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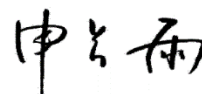
Title of the Invention-Creation: ANTIBODY-DRUG CONJUGATE

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Claims

1. An antibody-drug conjugate, a pharmaceutically acceptable salt thereof, a solvate thereof or a solvate of the salt, comprising an anti-epidermal growth factor receptor antibody covalently linked to a cytotoxic agent.

2. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of claim 1, wherein the anti-epidermal growth factor receptor antibody comprises a heavy chain and a light chain, wherein CDR1, CDR2 and CDR3 of a variable region of the heavy chain comprise sequences as shown in SEQ ID NOs: 5 to 7 or mutants thereof, respectively, and CDR1, CDR2 and CDR3 of a variable region of the light chain comprise sequences as shown in SEQ ID NOs: 12 to 14 or mutants thereof, respectively.

3. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of claim 2, wherein FR1, FR2, FR3 and FR4 regions of the variable region of the heavy chain of the anti-epidermal growth factor receptor antibody comprise sequences as shown in SEQ ID NOs: 8 to 11 or mutants thereof, respectively.

4. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of claim 2, wherein FR1, FR2, FR3 and FR4 regions of the variable region of the light chain of the anti-epidermal growth factor receptor antibody comprise sequences as shown in SEQ ID NOs: 15 to 18 or mutants thereof, respectively.

5. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of any one of claims 1 to 4, wherein the heavy chain of the anti-epidermal growth factor receptor antibody has a constant region selected from human IgG, IgM, IgA, IgD and IgA constant regions or mutants of the constant regions.

6. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of claim 5, wherein the IgG is selected from IgG1, IgG2, IgG3 and IgG4.

7. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of claim 5, wherein the constant region of the heavy chain of the anti-epidermal growth factor receptor antibody has an amino acid sequence comprising a sequence as shown in SEQ ID NO: 3, or comprising a sequence with identity of greater than 70%, for example, greater than 75%, 80%, 85%, 90%, 95%, or 99% to the sequence as shown in SEQ ID NO: 3.

8. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of any one of claims 1 to 7, wherein the light chain of the anti-epidermal growth factor receptor antibody has a constant region that is a human lambda constant region, a human kappa constant region, or a mutant thereof.

9. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of claim 8, wherein the constant region of the light chain of the anti-epidermal growth factor receptor antibody has an amino acid sequence comprising a sequence as shown in SEQ ID NO: 4, or comprising a sequence with identity of greater than 70%, for example, greater than 75%, 80%, 85%, 90%, 95%, or 99% to the sequence as shown in SEQ ID NO: 4.

10. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of any one of claims 1 to 9, having a structure represented by Formula I of $Ab-(L-D)_p$, where:

Ab represents the anti-epidermal growth factor receptor antibody;

L represents a linker;

D represents the cytotoxic agent;

p represents 1 to 9, for example 2 to 6, such as 3 to 5.

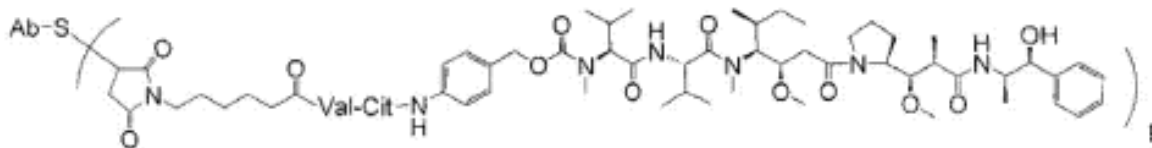
11. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of any one of claims 1 to 10, wherein the cytotoxic agent is selected from chemotherapeutic agents, toxins, radioisotopes, cytokines, antibiotics, enzymes, nanoparticles and biologically active peptides.

12. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of claim 11, wherein the cytotoxic agent is selected from Monomethyl auristatin E (MMAE), Monomethyl auristatin F (MMAF), maytansinoid alkaloids (e.g., Maytansine DM1, Maytansine DM4), calicheamicin, duocarmycin MGBA, doxorubicin, ricin, diphtheria toxin and other toxins, I131, interleukins, tumor necrosis factors, chemokines, nanoparticles, etc.

13. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of claim 10, wherein the linker is cleavable or non-cleavable.

14. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of claim 13, wherein the linker is selected from 6-maleimidocaproyl (MC), maleimidopropionyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-succinimidyl-4-(2-pyridylthio)valerate (SPP), N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC), N-succinimidyl(4-iodo-acetyl)aminobenzoate (SIAB), and 6-maleimidocaproyl-valine-citrulline-p-aminobenzyloxycarbonyl (MC-vc-PAB).

15. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of any one of claims 1 to 14, being represented by:



where Ab represents the anti-epidermal growth factor receptor antibody, and p is 1 to 8, for example 2 to 6, such as 3 to 5.

16. A composition, comprising the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of any one of claims 1 to 15, and optionally, further comprising at least one pharmaceutically acceptable carrier, diluent or excipient.

17. A use of the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of any one of claims 1 to 15 in preparation of a drug for

prophylaxis and/or treatment of a disease associated with an epidermal growth factor receptor (EGFR).

18. The use of claim 16, wherein the disease associated with the epidermal growth factor receptor (EGFR) is a tumor associated with EGFR, such as a tumor associated with over-expression of EGFR, which is selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, kidney cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma, for example.

19. A use of the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt of any one of claims 1 to 15 in preparation of a drug for inhibiting tumor angiogenesis, delaying tumor progression, inhibiting tumor growth, or inhibiting tumor cell proliferation.

20. The use of claim 19, wherein the tumor is selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

DESCRIPTION

ANTIBODY-DRUG CONJUGATE

Technical Field

The present invention relates to an antibody-drug conjugate, in particular to an antibody-drug conjugate in which the antibody is an anti-epidermal growth factor receptor antibody. The present invention further relates to a composition comprising the antibody-drug conjugate, and a pharmaceutical use of the antibody-drug conjugate.

Background Art

Epidermal growth factor receptor (Epidermal Growth Factor Receptor, EGFR, also known as HER1, c-ErbB1) is a cell surface receptor of epidermal growth factor family, is a transmembrane glycoprotein composed of 1186 amino acid residues, and has a molecular weight of 170 kD (Jorissen RN, Walker F, Pouliot N, et al. Epidermal growth factor receptor: mechanisms of activation and signaling. *Exp Cell Res*, 2003; 284:31-53). EGFR belongs to type I tyrosine kinase receptor subfamily ErbB (ErbB 1-4) and has tyrosine kinase activity. EGFR is stably expressed in many epithelial tissues, including the skin and hair follicles. Abnormal expression of epidermal growth factor receptor or activation caused by receptor mutation may lead to carcinogenesis. There are many solid tumors where over expression of epidermal growth factor receptor are found, such as colorectal cancer, head-neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer and esophageal cancer (Olayioye MA, Neve RM, Lane HA, et al. The EerbB Signaling network: receptor heterodimerization in development and cancer. *The EMBO J*, 2000; 19:3159-3167). Growth factors such as transforming growth factor α and epidermal growth factor are endogenous ligands for EGFR. These ligands bind to epidermal growth factor receptor and activate intracellular tyrosine protein kinase activity, initiate a lot of downstream signal transduction pathways, thereby regulating growth and differentiation of normal cells, enhancing invasiveness of tumor cells, promoting angiogenesis and inhibiting apoptosis of tumor cells (Ciardiello F, Tortora G.A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. *Clin Cancer Res*, 2001; 7:2958-2970). Epidermal growth factor receptor overexpression in tumor and its

important roles in the growth and differentiation of tumor cells make epidermal growth factor receptor a promising target for tumor therapy.

At present, there are two anti-epidermal growth factor receptor therapeutic antibodies on the market, one is human-mouse chimeric antibody C225 antibody (Erbix or Cetuximab, ImClone Company (now Eli Lilly Company)), which has a specific binding affinity to epidermal growth factor receptor, can block the binding of ligands such as EGF and TGF α to a epidermal growth factor receptor, inhibit its phosphorylation and downstream signal transduction, thereby inhibiting tumor cell growth, inducing apoptosis, reducing production of matrix metalloproteinases and vascular endothelial growth factor. The FDA of the United States approved the use of Erbix for treatment of colorectal cancer in 2004, and for treatment of head and neck cancer in 2006, and there are more clinical trials for other cancer indications currently. Clinically, the overall response rate (ORR) of the combination of Erbix and irinotecan in treatment of colorectal cancer is 23%, and the ORR of the combination of Erbix and chemotherapy drug such as fluoropyrimidine in treatment of head and neck cancer is 13% to 30%. Because of being a human-mouse chimeric antibody, Erbix induced an anti-therapeutic antibody response in 3.7% of the patients in the clinical trials .

