the present invention).

Unless otherwise specified, the term "CDR" or "CDR sequence" used herein encompasses CDR sequences determined by any one of the schemes described above.

Unless otherwise specified, residue positions of an antibody variable region (including heavy chain variable region residues and light chain variable region residues) in the present invention are positions numbered according to the Kabat numbering system (Kabat et al, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991))

In an embodiment, the heavy chain variable region CDR of the antibody of the present invention is determined according to the following schemes:

VH CDR1 is determined according to AbM scheme; and VH CDR2 and 3 are determined according to Kabat scheme.

In an embodiment, the light chain variable region CDR of the antibody of the present invention is determined according to the Kabat scheme.

In an embodiment, the heavy chain variable region CDR of the antibody of the present invention is determined according to the following schemes: VH CDR1 is determined according to the AbM schem; and VH CDR2 and 3 are determined according to Kabat scheme; and CDRs of light chain variable area are determined according to Kabat scheme.

It should be noted that boundaries of CDRs of variable regions of an antibody based on different assignment systems may differ. That is, CDR sequences of variable regions of an antibody defined by different assignment systems differ. Therefore, when it comes to defining an antibody with specific CDR sequences defined in the present invention, the scope of antibody also encompasses such antibodies whose variable region sequences comprise the specific CDR sequences, but having claimed CDR boundaries different from the specific CDR boundaries defined by the present invention due to different schemes (e.g., different assignment system schemes or their combinations) applied.

Antibodies with different specificities (i.e., different binding sites for different antigens) have different CDRs (under the same assignment system). However, although CDRs differ from antibody to antibody, only a limited

number of amino acid positions within the CDRs are directly involved in antigen binding. The smallest overlapping region can be determined using at least two of the Kabat, Chothia, AbM, Contact, and North methods, thereby providing a "minimal binding unit" for antigen binding. The minimal binding unit may be a sub-portion of the CDR. As will be clear to those skilled in the art, residues of the rest CDR sequences can be determined via antibody structure and protein folding. Therefore, any variants of the CDRs given herein are also contemplated in the present invention. For example, in a CDR variant, the amino acid residues in the minimal binding unit may remain unchanged, while the other CDR residues defined by Kabat or Chothia may be substituted by conservative amino acid residues.

The term "Fc region" is used herein to define a C-terminus region of an immunoglobulin heavy chain, which comprises at least one portion of a constant region. The term includes Fc regions of native sequences and variant Fc regions. "Fc domain" of a native immunoglobulin comprises two or three constant domains, namely a CH2 region and a CH3 region, and optionally comprises a CH4 region. For example, an immunoglobulin Fc domain in native antibody comprises the second and the third constant domains (a CH2 region and a CH3 region) derived from two heavy chains of IgG, IgA and IgD, or comprises the second and the third and the fourth constant domains (a CH2 region, a CH3 region and a CH4 region) derived from two heavy chains of IgM and IgE. Unless otherwise stated, the numbering of amino acid residues in the Fc region or constant region is based on an EU numbering system, which is also called EU index as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991. In the present invention, the term "Fc region" does not comprise 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 comprise the hinge region in the N-terminus of the heavy chain chain constant region in some cases.

"Antibody in IgG form" refers to the IgG form that the heavy chain constant region of the antibody belonging to. All antibodies of the same type have the same heavy chain constant region. Heavy chain constant regions of antibodies of different types are different. For example, an antibody in the form of IgG4 refers to its heavy chain constant region is derived from IgG4, or an antibody in the form of IgG1 refers to its heavy chain constant region is derived from IgG1.

As used herein, the term "binding" or "specific binding" means that binding interactions to antigen are selective and can be distinguished from unwanted or non-specific interactions. The ability of antigen binding sites to bind to specific antigens can be determined by enzyme-linked immunosorbent assay (ELISA) or conventional binding assay known in the art, such as radioimmunoassay (RIA), thin-layer biomembrane interference assay, MSD assay or surface plasmon resonance (SPR).

As used herein, "antibody-drug conjugate (ADC)" refers to a compound obtained by linking an antibody and a drug via a linker.

The term "site-specific conjugation" as used herein refers to conjugations by which a drug/an active substance is specifically linked to a specific position of an antibody through a linker.

The term "pharmaceutically acceptable salt" represents a salt that retains the biological effects and properties of the antibody-drug conjugate (ADC) of the present invention, and is not biologically or otherwise undesired. The ADC of the present invention may exist in the form of their pharmaceutically acceptable salts, including acid addition salts or base addition salts. In the present invention, a pharmaceutically acceptable non-toxic acid addition salt represents a salt formed by the ADC conjugate of the present invention and an organic or inorganic acid, including but not limited to hydrochloric acid, sulfuric acid, hydrobromic acid, hydriodic acid, phosphoric

acid, nitric acid, perchloric acid, acetic acid, oxalic acid, maleic acid, fumaric acid, tartaric acid, benzenesulfonic acid, methanesulfonic acid, salicylic acid, succinic acid, citric acid, lactic acid, propionic acid, benzoic acid, p-toluenesulfonic acid, malic acid, etc. The pharmaceutically acceptable non-toxic base addition salt represents a salt formed by the ADC conjugate of the present invention and an organic or inorganic base, including but not limited to an alkali metal salt, such as a lithium, a sodium or a potassium salt; an alkaline earth metal salt, such as a calcium or a magnesium salt; a salt of an organic base, such as an ammonium salt formed with a N group-containing organic base.

The term "drug-to-antibody ratio" or "DAR" refers to the ratio of small molecule drug moiety (D) conjugated to the Ab moiety as described herein to the Ab moiety. For example DAR may be 1 to 20, such as 2-18, 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 can also be calculated as the average DAR of the molecule population in the product, i.e., the overall ratio of the small molecule drug moiety (D) conjugated to the Ab moiety as described herein to the Ab moiety in the product, as measured by detection methods (e.g., by conventional methods such as mass spectrometry, ELISA assay, electrophoresis and/or HPLC), such DAR is referred to as the average DAR in the context. It should be understood that the overall ratio described above is molar ratio. In some embodiments, the conjugates of the invention have an average DAR value of 1 to 20, such as for example 2-18, 4-16, 5-12, 6-10, 2-8, 3-8, 2-6, 4-6, 6-10, for example 1.0-8.0, 2.0-6.0, for example 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, ranges with two of these values as endpoints.

The term "effective amount" refers to an amount or dose of the antibody-drug conjugate or a pharmaceutically acceptable salt thereof, antibody or fragment or composition or combination of the present invention, which will produce expected effects in patients or subjects in need of treatment or prevention after being administered to patients or subjects in a single dose or multiple doses.

"Therapeutically effective amount" refers to an amount that can effectively achieve desired therapeutic results at a required dose for a required period of time. The therapeutically effective amount is also such an amount, at which any toxic or harmful effect of the antibody-drug conjugate or a pharmaceutically acceptable salt thereof, antibody or antibody fragment or composition or combination is inferior to the therapeutic beneficial effect. "Therapeutically effective amount" preferably inhibits a measurable parameter (e.g., tumor volume) by at least about 30%, or even more preferably by at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or even 100% compared with untreated subjects.

"Prophylactically effective amount" refers to an amount that can effectively achieve desired prevention results at a required dose for a required period of time. Generally, since the prophylactic dose is administered 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 cells into which exogenous nucleic acids are introduced, including the descendants of such cells. Host cells include "transformants" and "transformed cells", which include primary transformed cells and descendants derived from them, regardless of the number of passages. The descendants may not be exactly the same as parent cells in term of nucleic acid content, and 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 agent to which it is conjugated or fused. The label itself can be detectable (for example, radioisotope label or fluorescent label) or can catalyze a chemical change of a detectable substrate compound or composition in the case of enzymatic labeling. The term is intended to cover direct labeling of a probe or an antibody by coupling (i.e., physically connecting) a detectable substance to the probe or antibody and indirect labeling of a probe or an antibody by reacting with another directly labeled reagent.

"Individual" or "subject" include mammals. Mammal include, but is not limited to, domestic animal (such as cattle, sheep, cat, dog and horse), primate (such as human and non-human primate, e.g. monkey), rabbit, and rodent (such as mouse and rat). In some embodiments, the individual or subject is a human.

An "Isolated" antibody or other molecule (e.g., ADC molecule) is such an antibody or molecule that has been separated from components in their natural environment or the environment in which it is expressed. In some embodiments, the antibody or ADC molecule is purified to more than 95% or 99% purity, as measured by, such as by electrophoresis (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatography (e.g., ion exchange or reverse phase HPLC).

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

The terms "tumor" and "cancer" are sometimes used interchangeably herein, covering solid tumors and blood tumors.

The terms "cancer" and "cancerous" refer to or describe physiological diseases in mammals characterized by unregulated cell growth. In some embodiments, cancer suitable for treatment by the antibody-drug conjugate of the present invention includes gastric cancer, pancreatic cancer, or gastroesophageal junction cancer, including metastatic forms of those cancers.

The term "tumor" refers to growth and proliferation of all neoplastic cells, whether malignant or benign, as well as all pre-cancerous and cancerous cells and tissues. The terms "cancer", "cancerous" and "tumor" are not mutually exclusive when mentioned herein.

The term “solid tumor” includes an advanced malignant solid tumor, a tumor associated with Claudin18.2, such as a Claudin18.2 positive tumor, for example selected from one or more of the following: gastric cancer, breast cancer, liver cancer, colon cancer, lung cancer, gallbladder cancer, esophageal cancer, gastric/gastro-esophageal junction adenocarcinoma, pancreatic ductal adenocarcinoma and biliary tract cancer (biliary tract cancer is classified as cholangiocarcinoma and gallbladder cancer). In some embodiments, the solid tumor may be a first-line (1L) gastric/gastro-esophageal junction adenocarcinoma, a posterior-line gastric/gastro-esophageal junction adenocarcinoma; preferably, the posterior-line gastric/gastro-esophageal junction adenocarcinoma is 2L and thereafter gastric/gastro-esophageal junction adenocarcinoma; more preferably, the posterior-line gastric/gastro-esophageal junction adenocarcinoma is 3L gastric/gastro-esophageal junction adenocarcinoma. In some embodiments, the solid tumor is a first-line (1L) gastric cancer, a posterior-line gastric cancer; preferably, the posterior-line gastric cancer is 2L and thereafter gastric cancer; more preferably, the gastric cancer is 3L gastric cancer. In some embodiments, the solid tumor is a first-line (1L) pancreatic ductal adenocarcinoma, a posterior-line pancreatic ductal adenocarcinoma; preferably, the posterior-line pancreatic ductal adenocarcinoma is 2L pancreatic ductal adenocarcinoma. Herein, gastric/gastro-esophageal

junction adenocarcinoma may refer to gastric cancer or gastro-esophageal junction adenocarcinoma.

The terms "first-line tumor", "1L tumor" or "first-line (1L) tumor" refer to tumors that have not been treated with a systemic anti-tumor treatment. For example, the term "first-line (1L) gastric/gastroesophageal junction adenocarcinoma" refers to gastric/gastroesophageal junction adenocarcinoma that has not been treated with a systemic anti-tumor treatment. The terms "first-line (1L) gastric cancer" and "first-line (1L)" pancreatic ductal adenocarcinoma" refer to gastric cancer and pancreatic ductal adenocarcinoma, respectively, that have not been treated with a systemic anti-tumor treatment.

The terms "second-line tumor" or "2L tumor" refer to tumors that have failed first-line treatment (e.g., are refractory or intolerant to first-line treatment). For example, second-line pancreatic ductal adenocarcinoma or 2L pancreatic ductal adenocarcinoma refers to pancreatic ductal adenocarcinoma that has failed first-line treatment, for example, is refractory or intolerant to first-line treatment.

The terms "third-line tumor" or "3L tumor" refer to tumors that have failed second-line treatment (e.g., are refractory or intolerant to second-line treatment). For example, "third-line (3L) gastric/gastroesophageal junction adenocarcinoma" refers to a gastric/gastroesophageal junction adenocarcinoma that has failed second-line treatment, for example, is refractory or intolerant to second-line treatment.

The term "posterior-line tumor" refers to a tumor for which first-line treatment and subsequent treatments (such as second-line treatment or third-line treatment) have failed (such as being refractory or intolerant to the treatment).

It should be understood that, for tumors, the meanings of first-line, second-line and third-line treatment or therapy and their treatment regimens are clear and well known to those skilled in the art. First-line treatment refers to the first round of treatment after tumor diagnosis; second-line treatment refers to the patient's tumor progression after first-line treatment, and resistance to the first-line treatment regimen, and need to be treated with a different treatment regimen; third-line treatment refers to the treatment using another regimen after the failure of second-line treatment; for example, refer to https://www.medsci.cn/article/show_article.do?id=1901e9517635. The treatment regimens are approved or recommended by authoritative agencies such as NMPA or FDA. For example, first-line treatment includes but is not limited to fluorouracils (fluorouracil or capecitabine) combined with platinum drugs (oxaliplatin or cisplatin). Second-line treatment includes but is not limited to paclitaxel combined with ramucirumab. Third-line treatment includes but is not limited to nivolumab, irinotecan, or trifluridine/tipiracil.

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

As used herein, "prevention" (or "prevent" or "preventing" or “prophylactic”) includes the inhibition of the onset or development 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 prophylactic regimens. Generally, in the context of cancer, the term "prevention" (or “prophylactic”) refers to an 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 may 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, 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.

The term "lyophilized formulation" refers to a composition obtained by a lyophilization process of a liquid formulation. Preferably, it is a solid composition having a water content of less than 5%, preferably less than 3%.

The term "buffer" refers to a pH buffer that can keep the pH of a solution stable. Preferably, the buffer can keep pH of the liquid formulation of the present invention or the liquid formulation obtained by reconstituting the lyophilized formulation of the present invention at about 5.5 to 7.5, for example about 6.0-6.5, about 5.5-7.2, about 6.0-7.0, or about 6.3-6.8. Preferably, the buffer is selected from one or more of histidine, citric acid, succinic acid, glutamic acid, phosphoric acid, acetic acid or a salt thereof. Wherein, the salt includes, but is not limited to a salt formed with acid, such as hydrochloride or sulfuric acid, a salt formed with alkali metal, such as lithium, sodium or potassium salt, for example histidine hydrochloride.

The term "stabilizer" refers to a chemical substance that can keep stability of a formulation in terms of chemical, physical and/or biological aspects. Preferably, the stabilizer is selected from one or more of carbohydrates, polyols. The carbohydrates can select from, but are not limited to: sucrose, trehalose, glucose, lactose, maltose, cyclodextrin, maltodextrin and dextran. The polyols can select from, but are not limited to: mannitol, sorbitol and xylitol. The carbohydrate or polyol may be in the form of hydrate.

