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(54) Title: EGFR AND LAG3 DUAL TARGETED BISPECIFIC ANTIBODY AND USES THEREOF

(57) Abstract: Disclosed herein are bispecific antibodies against EGFR and LAG3, nucleic acids comprising the antibodies, vectors comprising the nucleic acids, and host cell comprising the nucleic acids or the vectors. Also disclosed are pharmaceutical compositions and antibody-drug conjugates comprising the antibodies, and therapeutic methods for using the antibodies.

EGFR AND LAG3 DUAL TARGETED BISPECIFIC ANTIBODY AND USES THEREOF

FIELD OF THE INVENTION

The present invention is directed to bispecific antibodies targeting EGFR and LAG3, and uses of such antibodies, in particular their use in the treatment of cancers.

BACKGROUND OF THE INVENTION

Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein of 170 kDa that is encoded by the c-erbB1 proto-oncogene. It is consisted of a C-terminal intracellular region that possesses the kinase activity, an N-terminal extracellular ligand-binding site, and a hydrophobic transmembrane domain. EGFR is a receptor of tyrosine kinase (RTK). RTKs play an important role in the control of most fundamental cellular processes such as cell cycle, cell migration, cell metabolism and survival, as well as cell proliferation and differentiation. Via tyrosine kinase-mediated signal transduction pathways, EGFR can regulate numerous cellular processes. In addition, activation of EGFR leads to simultaneous activation of several signaling cascades including the MAPK pathway, the protein kinase C (PKC) pathway and the PI(3)K-activated AKT pathway.

EGFR is frequently overexpressed or mutated in human cancers, and contribute to neoplastic growth in humans. About 30% of solid tumors show gain-of-function genetic alteration of EGFR, including cancers of the bladder, brain, head and neck, pancreas, lung, breast, ovary, colon, prostate, and kidney. Some tumor cells exhibit “oncogene addiction,” a concept introduced by *Weinstein* in 2002, as they are dependent on EGFR signaling for survival and growth. Hence, targeting EGFR may be a great treatment modality with great results. Various strategies to target EGFR and block EGFR signaling pathways include cetuximab and panitumumab have been reported. All these confirm that targeting EGFR is an effective antitumor therapy.

Lymphocyte activation gene-3 (LAG3 or CD223) was initially discovered in an experiment designed to selectively isolate molecules expressed in an IL-2-dependent NK cell line. It is a 70kDa transmembrane protein with structural homology to CD4 with four extracellular glycosylation sites. LAG3 is not expressed on resting peripheral blood lymphocytes, but is expressed on activated T, NK, some B, and DC cells. Like CD4, the LAG3 protein binds to MHC class II molecules with a higher affinity, but unlike CD4, LAG3 does not interact with the human immunodeficiency virus

gp120 protein. Studies using a soluble LAG3 immunoglobulin fusion protein (sLAG3Ig) demonstrated direct and specific binding of LAG3 to MHC class II on the cell surface.

The cytoplasmic tail of LAG3 consists of three conserved motifs, one of the motif is a highly unique and conserved six amino acid sequence (KIEELE, SEQ ID NO: 20) that has been shown to be required for LAG3 to downregulate T cell function. Interaction of LAG3 with its ligand, the MHC class II expressed on antigen presenting cells like macrophages and DC, inhibits the activation of T and NK cells and therefore, suppress the ability of the cells to recognize and kill cancer cells. Furthermore, $CD4^+CD25^+$ regulatory T cells (T_{reg}) have been shown to express LAG3 upon activation and antibodies against LAG3 inhibit suppression by induced T_{reg} cells, both in vitro and in vivo, suggesting that LAG3 contributes to the suppressor activity of T_{reg} cells.

The individual roles of both EGFR and LAG3 in cancer is well established, while monotherapy targeting EGFR or LAG3 may initially be highly effective in cancer treatments, therapeutic resistance often follows. Accordingly, there exists a need for EGFR×LAG3 dual targeted bispecific antibody which is predicted to achieve effective cancer treatment by recognizing two kinds of antigens simultaneously.

SUMMARY OF THE INVENTION

The present disclosure provides EGFR×LAG3 recombinant bispecific antibody designed for simultaneous binding to a surface antigen on target cells and to certain immune cells such as T cells. A variety of functional assays have demonstrated the potent anti-tumor effect on various cancers (particularly, EGFR positive cancers, e.g., colon cancer, kidney cancer, colorectal cancer, lung cancer, gastric cancer, ovarian cancer, breast cancer, pancreatic cancer, prostate cancer, skin cancer, head and neck cancer, brain cancer, bladder cancer and liver cancer) of the engineered antibody in the form of the EGFR×LAG3 bispecific antibody.