Another anti-epidermal growth factor receptor therapeutic antibody is panitumumab (Vectibix, panitumumab, Amgen company), which is a fully humanized monoclonal antibody prepared by using transgenic mouse technology, and is free of mouse original protein sequence. The antibody targets the epidermal growth factor receptor (EGFR), and was approved by the FDA in September 2006 for the treatment of EGFR-positive metastatic colorectal cancer in combination with fluoropyrimidine, Oxaliplatin and Irinotecan or after chemotherapy. In 2006, FDA approved its monotherapy for the treatment of metastatic colorectal cancer (mCRC) with chemotherapy tolerance. However, panitumumab is an IgG2 subtype antibody. Compared with IgG1, IgG2 exhibits significantly decreased biological activities such as CDC activity and ADCC activity. In addition, IgG2 subtype antibodies usually have poor stability. These may be the main reasons that the fully humanized antibody panitumumab shows no significant advantages in clinical effects in comparison with Erbix, the chimeric antibody. The overall survival rate (OR) in the clinical treatment of colorectal cancer was merely 8% and the progression-free survival was only extended by 3.6 months.

At present, a large amount of clinical data showed that Erbitux and panitumumab had therapeutic effects only on the KRAS wild type with expression of EGFR, but had no tumor growth inhibitory activity to KRAS mutants. Therefore, the Guidelines published by the American Society of Clinical Oncology explicitly point out that anti-EGFR monoclonal antibody drugs are only applicable to KRAS wild type colorectal cancer patients (Allegra CJ, Jessup JM, Somerfield MR, Hamilton SR, Hammond EH, Hayes DF, et al. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor monoclonal antibody therapy. *J. Clin Oncol.* 2009; 27:2091-2096; Bardelli A, Siena S. Molecular mechanisms of resistance to cetuximab and panitumumab in Colorectal, cancer., *J, Clin, Oncol.* 2010; 28:1254-1261).

Therefore, there is an urgent need in the art for humanized anti-epidermal growth factor receptor antibody drugs with biological activity, especially antibody drugs, such as antibody-drug conjugates, with curative effects to KRAS mutants, so as to further improve therapeutic efficacy and reduce side effects.

In recent years, the rapid development of antibody-drug conjugates (ADC) has become one of the most advanced biopharmaceutical technologies in recent years due to the lead of monoclonal antibody cancer drugs.

An antibody-drug conjugate usually consists of three parts:

1. a monoclonal antibody with specific binding to a target;
2. a small molecule chemical drug with cytotoxicity; and
3. a linker that links the small molecule drug and the monoclonal antibody.

The antibody-drug conjugate kills tumor cells by utilizing the target specificity of the monoclonal antibody and the cytotoxicity of the chemical drug. Its mechanism of action is that: (1) the antibody-drug conjugate specifically binds to a target antigen expressed on a tumor cell surface by the monoclonal antibody; (2) the complex of the antibody-drug conjugate and the target antigen enters the cell via endocytosis mediated by the target antigen; (3) the antibody-drug conjugate degrades in the cell and releases the cytotoxic chemical drug; and (4) the cytotoxic chemical drug kills the tumor cell.

Although the mechanism of antibody-drug conjugate appears straightforward, it is highly complex and unpredictable whether an antibody-drug conjugate becomes a safe and effective drug depending on a variety of factors, such as:

1) characteristics of target: whether a target can be internalized or not, the expression level of the target, whether there is a sufficient difference in the expression level of the target between cancer cells and normal cells, and whether the target will shed an extracellular domain (ECD) into the blood;

2) characteristics of monoclonal antibody: whether the specificity of the monoclonal antibody to the target is good enough (no cross-reaction with other proteins), the stability of the monoclonal antibody, and whether the complex of the monoclonal antibody and the target can be internalized into the cells;

3) characteristics of small molecule drug: whether the cytotoxicity of the small molecule drug is strong enough, its stability in blood, and the toxicity of the in vivo metabolites of the small molecule drug after it becomes an ADC;

4) characteristics of linker: whether the linker is cleavable or non-cleavable, and the stability of the linker in the blood; and

5) characteristics of ADC: whether the complex of the ADC and the target can be internalized or not, the stability of the ADC in the blood, what linker is used on the ADC and how many chemical drugs are linked to the ADC, and the activity and biological toxicity of the ADC in killing cancer cells.

It can be seen that the research and development of ADC drugs requires a lot of experimental exploration and verification, and their safety and efficacy are hardly predictable before the experiments.

Summary of the Invention

The inventors of the present invention have prepared an anti-epidermal growth factor receptor antibody-drug conjugate through a large number of experiments and inventive work, and confirmed that it has good biological activities, thereby completing the present invention.

A first aspect of the present invention relates to an antibody-drug conjugate, a pharmaceutically acceptable salt thereof, a solvate thereof or a solvate of the salt, comprising an anti-epidermal growth factor receptor antibody covalently linked to a cytotoxic agent.

In an embodiment of the present invention, the anti-epidermal growth factor receptor antibody comprises a heavy chain and a light chain, wherein CDR1, CDR2 and CDR3 of a variable region of the heavy chain comprise sequences as shown in SEQ ID NOs: 5 to 7 or mutants thereof,

respectively, and CDR1, CDR2 and CDR3 of a variable region of the light chain comprise sequences as shown in SEQ ID NOs: 12 to 14 or mutants thereof, respectively.

In an embodiment of the present invention, the CDR1, CDR2 and CDR3 regions of the variable region of the light chain of the anti-epidermal growth factor receptor antibody comprise sequences as shown in SEQ ID NOs: 12 to 14, respectively, or comprise sequences with identity of greater than 70%, such as greater than 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% to the sequences as shown in SEQ ID NOs: 12 to 14, for example, sequences having 3, 2 or 1 mutations, deletions or addition of amino acids, respectively.

In an embodiment of the present invention, the CDR1, CDR2 and CDR3 regions of the variable region of the heavy chain of the anti-epidermal growth factor receptor antibody comprise sequences as shown in SEQ ID NOs: 5 to 7, respectively, or comprise sequences with identity of greater than 70%, such as greater than 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% to the sequences as shown in SEQ ID NOs: 5 to 7, for example, sequences having 3, 2 or 1 mutations, deletions or addition of amino acids, respectively.

In an embodiment of the present invention, FR1, FR2, FR3 and FR4 regions of the variable region of the heavy chain of the anti-epidermal growth factor receptor antibody comprise sequences as shown in SEQ ID NOs: 8 to 11 or mutants thereof, respectively.

In an embodiment of the present invention, FR1, FR2, FR3 and FR4 regions of the variable region of the heavy chain of the anti-epidermal growth factor receptor antibody comprise sequences as shown in SEQ ID NOs: 8 to 11, respectively, or comprise sequences with identity of greater than 70%, such as greater than 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% to the above sequences, respectively.

In an embodiment of the present invention, FR1, FR2, FR3 and FR4 regions of the variable region of the light chain of the anti-epidermal growth factor receptor antibody comprise sequences as shown in SEQ ID NOs: 15 to 18 or mutants thereof, respectively.

In an embodiment of the present invention, FR1, FR2, FR3 and FR4 regions of the variable region of the light chain of the anti-epidermal growth factor receptor antibody comprise sequences as shown in SEQ ID NOs: 15 to 18, respectively, or comprise sequences with identity of greater than 70%, such as greater than 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% to the above sequences, respectively.

In an embodiment of the present invention, the variable region of the heavy chain of the anti-epidermal growth factor receptor antibody has a sequence as shown in SEQ ID NO: 1.

In an embodiment of the present invention, the variable region of the light chain of the anti-epidermal growth factor receptor antibody has a sequence as shown in SEQ ID NO: 2.

In an embodiment of the present invention, the heavy chain of the anti-epidermal growth factor receptor antibody has a constant region selected from human IgG, IgM, IgA, IgD and IgA constant regions or mutants of the constant regions.

In an embodiment of the present invention, the IgG is selected from IgG1, IgG2, IgG3 and IgG4.

In an embodiment of the present invention, the constant region of the heavy chain of the anti-epidermal growth factor receptor antibody has an amino acid sequence comprising a sequence as shown in SEQ ID NO: 3, or comprising a sequence with identity of greater than 70%, for example, greater than 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% to the sequence as shown in SEQ ID NO: 3.

In an embodiment of the present invention, the light chain of the anti-epidermal growth factor receptor antibody has a constant region that is a human lambda constant region, a human kappa constant region, or a mutant thereof.

In an embodiment of the present invention, the constant region of the light chain of the anti-epidermal growth factor receptor antibody has an amino acid sequence comprising a sequence as shown in SEQ ID NO: 4, or comprising a sequence with identity of greater than 70%, for example, greater than 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% to the sequence as shown in SEQ ID NO: 4.

In an embodiment of the present invention, the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt has a structure represented by Formula I of $Ab-(L-D)_p$, where:

Ab represents the anti-epidermal growth factor receptor antibody;

L represents a linker;

D represents the cytotoxic agent;

p represents 1 to 8, for example 2 to 6, such as 3 to 5.

In an embodiment of the present invention, the cytotoxic agent is selected from chemotherapeutic agents, toxins (e.g., bacterial, fungal, plant, or animal-derived enzymatically

active toxins or fragments thereof), radioisotopes, cytokines, antibiotics, enzymes, nanoparticles and biologically active peptides.