The term "surfactant" refers to a substance that can significantly reduce the surface tension of a solution system. The concentration of the surfactant in the liquid formulation is, for example, about 0.05-1, about 0.05-2, about 0.1-0.5, about 0.1-0.4, about 0.1-0.3, about 0.2-0.4 mg/ml. Preferably, the surfactant is a nonionic surfactant, includes but is not limited to alkyl poly(ethylene oxide); polysorbates, such as polysorbate-20, polysorbate-80, polysorbate-60, or polysorbate-40; pluronics; or a combination thereof, and the like.

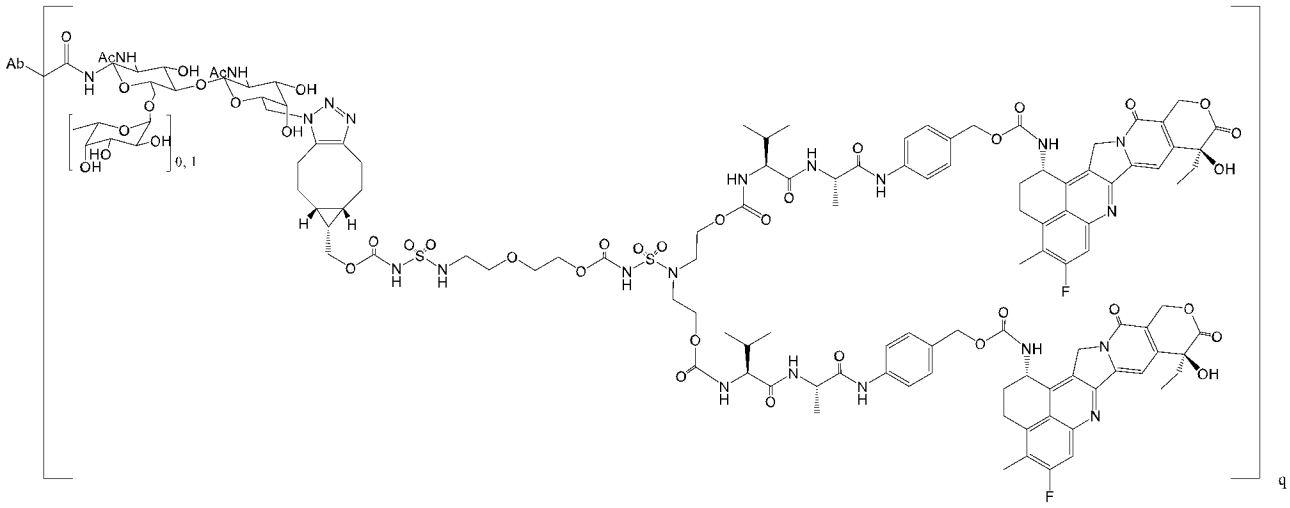
The term "reconstituted" formulation means the liquid formulation obtained by dissolving and/or suspending a solid formulation (e.g., a lyophilized formulation) in a physiologically acceptable solution. Herein, in some cases, "reconstituted" and "redissolved" and the like are used interchangeably in term of the formulation.

As used herein, "w/v" refers to "weight/volume", for example, "1% w/v" means 1 g/100 mL = 0.01 g/mL = 10 mg/mL.

Method of treatment

The present invention provides a method for preventing or treating a solid tumor in a subject, which comprises administering to a subject in need thereof an effective amount of an antibody-drug conjugate targeting Claudin18.2 or a pharmaceutically acceptable salt thereof.

In an embodiment of the invention, the antibody-drug conjugate targeting Claudin18.2 is selected from



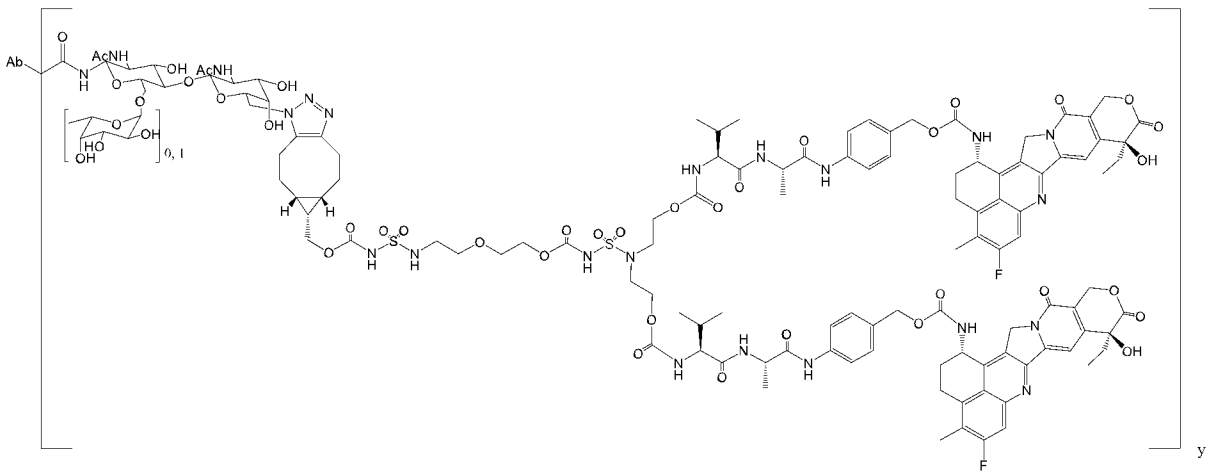
IA

wherein Ab is an antibody or antigen-binding fragment thereof targeting Claudin18.2;

q represents an average DAR, and is 2 to 11; for example, q is from 2 to 5, e.g., q is a number from 3 to 5, 3.5 to 4.5, 3.2 to 4.2, or 3 to 4, more specifically q is 2, 2.5, 3, 3.5, 4, 4.8, or 5.

According to the context, it should be understood that when the variable after the square brackets in the ADC structural formula is designated as an average DAR, e.g., q in formula IA, it represents the ratio of drug moiety (i.e., Exatecan) to Ab as determined by a conventional determination method, as described in the Definitions section herein. The average DAR may be a decimal. For average DAR, for example, when referring to "q is 3-5" or "q is a number from 3 to 5," it means that q is any number selected from between 3 and 5, including integers and decimals.

In an embodiment of the invention, the antibody-drug conjugate targeting Claudin18.2 is selected from



IB

wherein Ab is an antibody or antigen-binding fragment thereof targeting Claudin18.2;

wherein y is an integer of 1 or 2.

It should be understood that, when the variable after the square brackets of the ADC structural formula is not specified as an average DAR, such as y in Formula IB, it does not represent an average DAR, but refers to the number of Linker-payloads (i.e., the structures in the square brackets of Formula IB) linked to each Ab in said ADC molecule, unless otherwise specified or contradictory according to the context. For example, when y is 2, it means that one Ab is connected to two Linker-payloads in the ADC molecule.

In a further embodiment of the present invention, the antibody or antigen-binding fragment thereof targeting Claudin18.2 comprise a HCDR1, a HCDR2, a HCDR3 respectively consisting of amino acid sequences set forth in SEQ ID NO:1(GFTFSSYVMS), SEQ ID NO:2(TISHSGGSTYYADSVKG) and SEQ ID NO:3(DAPYYDILTGYRY), as well as an LCDR1, an LCDR2 and an LCDR3 respectively consisting of amino acid sequences set forth in SEQ ID NO:6(RASQSISSWLA), SEQ ID NO:7(KASSLES) and SEQ ID NO:8(QQYNSYSYT).

In a further embodiment of the present invention, the antibody or antigen-binding fragment thereof targeting Claudin18.2 comprises a heavy chain variable region and/or a light chain variable region, wherein the heavy chain variable region

(i) comprises or consists of an amino acid sequence set forth in SEQ ID NO: 4; or

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

(iii) comprises or consists of an amino acid sequence having one or more (preferably no more than 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid replacements, and more preferably conservative amino acid replacements) compared with an amino acid sequence set forth in SEQ ID NO: 4, preferably, the amino acid alterations do not occur in the CDRs; and/or

the light chain variable region

(i) comprises or consists of an amino acid sequence set forth in SEQ ID NO: 9; or

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

(iii) comprises or consists of an amino acid sequence having one or more (preferably no more than 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid replacements, and more preferably conservative amino acid replacements) compared with an amino acid sequence set forth in SEQ ID NO: 9, preferably, the amino acid alterations do not occur in the CDRs.

In a further embodiment of the present invention, the antibody or antigen-binding fragment thereof targeting Claudin18.2 comprises a heavy chain and a light chain,

wherein the heavy chain

(i) comprises or consists of an amino acid sequence set forth in SEQ ID NO:11 as shown below:

EVQLLDSGGGLVQPGGSLRLSCAASGFTFSSYVMSWVRQAPGKGLNWVSTISHSGGSTYYADSVKG
RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAIDAPYYDILTGYRYWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL

PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK;

or

(ii) comprises or consists an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid set forth in SEQ ID NO:11; or

(iii) comprising or consists of an amino acid sequence having one or more (preferably no more than 20 or 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid replacements, and more preferably conservative amino acid replacements) compared with an amino acid sequence set forth in SEQ ID NO: 11; and/or

the light chain

(i) comprises or consists of an amino acid sequence set forth in SEQ ID NO:12 as shown below:

DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYKASSLESGVPSRFSGSGSG
TEFTLTISSLQPDDFATYYCQQYNSYSYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS
PVTKSFNRGEC;

or

(ii) comprises or consists an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid set forth in SEQ ID NO:12; or

(iii) comprises or consists of an amino acid sequence having one or more (preferably no more than 20 or 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid replacements, and more preferably conservative amino acid replacements) compared with an amino acid sequence set forth in SEQ ID NO: 12.

In an embodiment of the invention, N-acetylglucosamine (GlcNAc) connected to Ab is present at Asn 297-glycosylation sites of both heavy chains of the antibody.

In an embodiment of the invention, the antibody-drug conjugate targeting Claudin18.2, or the pharmaceutically acceptable salt thereof, is formulated as a formulation for administration.

In an embodiment of the invention, the formulation of the antibody-drug conjugate targeting Claudin18.2, or the pharmaceutically acceptable salt thereof, is a liquid formulation comprising:

about 15-60 mg/mL, e.g., about 20-40 mg/mL, of the antibody-drug conjugate targeting Claudin18.2, or the pharmaceutically acceptable salt thereof;

about 5-50mM, e.g., about 10-30mM of a buffer;

about 50-100 mg/ml, such as about 60-90 mg/ml of a stabilizer; and

about 0.05-1 mg/ml, such as about 0.1-0.3 mg/ml of a surfactant;

the pH of the formulation is about 5.5-7.5, preferably about 6.0-6.5, more preferably about 6.5.

In an embodiment of the invention, the buffer is a combination of histidine and histidine hydrochloride or is histidine;

the stabilizer is sucrose; and

the surfactant is polysorbate 80.

In an embodiment of the invention, the formulation of the antibody-drug conjugate targeting Claudin18.2, or the pharmaceutically acceptable salt thereof has the following composition or formula: 20 mg/mL of the antibody-drug conjugate targeting Claudin18.2, or the pharmaceutically acceptable salt thereof, 20mM histidine buffer, 8% sucrose (w/v), 0.02% polysorbate 80(w/v), pH 6.5.

It is understood that, unless otherwise specified, when the formulation of the present invention is a liquid formulation, it further comprises a vehicle, including, but not limited to, for example, purified water such as ultrapure water, water for injection, sterile water, double distilled water, and the like. Vehicles that can be used to reconstitute the lyophilized formulation of the present invention include, but are not limited to, for example, purified water such as ultrapure water, water for injection, sterile water, double distilled water, physiological saline, ringer's solution, glucose injection solution, and the like.

In a further embodiment of the invention, the formulation of the antibody-drug conjugate targeting Claudin18.2, or the pharmaceutically acceptable salt thereof is a lyophilized formulation. The lyophilized formulation is prepared by lyophilizing the pharmaceutical formulation of the present invention. For example, the lyophilized formulation is prepared by lyophilizing the liquid pharmaceutical formulation described in the above embodiments.

In an embodiment of the invention, the lyophilized formulation is reconstituted prior to administration to a subject.

In an embodiment of the invention, in the method for preventing or treating a solid tumor in a subject according to the present invention, the solid tumor is an advanced malignant solid tumor, and/or

the solid tumor may be a tumor associated with Claudin18.2, such as a Claudin18.2 positive tumor, for example selected from one or more of the following: gastric cancer, breast cancer, liver cancer, colon cancer, lung cancer, gallbladder cancer, esophageal cancer, gastric/gastro-esophageal junction adenocarcinoma, pancreatic ductal adenocarcinoma and biliary tract cancer (biliary tract cancer is classified as cholangiocarcinoma and gallbladder cancer).

In an embodiment of the invention, the advanced solid tumor is a tumor that has not been treated with a systemic anti-tumor treatment, a tumor that has failed at least one systemic anti-tumor treatment, a tumor that has failed first-line treatment (such as being refractory or intolerant to first-line treatment), a tumor that has failed second-line treatment (such as being refractory or intolerant to second-line treatment).

In an embodiment of the invention, the advanced malignant solid tumor is a locally advanced unresectable or metastatic solid tumor.

In an embodiment of the invention, the advanced solid tumor is gastric cancer or gastro-esophageal junction adenocarcinoma.

The solid tumor is a first-line (1L) gastric/gastro-esophageal junction adenocarcinoma, a posterior-line gastric/gastro-esophageal junction adenocarcinoma; preferably, the posterior-line gastric/gastro-esophageal junction adenocarcinoma is 2L and thereafter gastric/gastro-esophageal junction adenocarcinoma; more preferably, the posterior-line gastric/gastro-esophageal junction adenocarcinoma is 3L gastric/gastro-esophageal junction adenocarcinoma. The solid tumor is a first-line (1L) gastric cancer, a posterior-line gastric cancer; preferably, the posterior-line gastric cancer is 2L and thereafter gastric cancer; more preferably, the gastric cancer is 3L gastric cancer. The solid tumor is a first-line (1L) pancreatic ductal adenocarcinoma, a

posterior-line pancreatic ductal adenocarcinoma; preferably, the posterior-line pancreatic ductal adenocarcinoma is 2L pancreatic ductal adenocarcinoma.

In an embodiment of the invention, the advanced malignant solid tumor is a locally advanced unresectable or metastatic gastric/gastro-esophageal junction adenocarcinoma.

In an embodiment of the invention, the advanced malignant solid tumor is Her-2 negative.

In an embodiment of the invention, the subject has previously received treatment for said solid tumor.