In an aspect, the present disclosure provides a bispecific antibody or an antigen binding fragment thereof, comprising a first antigen binding region binding to EGFR comprising a first light chain variable region (VL1) and a first heavy chain variable region (VH1) and a second antigen binding region binding to LAG3 comprising a second light chain variable region (VL2) and a second heavy chain variable region (VH2), wherein the VL1 comprises LCDRs 1-3 having the amino acid sequences as shown in SEQ ID NOs: 1-3 respectively; the VH1 comprises HCDRs 1-3 having the amino acid sequences as shown in SEQ ID NOs: 5-7 respectively; the VL2

comprises LCDRs 1-3 having the amino acid sequences as shown in SEQ ID NOs: 9-11 respectively; and the VH2 comprises HCDRs 1-3 having the amino acid sequences as shown in SEQ ID NOs: 13-15 respectively.

In some embodiments of the bispecific antibody or the antigen binding fragment thereof disclosed herein, the VL1 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4; the VH1 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 8; the VL2 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 12; and the VH2 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 16.

In some embodiments, the VL1 comprises an amino acid sequence as set forth in SEQ ID NO: 4; the VH1 comprises an amino acid sequence as set forth in SEQ ID NO: 8; the VL2 comprises an amino acid sequence as set forth in SEQ ID NO: 12; and the VH2 comprises an amino acid sequence as set forth in SEQ ID NO: 16.

In some embodiments, the bispecific antibody comprises: a first polypeptide chain comprising from the N terminal to C terminal: VH2-CH1-CH2-CH3-L1-VH1-L2-VL1; and a second polypeptide chain comprising from the N terminal to C terminal: VL2-CL; wherein CH1 represents the first domain of constant region of an immunoglobulin heavy chain; CH2 represents the second domain of constant region of an immunoglobulin heavy chain; CH3 represents the third domain of constant region of an immunoglobulin heavy chain; CL represents constant region of an immunoglobulin light chain; and L1 and L2 each independently represents an optional linker.

In some embodiments, the bispecific antibody comprises two copies of the first polypeptide chain and two copies of the second polypeptide chain.

In some embodiments, each of the CH1, CH2 and CH3 is independently derived from immunoglobulin isotype IgG (e.g. human IgG), preferably derived from IgG subtype selected from the group consisting of IgG1, IgG2 and IgG4 (e.g. human IgG1, IgG2 and IgG4).

In some embodiments, the CL is derived from λ light chain or κ light chain.

In some embodiments, the linker comprises an amino acid sequence of (G4S)n, wherein n is an integer selected from 1-5, preferably the linker comprises an amino acid sequence as shown in SEQ ID NO: 19.

In some embodiments, the first polypeptide chain comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 17; and the second polypeptide chain comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 18.

In some embodiments, the first polypeptide chain comprises an amino acid sequence as shown in SEQ ID NO: 17; and the second polypeptide chain comprises an amino acid sequence as shown in SEQ ID NO: 18.

In another aspect, the present disclosure provides a nucleic acid comprising a nucleotide sequence encoding the bispecific antibody or the antigen binding fragment thereof disclosed herein.

In still another aspect, the present disclosure provides a vector comprising the nucleic acid disclosed herein.

In yet another aspect, the present disclosure provides a host cell comprising the nucleic acid disclosed herein or the vector disclosed herein.

In another aspect, the present disclosure provides a pharmaceutical composition comprising the bispecific antibody or the antigen binding fragment thereof disclosed herein, and a pharmaceutically acceptable carrier or excipient.

In some embodiments of the pharmaceutical composition disclosed herein, the pharmaceutical composition further comprises a second therapeutic agent. In some embodiments, the second therapeutic agent is selected from an antibody, a chemotherapeutic agent and a small molecule drug. In some embodiments, the second therapeutic agent is selected from a Bruton's tyrosine kinase (BTK) inhibitor, a PI3K inhibitor, a HDAC inhibitor, an ERK inhibitor, a MAPK inhibitor, a PD-1/PD-L1 inhibitor, a CTLA-4 inhibitor, a TIGIT inhibitor, a TIM3 inhibitor, a VEGF inhibitor, and glucocorticoid.

In yet another aspect, the present disclosure provides a conjugate comprising the bispecific antibody or the antigen binding fragment thereof disclosed herein, and a chemical moiety conjugated thereto.

In some embodiments of the conjugate disclosed herein, the chemical moiety is selected from the group consisting of a therapeutic agent, a detectable moiety, and an immune stimulatory molecule.

In still another aspect, the present disclosure provides a method of treating a cancer in a subject, comprising administering to the subject an effective amount of the bispecific antibody or the antigen binding fragment thereof disclosed herein, the pharmaceutical composition disclosed herein, or the conjugate disclosed herein.

In some embodiments of the method disclosed herein, the cancer is an EGFR positive cancer. In some embodiments, the cancer is selected from the group consisting of colon cancer, kidney cancer, colorectal cancer, lung cancer, gastric cancer, ovarian cancer, breast cancer, pancreatic cancer, prostate cancer, skin cancer, head and neck cancer, brain cancer, bladder cancer and liver cancer. In preferred embodiments, the cancer is selected from the group consisting of colorectal cancer, head and neck cancer, kidney cancer, and skin cancer.