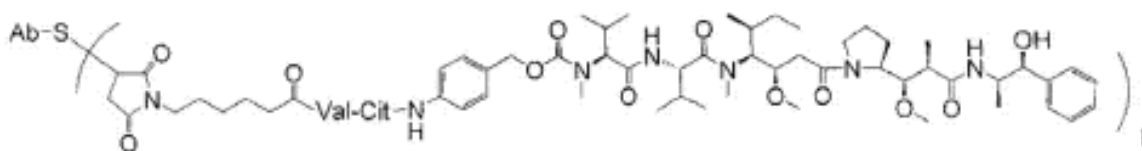
In an embodiment of the present invention, the cytotoxic agent is selected from Monomethyl auristatin E (MMAE), Monomethyl auristatin F (MMAF), maytansinoid alkaloids (e.g., Maytansine DM1, Maytansine DM4), calicheamicin, duocarmycin MGBA, doxorubicin, ricin, diphtheria toxin and other toxins, I131, interleukins, tumor necrosis factors, chemokines, nanoparticles, etc.

In an embodiment of the present invention, the cytotoxic agent is MMAE.

In an embodiment of the present invention, the linker is cleavable or non-cleavable.

In an embodiment of the present invention, the linker is selected from 6-maleimidocaproyl (MC), maleimidopropionyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-succinimidyl-4-(2-pyridylthio)valerate (SPP), N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC), N-succinimidyl(4-iodo-acetyl)aminobenzoate (SIAB), and 6-maleimidocaproyl-valine-citrulline-p-aminobenzyloxycarbonyl (MC-vc-PAB).

In a specific embodiment of the present invention, the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt is represented by:



where Ab represents the anti-epidermal growth factor receptor antibody, and p is 1 to 8, for example 2 to 6, such as 3 to 5.

A second aspect of the present invention relates to a composition (e.g., a pharmaceutical composition) comprising the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of items of the first aspect of the present invention, optionally, further comprising at least one pharmaceutically acceptable carrier, diluent or excipient.

In an embodiment of the present invention, the composition further comprises a known chemotherapeutic agent for the treatment of a tumor, such as Adriamycin, cyclophosphamide and taxane [Taxol and Taxotere], Xeloda, Gemzar, Navelbine, Tamoxifen, aromatase inhibitors (Ruining, Fulong, Arnoldin), 5-FU plus folinic acid, camptosar, oxaliplatin, cisplatin, carboplatin, estramustine, Novantrone, prednisone, vincristine (Oncovin), etc., or a combination thereof.

The present invention further relates to a use of the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of items of the first aspect of the present invention in the preparation of a drug for prophylaxis and/or treatment of a disease associated with an epidermal growth factor receptor (EGFR).

In an embodiment of the present invention, the disease associated with the epidermal growth factor receptor (EGFR) is a tumor associated with EGFR, such as a tumor associated with overexpression of EGFR, e.g., selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, kidney cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

The present invention further relates to a use of the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of items of the first aspect of the present invention in the preparation of a drug for inhibiting tumor angiogenesis, delaying tumor progression, inhibiting tumor growth, or inhibiting tumor cell proliferation.

In an embodiment of the present invention, the tumor is selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

The present invention further relates to a method for prophylaxis and/or treatment of a disease associated with an epidermal growth factor receptor (EGFR), wherein the method comprises a step of administering to a subject in need a prophylactically and/or therapeutically effective amount of the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of items of the first aspect of the present invention.

The present invention further relates to a method for inhibiting tumor angiogenesis, delaying tumor progression, inhibiting tumor growth, or inhibiting tumor cell proliferation, wherein the method comprises a step of administering to a subject in need a prophylactically and/or therapeutically effective amount of the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to the first aspect of the present invention.

In an embodiment of the present invention, the disease associated with epidermal growth factor receptor (EGFR) is a tumor associated with EGFR, such as a tumor associated with over-expression of EGFR, which is selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, kidney cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma, for example.

The anti-epidermal growth factor receptor antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to the present invention has a good inhibition activity on tumor cell growth in vivo and in vitro, especially significant inhibition activity on tumor growth of tumor cells with medium and low EGFR expression, and has low cytotoxicity, so that it has good application prospects.

The present invention will be further described below:

In the present invention, the scientific and technical terms used herein have the same meaning as commonly understood by a person skill in the art, unless otherwise indicated. Also, the terms and laboratory procedures related to chemistry of proteins and nucleic acids, molecular biology, cell and tissue culture, microbiology and immunology are all terms and conventional steps that are widely used in the corresponding fields. Meanwhile, for the sake of better understanding the present invention, definitions and explanations of related terms will be provided below.

In the present invention, the term "antibody" refers to an immunoglobulin molecule that typically consists of two pairs of identical polypeptide chains (each pair having a "light" (L) chain and a "heavy" (H) chain). Light chains of the antibodies may be divided into two types: κ and λ . Heavy chains may be divided into five types: μ , δ , γ , α or ϵ , and according to different types of heavy chains, the antibodies can be divided into five types: IgM, IgD, IgG, IgA and IgE. Within the light and heavy chains, variable and constant regions are linked by a "J" region of about 12 or

more amino acids, and the heavy chain further comprises a "D" region of about 3 or more amino acids. Each heavy chain consists of a heavy chain variable region (V_H) and a heavy chain constant region (C_H). The heavy chain constant region consists of three structural domains (C_{H1}, C_{H2} and C_{H3}). Each light chain consists of a light chain variable region (V_L) and a light chain constant region (C_L). The light chain constant region consists of one structural domain C_L. The constant regions of antibody may mediate the binding of immunoglobulin to a host tissue or factor, including various cells (e.g., effector cells) of the immune system and component C1q of the complement system. The V_H and V_L regions may also be subdivided into regions with high variability (referred to as complementarity determining regions (CDR)) among which more conserved regions referred to as framework regions (FR) are interspersed. Each of V_H and V_L consists of three CDRs and 4 FRs arranged from the amino terminus to the carboxy terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The variable regions (V_H and V_L) of each heavy chain/light chain pair form antibody binding sites, respectively. The distribution of amino acids to respective regions or structural domains follows the definitions of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk (1987) J. Mol. Biol. 196:901-917; and Chothia et al. (1989) Nature 342:878-883. The term "antibody" is not limited by any particular method of producing an antibody. For example, it comprises, in particular, recombinant antibodies, monoclonal antibodies, and polyclonal antibodies. The antibodies may be different types of antibodies, for example, IgG (e.g., IgG1, IgG2, IgG3 or IgG4 subtype), IgA1, IgA2, IgD, IgE or IgM antibodies.

In the present invention, the IgG heavy chain constant region comprises IgG1, IgG2, IgG3 or IgG4. In an embodiment of the present invention, the IgG heavy chain constant region is an IgG1 type.

In the present invention, the κ light chain constant region comprises various allotypes, such as Km1, Km1.2 or Km3.

With regard to amino acid sequence of antibody

In an embodiment of the present invention, the amino acid sequence of the heavy chain variable region of the anti-epidermal growth factor receptor antibody is SEQ ID NO: 1. In an embodiment of the present invention, the amino acid sequence of the light chain variable region of the anti-epidermal growth factor receptor antibody is SEQ ID NO: 2.

In another aspect, the amino acid sequence of the heavy chain variable region of the antibody according to the present invention has at least 70%, preferably at least 75%, preferably at least 80%, preferably 85% more preferably at least 90%, and most preferably at least 95% similarity to the sequence of SEQ ID NO: 1.

In another aspect, the amino acid sequence of the light chain variable region of the antibody according to the present invention has at least 70%, preferably at least 75%, preferably at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% similarity to the sequence of SEQ ID NO: 2.

In an embodiment of the present invention, the amino acid sequences of CDRs of the heavy and light chain variable regions of the anti-epidermal growth factor receptor antibody are determined as follows:

the amino acid sequences of CDR1, CDR2 and CDR3 of the heavy chain are SEQ ID NO: 5 to 7, respectively; the amino acid sequences of CDR1, CDR2 and CDR3 of the light chain are SEQ ID NO: 12 to 14, respectively.

In another aspect, the amino acid sequences contained in the CDRs of the heavy chains of the anti-epidermal growth factor receptor antibody may have one or more mutations or additions or deletions of amino acids in SEQ ID NOs: 5 to 7. Preferably, the amino acids for the mutations, additions or deletions are not more than 3 amino acids. More preferably, the amino acids for the mutations, additions or deletions are not more than 2 amino acids. Most preferably, the amino acids for the mutations, additions or deletions are not more than 1 amino acid.

In another aspect, the amino acid sequences contained in the CDRs of the light chains of the anti-epidermal growth factor receptor antibody may have one or more mutations or additions or deletions of amino acids in SEQ ID NOs: 12 to 14. Preferably, the amino acids for the mutations, additions or deletions are not more than 3 amino acids. More preferably, the amino acids for the mutations, additions or deletions are not more than 2 amino acids. Most preferably, the amino acids for the mutations, additions or deletions are not more than 1 amino acid.

In an embodiment of the present invention, the amino acid sequences of FRs of the heavy and light chain variable regions of the anti-epidermal growth factor receptor antibody are determined as follows:

the sequences of the heavy chain variable regions FR1, FR2, FR3 and FR4 are SEQ ID NO: 8 to 11, respectively. The sequences of the light chain variable regions FR1, FR2, FR3 and FR4 are SEQ ID NO: 15 to 18, respectively.