In an embodiment of the invention, the method for preventing or treating advanced malignant solid tumors in a subject of the invention comprises administering to the subject 0.1mg/kg-100mg/kg of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof; further, the method comprises administering to the subject 0.2mg/kg-50mg/kg of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof; still further, the method comprises administering to the subject 0.3mg/kg-30mg/kg of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof. For example: the method comprises administering to the subject 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 4.5 mg/kg, 6 mg/kg, 7.5 mg/kg, 8 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, 13.5 mg/kg, or 15 mg/kg of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof. Still further, the method comprises administering to the subject 0.3mg/kg-10mg/kg such as 2 mg/kg- 9 mg/kg, 4 mg/kg- 8 mg/ kg, or 5 mg/kg- 7 mg/ kg of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof. For example: the method comprises administering to the subject 6 mg/kg of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof.

In an embodiment of the invention, the method for preventing or treating advanced malignant solid tumors in a subject according to the present invention comprises administering to the subject 10 mg to 1500 mg, such as 50 mg to 1000 mg, 100 mg to 800 mg, 200 mg to 600 mg, or 300 mg to 500 mg of the antibody-drug conjugate targeting Claudin18.2, or the pharmaceutically acceptable salt thereof.

In an embodiment of the invention, the antibody-drug conjugate targeting Claudin18.2, or a pharmaceutically acceptable salt thereof is administered according to an administration cycle.

In an embodiment of the invention, the administration cycle of the method is 2 weeks/14 days (Q2W) to 5 weeks/35 days (Q5W), such as 18-24 days, 16-28 days or 15-30 days; e.g., 19, 20, 21, 22 or 23 days, administering once per administration cycle; further, the administration cycle of the method is 2 weeks/14 days (Q2W), 3 weeks/21 days (Q3W), 4 weeks/28 days (Q4W), or 5 weeks/35 days (Q5W), administering once per administration cycle, i.e., once every two weeks, once every three weeks, once every four weeks, or once every five weeks; preferably administering once on day 1 per administration cycle.

In a further embodiment of the invention, the method comprises administering to the subject 6 mg/kg of the antibody-drug conjugate targeting Claudin18.2, or the pharmaceutically acceptable salt thereof, at a frequency of once every 3 weeks/21 days (administering once on day 1 per administration cycle).

In an embodiment of the invention, the mode of administration of the method of the invention includes intravenous infusion, such as intravenous drip or intravenous bolus; wherein the intravenous drip may be performed through a peripheral vein, central venous catheter, or infusion port.

Antibody-drug conjugates or pharmaceutically acceptable salts thereof for use in therapy

The invention provides an antibody-drug conjugate targeting Claudin18.2 or a pharmaceutically acceptable salt thereof for use in the prevention or treatment of a solid tumor in a subject;

wherein the antibody-drug conjugate targeting Claudin18.2 is selected from the antibody-drug conjugate of formula IA or formula IB as defined herein, for example, in the "Method of treatment" section.

In an embodiment of the invention, the antibody or antigen-binding fragment thereof targeting Claudin18.2 is as defined herein, e.g., in the "Method of treatment" section.

In an embodiment of the invention, the solid tumor is as defined herein, e.g., in the "Method of treatment" section.

In an embodiment of the invention, the antibody-drug conjugate targeting Claudin18.2, or the pharmaceutically acceptable salt thereof, is administered to the subject at a dose as described herein, e.g., in the "Method of treatment" section.

In an embodiment of the invention, the antibody-drug conjugate targeting Claudin18.2, or the pharmaceutically acceptable salt thereof, is administered in a dosing regimen as described herein, e.g., in the "Method of treatment" section.

Pharmaceutical use

The invention provides a use of an antibody-drug conjugate targeting Claudin18.2 or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for preventing or treating a solid tumor in a subject,

wherein the antibody-drug conjugate targeting Claudin18.2 is selected from the antibody-drug conjugate of formula IA or formula IB as defined herein, for example, in the "Method of treatment" section.

In an embodiment of the invention, the antibody or antigen-binding fragment thereof targeting Claudin18.2 is as defined herein, e.g., in the "Method of treatment" section.

In an embodiment of the invention, the solid tumor is as defined herein, e.g., in the "Method of treatment" section.

In an embodiment of the invention, the antibody-drug conjugate targeting Claudin18.2, or the pharmaceutically acceptable salt thereof, is administered to the subject at a dose as described herein, e.g., in the "Method of treatment" section.

In an embodiment of the invention, the antibody-drug conjugate targeting Claudin18.2, or the pharmaceutically acceptable salt thereof, is administered in a dosing regimen as described herein, e.g., in the "Method of treatment" section.

Single dosage unit

A single dosage unit comprising an effective amount of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof according to the present invention. Preferably, a fixed dose of 1-500mg such as 100-500mg of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof is comprised. For example, a fixed dose of 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200 or 500 mg of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable

salt thereof is comprised.

In an embodiment of the invention, the single dosage unit is a unit dosage form.

Kit of parts

A kit of parts comprising an effective amount of the antibody-drug conjugate targeting Claudin18.2, or the pharmaceutically acceptable salt thereof according to the invention;

Preferably, a fixed dose of 1-500mg, e.g., 100-500mg, of the antibody-drug conjugate targeting Claudin18.2, or the pharmaceutically acceptable salt thereof, is comprised. For example, a fixed dose of 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, or 500mg of the antibody-drug conjugate targeting Claudin18.2, or the pharmaceutically acceptable salt thereof, is comprised.

Preferably, the kit of parts further comprises a package insert on which instructions for using the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof to prevent or treat an advanced malignant solid tumor in a subject are printed.

Application

Use of the single dosage unit or kit of parts according to the invention in the manufacture of a medicament for the prevention or treatment of an advanced malignant solid tumor, including but not limited to gastric cancer, breast cancer, liver cancer, colon cancer, lung cancer, gallbladder cancer, esophageal cancer, gastric/gastro-esophageal junction adenocarcinoma, pancreatic ductal adenocarcinoma, biliary tract cancer (biliary tract cancer is classified as cholangiocarcinoma and gallbladder cancer).

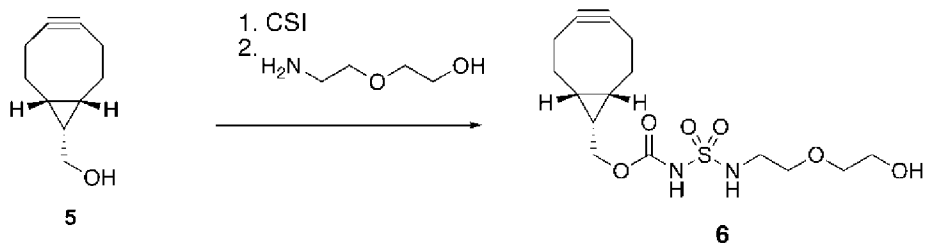
Examples

Example 1 Preparation of antibody-drug conjugates targeting Claudin18.2

Anti-CLDN18.2 monoclonal antibody HB37A6 (see CN202010570517.X (corresponding to PCT application PCT/CN2021/100870)) and control antibody zolbetuximab (Zmab for short, sequence derived from INN117) were expressed in HEK293 cells (Invitrogen, A14527) in the form of full-length monoclonal antibodies.

Based on HB37A6, a ADC (IEX019-02) was further designed and synthesized as described below. The specific synthesis steps may also refer to the preparation steps of IEX019-02 in PCT/CN2022/139637 (which is incorporated herein as a reference in its entirety).

1). Preparation of compound 6

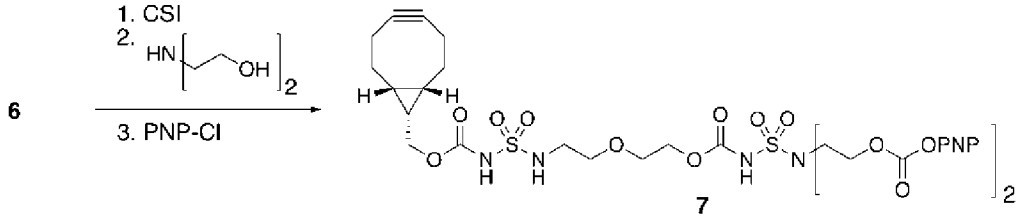


5 6

Under N_2 atmosphere, chlorosulfonyl isocyanate(CSI) (0.87 mL, 1.4 g, 10 mmol), Et_3N (2.8 mL, 2.0 g, 20 mmol) and 2-(2-aminoethoxy)ethanol (1.2 mL, 1.26 g, 12 mmol) were added to a solution of BCN-OH (5, 1.5 g, 10 mmol) in DCM (150 mL). The mixture was stirred for 10 minutes and quenched by adding NH_4Cl

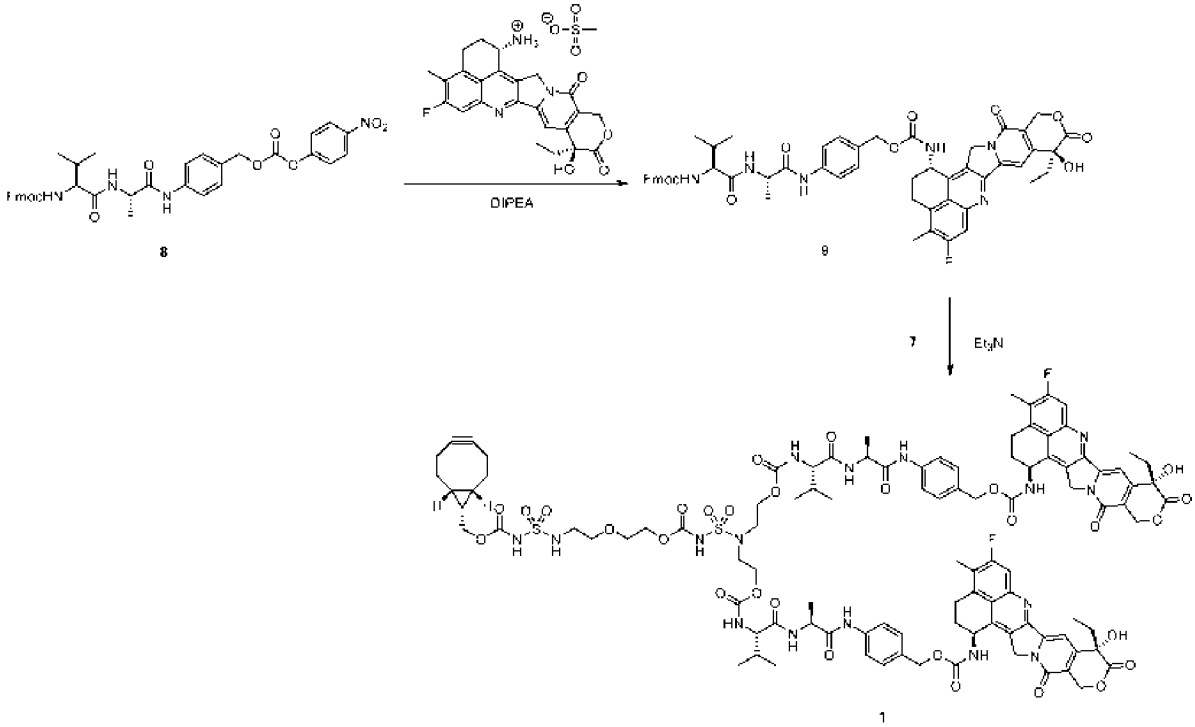
aqueous solution (saturated, 150mL). The aqueous layer was extracted with DCM (150mL) after separation. The combined organic layer was dried (Na_2SO_4) and concentrated. The residues were purified by column chromatography. The product 6 (2.06 g, 5.72 mmol, 57%) was obtained as light yellow thick oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.0 (bs, 1H), 4.28 (d, J = 8.2 Hz, 2H), 3.78–3.73 (m, 2H), 3.66–3.61 (m, 2H), 3.61–3.55 (m, 2H), 3.34 (t, J = 4.9 Hz, 2H), 2.37–2.15 (m, 6H), 1.64–1.48 (m, 2H), 1.40 (quintet, J = 8.7 Hz, 1H), 1.05–0.92 (m, 2H).

2) Preparation of compound 7



CSI (11 µL, 18 mg, 0.13 mmol) was added to a stirred solution of 6 (47 mg, 0.13 mmol) in DCM (10 mL). After 30 minutes, a solution of Et_3N (91 µL, 66 mg, 0.65 mmol) and diethanolamine (16 mg, 0.16 mmol) in DMF (0.5 mL) was added. After 30 minutes, p-nitrophenyl chloroformate (52 mg, 0.26 mmol) and Et_3N (54 µL, 39 mg, 0.39 mmol) were added. After additional 4.5 hours, the reaction mixture was concentrated. The residue was purified by gradient column chromatography(33 → 66% EtOAc/heptane(1% AcOH)) to obtain 7 (88 mg, 0.098 mmol, 75%), as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.28–8.23 (m, 4H), 7.42–7.35 (m, 4H), 4.52 (t, J = 5.4 Hz, 4H), 4.30 (d, J = 8.3 Hz, 2H), 4.27–4.22 (m, 2H), 3.86 (t, J = 5.3 Hz, 4H), 3.69–3.65 (m, 2H), 3.64–3.59 (m, 2H), 3.30–3.22 (m, 2H), 2.34–2.14 (m, 6H), 1.62–1.46 (m, 2H), 1.38 (quintet, J = 8.7 Hz, 1H), 1.04–0.92 (m, 2H).

3). Preparation of compound 9



Compound 8 (163 mg, 240 µmol) was added to a mixture of Exatecan mesylate(125 mg, 235 µmol) and DIPEA

(61 mg, 82 μL, 0.47 mmol) in dry DMP(0.9 mL). After 20 hours, the reaction mixture was diluted into 9mL of DCM and purified by gradient column chromatography(0 → 40% MeOH/DCM) to obtain 9 (155 mg, 159 μmol, 68%). LCMS (ESI+) $C_{55}H_{54}FN_6O_{10}^+$ $(M+H)^+$ calculated value: 977.39; found: 977.80.

4) Preparation of compound 1

A solution of Et_3N (73 mg, 101 μL, 0.72 mmol) and compound 7 (65 mg, 72 μmol) in DMF(1.4 mL) was added to a soluiton of compound 9 (155 mg, 159 μmol) in DMF(1.6 mL). The reaction mixture was sitrred for 24 hours, diluted with DCM (20 mL) and purified by gradient column chromatography(0 → 40% MeOH/DCM) to obtain compound 1 (94 mg, 44 μmol, 28%), as a pale yellow solid. LCMS (ESI+) $C_{102}H_{118}F_2N_{16}O_{29}S_2^{2+}$ $(M/2+H)^+$ calculated value: 1066.88; found: 1067.12.

5) HB37A6 was enzymatically reconstituted to HB37A6-(GlcNAc(Fuc)$_1$-6-N_3-GalNAc)$_2$

HB37A6 was expressed in HEK293 cells and purified. The obtained HB37A6 (16.4 mg/mL) was incubated with EndoSH (1% w/w) as described in PCT/EP2017/052792 to obtain trimmed HB37A6 with -GlcNAc or with GlcNAc (Fuc) at the Asn297 position. The above trimmed HB37A6 was incubated with enzymes His-TnGalNAcT (4.5% w/w) disclosed in PCT/EP2016/059194 and 6-azide-GalNAc-UDP (25eq (equivalent) compared with antibody) disclosed in PCT/EP2016/059194 (in a solution of histidine (20 mM) + NaCl (150 mM) containing 6 mM $MnCl_2$) at 30 °C for 16 h.