On the other hand, the amino acid sequences of the heavy or light chain variable regions FRs of the anti-epidermal growth factor receptor antibody may have one or more mutations or additions or deletions of amino acids in SEQ ID NOs: 8 to 11 and SEQ ID Nos: 15 to 18. Preferably, the amino acids for the mutations, additions or deletions are not more than 3 amino acids. More preferably, the amino acids for the mutations, additions or deletions are not more than 2 amino acids. Most preferably, the amino acids for the mutations, additions or deletions are not more than 1 amino acid.

The variants with the mutations, additions or deletions of amino acids in the above antibody or CDR regions or framework regions still retain the ability of specific binding to EGFR.

In an embodiment of the present invention, the amino acid sequence of the heavy chain constant region of the anti-epidermal growth factor receptor antibody is SEQ ID NO: 3. In an embodiment of the present invention, the amino acid sequence of the light chain constant region of the anti-epidermal growth factor receptor antibody is SEQ ID NO: 4.

In another aspect, the amino acid sequence of the heavy chain constant region of the antibody according to the present invention has at least 70%, preferably at least 75%, preferably at least 80%, preferably at least 85%, more preferably at least 90%, most preferably at least 95%, such as 96%, 97%, 98%, 99% similarity to SEQ ID NO: 3.

In another aspect, the amino acid sequence of the light chain constant region of the antibody according to the present invention is at least 70%, preferably at least 75%, preferably at least 80%, preferably at least 85%, more preferably at least 90%, most preferably at least 95%, such as 96%, 97%, 98%, 99% similarity to SEQ ID NO: 4.

The monoclonal antibody variants according to the present invention may be obtained by a conventional genetic engineering method. A person skilled in the art is fully aware of the methods for transforming DNA molecules using nucleic acid mutations. In addition, nucleic acid molecules encoded with heavy and light chain variants may also be obtained by means of chemical synthesis.

In the present invention, algorithms for determining the percentage of sequence identity (homology) and sequence similarity are, for example, BLAST and BLAST 2.0 algorithms, which are separately described in Altschul et al. (1977) Nucl. Acid. Res. 25: 3389-3402 and Altschul et

al. (1990) J. Mol. Biol. 215: 403-410. BLAST and BLAST 2.0 may be used to determine the identity percentages of the amino acid sequences of the present invention by using, for example, parameters as described in the documents or default parameters. The software that performs BLAST analysis can be publicly obtained through the National Biotechnology Information Center.

In the present invention, the amino acid sequences having at least 70% sequence identity to the amino acid sequence comprises polypeptide sequences substantially identical to the amino acid sequence, for example, when the method described herein (e.g., BLAST analysis using standard parameters) is used, these amino acid sequences with at least 70% sequence identity in comparison with the polypeptide sequence of the present invention, preferably at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater sequence identity to the polypeptide sequence of the present invention.

In the present invention, the toxins used for the antibody-drug conjugate comprise diphtheria toxin A chain, non-binding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, α -sarcin, Aleutites fordii toxic protein, dianthin toxic protein, Phytolacaamericana toxic proteins (PAPI, PAPII and PAP-S), Momordica charantia inhibitors, curcin, crotin, sapaonaria officinalis inhibitors, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and trichothecenes. See WO 93/21232 published on October 28, 1993, for example.

A variety of radionuclides may be used to generate the antibody-drug conjugate. Examples of the radionuclides comprise ^{212}Bi , ^{131}I , ^{131}In , ^{90}Y , and ^{186}Re .

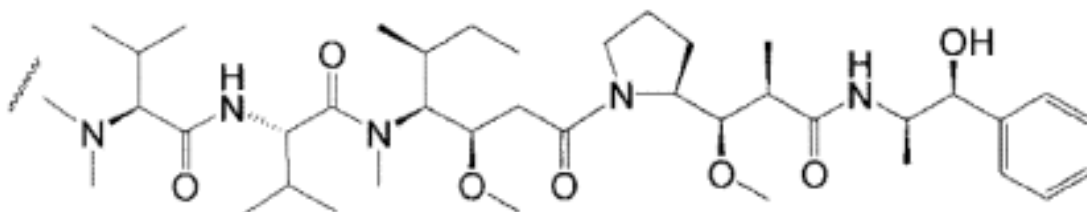
An antibody-cytotoxic agent conjugate may be prepared using a variety of bifunctional protein coupling agents, for example, bifunctional derivatives, such as N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), iminothiolane (IT), imidates (e.g., dimethyl adipimidate dihydrochloride), active esters (e.g., disuccinimidyl suberate), aldehydes (e.g., glutaraldehyde), bisazide compounds (e.g., bis(p-diazidobenzoyl)hexamethylenediamine), bisdiazo compounds (e.g., bis(p-diazobenzoyl)-ethylenediamine), diisothiocyanates (e.g., toluene-2,6-diisocyanate), and double active fluorine compounds (e.g., 1,5-difluoro-2,4-dinitrobenzene). For example, it may be a ricin-containing immunotoxin prepared as described by Vitetta et al. (1987) Science, 238: 1098. Carbon-14 labeled 1-isothiocyanic acid benzyl-3-methyldiethylenetriamine pentaacetic acid

(MX-DTPA) is an exemplary chelating agent for coupling radioactive nucleotides with antibodies (WO94/11026).

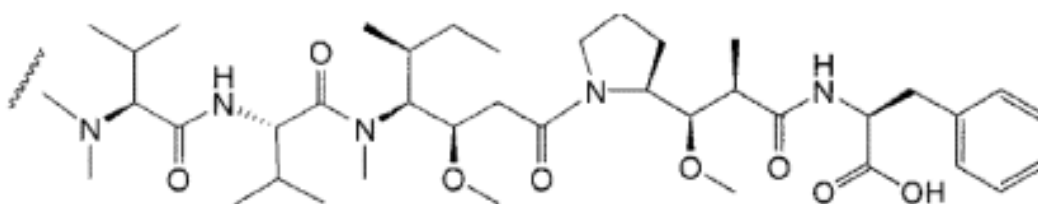
The present invention further comprises a conjugate of an antibody and one or more small molecule toxins (e.g., calicheamicin, maytansinoid, dolastatin, auristatin, trichothecene, and CC 1065, as well as toxic derivatives of these toxins).

In some embodiments, the antibody-drug conjugate comprises an anti-epidermal growth factor receptor antibody coupled to dolastatin or a dolastatin peptide analogue and a derivative auristatin (U.S. Pat. Nos. 5,635,483; and 5,780,588). Dolastatin and auristatin have shown activities of interfering with microtubule kinetics, GTP hydrolysis, and nuclear and cell division (Woyke et al. (2001) *Antimicrob. Agents and Chemother.* 45 (12): 3580-3584), and have anticancer activity (U.S. Pat. No. 5,663,149) and antifungal activity (Pettit et al. (1998) *Antimicrob. Agents Chemother.* 42: 2961-2965). The drug modules of dolastatin or auristatin may be attached to an antibody via N (amino) terminus or C (carboxy) terminus of the peptide drug modules (WO 02/088172).

In the present invention, the structure of MMAE is:



In the present invention, the structure of MMAF is:



In the present invention, an enzyme as cytotoxic agent may be a compound having nucleic acid degradation activity (e.g., ribonuclease or DNA endonuclease, such as deoxyribonuclease; and DNA enzyme).

In the present invention, drug loading is represented by p , i.e., an average number of drug modules (i.e., cytotoxic agents) per antibody in the molecule of Formula I: $Ab-(L-D)_p$. The drug loading may range from 1 to 20 drug modules (D) per antibody. The ADC of Formula I comprises

a collection of antibodies conjugated to a range of (1-20) drug modules. The average number of drug modules per antibody from the ADC preparation of coupling reaction may be verified by conventional means, such as mass spectrometry, ELISA assay, and HPLC. The quantitative distribution of ADCs in respect of p may also be determined. In some cases, homogeneous ADCs with p of certain value may be isolated from ADCs with other drug loadings, and purification and validation may be achieved by means of reverse phase HPLC or electrophoresis, for example.

For some antibody-drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, if the attachment site is a cysteine thiol, the antibody may have only one or several cysteine thiol groups, or may have only one or more thiol groups with sufficient reactivity to which linkers may be attached. In certain embodiments, higher drug loading, such as $p > 5$, may cause aggregation, insolubility, toxicity, or loss of cell permeability of certain antibody-drug conjugates.

In certain embodiments, the drug loading of ADCs of the present invention is in the range from 1 to about 8; from about 2 to about 6; from about 3 to about 5; from about 4 to about 5; or from about 3.5 to about 4.5; or is about 4. In fact, it has been shown that some ADCs might have an optimal ratio of drug modules per antibody of less than 8, or from about 2 to about 5. See US2005-0238649A1 (which is fully incorporated herein by reference).

In certain embodiments, drug modules less than the theoretical maximum are conjugated to the antibody in the coupling reaction. The antibody may comprise, for example, a lysine residue that does not react with a drug-linker intermediate or a linker reagent. In general, the antibody does not contain a number of free and reactive cysteine thiol groups, which may be linked to a drug module; in fact, most of the cysteine thiol groups in the antibody are present in the form of a disulfide bridge. In certain embodiments, the antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarboxyl ethyl phosphine (TCEP) under partial or complete reductive conditions to produce a reactive cysteine thiol group. In certain embodiments, the antibody is placed under denaturing conditions to expose a reactive nucleophilic group, such as lysine or cysteine.

The loading (drug/antibody ratio) of the ADC may be controlled in different ways, for example, by: (i) limiting the mole number of drug-linker or linker reagent relative to the antibody, (ii) limiting the time or temperature of the coupling reaction, (iii) modifying cysteine thiol moieties or restricting reduction conditions, (iv) performing engineering reconstruction of amino acid

sequences of the antibodies by recombinant techniques, such that the number and location of cysteine residues are changed in order to control the number and/or location of the linker-drug attachments. It should be understood that if more than one nucleophilic group is reacted with a drug-linker intermediate or with a linker reagent and a subsequent drug module reagent, the resulting product is an ADC compound mixture having one or more drug modules attached to the antibody. The average number of drug modules per antibody can be calculated from the mixture by an antibody-specific and drug-specific double-ELISA antibody assay. The various ADC molecules in the mixture can be identified by mass spectrometry and separated by HPLC, for example, hydrophobic interaction chromatography. In certain embodiments, a homogeneous ADC with a single loading value may be isolated from the coupling mixture by electrophoresis or chromatography.

In the present invention, the pharmaceutically acceptable salts of the antibody-drug conjugates include acid addition salts of inorganic acids, carboxylic acids and sulfonic acids, for example, salts of the following acids: hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, toluenesulfonic acid, naphthalene disulfonic acid, acetic acid, trifluoroacetic acid, propionic acid, lactic acid, tartaric acid, malic acid, citric acid, fumaric acid, maleic acid and benzoic acid.

The pharmaceutically acceptable salts of the antibody-drug conjugates of the present invention also include salts of conventional bases, for example (merely exemplified and preferred), alkali metal salts (e.g., sodium salts and potassium salts), alkaline earth metal salts (e.g., calcium salts and magnesium salts) and ammonium salts derived from ammonia or organic amines containing from 1 to 16 carbon atoms, in which the organic amines are, for example (merely exemplified and preferred), ethylamine, diethylamine, triethylamine, ethyl diisopropylamine, monoethanolamine, diethanolamine, triethanolamine, dicyclohexylamine, dimethylaminoethanol, procaine, dibenzamide, N-methylpiperidine, N-methylmorpholine, arginine, lysine and 1,2-ethylenediamine.

In the present invention, the solvate represents these forms of the antibody-drug conjugate of the present invention: complexes in solid or liquid form that are formed by coordination of the antibody-drug conjugate with solvent molecules. Hydrate is a specific form of the solvate which has coordinating water molecules. In the present invention, the hydrate is a preferred solvate.

The EGFR-associated tumors that may be preferably treated with the antibody-drug conjugates of the present invention include tumors with EGFR overexpression, respiratory tract tumors (e.g.,

small cell carcinoma and non-small cell carcinoma, bronchial carcinoma), wherein non-small cell lung cancer is particularly preferred; tumors of digestive tracts (e.g., esophagus, stomach, gallbladder, small intestine, large intestine, rectum), wherein intestinal tumor is particularly preferred; tumors of endocrine and exocrine glands (e.g., thyroid and parathyroid, pancreas and salivary glands), wherein pancreatic tumor is particularly preferred; tumors of head and neck regions (e.g., larynx, hypopharynx, nasopharynx, oropharynx, lips, mouth, tongue and esophagus); and/or brain gliomas.

The antibody-drug conjugates of the present invention may be used in combination with a known chemotherapeutic agent for the treatment of tumors, the chemotherapeutic agent can be, for example, Adriamycin, cyclophosphamide and taxane [Taxol and Taxotere], Xeloda, Gemzar, Navelbine, Tamoxifen, aromatase inhibitors (Arimidex, Femara, Aromasin), 5-FU plus folinic acid, camptosar, oxaliplatin, cisplatin, carboplatin, estramustine, Novantrone, prednisone, Oncovin, etc., or a combination thereof.

In the present invention, "treatment" refers to clinical intervention that attempts to alter the natural course of a treated individual or cell, either for prevention or in the course of clinical pathology. The desired effect of treatment comprises the prevention of recurrence or relapse of disease, the alleviation of symptoms, the weakening of any direct or indirect pathological consequences of disease, the prevention of metastasis, the reduction of disease progression rate, the improvement or alleviation of disease status, and the elimination or improvement of prognosis. In some embodiments, the antibody or antibody-drug conjugate of the present invention is used to delay the onset of a disease or condition or to slow down the progression of a disease or condition. The above parameters used to assess the successful treatment and improvement of disease can be easily measured by conventional procedures familiar to physicians. For cancer treatment, efficacy can be measured by, for example, assessing time to progress (TTP) and/or measuring response rate (RR).

In the present invention, a "subject" refers to a vertebrate. In certain embodiments, the vertebrate refers to a mammal. The mammal includes, but is not limited to, livestock (such as cattle), pets (such as cats, dogs, and horses), primates, mice and rats. In certain embodiments, the mammal refers to a human.

In the present invention, an "effective amount" refers to an amount effective to achieve the desired therapeutic or prophylactic effect at the desired dose and time. An "therapeutically

effective amount" of a substance/molecule of the present invention may vary depending on factors such as disease state, age, gender and body weight of an individual and the ability of the substance/molecule to elicit a desired response in the individual. The therapeutically effective amount also covers an amount of the substance/molecule of which beneficial effects are superior to any toxic or detrimental effect. A "prophylactically effective amount" refers to an amount effective to achieve the desired prophylactic effect at the desired dose and time. It is generally but not necessary, however, that the prophylactically effective amount will be lower than the therapeutically effective amount since the prophylactic dose is administered to the subject prior to the onset of the disease or early in the disease. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor volume; inhibit (i.e., slow down to some extent, preferably stop) the cancer cells infiltrating into the surrounding organs; inhibit (i.e., slow down to some extent, preferably stop) tumor metastasis; inhibit to some extent the growth of tumor; and/or alleviate to some extent one or more symptoms associated with cancer.

For the prophylaxis or treatment of the disease, the appropriate dosage of the antibody-drug conjugate of the present invention (when used alone or in combination with one or more other therapeutic agents such as chemotherapeutic agents) will depend on the type of disease to be treated, the type of the antibody-drug conjugate, the severity and progression of the disease, the administration of the antibody-drug conjugate that is for the purpose of prevention or treatment, the previous therapy, the patient's clinical history and reactivity with the antibody-drug conjugates, judgment of physicians. It is appropriate that the antibody-drug conjugate is administered to a patient either once or through a series of treatments. According to the type and severity of the disease, the initial candidate dose administered to the patient may be about 1 $\mu\text{g}/\text{kg}$ to 100 mg/kg (e.g., 0.1 mg/kg to 20 mg/kg) of the antibody-drug conjugate, for example or by one or more separate administrations or by continuous infusion. According to the factors described above, the typical daily dose may range from about 1 $\mu\text{g}/\text{kg}$ to 100 mg/kg or more. For repeated administrations for several days or more, according to the conditions, the treatment is usually continued until the desired inhibition of symptoms appears. An exemplary dose of the antibody-drug conjugate may range from about 0.05 mg/kg to about 10 mg/kg . As such, the antibody-drug conjugate of one or more doses of about 0.5 mg/kg , 2.0 mg/kg , 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, for example, weekly or every three weeks (e.g., such that the patient receives about

2 to about 20 doses, or, for example, about 6 doses of the antibody-drug conjugate). A higher initial loading dose may be administered, followed by one or more doses at a lower dose. The process of this therapy is easily monitored by conventional techniques and assays. "Long-term" administration refers to administration of the drug in a continuous mode in contrast to a short-term mode, so that the initial therapeutic effect (activity) is maintained for a longer period of time. "Intermittent" administration refers to treatment that is not continuous without interruption, but is essentially periodic. Administration in "combination" with one or more other therapeutic agents includes simultaneous (co-)administration and sequential administration in any order.

A "pharmaceutically acceptable carrier" includes, when used in the present invention, pharmaceutically acceptable carriers, excipients or stabilizers, which are non-toxic to the cells or mammals to which they are exposed at the dosage and concentration used. Typically, a physiologically acceptable carrier is a pH buffered aqueous solution. Examples of the physiologically acceptable carrier include buffers such as phosphates, citrates and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides and other carbohydrates, including glucose, mannose, sucrose, trehalose or dextrin; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG) and PLURONICS™.

In the present invention, 20 conventional amino acids and their abbreviations follow their conventional usages. See Immunology-A Synthesis (2nd ed., E. S. Golub and D. R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference.

Brief Description of the Drawings

FIG. 1 is a HIC-HPLC plot for determining a drug/antibody ratio of an antibody-drug conjugate.

FIG. 2 shows determination of in-vitro cell activity of a monoclonal antibody and an antibody-drug conjugate, wherein ○ represents JMT101 monoclonal antibody, ▲ represents MRG003 antibody-drug conjugate.