Then, the obtained incubation mixture was purified using a 50 mL protA column (Hitrap Mabselect Sure, GE, 11-0034-95). The incubation mixture obtained above was introduced to the column, and the column was washed with TBS + 0.2% Triton and TBS. The column was then eluted with 0.1 M acetate buffer solution(pH 2.9), and neutralized with 2.5 M Tris-HCl (pH 7.2). After dialysis with PBS for 3 times, the obtained (modified) glycosylated antibody was concentrated to 32.6 mg/mL using a Vivaspin Turbo 15 ultrafiltration device (Sartorius).

The obtained glycosylated antibody was analyzed by mass spectrometry, the main steps are as follows: the glycosylated antibody was treated with IdeS prior to mass spectrometry analysis to analyze the Fc/2 fragment. 20 μg of (modified) glycosylated antibody solution was inculated with IdeS (Fabricator™) (1.25 U/μL) in PBS (pH 6.6) at a total volume of 10 μL for 1 hour at 37°C. The sample was diluted to 80 μL and analyzed on JEOL AccuTOF (ESI-TOF), and deconvoluted spectra were obtained by Magtran software. Mass spectrometry analysis of the IdeS-digested sample revealed that the main product corresponded to the obtained HB37A6-(GlcNAc(Fuc)$_1$-6-N_3-GalNAc)$_2$, with an observed mass of 24,330.4.

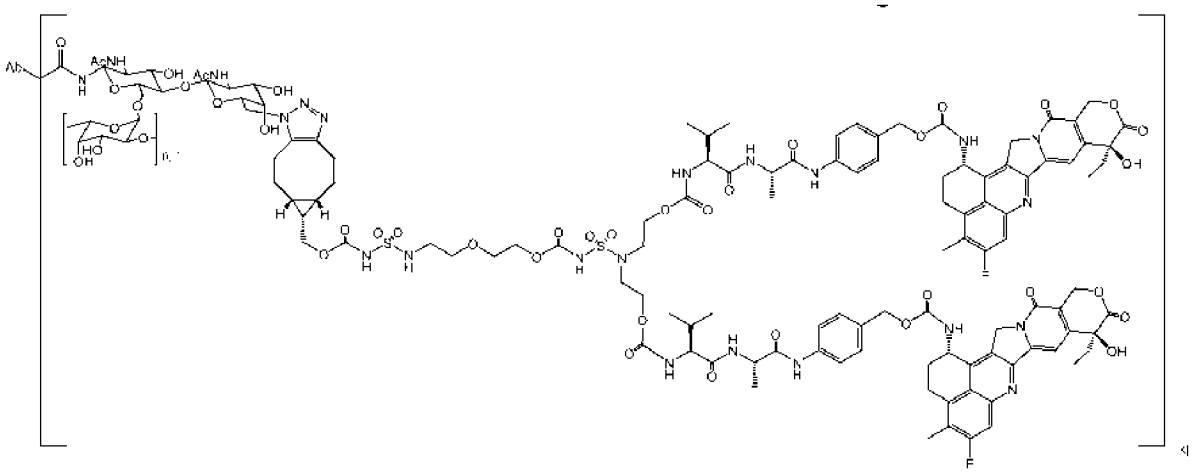
Thus, the above results demonstrated that HB37A6-(GlcNAc(Fuc)$_1$-6-N_3-GalNAc)$_2$, in which GlcNAcs at Asn297s in both heavy chains were substituted by 6-azido-GalNAc (substituted at position 4) was obtained.

6) Preparation of conjugate HB37A6-SYNtecan E (IEX019-02)

The bioconjugate IEX019-02 of the present invention was prepared by conjugating compound 1 (linker-payload) as the linker-conjugate to azide-modified HB37A6-(GlcNAc(Fuc)$_1$-6-N_3-GalNAc)$_2$ asthe biomolecule. Thus, to a solution of HB37A6-(GlcNAc(Fuc)$_1$-6-N_3-GalNAc)$_2$ (10.8 mL, 350 mg, 32.6 mg/ml, PBS pH 7.4) were added PBS pH 7.4 (808 μL), and 1,2-propylene glycol (11.3 mL) and compound 1 (350 μL, 40 mM DMF solution). The reaction was incubated overnight at room temperature, then filtered and dialyzed to PBS pH 7.4. The residual free payload was removed by adding Charcoal (350mg) and spinning overnight at room temperature. The charcoal was removed by centrifugation and filteration. The ADC was purified by AKTA Purifier-10 (GE Healthcare) with Superdex200 26/600 SEC (GE Healthcare) column.

Mass spectrometry analysis of the IdeS-digested samples revealed that the two main products corresponded to the obtained ADC, namely HB37A6-SYNtecan E conjugate. 1st peak: the observed mass is 26469 Da (calculated mass is 26465 Da), corresponding to the conjugated Fc/2 fragment (2x blocked lactone form of the payload). 2ed peak: the observed mass of 26499 Da (calculated mass 26501 Da), corresponding to the bound Fc/2 fragment (2x open carboxylate form of the payload).

The resulting structure is as follows. The concentration, DAR value and SEC purity of ADC products were determined by UV, SEC, RP-HPLC and LC-MS. The purity of the monomer detected by SE-HPLC was >99%, and the concentration was 6.12 mg/ml.



wherein q represents an average DAR, for example 3-5, 3.2-4.8, or 3.5-4.5, such as 3.52.

wherein Ab is HB37A6.

It should be understood that the average DAR is the DAR measured for the prepared ADC product, and the average DAR of different batches of products may vary.

Example 2. Production of the formulation

20 mg/mL IEX019-02 ADC, 20 mM histidine solution, 8% (w/v) sucrose, 0.02% (w/v) polysorbate 80, pH 6.5 were freeze-dried (the freeze-drying process can be prepared according to the conventional process in the art), and the freeze-drying process parameters are shown in Table 1.

Table 1

Steps	Temperature / ℃	rate / min	Holding time / min	pressure/ mbar
Loading temperature	5	30	60	1000
Freezing	-20	40	120	1000
	-45	90	180	1000
	-7	40	180	1000
	-45	40	180	1000
First drying	-15	30	2520	0.1

Secondary drying	35	250	720	0.1

Example 3 Phase Ia and Ib clinical trials

1. Test antibodies and other medicaments

Investigational drug: Antibody-drug conjugate targeting Claudin 18.2 of the present invention (IEX019-02)

Strength: 100 mg/vial

2. Criteria for inclusion/exclusion of subjects

Inclusion criteria:

To be eligible, the subjects must meet all of the following conditions:

The inclusion criteria required for both Phase Ia and Phase Ib:

1. Signing an informed consent form (ICF), and willing and able to comply with the visits and related procedures set in the plan.

2. Dose escalation stage in Phase Ia: According to the response evaluation criteria in solid tumor RECIST v1.1, there should be at least one evaluable lesion; Dose expansion stage in Phase Ia, and Phase Ib: According to the response evaluation criteria in solid tumor RECIST v1.1, there should be at least one measurable lesion.

3. The age was ≥18 years old, regardless of gender.

4. According to the Eastern Cooperative Oncology Group Performance Status (ECOG PS), the score is 0 or 1.

5. The expected survival was ≥ 12 weeks.

6. Having sufficient bone marrow and organ functions:

- Blood routine: ANC ≥ 1.5×10^9/L; Platelet count ≥ 100×10^9/L; Hemoglobin content ≥ 9.0 g/dL, the subject cannot receive treatment with blood transfusion products (including red blood cell suspension, apheresis platelets, cryoprecipitation, etc.), erythropoietin (EPO), G-colony-stimulating factor (G-CSF), or granulocyte macrophage colony-stimulating factor (GM-CSF) within 7 days before blood collection;
- Liver function: TBIL ≤ 1.5 × ULN (allowing TBIL ≤ 3 × ULN in subjects with Gilbert syndrome); ALT and AST ≤ 2.5 × ULN in subjects without liver metastasis, as well as ALT and AST ≤ 5 × ULN in subjects with liver metastasis; Albumin ≥ 28 g/L;
- Renal function: serum creatinine ≤ 1.5 × ULN or creatinine clearance ≥ 60 mL/min (using Cockcroft-Gault formula); Urinary protein < 2+ or 24 h total urine protein < 1 g;
- Coagulation function: International normalized ratio (INR) ≤1.5 and activated partial thromboplastin time (APTT) ≤ 1.5 × ULN (subjects who have received anticoagulant treatment and have coagulation function within the above range were allowable).

7. Female subjects of childbearing age or male subjects whose partners are at childbearing age were required to take effective contraceptive measures throughout the entire treatment period and within 6 months after the treatment period.

Inclusion criteria for dose expansion stage in Phase Ia:

1. Subjects with unresectable, locally advanced or metastatic G/GEJ AC, PDAC, BTC, or other solid tumors diagnosed by histopathology with standard treatment failure or intolerance, or without standard treatment.

2. *CLDN18.2-positive confirmed by histopathological testing (high **CLDN18.2 expression subjects were preferably enrolled for G/GEJ AC, while medium and high **CLDN18.2 expression subjects were preferably enrolled for PDAC).

Inclusion criteria for cohort A in Phase Ib:

1. Unresectable, locally advanced or metastatic G/GEJ AC confirmed by histopathology.

2. Receiving at least two systemic treatments (anti-PD-(L)1 + a platinum chemotherapeutic agent, fluorouracils, paclitaxel/docetaxel, or irinotecans; subjects with HER2 overexpression (defined as immunohistochemical results of 3+ or 2+ and ISH+) must have received anti-HER2 treatment, and subjects who had not previously received anti-PD-(L)1 or anti-HER2 treatment must have contraindications or reasonable reasons for non-benefit) and experiencing disease progression.

3. High **CLDN18.2 expression confirmed by histopathological testing.

Inclusion criteria for cohort B in Phase Ib:

1. Unresectable, locally advanced or metastatic G/GEJ AC diagnosed by histopathology.

2. Disease progression occurred after first-line standard treatment (Subjects with HER2 overexpression must have received anti-HER2 treatment, unless there were contraindications or reasonable reasons for non-benefit).

3. *CLDN18.2-positive confirmed by histopathological testing.

Inclusion criteria for cohort C in Phase Ib:

1. Unresectable, locally advanced or metastatic PDAC diagnosed by histopathology.

2. Disease progression occurred after receiving at least one systemic treatment.

Inclusion criteria for cohort D in Phase Ib:

1. Unresectable, locally advanced or metastatic BTC diagnosed by histopathology.

2. Disease progression occurred after receiving at least one systemic treatment.

3. *CLDN18.2-positive confirmed by histopathological testing.

Notes:

*CLDN18.2-positive: defined as the proportion of tumor cells with any degree of membrane staining in tumor tissue detected by immunohistochemistry being ≥1%, and previous test results, test results of research center and test results of central laboratory were acceptable.

**High CLDN18.2 expression: The intensity of Claudin18.2 immunohistochemical membrane staining was ≥2+ in ≥75% of tumor cells.

*** Medium and high CLDN18.2 expression: The intensity of Claudin18.2 immunohistochemical membrane staining was ≥2+ in ≥50% of tumor cells.

Exclusion criteria:

Subjects meeting any one of the following criteria were not selected for this study:

Common exclusion criteria for Phases Ia and Ib:

1. Being participating in another interventional clinical study, excluding observational (non-interventional) clinical studies or in the survival follow-up stage of interventional studies.

2. Receiving the last anti-tumor treatment within 4 weeks prior to the first administration of the investigational drug or within 5 half-lives of the anti-tumor treatment drug (whichever was shorter).

3. Being expected to receive other anti-tumor treatments during the therapy with the investigational drug [allowing for palliative radiotherapy with the aim of relieving symptoms (such as pain) without affecting efficacy evaluation].

4. Receiving treatment with a strong inhibitor of cytochrome P450 3A4 (CYP3A4) within 2 weeks or 5 half-lives (whichever is longer) prior to the first administration of the investigational drug.

5. Inoculated any live vaccine within 4 weeks prior to the first administration of the investigational drug or planned to inoculate any live vaccine during the study.

6. Prior to the first administration of the investigational drug, there were toxicity that had not been restored to 0 or 1 level in NCI CTCAE v5.0 due to previous treatments (excluding hair loss, fatigue, pigmentation, and other situations judged by the investigator to have no safety risk).

7. Within 4 weeks prior to the first administration of the investigational drug, having major surgy (craniotomy, thoracotomy, or laparotomy, or others as defined by the investigator, excluding biopsy) or unhealed wounds, ulcers, or fractures; or planning to undergo major surgery during the study; Note: for the purpose of palliative treatment, local surgical treatment for isolated lesions is acceptable.

8. Previously receiving whole pelvic radiotherapy.

9. Presence of pyloric obstruction and/or persistent recurrent vomiting (vomiting $\geq$3 times within 24 hours).

10. Within 6 months prior to the first administration of the investigational drug, there was a history of gastrointestinal perforation and/or fistula that have not been cured by surgical treatment.

11. Symptomatic metastasis to central nervous system. For patients with asymptomatic brain metastases (i.e. no neurological symptoms, no glucocorticoid treatment required, all brain metastases being $\leq$1.5 cm) or stable symptoms after treatment of brain metastases, all of the following criteria must be met to be enrolled in this study: no metastasis to midbrain, pons, cerebellum, meninges, medulla oblongata, or spinal cord; maintaining a stable clinical state for at least 4 weeks, with clinical evidence confirming the absence of new or enlarged brain metastases, and discontinuing corticosteroid and anticonvulsant therapy for at least 2 weeks before the first administration of the investigational drug. Note: The central nervous system is not a target lesion.

12. Having a history of pneumonia and requiring hormone therapy, or having a history of interstitial lung disease, non-infectious pneumonia, severely impaired lung function or uncontrolled lung diseases, such as pulmonary fibrosis, severe radiation pneumonia, acute lung injury, etc., or suspected to have the aforementioned diseases during screening.