FIG. 3 shows inhibition activity of MRG003 on the growth of colon cancer cell HT-29, wherein ○ represents JMT101 monoclonal antibody and ▲ represents MRG003 antibody-drug conjugate.

FIG. 4 shows inhibition activity of MRG003 on the growth of brain glioma cell U87-MG, where ○ represents JMT101 monoclonal antibody and ▲ represents MRG003 antibody-drug conjugate.

FIG. 5 shows inhibition activity of MRG003 on the growth of lung cancer cell A549, wherein ○ represents JMT101 monoclonal antibody, and ▲ represents MRG003 antibody-drug conjugate.

FIG. 6 shows inhibition activity of MRG003 on the growth of KRAS mutant colon cancer cell LoVo, in which ○ represents Erbitux monoclonal antibody and ▲ represents MRG003 antibody-drug conjugate.

FIG. 7 shows effects of a monoclonal antibody and an antibody-drug conjugate on volume of HT-29 colon cancer xenografted tumor in mice. The data in the figure is expressed as mean±standard deviation; as compared with a buffer control group, * indicates $P < 0.05$, ** indicates $P < 0.01$, and *** indicates $P < 0.001$.

FIG. 8 shows effects of a monoclonal antibody and an antibody-drug conjugate on body weight of an HT-29 colon cancer xenografted tumor model for mice.

Detailed Description of the Embodiments

Embodiments of the present invention will be described in details with reference to the following examples. However, it will be understood by a person skilled in the art that the following examples are only used to describe the present invention and should not be considered as limiting the scope of the present invention. If specific conditions are not specified in the examples, the routine conditions or the conditions recommended by the manufacturers are followed. When reagents or instruments as used are not indicated with the manufacturers, they are conventional products commercially available in the market.

The antibody JMT101 of the present invention is BA03 as described in the Chinese invention patent application CN 103772504A, and for its preparation method, reference is made to Example 3 of this patent application. The sequences of respective parts of the antibody are as follows:

The sequence of a variable region of a heavy chain is:

QVQLQESGPGLVKPSSETLSLTCTVSGFSLSNYDVHWVRQAPGKGLEWLGVIWSSGGN
TDYNTPFTRSRLTISVDTSKNQFSLKLSSVTAADTAVYYCARALDYDYEFAYWGQGT
LTVSS (SEQ ID NO: 1).

In the sequence, the underlined parts are CDR1 (SEQ ID NO: 5), CDR2 (SEQ ID NO: 6), and CDR3 (SEQ ID NO: 7), respectively; and

the non-underlined parts are FR1 (SEQ ID NO: 8), FR2 (SEQ ID NO: 9), FR3 (SEQ ID NO: 10), and FR4 (SEQ ID NO: 11), respectively.

The sequence of a variable region of a light chain is:

EIVLTQSPDFQSVTPKEKVTITCRASQSIGTNIHWYQQKPDQSPKLLIKYASESISGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQNNEWPTSFGQGTKLEIK (SEQ ID NO: 2).

In the sequence, the underlined parts are CDR1 (SEQ ID NO: 12), CDR2 (SEQ ID NO: 13), and CDR3 (SEQ ID NO: 14), respectively; and

the non-underlined parts are FR1 (SEQ ID NO: 15), FR2 (SEQ ID NO: 16), FR3 (SEQ ID NO: 17), and FR4 (SEQ ID NO: 18), respectively.

The sequence of a constant region of the heavy chain is:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSQVHTEPAAVL
QSSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYITLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLT
VDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 3).

The sequence of a constant region of the light chain is:

RTVAAPSVEFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT
EQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:
4).

Example 1: Preparation of Antibody-Drug Conjugate

10 mg of JMT101 antibody was taken and subjected to buffer exchange into a reduction buffer (25 mM sodium borate, pH 8.0, 25 mM NaCl, and 5 mM EDTA) using a 15 mL 30 KD ultrafiltration device for a total of three times; the final volume was about 1 mL, transferred to a new Eppendorf centrifuge tube (weighed), and weighed; the protein concentration was measured

and the total amount of protein was calculated. 2.5 times the molar amount of DTT was added to the antibody, and they were incubated at room temperature for 2 hours and continuously mixed. The mixture was subjected to buffer exchange into a coupling buffer (50 mM Tris, pH 7.2, 150 mM NaCl, and 5 mM EDTA) using a 15 ml 30 KD ultrafiltration device for a total of three times. A concentrated solution, which was measured by A280 to determine the protein concentration, was taken and weighed, and the total amount of protein was calculated. A 10 µl sample was taken and measured by Ellman's method to determine number of free thiol groups.

In addition, the molar concentration of its free thiol groups was calculated by the following formula:

$$C_{\text{thiol}} = \frac{A_{412} \times 11.2}{b \times 24150} \text{ (M)}$$

b: optical path length of cuvette (usually 1 cm).

The mole number of free thiol groups was calculated from the molar concentration of free thiol groups and the total volume of protein solution.

1.1 times the mole number of free thiol groups of vc-MMAE (purchased from Haoyuan Chemical Technology Co., Ltd., No. HY-15575) (dissolved in DMSO) was added to the reduced antibody. The mixture was mixed, then reacted at room temperature for 2 hours, and intermittently mixed. N-acetylcysteine in an amount of 20 times the mole number of vc-MMAE was added to the reaction system in the reaction solution, and mixed, and the mixture was allowed to stand for 5 minutes. The mixture was subjected to buffer exchange into a conjugate stock solution (20 mM sodium citrate (Na-citrate), 0.3% NaCl, 5% Trehalose, 0.05% Tween-80, pH 6.0) using a 15 ml 30 KD ultrafiltration device for a total of 3 times. The obtained antibody-drug conjugate MRG003 sample was stored at 4° C.

Determination of Drug/Antibody Ratio:

The prepared antibody-drug conjugate MRG003 was subjected to HIC-HPLC analysis (Jun Ouyang, Drug-To-Antibody (DAR) Ratio and Drug Distribution by Hydrophobic Interaction Chromatography and Reverse Phase High Performance Chromatography, Laurent Ducry (ed.), Antibody Drug Conjugates, Chapter 17, Methods in Molecular Biology, Vol 1045, p 275-283) to determine a drug antibody ratio (DAR). See FIG. 1, and the average drug loading number DAR was calculated as 4.1 according to the peak area of the spectrum.

Example 2: Determination of In-vitro Cell Activity of Antibody-Drug Conjugate MRG003

Method for Determination of Cell Activity:

1.1 After 3-4 generations of passages of thawed cell lines, culture media were firstly discarded. Cells were rinsed with 5 mL of DPBS once, then digested with 3 mL of trypsin, resuspended respectively with media, and centrifuged with a centrifuge, and the supernatants were discarded. Next, the cells were resuspended again with the media, and 0.5 mL samples were taken and counted with a cell counter. The cells were plated on 96-well cell plates (DiFi cells at 10,000 cells/well, HT-29 cells at 5000 cells/well, A549 cells at 2000 cells/well, U87-MG cells at 3000 cells/well, and LoVo cells at 4000 cells/well), cultured for 24 hours, then monoclonal antibody JMT101 and antibody-drug conjugate MRG003 as diluted in a series of concentrations were added and incubated in a cell culture incubator for 72 hours. Thereafter, 20 μ l of CCK8 color-producing reagent was added to each well. OD450-650 was detected with an enzyme reader at a wavelength of 450-650 nm, and four-parameter fitting was performed.

Experimental results of In-vitro Cell Activity:

The following cell lines were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences.

Regarding the activities in DiFi cells (human colorectal cancer cells) with high EGFR expression: MRG003 showed significantly increased cell growth inhibitory activity than monoclonal antibody JMT101, and EC50 was reduced by about 10 times (EC50 of JMT101 was 51.9 ng/ml, and EC50 of MRG003 was 5.1 ng/ml), as shown in FIG. 2.

Regarding the activities in other tumor cells with medium and low EGFR expression: MRG003 showed significant cell growth inhibitory activity relative to monoclonal antibody itself in cancer cells (human colon cancer cells HT29, human lung cancer cell A549, human brain astrocytoma cell line U87-MG) with medium and low EGFR expression (as shown in FIGS. 3, 4, and 5), wherein EC50 of HT-29 was 611 ng/ml, EC50 of A549 was 28.3 μ g/ml, and EC50 of U87-MG was 5.3 μ g/ml.

In addition, the activities in KRAS mutant colon cancer cells LoVo (Dunn EF, Iida M, Myers RA, Hintz KA, Campbell DA, Armstrong EA, Li C and Wheeler DL. Dasatinib sensitizes KRAS mutant colorectal tumors to cetuximab. *Oncogene* 2011; 30:561-574) with medium EGFR expression were also tested, and it was found that MRG003 showed significant tumor growth

inhibitory activity against KRAS mutant colon cancer cell LoVo (as shown in FIG. 6, EC50 was 3.2 µg/ml).

Example 3: In-vivo Xenografted Tumor Experiment in Mice

Method of In-vivo Xenografted Tumor Experiment in Mice:

HT-29 colon cancer cells were cell lines with relatively low EGFR expression and BRAF mutations, and Erbitux, the EGFR targeting monoclonal antibody currently available on the market for the treatment of colorectal cancer had no growth inhibitory activity against HT-29 cell lines.