13. Having uncontrollable diseases, such as:

- Having infections with poor control and the need for systemic anti-infective drugs (antibiotics, antiviral drugs, or antifungal drugs) within 4 weeks prior to the first administration of the investigational drug.
- Human immunodeficiency virus (HIV) infected individuals (with positive HIV 1/2 antibody).
- Acute or chronic active hepatitis B (defined as positive hepatitis B surface antigen (HBsAg) and/or hepatitis B core antibody (HBcAb) and hepatitis B virus DNA copy numbers ≥104 copies/mL or ≥2000 IU/mL or higher than the detection limit) or acute or chronic active hepatitis C [with positive hepatitis C virus antibody (HCVAb), HCV RNA >103 copies/mL]. Subjects who had undergone nucleotides antiviral therapy and were below the above standards, as well as those who were HCV antibody positive but RNA test negative, were allowed to be enrolled.
- Active COVID-19 infection.
- Active pulmonary tuberculosis, being receiving anti-tuberculosis treatment or having received anti-tuberculosis treatment within one year prior to the first administration of the investigational drug.
- Active or latent syphilis required to be treatment.
- Symptomatic congestive heart failure (New York Heart Association NYHA grades II-IV), with symptomatic or poorly controlled arrhythmias, QTc interval >480 ms, or a personal or family history of congenital long/short QT syndrome.
- Poorly controlled hypertension by standardized treatment (systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥100 mmHg).

14. Having any arterial thromboembolic event within 6 months prior to the first administration of the investigational drug, including myocardial infarction, unstable angina, cerebrovascular accidents, transient ischemic attacks, etc.

15. Tumor invasion of important surrounding structures (such as large blood vessels, trachea, etc.) or risk of gastrointestinal/respiratory tract fistula.

16. After tracheal or digestive tract stent implantation surgery.

17. Symptomatic pleural effusion, ascites, or pericardial effusion that required intervention (such as drainage).

18. Esophageal or gastric varices that required immediate intervention (such as bandaging or sclerotherapy), or subjects who, based on the opinion of the investigator or consultation with gastroenterologists or hepatologists, had a higher risk of bleeding, evidence of portal hypertension (including splenomegaly detected by imaging), or a history of variceal bleeding must undergo endoscopic evaluation within 3 months before the first administration of the investigational drug.

19. Any life-threatening bleeding event or grade 3 or 4 gastrointestinal/variceal bleeding event requiring blood transfusion, endoscopy, or surgical treatment occurring within 3 months prior to the first administration of the investigational drug.

20. A medical history of deep vein thrombosis, pulmonary embolism, or any other severe venous thromboembolism within 3 months prior to the first administration of the investigational drug (implantable venous access port or catheter-related thrombosis, or superficial venous thrombosis was not considered as "severe" venous thromboembolism).

21. Hepatic encephalopathy, hepatorenal syndrome, or Child-Pugh B grade or more severe cirrhosis.

22. Having a risk of intestinal obstruction or perforation (including but not limited to a history of acute diverticulitis and abdominal abscess) or a history of the following diseases: inflammatory bowel disease or extensive bowel resection (partial colectomy or extensive small bowel resection complicated with chronic diarrhea), Crohn's disease, ulcerative colitis, or chronic diarrhea.

23. Significant malnutrition, if intravenous supplementation of nutrient solution was required. Excluding those whose malnutrition had been corrected for more than 4 weeks before the first administration of the investigational drug.

24. Other acute or chronic diseases or laboratory abnormalities that might lead to the following results: increasing the risk of study participation or investigational drug administration, or interfering with the interpretation of study results, and the subjects was regarded as ineligible to participate in this study based on the investigator's judgment.

25. Neurological, psychiatric, or social conditions that met the following characteristics: affecting compliance with study requirements, significantly increasing AE risk, or influencing the ability of subjects to provide written ICF.

26. History of other primary malignant tumors, except for the following:

- Malignant tumors that had been cured, with no known active diseases for at least 2 years prior to enrollment in the study and an extremely low risk of recurrence;
- Non-melanoma skin cancer or lentigo maligna that had been adequately treated and had no evidence of disease recurrence;
- Carcinoma in situ with sufficient treatment and no evidence of disease recurrence.

27. Known history of immunodeficiency.

28. History of allogeneic organ transplantation and history of allogeneic hematopoietic stem cell transplantation.

29. Previous severe allergic reactions to other monoclonal antibodies and/or allergic reactions to any formulation component of the antibody-drug conjugate targeting Claudin 18.2 of the present invention.

30. Pregnant or lactating female subjects.

31. Other conditions ineligible to be enrolled in this study based on the investigator's judgment.

3. Mode of administration and procedures

Dosage: 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 4.5 mg/kg, 6 mg/kg, 8 mg/kg, 10 mg/kg. The actual dosage (mg) would be calculated based on the subject's weight (kg). The initial dose was calculated based on the baseline weight of the subjects. If the weight of the subject changed by less than ±10% from the baseline (the weight for the first administration during the study), the baseline weight would be used to calculate the dosage. Otherwise, the actual dosage would be calculated based on the weight on the pre-determined dosing day or the day before dosing.

Mode of administration: Intravenous drip (peripheral vein, central venous catheter or infusion port). If there were indications, an infusion pump could be used.

Dosing frequency: The administration cycle was 3 weeks/21 days (Q3W), with one administration on the first day of each cycle.

4. Experimental results

Safety assessment

• The incidence rate, the correlation with the investigational drug, and the severity of all TEAEs, AESIs, and SAEs.

• The changes in vital signs, physical examination, and laboratory test results before, during, and after investigational drug treatment.

• Immunogenicity: ADA assay would be conducted on all subjects, and neutralizing antibody (NAb) assay would be performed on ADA positive serum samples (if necessary).

PK assessment

• The PK characteristics of drugs in the human body after single and multiple administration, determining key PK parameters, including but not limited to: AUC, Tmax, Ctrough, Cmax, CL, V, and $t_{1/2}$.

Efficacy assessment

• The efficacy assessment of solid tumors followed RECIST v1.1, and the evaluation indicators included: ORR, DoR, DCR, TTR, and PFS.

• OS.

Biomarker assessment

• Evaluating the relationship between the expression level of CLDN18.2 in tumor tissue before treatment and the therapeutic effect.

Sample size

Dose escalation stage (Phase Ia): Approximately 14-30 DLT evaluable subjects were planned to be enrolled to accurately assess MTD.

Dose expansion stage (Phase Ia): For each tumor type: 2-4 doses were intended to be selected for expansion, with approximately 6-60 subjects enrolled for each expansion dose, totaling approximately 40-140 cases.

Phase Ib: Approximately 261 subjects were planned to be enrolled.

Cohort A: According to the main estimation target of this cohort, assuming that the ORR after receiving the antibody-drug conjugate targeting Claudin 18.2 monotherapy could reach 25%, at a unilateral level of 0.025, 100 samples can be used to observe with approximately 90% confidence that after receiving the antibody-drug conjugate targeting Claudin 18.2 therapy of the present invention, the lower limit of the 95% confidence interval for ORR was 12%. Assuming an expulsion rate of 10%, 111 subjects needed to be enrolled.

Cohort B: Approximately 50 subjects were planned to be enrolled to evaluate the efficacy of the antibody-drug

conjugate targeting Claudin 18.2 of the present invention monotherapy.

Cohorts C and D: Approximately 50 subjects were planned to be enrolled for each cohort (including the subjects who received RP2D during the dose expansion phase (Phase Ia)), to evaluate the efficacy of monotherapy of the antibody-drug conjugate targeting Claudin 18.2 of the present invention.

Statistical method

• Descriptive statistics were used to summarize all safety, PK, immunogenicity, and efficacy data. For continuous variables, descriptive statistics (including number of cases, mean, standard deviation, median, minimum and maximum values; PK indicators also included geometric mean and geometric coefficient of variation) were used to summarize; for discrete variables, frequency and percentage were used for summarization.

• The anti-tumor efficacy indicators included:

- The best overall efficacy [complete response (CR), (partial response) PR, stable disease (SD) or progressive disease (PD)]

- ORR (CR+PR) and DCR (CR+PR+SD)

-DoR or TTR

-PFS

-OS

• If being allowed by data, ORR and DCR and their 95% CI would be provided, and the proportion of subjects with the best response of CR, PR, SD, or PD would also be calculated. The Kaplan-Meier method would be used to calculate the median time and 95% CI of DoR, TTR, PFS, and OS, as well as the rate and 95% CI at specified time points (if applicable, e.g. 6th, 12th month and every 6 months thereafter).

The antibody-drug conjugate targeting Claudin18.2 of the present invention, as an ADC with bystander effect, had a significant tumor inhibitory effect in mouse tumor models with high and low expression of CLDN18.2. To verify the above conclusion, a drug regimen exploration experiment was conducted on subjects with multiple tumor types and different levels of CLDN18.2 expression in clinical studies of the present invention. It was estimated that the monotherapy of the antibody-drug conjugate targeting Claudin18.2 of the present invention could achieve an ORR of 25% for gastric/gastro-esophageal junction adenocarcinoma with high expression of CLDN18.2, which had failed previous second-line systemic treatment, improve PFS and OS while also exhibit good tolerability.

Eligible patients (pts) with advanced solid tumors who failed or intolerant to standard therapy were enrolled. During dose escalation, 6 dose levels of the antibody-drug conjugate targeting Claudin 18.2 (0.3/1/3/6/8/10 mg/kg intravenous Q3W) were evaluated. Selected dose levels were expanded in gastric/gastro-esophageal junction adenocarcinoma (G/GEJ AC) pts with positive CLDN18.2 expression, defined as ≥1% tumor cells with membranous staining of any intensity (1+/2+/3+) by immunohistochemistry. Primary endpoint was safety. Secondary endpoints included objective response rate (ORR), duration of response (DoR), disease control rate (DCR) and progression free survival (PFS) assessed by investigator per RECIST v1.1.

As of January 15, 2024, 159 pts in China and Australia were enrolled (males: 61.0%; median age: 58.0 years; ECOG PS of 1: 82.4%; stage IV: 96.8%; median lines of prior treatment: 2; G/EJ AC: 66.7%) with 79 pts at

6mg/kg and 51 pts at 8mg/kg. Median treatment duration was 13.7 weeks (range: 3.0-40.6). Dose-limiting toxicities (DLTs) were observed in 2 of 6 pts at 10 mg/kg (1 pt with G4 myelosuppression, 1 pt with G4 neutrophil decreased and G3 febrile neutropenia). In all pts, treatment-emergent adverse events (TEAEs) occurred in 149 pts (93.7%) with ≥G3 TEAEs in 79 pts (49.7%). Most common TEAEs were anemia (54.7%), white blood cell count decreased (47.8%), neutrophil count decreased (46.4%). Low incidence of gastrointestinal toxicities ≥G3 were observed including vomiting (1.9%), nausea (1.3%) and decreased appetite (1.3%). Hypoalbuminemia occurred in 24.5% pts and no pt had ≥G3 event. Treatment-related adverse events (TRAEs) leading to treatment discontinuation occurred in 2 (1.3%) pts. No TRAE led to death. As of February 2, 2024, efficacy was evaluable in CLDN18.2-positive (1+/2+/3+ ≥1%) pts with G/GEJ AC (n=95). The overall ORR and DCR were 29.5% (95% CI: 20.6-39.7) and 73.7% (63.6-82.2). In high CLDN18.2 expression (2+/3+ ≥75%) pts, ORR was 43.5% (95% CI: 23.2-65.5) at 6 mg/kg (n=23) and 47.1% (95% CI: 23.0-72.2) at 8 mg/kg (n=17). In high CLDN18.2 expression pts at 6 mg/kg, median PFS was 6.8 months (95% CI: 4.2-NC) with events occurred in 13 pts. PFS of other pts and DoR were immature.

Eligible pts who failed or were intolerant to standard treatment were enrolled. The antibody-drug conjugate targeting Claudin 18.2 was intravenously administered at 6 mg/kg or 8 mg/kg Q3W. In dose escalation, pts were enrolled regardless of CLDN18.2 expression. In dose expansion, pts were required to have CLDN18.2 expression ≥40% (1+/2+/3+ staining intensity by immunohistochemistry). Primary endpoint was safety. Secondary endpoints included objective response rate (ORR), disease control rate (DCR), duration of response (DoR) and progression free survival (PFS) assessed by investigator per RECIST v1.1.

As of December 19, 2023, 35 pts (1 pt in dose escalation and 34 pts in dose expansion) were enrolled from China and Australia (males: 57.1%, median age: 58.0 years, ECOG PS 1: 71.4%, stage IV: 91.4%, median lines of prior treatment: 2) including 28 PDAC pts and 7 BTC pts. Pts received the antibody-drug conjugate targeting Claudin 18.2 at 6 mg/kg (n=17) or 8 mg/kg (n=18). Median duration of treatment was 7.0 weeks (range: 3.0-23.6) with 23 (65.7%) pts still on treatment. In all pts, treatment-related adverse events (TRAEs) occurred in 28 (80.0%) pts including grade ≥3 TRAEs in 9 (25.7%) pts. Common TRAEs (≥20%) were anemia (42.9%), neutrophil count decreased (28.6%), nausea (25.7%), vomiting (25.7%) and white blood cell count decreased (22.9%). Serious TRAEs occurred in 4 (11.4%) pts. TRAEs leading to dose interruption and treatment discontinuation occurred in 7 (20.0%) pts and 1 (2.9%) pt respectively. No TRAE led to death. Safety profiles of the antibody-drug conjugate targeting Claudin 18.2 in PDAC and BTC were comparable with the whole study cohort and no new safety signal was observed. As of January 15, 2024, 25 pts at 6 mg/kg and 8 mg/kg were efficacy evaluable. Partial response (PR) was observed in 7 pts (5 PDAC and 2 BTC). The ORR was 28.0% (95%CI: 12.1-49.4) and DCR was 80.0% (95%CI: 59.3-93.2). In evaluable pts at 6 mg/kg with CLDN18.2 expression ≥60% (1+/2+/3+, n=13), 5 pts had PR with ORR of 38.5% (95%CI: 13.9-68.4) and DCR of 84.6% (95%CI: 54.6-98.1). Among 10 PDAC pts in this subgroup, ORR was 40% (95%CI: 12.2-73.8). DoR and PFS data were immature.

5. Study of dosing regimen

Based on the comprehensive analysis of the clinical safety, efficacy and PK data of the antibody-drug conjugate targeting CLAUDIN18.2 of the present invention, a dose of 6 mg/kg Q3W was selected as the recommended dosing regimen for the key clinical study of the antibody-drug conjugate targeting CLAUDIN18.2 of the present invention:

Pharmacokinetics

The half-life of the antibody-drug conjugate targeting CLAUDIN18.2 of the present invention in tumor patients was about 2 weeks (NCA method), which supported a 3-week dosing interval.

Clinical safety

The results of the exposure-safety analysis showed that: in the dose range of 1~10 mg/kg, the clinical safety risk of the antibody-drug conjugate targeting CLAUDIN18.2 of the present invention increased with increasing dose. In the dose range of 1~6 mg/kg, the exposure-safety relationship was relatively flat, and the overall safety of 6 mg/kg was good. When the dose was greater than 6 mg/kg, the safety risk increased significantly.

Clinical effectiveness

The results of the exposure-effectiveness analysis showed that: In the dose range of 3~6 mg/kg, the PR rate had no obvious exposure-dependent trend, and the PR rate reached a platform. DC (Disease control, SD+PR) had a significant exposure-dependent trend, and DCR reached a plateau at a dose of 6 mg/kg. 6 mg/kg was the best efficacy dose.

In summary, the 6 mg/kg Q3W dosing regimen was the best dosing regimen that balances clinical benefits and safety.

Example 4 Phase III Clinical Trial

1. Investigational drug

Antibody-drug conjugate targeting Claudin 18.2 of the present invention (IEX019-02) for injection

Irinotecan

Paclitaxel

2. Study objectives

2.1 Primary study objective

Comparing the progression free survival (PFS), overall survival (OS), and other efficacy indicators of monotherapy of the antibody-drug conjugate targeting CLAUDIN18.2 of the present invention (test group) and the treatments chosen by the investigators (control group) in subjects on gastric cancer or gastro-esophageal junction adenocarcinoma as a posterior-line treatment.

2.2. Other study objectives

To compare the other efficacy indicators (including ORR, DCR, DoR, TTR) of the antibody-drug conjugate targeting CLAUDIN18.2 of the present invention (test group) and the treatments selected by the investigator (control group) on subjects with gastric cancer or gastroesophageal junction adenocarcinoma as posterior-line treatment.

To evaluate the quality of life of the two groups of subjects.

To further evaluate the safety and tolerability of the antibody-drug conjugate targeting CLAUDIN18.2 of the present invention (test group) in subjects.

To evaluate the overall pharmacokinetic (PK) characteristics of the CLDN18.2 antibody-drug conjugate and

exatecan.

To evaluate the immunogenicity of the antibody-drug conjugate targeting CLAUDIN18.2 of the present invention.

3 Criteria for inclusion/exclusion of subjects

Inclusion criteria:

To be eligible, the subjects must meet all of the following conditions:

Common inclusion criteria for safe introduction stage and primary study stage:

1. Signing an informed consent form (ICF), and willing and able to comply with the visitation and related procedures set in the plan.

2. The age met the definition of adult according to local regulations (such as in the United States, ≥18 years old) on the day of signing the ICF, regardless of gender.

3. According to the Eastern Cooperative Oncology Group Performance Status (ECOG PS), the score was 0 or 1.

4. The expected survival was ≥ 12 weeks.

5. Having sufficient bone marrow and organ functions:

• Blood routine: ANC ≥ 1.5×10^9/L; Platelet count ≥ 100×10^9/L; Hemoglobin content ≥ 9.0 g/dL, the subject should not receive treatment with blood transfusion products (including red blood cell suspension, apheresis platelets, cryoprecipitation, etc.), erythropoietin (EPO), Granulocyte-colony-stimulating factor (G-CSF), or granulocyte macrophage colony-stimulating factor (GM-CSF) within 7 days before blood collection;

• Liver function: TBIL ≤ 1.5 × ULN (allowing TBIL ≤ 3 × ULN in subjects with Gilbert syndrome); ALT and AST ≤ 2.5 × ULN in subjects without liver metastasis, as well as ALT and AST ≤ 5 × ULN in subjects with liver metastasis; Albumin ≥ 28 g/L (Subjects were not allowed to receive treatment with human serum albumin infusion within 7 days prior to blood collection);

• Renal function: creatinine clearance ≥ 60 mL/min (using Cockcroft-Gault formula); Urinary protein < 2+ or 24 h total urine protein < 1 g;

• Coagulation function: International normalized ratio (INR) ≤1.5 and activated partial thromboplastin time (APTT) ≤ 1.5 × ULN (subjects were allowed to receive anticoagulant treatment and had coagulation function within the above range).

6. Female subjects must agree not to breastfeed from screening to 6 months after the last administration.

7. Female subjects of childbearing age or male subjects whose partners were in childbearing age were required to take effective contraceptive measures from screening to the entire treatment period and within 6 months after the treatment period.

• Male subjects must agree not to donate sperm from screening to the entire study period and within 6 months after the last administration;

• Female subjects must agree not to donate oocytes from screening to the entire study period and within 6 months after the last administration;

- Female subjects must agree not to breastfeed from screening to the entire treatment period and within 6 months after the last administration.

Additional inclusion criteria for primary study stage:

8. Unresectable, locally advanced or metastatic Gastric/gastro-Esophageal Junction Adenocarcinoma (G/GEJA) diagnosed by histopathology.

9. Progressive locally advanced or metastatic gastric/gastroesophageal junction adenocarcinoma confirmed by imaging.

10. Suffering from disease lesions (measurable and/or unmeasurable diseases) that could be evaluated by radiological image according to RECIST 1.1 standards. For subjects with only one radiological assessable lesion, if the lesion had previously been treated with local radiotherapy, the progression after radiotherapy must be clearly clarified.

11. Having received at least two systemic treatments [previous treatments must include fluorouracil, platinum chemotherapeutic agent, and paclitaxel/docetaxel, irinotecans, or taxanes] and experienced disease progression. The previous systemic treatment lines were ≤4.

a) The period from the end of previous (new) adjuvant systemic treatment to disease recurrence/progression of ≤6 months was considered as one-line treatment;

b) If a certain drug was discontinued due to AE in previous combination therapy, but any other drug in combination was still used, it would be considered as the same treatment regimen;

c) No disease progression had occurred, and the same dosage form had been changed, which was considered as the same treatment regimen.

12. The positive expression of CLDN18.2* was confirmed by pathological tissue testing in the central laboratory. For subjects who had previously received any anti-CLDN18.2 treatment, tumor samples should be collected again after the relevant anti-CLDN18.2 treatment was completed.

Note:

*CLDN18.2-positive: The degree of Claudin18.2 immunohistochemical membrane staining was ≥2+ in ≥75% of tumor cells.

Additional inclusion criteria for the safe introduction stage:

13. Locally advanced, unresectable or metastatic solid tumors confirmed by histopathology, with standard treatment failure or intolerance or no standard treatment.

Exclusion criteria:

Subjects meeting any one of the following criteria were not selected for this study:

1. HER2 positivity (immunohistochemistry [IHC] 3+, or IHC 2+ and in situ hybridization positivity). HER2 positive subjects who had received anti-HER2 treatment in the past did not meet the exclusion criteria if HER-2 detection was negative in tissue samples taken after anti-HER2 treatment. (For primary study stages)

2. Being participating in another interventional clinical study, excluding observational (non-interventional) clinical studies or in the survival follow-up stage of interventional studies.

3. Previously receiving the antibody-drug conjugate therapy based on topoisomerase inhibitors.

4. Receiving the last anti-tumor treatment within 4 weeks prior to the first administration of the investigational drug or within 5 half-lives of the anti-tumor treatment drug (Including traditional Chinese medicine with clear indications for gastric cancer in the instruction, but not including herbal formulas) (whichever was shorter) (Drugs without an exact half-life needed to be washed out for 4 weeks).

5. Being expected to receive other anti-tumor treatments during the treatment with the investigational drug [allowing for palliative radiotherapy with the aim of relieving symptoms (such as pain) without affecting efficacy evaluation].

6. Receiving treatment with a strong inhibitor of cytochrome P450 3A4 (CYP3A4) within 2 weeks or 5 half-lives (whichever is longer) prior to the first administration of the investigational drug.

7. Inoculated any live vaccine within 4 weeks prior to the first administration of the investigational drug or being expected to inoculate any live vaccine during the study.

8. Prior to the first administration of the investigational drug, there were toxicity that had not been restored to 0 or 1 level in NCI CTCAE v5.0 due to previous treatments (excluding hair loss, fatigue, pigmentation, and other situations judged by the investigator to have no safety risk).

9. Within 4 weeks prior to the first administration of the investigational drug, having major surgy (craniotomy, thoracotomy, or laparotomy, or others as defined by the investigator, excluding biopsy) or unhealed wounds, ulcers, or fractures; or underwent laparoscopic exploration surgery within 2 weeks prior to the first dose of study treatment; or plan to undergo major surgery during the study; Note: for the purpose of palliative treatment, local surgical treatment for isolated lesions was acceptable.

10. Patients with bone metastases at risk of paraplegia.

11. Previously receiving whole pelvic radiotherapy.

12. Received radiation therapy within 3 weeks prior to initial administration. For subjects who received radiation therapy three weeks before the first administration, all of the following conditions must be met before enrollment: there were currently no radiation-related toxic reactions (including but not limited to: no need to take glucocorticoids; excluding radiation pneumonia, radiation hepatitis, radiation enteritis, intestinal dysfunction, etc.), and no need to take glucocorticoids.

13. Obstruction of the cardia and pylorus that affected eating or gastric emptying, and/or persistent recurrent vomiting (vomiting $\geq$3 times within 24 hours).

14. Within 6 months prior to the first administration of the investigational drug, there was a history of gastrointestinal perforation and/or fistula that had not been cured by surgical treatment.

15. Symptomatic metastasis to central nervous system and/or spinal compression symptoms. For subjects with asymptomatic brain metastases (i.e. no neurological symptoms, no glucocorticoid treatment required, all brain metastases being $\leq$1.5 cm) or subjects with stable symptoms after treatment of brain metastases, all of the following criteria must be met to be enrolled in this study: no metastasis to midbrain, pons, cerebellum, meninges, medulla oblongata, or spinal cord; maintaining a stable clinical state for at least 4 weeks, with clinical evidence confirming the absence of new or enlarged brain metastases, and discontinuing corticosteroid and anticonvulsant therapy for at least 2 weeks before the first administration of the investigational drug. Note: The central nervous system was not a target lesion.

16. Having a history of interstitial lung disease, non-infectious pneumonia, severely impaired lung function or uncontrolled lung diseases, such as pulmonary fibrosis, severe radiation pneumonia, acute lung injury, etc., or suspected to have the aforementioned diseases during screening.

17. Having uncontrollable diseases, such as:

- Having serious infections with activity or poor clinical control, requiring treatment with systemic anti-infective drugs (antibiotics, antiviral drugs, or antifungal drugs), including but not limited to respiratory, urinary, and biliary systems, within one week prior to the first administration of the investigational drug.
- Human immunodeficiency virus (HIV) infected individuals (with positive HIV 1/2 antibody).
- Acute or chronic active hepatitis B [Subjects who are positive for hepatitis B surface antigen (HBsAg) and/or hepatitis B core antibody (HBcAb) need to undergo further hepatitis B virus (HBV) DNA testing. If the copy number of HBV DNA is 104 ≤copies/mL or ≥2000 IU/mL or lower than the detection limit, the subject can be included in the study.] or acute or chronic active hepatitis C [with positive hepatitis C virus antibody (HCVAb), HCV RNA >103 copies/mL]. Subjects who had undergone nucleotides antiviral therapy and were below the above standards, as well as those who were HCV antibody positive but RNA test negative, were allowed to be enrolled.
- Active pulmonary tuberculosis, being receiving anti-tuberculosis treatment or having received anti-tuberculosis treatment within one year prior to the first administration of the investigational drug.
- Active or latent syphilis required treatment.
- Symptomatic congestive heart failure (New York Heart Association NYHA grades II-IV), with symptomatic or poorly controlled arrhythmias, QTc interval >480 ms, or a personal or family history of congenital long/short QT syndrome.
- Poorly controlled hypertension (systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥100 mmHg).

18. Having any arterial thromboembolic event within 6 months prior to the first administration of the investigational drug, including myocardial infarction, unstable angina, cerebrovascular accidents, transient ischemic attacks, etc.

19. A history of deep vein thrombosis, pulmonary embolism, or any other severe venous thromboembolism within 3 months prior to the first administration of the investigational drug (implantable venous infusion port or catheter-related thrombosis, or superficial venous thrombosis was not considered "severe" venous thromboembolism).

20. Tumor invasion of important surrounding structures or organs (such as large blood vessels, trachea, etc.) or risk of gastrointestinal/respiratory tract fistula.

21. After tracheal or gastrointestinal [Denoting the muscular tracts from the oral cavity to the anal canal, including the oral cavity, pharynx, esophagus, stomach, small intestine (duodenum, jejunum, ileum), large intestine (cecum, appendix, colon, rectum), anal canal, etc.] stent implantation surgery.

22. Symptomatic pleural effusion, ascites, or pericardial effusion that requires intervention (such as drainage, peritoneal shunt or cell-free and concentrated ascites reinfusion therapy [CART]). Only the subjects with a small amount of pleural effusion, ascites, and pericardial effusion displayed on imaging and the asymptomatic

subjects could be selected (drainage and CART treatment were not allowed within two weeks before the screening).

23. Esophageal or gastric varices that required immediate intervention (such as bandaging or sclerotherapy), or subjects who, based on the opinion of the investigator or consultation with gastroenterologists or hepatologists, had a higher risk of bleeding, evidence of portal hypertension (including splenomegaly detected by imaging), or a history of variceal bleeding must undergo endoscopic evaluation within 3 months before the first administration of the investigational drug.

24. Any life-threatening bleeding event or grade 3 or 4 gastrointestinal/variceal bleeding event requiring blood transfusion, endoscopy, or surgical treatment occurring within 3 months prior to the first administration of the investigational drug.

25. Hepatic encephalopathy, hepatorenal syndrome, or Child-Pugh B grade or more severe cirrhosis.

26. Having complete or incomplete intestinal obstruction during the screening, or a history of complete or incomplete intestinal obstruction or a risk of intestinal perforation (including but not limited to a history of acute diverticulitis and abdominal abscess) or a history of the following diseases within 3 months prior to the first administration: inflammatory bowel disease or extensive bowel resection (partial colectomy or extensive small bowel resection complicated with chronic diarrhea), Crohn's disease, ulcerative colitis, or chronic diarrhea.

27. Significant malnutrition (e.g. requiring intravenous supplementation of nutrient solution; from the day of signing ICF, weight loss exceeding 5% within one month, weight loss exceeding 15% within 3 months, or food intake reduction of 1/2 or more within 1 week). Excluding those whose malnutrition had been corrected for more than 4 weeks before the first administration of the investigational drug.

28. Other acute or chronic diseases or laboratory abnormalities that might lead to the following results: increasing the risk of study participation or investigational drug administration, or interfering with the interpretation of study results, and the subjects was regarded as ineligible to participate in this study based on the investigator's judgment.

29. Uncontrolled metabolic disorders or other non-malignant organ or systemic diseases or secondary reactions to cancer (such as leukemoid reaction) could lead to higher medical risk and/or uncertainty in survival assessment.

30. Neurological, psychiatric, or social conditions that met the following characteristics: affecting compliance with study requirements, significantly increasing AE risk, or influencing the ability of subjects to provide written ICF.

31. History of other primary malignant tumors, except for the following:

- Malignant tumors that had been cured, with no known active diseases for at least 2 years prior to enrollment in the study and an extremely low risk of recurrence;
- Non-melanoma skin cancer or lentigo maligna that had been adequately treated and had no evidence of disease recurrence;
- Carcinoma in situ with sufficient treatment and no evidence of disease recurrence.