HT-29 cell xenograft model: the tumor cells at a logarithmic growth phase were collected and counted, then resuspended in 1×PBS. The cell suspension concentration was adjusted to 3×10^7 /ml. The tumor cells were inoculated subcutaneously on the right side of back of nude mice with a 1 ml syringe (4-gauge needle), 3×10^6 /0.1 ml/mouse. When the tumor volume reached 150-200 mm³, the mice were grouped by a randomized block method, 8 mice per group, so as to ensure that the tumor volume and body weight of mice between the groups were uniform. The difference between the mean value of tumor volume in each group and the mean value of tumor volume of all experimental animals was not more than ±10%. Tail vein administration was performed, once every four days (the 1st, 5th, 9th and 13th day), for a total of 4 times, and the tumor volumes and body weights of mice were regularly measured. There were 8 mice in each administration group.

Experimental Results of Xenografted Tumor in Mice

HT-29 colon cancer xenografted tumor experiment in mice: there were 5 groups in the experiment, including a buffer solution group (20 mM sodium citrate, 0.3% sodium chloride, 5% trehalose, 0.05% Tween 80, and pH 6), a JMT101 monoclonal antibody group (5 mg/kg), an MRG003 group (1 mg/kg), an MRG003 group (5 mg/kg) and a non-binding ADC group (5 mg/kg) (human IgG-vcMMAE conjugate, in which IgG was IgG obtained by purification from human serum, and this conjugate was prepared by the same method as MRG003). The volume of the xenografted tumor in the mice administrated with MRG003 was significantly lower than that of the control group, showing a significant anti-tumor growth effect (FIG. 7). On the 18th day, for the MRG003 group at the dose of 5 mg/Kg, its tumor growth inhibition rate was up to 54% as compared with the buffer solution group, and its tumor growth inhibition rate was up to 46% as compared with the JMT101 monoclonal antibody group at the same dose, and its tumor growth inhibition rate was up to 42% as compared with the non-binding ADC.

Body weight of mice: the body weight of mice administered with MRG003 showed no significant change as compared with the control group (see FIG. 8), indicating that MRG003 had not toxic effect of reducing the body weight of mice.

While specific embodiments of the present invention have been described in detail, a person skilled in the art will understand that various modifications and substitutions can be made to these details according to all teachings that have been disclosed, and all of these changes fall within the scope of protection of the present invention. The full scope of the present invention is given by the appended claims and any equivalents thereof.