32. Known history of immunodeficiency.

33. History of allogeneic organ transplantation and history of allogeneic hematopoietic stem cell transplantation.

34. Previous severe allergic reactions to other monoclonal antibodies and/or allergic reactions to any formulation component of the antibody-drug conjugate targeting Claudin 18.2 of the present invention, irinotecan, and paclitaxel.

35. Other conditions ineligible to be enrolled in this study based on the investigator's judgment.

4 Mode of administration and procedures

According to the research program, the subjects were randomly divided into the test group or control group in a 1:1 ratio. Stratified factors included: region (Asian countries/regions except Japan vs. Japan vs. Europe and America), primary site of cancer (stomach vs. gastro-esophageal junction), and primary lesion resection (yes vs. no). The subjects in the test group received antibody-drug conjugates targeting CLAUDIN18.2 at a dose of 6 mg/kg IV D1, Q3W, every three weeks as one cycle. The control group received the treatment selected by the investigators, including: irinotecan 125 mg/m^2 IV D1, D8, Q3W every three weeks as one cycle (note: 150 mg/m^2 IV D1, Q2W every two weeks as one cycle in Europe and Japan), or paclitaxel 80 mg/m^2 IV D1, D8, D15, Q4W every four weeks as one cycle. Investigators need to choose the treatment method before randomizing the subjects.

The subjects would receive continuous treatment until the disease progressed and/or the investigator evaluated that the subject no longer benefited, toxicity was intolerable, ICF was withdrawn, other reasons for discontinuing study treatment occurred, or the treatment duration reached 24 months (whichever occurred first). Subjects terminated treatment standard. After termination of treatment, safety and survival follow-up would be conducted on the subjects. After disease progression, the subjects in the control group were not allowed to cross over to the group of antibody-drug conjugate targeting CLAUDIN18.2 for treatment.

This study established an Independent Data Monitoring Committee (IDMC), which would review the accumulated safety data of the study, to ensure the safety of the subjects and the scientificity of the experiment. Moreover, based on the safety data, IDMC would provide recommendations to the sponsor for the continuation, revision, or cessation of the experiment. For mid-term analysis, IDMC would review the results of safety and efficacy data, make judgments on the validity of the trial based on the preset validity threshold, and provide recommendations to the sponsor. The responsibilities and related procedures of IDMC members would be defined separately in the IDMC Charter. The final version of the IDMC charter needed to be approved by IDMC and the sponsor before analysis.

For Japan and the United States, a safe introduction phase will be set up before entering critical clinical practice. Each country will enroll 3-6 subjects to evaluate safety and PK characteristics. In the safe introduction stage, three subjects will first be enrolled and treated with 6 mg/kg of the antibody-drug conjugate targeting CLAUDIN18.2. The DLT observation period is 21 days after the first administration in the first cycle. If all three subjects do not experience DLT during the observation period of DLT, critical clinical enrollment will begin. If one of the first three subjects experiences DLT, three more subjects will be included in the safe introduction phase; If there is no occurrence of DLT in the 3 additional subjects included, critical clinical enrollment will begin.

Antibody-drug conjugate targeting CLAUDIN18.2 of the present invention

Dosage form: freeze-dried injection preparation

Strength: 100 mg/vial

Mode of administration: 6 mg/kg, D1, Q3W, intravenous infusion (IV).

Irinotecan

Mode of administration:

125 mg/m^2, D1, D8, Q3W, IV

150 mg/m^2, D1, Q2W, IV (Applicable only to research centers in the European Union and Japan).

Paclitaxel

Mode of administration: 80 mg/m^2, D1, D8, D15, Q4W, IV.

5 Analysis of experimental results

Efficacy assessment

- OS.
- The efficacy assessment of solid tumors followed RECIST v1.1, evaluated by IRRC and investigators, and the evaluation indicators included: PFS, ORR, DCR, DOR, and TTR.

Quality of life

- EORTC QLQ-C30 score, EORTC QLQ-OG25 score, EQ-5D-5L score.

Safety assessment

- The incidence rate, the correlation with the investigational drug, and the severity of all TEAEs, AESIs, and SAEs.
- The changes in vital signs, ECG physical examination, and laboratory test results before, during, and after investigational drug treatment.
- Immunogenicity: ADA assay would be conducted on all subjects, and NAb assay would be performed on ADA positive serum samples (if necessary).

PK assessment

- The PK parameters included but were not limited to: AUC, Ctrough, Cmax, CL, V, and t1/2.

Biomarker assessment

- Evaluating the relationship between the expression level of CLDN18.2 in tumor tissue before treatment and the therapeutic effect.

Hypothesis testing:

The main efficacy endpoints of this study were PFS and OS. Type I error was controlled at 0.025 on one-side and assigned to two endpoints: PFS(α=0.005) and OS(α=0.02). Firstly, hypothesis testing on PFS was

performed. If a statistically significant difference was reached at the level of one-side α=0.005, α will be recovered and all would be passed on to OS. Otherwise, OS would be analyzed at one-side α=0.0199. During the final analysis of PFS, OS unbound futility interim analysis would be carried out, and α=0.0001 would be allocated for OS futility interim analysis.

The hypothesis testing for the superiority of PFS and OS was as follows:

Null hypothesis H0: HR≥1; Alternative hypothesis Ha: HR<1

Sample size calculation:

Rank test would be used to compare the differences between groups, and subjects would be randomly assigned to two treatment groups in a 1:1 ratio. Assuming an exponential distribution of PFS and OS for each treatment group, with a PFS HR of 0.5 (median PFS was 3.5 months in the control group, while median PFS was 7.0 months in the test group), a total of approximately 179 PFS events needed to be observed at a unilateral level of α=0.005, with approximately 98% confidence for final analysis. Assuming HR was 0.71 (median OS was 6.5 months in the control group, while median OS was 9.2 months in the test group), a total of approximately 371 OS events needed to be observed at a unilateral level of α=0.0199, with approximately 88% confidence for final analysis. Assuming the enrollment rate was as follows: 29 cases per month in the first 4 months, 37 cases per month from 5 to 8 months, 46 cases per month from 9 to 12 months, and 17 cases per month thereafter. Assuming an annual dropout rate of 15% and 5% for PFS and OS in each group, 450 subjects would be expected to observe 179 PFS events in the 11th month after the first subject was randomized, and 371 OS events could be observed in the 27th month after the first subject was randomized.

Interim analysis

This study plans to conduct one PFS final analysis, two OS interim analyses, and one OS final analysis. The first OS futility interim analysis would be conducted simultaneously with the final analysis of PFS (179 PFS events), the interim analysis time would be driven by the number of events required for PFS final analysis, and it was expected that approximately 37% (138) of OS events would occur during the interim analysis (HR futility boundary ≥1.005); the second interim analysis of OS validity would be conducted at 75% (278) events, with a minimum detectable difference (MDD) of HR=0.745 and a unilateral level of 0.0072. The final analysis of OS was expected to be conducted at 100% (371 cases) of events, with a minimum detectable difference (MDD) of HR=0.804 and a unilateral level of approximately 0.0177. The overall significance level of OS would be adjusted as follows: the first OS futility interim analysis would conduct by two sets of unbound futility tests based on the Gamma Family cost function with parameter -2. The α margin of the second validity interim analysis and final analysis of OS were approximated by the Lan-Demets method to the O'brien-Fleming boundary allocation and PFS final analysis results (determining whether to recover α of PFS).

Statistical methods:

The descriptive summary of continuous variables would include number of cases, mean, standard deviation, median, minimum and maximum values. The descriptive summary of categorical variables would include the number of cases and percentages.

Primary analysis:

The primary analysis for comparing PFS and OS groups was conducted using a stratified log-rank test. The Kaplan-Meier method was used to estimate the median PFS and OS, as well as their 95% confidence interval (CI), and survival curves were plotted. Simultaneously, a stratified Cox proportional hazard model was used

to estimate inter group HR and its 95% CI, and the stratification factor was a random stratification factor.

Minority analysis:

The primary analysis for the comparison between ORR and DCR groups was performed by testing via the CMH method with added stratification factors, and the Miettinen &Nurminen method with added stratification factors was used to estimate the difference in ORR and DCR rates between groups and their CI. Kaplan-Meier method was used to estimate median DoR, TTR, and their 95% CI, and the survival curves were plotted.

Descriptive statistical analysis was conducted on the scale scores for quality of life.

Descriptive summary was performed for safety and tolerability. Unless otherwise specified, no formal hypothesis testing was conducted on the data of safety and tolerability.

Descriptive statistical analysis was carried out on drug concentration and related PK parameter indicators.

Descriptive summary was performed for CLDN18.2 baseline expression levels. If the data were applicable, exploratory analysis was conducted on the relationship between the expression level of CLDN18.2 and the efficacy, safety, and PK (if applicable) indicators.

Descriptive summary of safe introduction stage would be provided separately.

The data currently obtained show that, as confirmed by Phase Ia and Ib trials, the antibody-drug conjugate targeting CLAUDIN18.2 of the present invention has a good safety and excellent therapeutic efficacy as shown by, for example, PFS, OS, ORR and/or DCR.

Abbreviations and Definitions of Terms

Abbreviations	Definitions
ADA	anti-drug antibody
ADC	antibody-drug conjugate
ADCC	antibody dependent cell-mediated cytotoxicity
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
AUC	area under curve
CDC	complement dependent cytotoxicity
CI	confidence interval
CR	complete response
CRA	clinical research associate
CRO	contract research organization
CSR	clinical study report
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DCR	disease control rate
DLT	dose limiting toxicity
DoR	duration of response
EC	ethics committee
ECOG PS	Eastern Cooperative Oncology Group Performance Status
eCRF	electronic case report form
EDC	electronic data capture

EEP	efficacy evaluable population
FDA	Food and Drug Administration
FIH	first in human
GCP	good clinical practice
G/GEJ AC	gastric/gastro-esophageal junction adenocarcinoma
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HR	hazard ratio
ICF	informed consent form
INR	international normalized ratio
IRR	infusion related reaction
IV	intravenously
MAD	maximum ascending dose
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NAb	neutralizing antibody
NSAID	non-steroidal anti-inflammatory drug
ORR	overall response rate
OS	overall survival
PAD	pharmacologically active dose
PD	progressive disease
PDAC	pancreatic ductal adenocarcinoma
PFS	progression free survival
PK	pharmacokinetics
PR	partial response
SAE	serious adverse event
SAP	statistical analysis plan
SD	stable disease
SP	safety population
TEAE	treatment emergent adverse event
TGI	tumor growth inhibition
TRAE	treatment related adverse event
TTR	time to response
ULN	upper limit of normal value

Sequence information:

SEQ ID NO		HB37A6 (HCDR1 is defined by AbM rules, and HCDR2, HCDR3 and LCDR1, 2 and 3 are defined by Kabat rules)
1	HCDR1	GFTFSSYVMS
2	HCDR2	TISHSGGSTYYADSVKG
3	HCDR3	DAPYYDILTGYRY
4	Heavy chain variable region	EVQLLDSGGGLVQPGGSLRLSCAASGFTFSSYVMSWVRQAPGKGLNWV STISHSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAI DAPYYDILTGYRYWGQGTLVTVSS

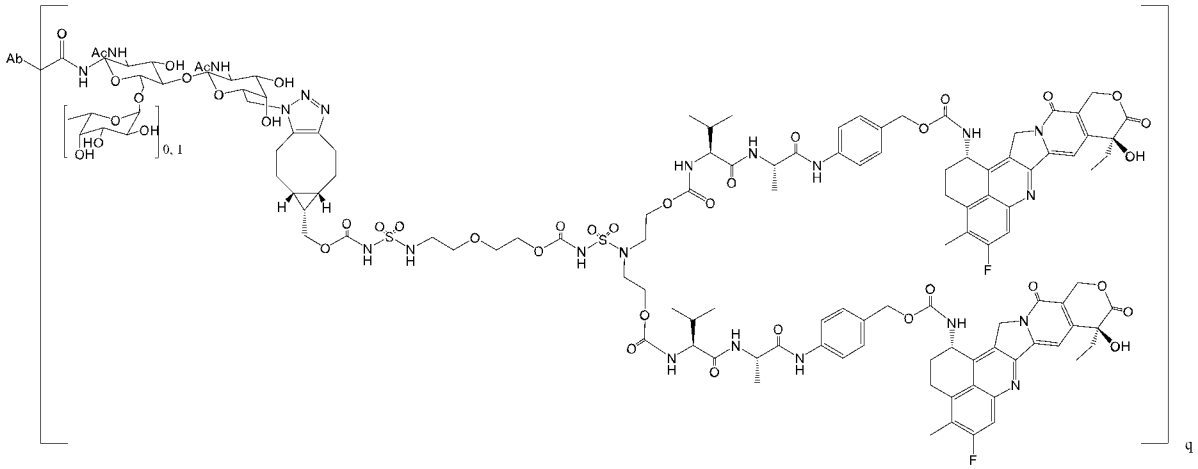
	(VH)	
5	Heavy chain constant region (HC)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
6	LCDR1	RASQSISSWLA
7	LCDR2	KASSLES
8	LCDR3	QQYNSYSYT
9	light chain variable region (VL)	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYK ASSLESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYNSYSYTFGQG TKLEIK
10	Light chain constant region (LC)	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS GNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC
11	Heavy chain	EVQLLDSGGGLVQPGGSLRLSCAASGFTFSSYVMSWVRQAPGKGLNWV STISHSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAI DAPYYDILTGYRYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK
12	Light chain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYK ASSLESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYNSYSYTFGQG TKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC

The exemplary embodiments of the present invention are described above, and it should be understood by those skilled in the art that these disclosures are only exemplary, and various other substitutions, adaptations and modifications can be made within the scope of the present invention. Therefore, the present invention is not limited to the specific embodiments listed herein.