Drawings

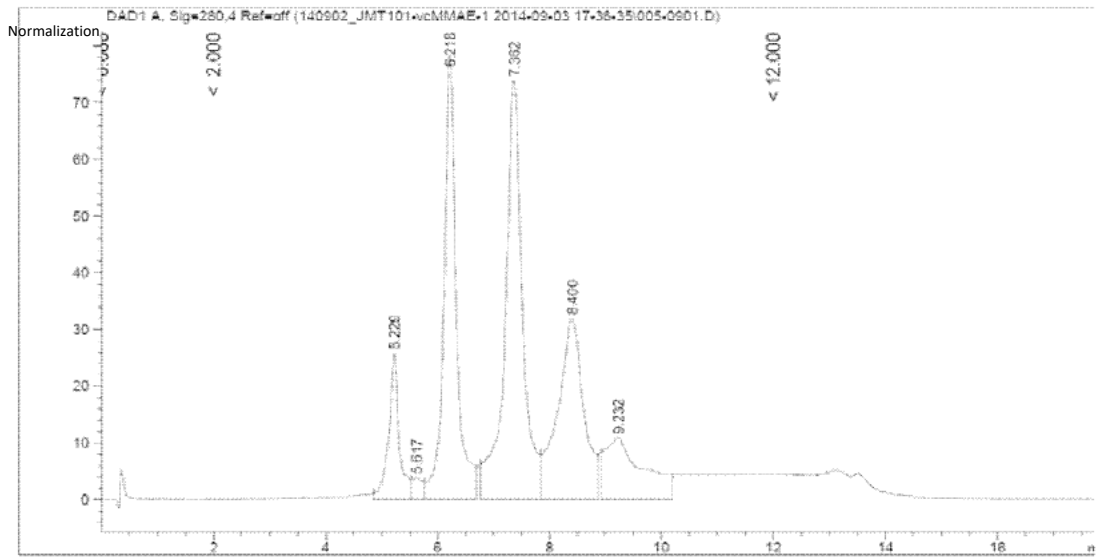


Fig. 1

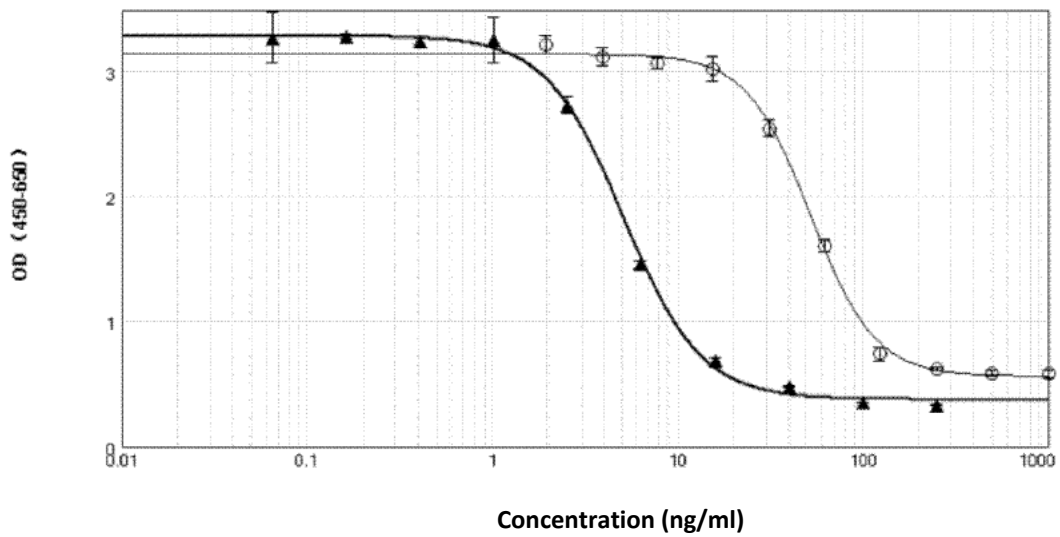


Fig. 2

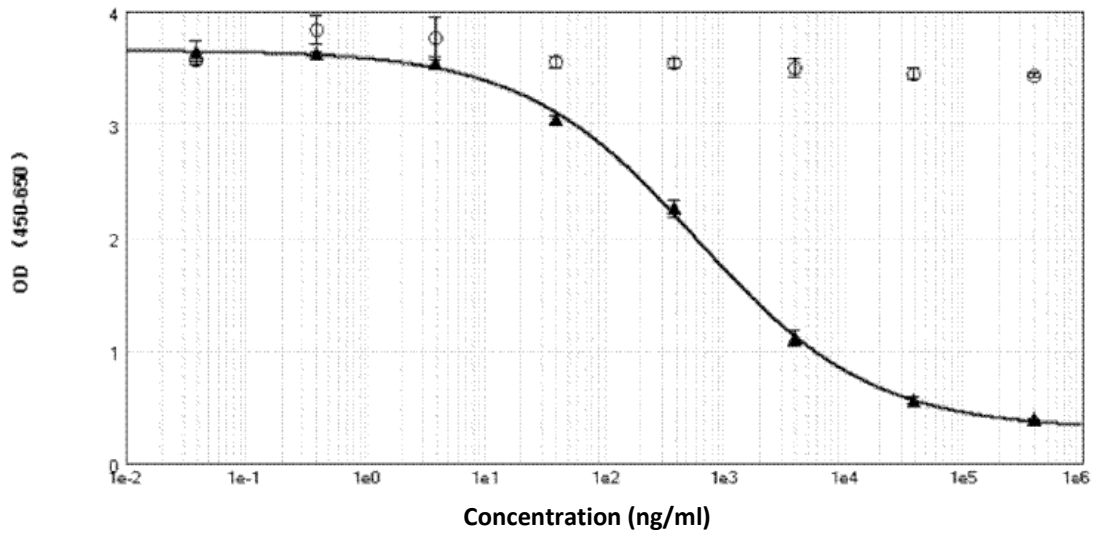


Fig. 3

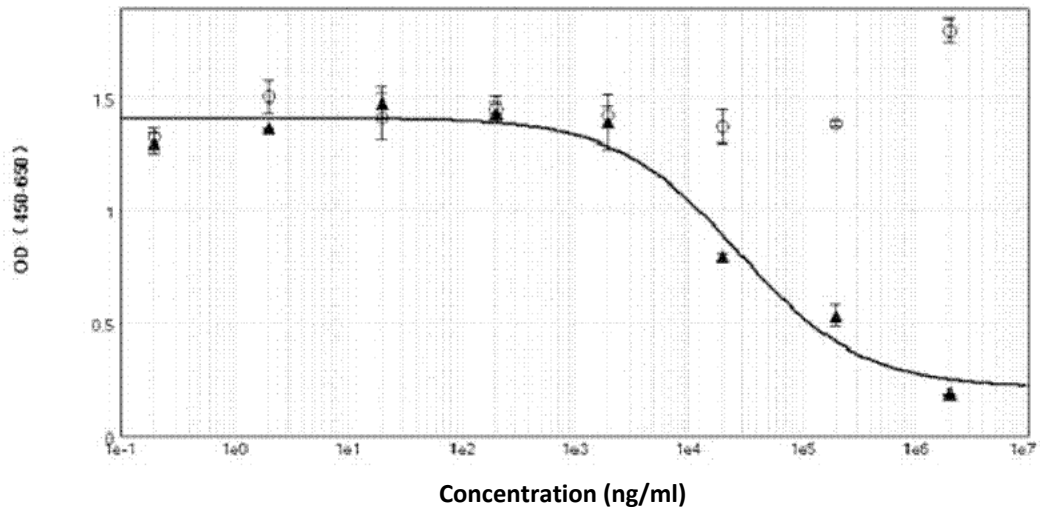


Fig. 4

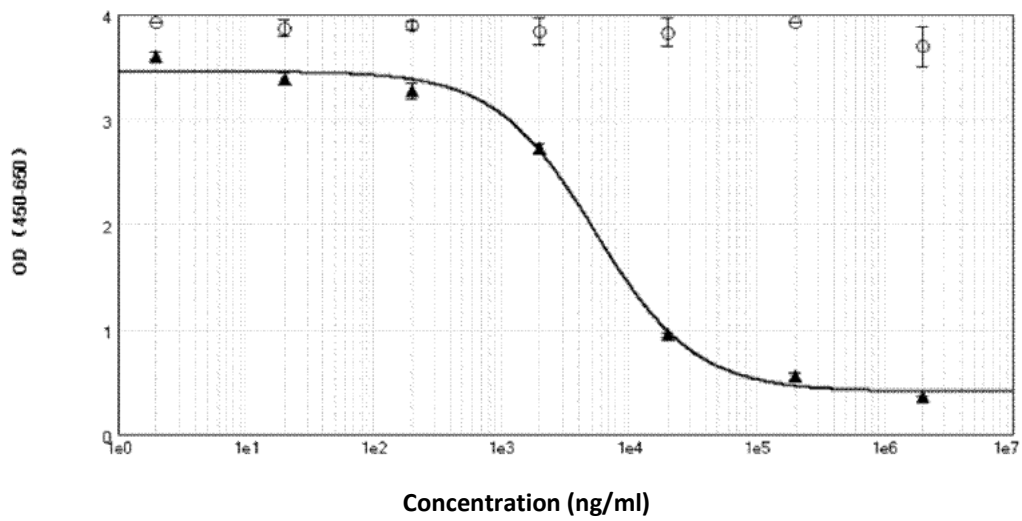


Fig. 5

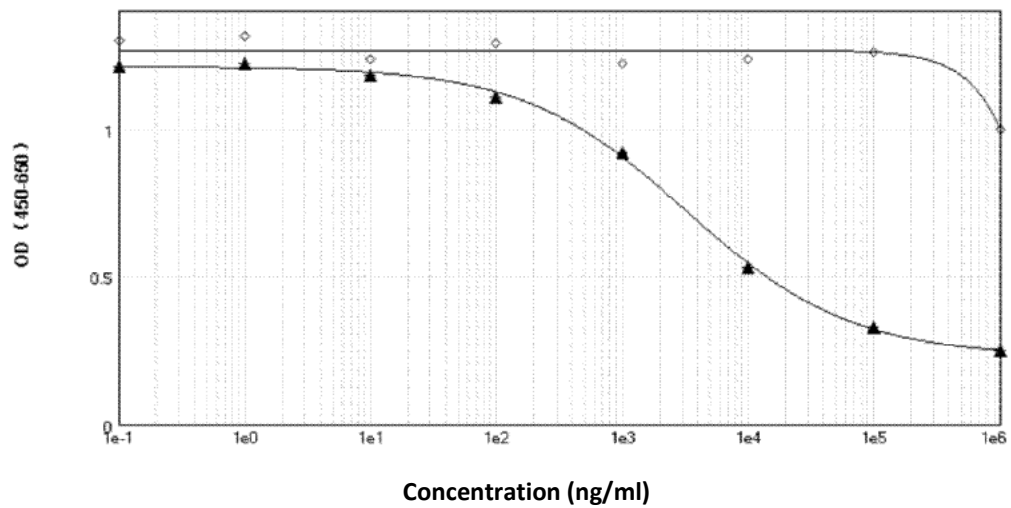


Fig. 6

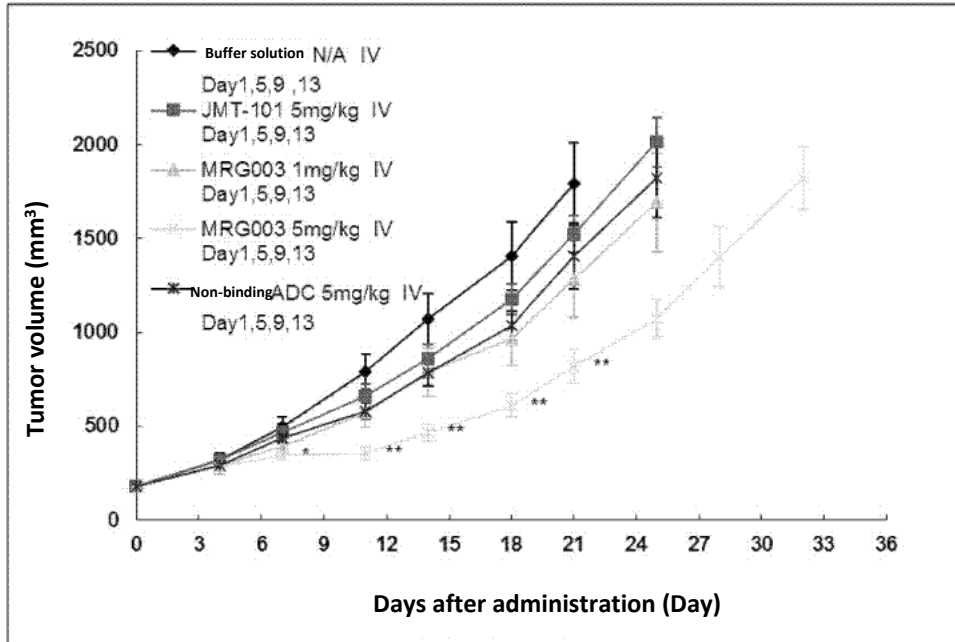


Fig. 7

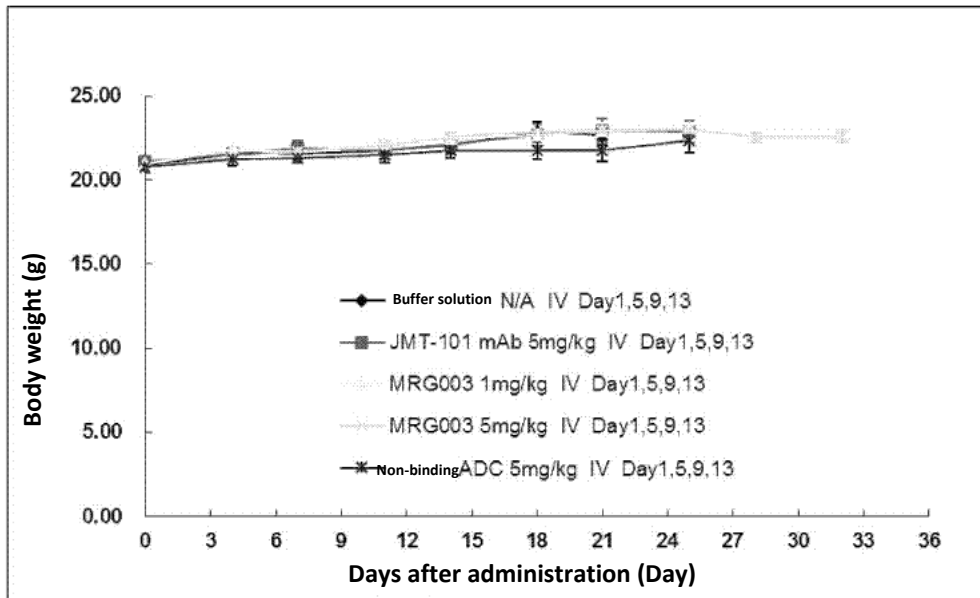


Fig. 8

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TRANSLATION CERTIFICATION

Date: February 26, 2025

To whom it may concern:

This is to certify that the attached translation is an accurate representation of the documents received by this office. The translation was completed from:

- Chinese (PRC)

To:

- English (USA)

The documents are designated as:

- Ex. 1030 CN201510085038.8-C

Emily Paras, Project Manager in this company, attests to the following:

“To the best of my knowledge, the aforementioned documents are a true, full and accurate translation of the specified documents.”

A handwritten signature in black ink that reads "Emily Paras". The signature is written in a cursive style with a horizontal line underneath it.

Signature of Emily Paras