CLAIMS

1. A method for preventing or treating a solid tumor in a subject, which comprises administering to a subject in need thereof an effective amount of an antibody-drug conjugate targeting Claudin18.2 or a pharmaceutically acceptable salt thereof;

wherein, the antibody-drug conjugate targeting Claudin18.2 is selected from antibody-drug conjugate of formula IA:



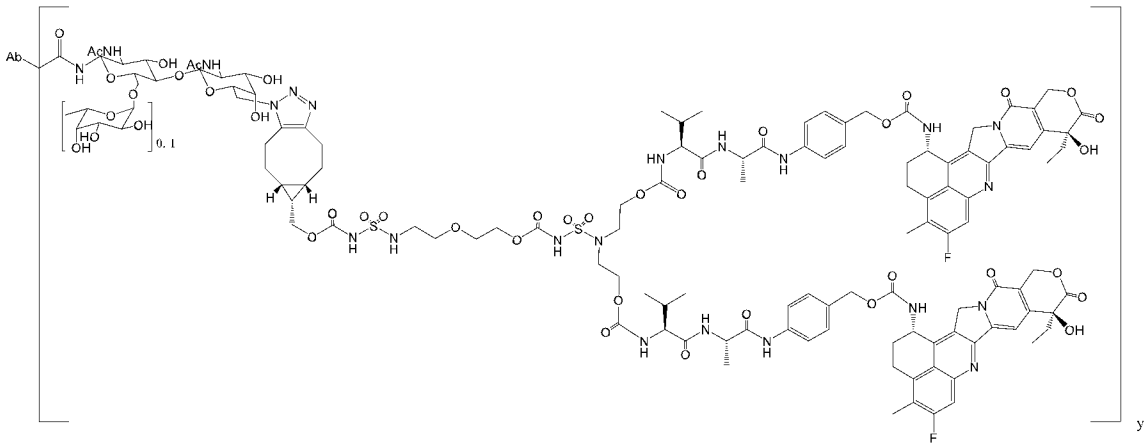
IA

wherein Ab is an antibody or antigen-binding fragment thereof targeting Claudin18.2;

wherein q represents an average DAR, and is a number from 2 to 11, for example a number from 2 to 5, preferably a number from 3 to 5, more preferably a number from 3 to 4.

2. A method for preventing or treating a solid tumor in a subject, which comprises administering to a subject in need thereof an effective amount of an antibody-drug conjugate targeting Claudin18.2 or a pharmaceutically acceptable salt thereof;

wherein, the antibody-drug conjugate targeting Claudin18.2 is selected from antibody-drug conjugate of formula IB:



IB

wherein Ab is an antibody or antigen-binding fragment thereof targeting Claudin18.2;

wherein y is an integer of 1 or 2.

3. The method according to claim 1 or 2, wherein the antibody or antigen-binding fragment thereof targeting to Claudin18.2 comprises a HCDR1, a HCDR2, a HCDR3 respectively consisting of amino acid sequences set forth in SEQ ID NO:1, 2 and 3, as well as an LCDR1, an LCDR2 and an LCDR3 respectively consisting of amino acid sequences set forth in SEQ ID NO: 6, 7 and 8.

4. The method according to any one of claims 1 to 3, wherein the antibody or antigen-binding fragment thereof targeting to Claudin18.2 comprises a heavy chain variable region and/or a light chain variable region, wherein the heavy chain variable region

(i) comprises or consists of an amino acid sequence set forth in SEQ ID NO: 4; or

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

(iii) comprises or consists of an amino acid sequence having one or more (preferably no more than 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid replacements, and more preferably conservative amino acid replacements) compared with an amino acid sequence set forth in SEQ ID NO: 4, preferably, the amino acid alterations do not occur in the CDRs; and/or

the light chain variable region

(i) comprises or consists of an amino acid sequence set forth in SEQ ID NO: 9; or

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

(iii) comprises or consists of an amino acid sequence having one or more (preferably no more than 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid replacements, and more preferably conservative amino acid replacements) compared with an amino acid sequence set forth in SEQ ID NO: 9, preferably, the amino acid alterations do not occur in the CDRs.

5. The method according to any one of claims 1 to 4, wherein the antibody or an antigen-binding fragment thereof targeting to Claudin18.2 comprises a heavy chain and a light chain, wherein the heavy chain

(i) comprises or consists of an amino acid sequence set forth in SEQ ID NO:11; or

(ii) comprises or consists an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid set forth in SEQ ID NO:11; or

(iii) comprising or consists of an amino acid sequence having one or more (preferably no more than 20 or 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid replacements, and more preferably conservative amino acid replacements) compared with an amino acid sequence set forth in SEQ ID NO: 11; and/or

the light chain

(i) comprises or consists of an amino acid sequence set forth in SEQ ID NO:12; or

(ii) comprises or consists an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid set forth in SEQ ID NO:12; or

(iii) comprises or consists of an amino acid sequence having one or more (preferably no more than 20 or 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid replacements, and more preferably conservative amino acid replacements) compared with an amino acid sequence set forth in SEQ ID NO: 12.

6. The method according to any one of claims 1-5, wherein the solid tumor is an advanced malignant solid tumor selected from one or more of: gastric cancer, breast cancer, liver cancer, colon cancer, lung cancer, gallbladder cancer, esophageal cancer, gastro-esophageal junction adenocarcinoma, pancreatic ductal adenocarcinoma and biliary tract cancer;

preferably, the advanced solid tumor is a tumor that has not been treated with a systemic anti-tumor treatment, a tumor that has failed at least one systemic anti-tumor treatment, a tumor that has failed first-line treatment (e.g., being refractory or intolerant to first-line treatment), a tumor that has failed second-line treatment (e.g., being refractory or intolerant to second-line treatment); and/or

preferably, the advanced malignant solid tumor is a locally advanced unresectable or metastatic solid tumor.

7. The method according to claim 6, wherein the solid tumor is a posterior-line gastric cancer or gastro-esophageal junction adenocarcinoma;

preferably, the posterior-line gastric cancer or gastro-esophageal junction adenocarcinoma is 2L and thereafter gastric cancer or gastro-esophageal junction adenocarcinoma.

8. The method according to claim 7, wherein the posterior-line gastric cancer or gastro-esophageal junction adenocarcinoma is 3L gastric cancer or gastro-esophageal junction adenocarcinoma.

9. The method according to any one of claims 1-8, which comprises administering to the subject 0.1mg/kg-100mg/kg of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof.

10. The method according to claim 9, which comprises administering to the subject 0.2mg/kg-50mg/kg of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof.

11. The method according to claim 10, which comprises administering to the subject 0.3mg/kg-30mg/kg of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof.

12. The method according to any one of claims 1 to 11, which comprises administering to the subject 4 mg/kg-8 mg/kg of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof.

13. The method according to any one of claims 1 to 12, which comprises administering to the subject 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 4.5 mg/kg, 6 mg/kg, 7.5 mg/kg, 8 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, 13.5 mg/kg, or 15 mg/kg of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof.

14. The method according to any one of claims 1-13, wherein the administration cycle of the method is 2 weeks-5 weeks, such as 18-24 days, such as 19, 20, 21, 22 or 23 days, administering once per administration cycle.

15. The method according to any one of claims 1-14, wherein the administration cycle of the method is 3 weeks, administering once on day 1 per administration cycle.

16. The method according to any one of claims 1-15, which comprises administering to the subject 6 mg/kg of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof once every 3 weeks.

17. The method according to any one of claims 1-16, wherein the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof is administered by intravenous infusion.

18. A single dosage unit comprising an effective amount of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof according to any one of claims 1 to 17.

19. A kit of parts comprising an effective amount of the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof according to any one of claims 1 to 17;

preferably, also comprising a package insert on which instructions for using the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof to prevent or treat an advanced malignant solid tumor in a subject are printed.

20. The single dosage unit according to claim 18 or kit of parts according to claim 19 in the manufacture of a medicament for preventing or treating an advanced malignant solid tumor.

21. An antibody-drug conjugate targeting Claudin18.2 or a pharmaceutically acceptable salt thereof, for use of prevention or treatment of a solid tumor in a subject;

wherein, the antibody-drug conjugate targeting Claudin18.2 is selected from the antibody-drug conjugate of formula IA as defined in claim 1 or is selected from the antibody-drug conjugate of formula IB as defined in claim 2.

22. The antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof according to claim 21, wherein the antibody targeting Claudin18.2 or antigen-binding fragment thereof is as defined in any one of claims 3-5, and/or

wherein the solid tumor is as defined in any one of claims 6-8; and/or

wherein the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof is administered to the subject at a dose as defined in any one of claims 9 to 13; and/or

wherein the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof is administered according to the dosing regimen as defined in any one of claims 14 to 17.

23. Use of an antibody-drug conjugate targeting Claudin18.2 or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for preventing or treating a solid tumor in a subject;

wherein, the antibody-drug conjugate targeting Claudin18.2 is selected from the antibody-drug conjugate of formula IA as defined in claim 1 or is selected from the antibody-drug conjugate of formula IB as defined in claim 2.

24. The use according to 23, wherein the antibody targeting Claudin18.2 or antigen-binding fragment thereof is as defined in any one of claims 3-5, and/or

wherein the solid tumor is as defined in any one of claims 6-8; and/or

wherein the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof is administered to the subject at a dose as defined in any one of claims 9 to 13; and/or

wherein the antibody-drug conjugate targeting Claudin18.2 or the pharmaceutically acceptable salt thereof is administered according to the dosing regimen as defined in any one of claims 14 to 17.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2024/099382

A. CLASSIFICATION OF SUBJECT MATTER

A61K 47/68 (2017.01)i; A61P35/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS, CNTXT, DWPI, WOTXT, EPTXT, USTXT, CNKI, PUBMED, ISI_Web of Science, Science Direct, NCBI, STNext: FORTVITA BIOLOGICS (SINGAPORE) PTE.LTD.,LUOYang, antibody-drug conjugate, claudin 18.2, structure search, SEQ ID NO:1-4, 6-9, 11-12

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2022058395 A1 (SYNAFFIX B.V.) 24 March 2022 (2022-03-24) description paragraphs 0001, 0135, claims 1-18	1-24
Y	WO 2022237666 A1 (REMEGEN CO., LTD.) 17 November 2022 (2022-11-17) abstract, claims 1-25	1-24
Y	WO 2022136642 A1 (SOTIO BIOTECH A. S.) 30 June 2022 (2022-06-30) claims 1-18	1-24
Y	CN 115969997 A (CHINA RESOURCES BIOPHARM CO., LTD.) 18 April 2023 (2023-04-18) claims 1-20	1-24
Y	WO 2016165762 A1 (GANYMED PHARMACEUTICALS AG. et al.) 20 October 2016 (2016-10-20) claims 1-68	1-24
Y	WO 2021228141 A1 (SICHUAN KELUN-BIOTECH BIOPHARMACEUTICAL CO., LTD.) 18 November 2021 (2021-11-18) claims 1-24	1-24

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 September 2024	**25 September 2024**

Name and mailing address of the ISA/CN	Authorized officer
CHINA NATIONAL INTELLECTUAL PROPERTY ADMINISTRATION **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088, China**	**JIAO,ShiYong** Telephone No. **(+86) 010-53961915**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2024/099382

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed.
   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2024/099382

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **1-17**
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 1-17 direct to a method for preventing or treating a solid tumor in a subject, and do not meet the criteria set out in PCT Rules 39.1(iv). The search report has been made and based on the use of antibody-drug conjugate of formula IA for the manufacturing of medicament for preventing or treating a solid tumor in a subject.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2024/099382

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
WO 2022058395 A1	24 March 2022	EP 4213885 A1	26 July 2023
		JP 2023541637 A	03 October 2023
		US 2023330245 A1	19 October 2023
WO 2022237666 A1	17 November 2022	EP 4335873 A1	13 March 2024
		US 2023272109 A1	31 August 2023
		CA 3184403 A1	17 November 2022
		BR 112023009912 A2	28 November 2023
		KR 20230135653 A	25 September 2023
		JP 2023552733 A	19 December 2023
		AU 2022275043 A1	16 February 2023
		TW 202246344 A	01 December 2022
		TWI 832235 B	11 February 2024
WO 2022136642 A1	30 June 2022	CA 3199830 A1	30 June 2022
		EP 4267194 A1	01 November 2023
		AU 2021405049 A1	22 June 2023
		US 2024100180 A1	28 March 2024
		JP 2024500242 A	05 January 2024
		TW 202241519 A	01 November 2022
		KR 20230124037 A	24 August 2023
		PE 20231561 A1	03 October 2023
		MX 2023007644 A	07 July 2023
		CL 2023001800 A1	11 December 2023
		IL 302894 A	01 July 2023
		ZA 202305221 B	26 June 2024
CN 115969997 A	18 April 2023	None	
WO 2016165762 A1	20 October 2016	IL 254085 A0	31 October 2017
		MA 41937 A	28 February 2018
		MA 41937 B1	29 December 2023
		SG 10202105142 VA	29 June 2021
		HK 1247210 A1	21 September 2018
		JP 2021038255 A	11 March 2021
		JP 7024152 B2	24 February 2022
		RU 2017139490 A	15 May 2019
		RU 2017139490 A3	17 January 2020
		KR 20180082325 A	18 July 2018
		KR 102336566 B1	23 December 2021
		ZA 201705923 B	26 June 2019
		RU 2021131990 A	04 February 2022
		JP 2018513146 A	24 May 2018
		JP 6800883 B2	16 December 2020
		SG 11201708271 TA	29 November 2017
		CA 2982401 A1	20 October 2016
		CA 2982401 C	11 June 2024
		KR 20240011250 A	25 January 2024
		PT 3283521 T	18 December 2023
		MD 3283521 T2	31 March 2024
		DK 3283521 T3	18 December 2023
		PL 3283521 T3	29 January 2024
		AU 2016249782 A1	02 November 2017

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2024/099382

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
		AU	2016249782	B2	28 April 2022
		LT	3283521	T	27 December 2023
		US	2018117174	A1	03 May 2018
		US	11541127	B2	03 January 2023
		UA	127469	C2	06 September 2023
		EP	4331578	A2	06 March 2024
		EP	4331578	A3	05 June 2024
		WO	2016166122	A1	20 October 2016
		EP	3283521	A1	21 February 2018
		EP	3283521	B1	15 November 2023
		ES	2964813	T3	09 April 2024
		US	2023270878	A1	31 August 2023
		JP	2024036444	A	15 March 2024
		KR	20210150612	A	10 December 2021
		KR	102401299	B1	24 May 2022
		MX	2023013476	A	15 December 2023
		HUE	064753	T2	28 April 2024
		JP	2022031979	A	22 February 2022
		FI	3283521	T3	12 December 2023
		HRP	20231609	T1	15 March 2024
		MX	2017013075	A	19 February 2018
		RS	64951	B1	31 January 2024
		BR	112017018521	A2	17 April 2018
		SI	3283521	T1	30 April 2024
		KR	20220072874	A	02 June 2022
		KR	102626316	B1	18 January 2024
WO 2021228141 A1	18 November 2021	CR	20220580	A	11 April 2023
		MX	2022014332	A	13 December 2022
		IL	298184	A	01 January 2023
		DOP	2022000251	A	16 April 2023
		EP	4151235	A1	22 March 2023
		US	2023338565	A1	26 October 2023
		JP	2024037960	A	19 March 2024
		AU	2021270940	A1	15 December 2022
		CO	2022016208	A2	27 February 2023
		CL	2022003170	A1	19 May 2023
		KR	20230012000	A	25 January 2023
		JP	2023525120	A	14 June 2023
		JP	7407973	B2	04 January 2024
		PE	20230373	A1	06 March 2023
		CA	3178406	A1	18 November 2021
		ECSP	22089507	A	28 February 2023