In some embodiments, the method further comprises administering to the subject a second therapeutic agent. In some embodiments, the second therapeutic agent is selected from an antibody, a chemotherapeutic agent and a small molecule drug. In some embodiments, the second therapeutic agent is selected from a Bruton's tyrosine kinase (BTK) inhibitor, a PI3K inhibitor, a HDAC inhibitor, an ERK inhibitor, a MAPK inhibitor, a PD-1/PD-L1 inhibitor, a CTLA-4 inhibitor, a TIGIT inhibitor, a TIM3 inhibitor, a VEGF inhibitor, and glucocorticoid.

In another aspect, the present disclosure provides use of the bispecific antibody or the antigen binding fragment thereof disclosed herein, the pharmaceutical composition disclosed herein, or the conjugate disclosed herein in the manufacture of a medicament for treating a cancer in a subject.

In still another aspect, the present disclosure provides the bispecific antibody or the antigen binding fragment thereof disclosed herein, the pharmaceutical composition disclosed herein, or the conjugate disclosed herein for use in treating a cancer in a subject.

In some embodiments of the use disclosed herein, the cancer is an EGFR positive cancer. In some embodiments, the cancer is selected from the group consisting of colon cancer, kidney cancer, colorectal cancer, lung cancer, gastric cancer, ovarian cancer, breast cancer, pancreatic cancer, prostate cancer, skin cancer, head and neck cancer, brain cancer, bladder cancer and liver cancer. In preferred embodiments, the cancer is selected from the group consisting of colorectal cancer, head and neck cancer, kidney cancer, and skin cancer. In some embodiments, the bispecific

antibody or the antigen binding fragment thereof disclosed herein, the pharmaceutical composition disclosed herein, or the conjugate disclosed herein is in combination with a second therapeutic agent. In some embodiments, the second therapeutic agent is selected from an antibody, a chemotherapeutic agent and a small molecule drug. In some embodiments, the second therapeutic agent is selected from a Bruton's tyrosine kinase (BTK) inhibitor, a PI3K inhibitor, a HDAC inhibitor, an ERK inhibitor, a MAPK inhibitor, a PD-1/PD-L1 inhibitor, a CTLA-4 inhibitor, a TIGIT inhibitor, a TIM3 inhibitor, a VEGF inhibitor, and glucocorticoid.

BRIEF DESCRIPTION OF THE DRAWINGS

An understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

Figure 1 schematically shows the structure of EGFR×LAG3 BsAb CMD005.

Figure 2A shows binding of EGFR×LAG3 BsAb CMD005 against recombinant human EGFR as measured by ELISA.

Figure 2B shows binding of EGFR×LAG3 BsAb CMD005 against recombinant human LAG3 as measured by ELISA.

Figure 3A shows binding of EGFR×LAG3 BsAb CMD005 against EGFR expressing cell line Caco-2 as measured by flow cytometry.

Figure 3B shows binding of EGFR×LAG3 BsAb CMD005 against LAG3 on the surface of activated T cells as measured by flow cytometry.

Figure 4A shows ability of EGFR×LAG3 BsAb CMD005 in blocking the interaction between EGFR and EGF as measured by ELISA.

Figure 4B shows ability of EGFR×LAG3 BsAb CMD005 in blocking the interaction between LAG3 and FGL1 as measured by ELISA.

Figure 5 shows ability of EGFR×LAG3 BsAb CMD005 in blocking the interaction between LAG3 and MHC II expressed on Raji cells as measured by flow cytometry.

Figure 6A shows growth inhibition of A431 cells by EGFR×LAG3 BsAb CMD005 *in vitro*.

Figure 6B shows growth inhibition of Caco-2 cells by EGFR×LAG3 BsAb CMD005 *in vitro*.

Figure 6C shows growth inhibition of Achn cells by EGFR×LAG3 BsAb CMD005 *in vitro*.

Figure 7 shows concentration of EGFR×LAG3 BsAb CMD005 in serum from CMD005-treated mice at 15hr, 39hr, 63hr, and 87hr after treatment.

Figure 8A shows inhibition of MC38-hEGFR tumor growth in hLAG3-c57 BL/6 mice by EGFR×LAG3 BsAb CMD005 measured by tumor volume.

Figure 8B shows body weight of hLAG3-c57 BL/6 mice of all groups in the tumor growth inhibition assay.

DETAILED DESCRIPTION OF THE INVENTION

The aforementioned features and advantages of the invention as well as additional features and advantages thereof will be more clearly understood hereafter as a result of a detailed description of the following embodiments when taken in conjunction with the drawings.

The embodiments described herein with reference to drawings are explanatory, illustrative, and used to generally understand the present invention. The embodiments shall not be construed to limit the scope of the present invention. The same or similar elements and the elements having same or similar functions are denoted by like reference numerals throughout the descriptions.

Unless indicated or defined otherwise, all terms used have their usual meaning in the art, which will be clear to the skilled person. Reference is for example made to the standard handbooks, such as Leuenberger, H.G.W, Nagel, B. and Klbl, H. eds., "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", Helvetica Chimica Acta (1995), CH-4010 Basel, Switzerland; Sambrook et al, "Molecular Cloning: A Laboratory Manual" (2nd Ed.), Vols. 1-3, Cold Spring Harbor Laboratory Press (1989); F. Ausubel et al, eds., "Current protocols in molecular biology", Green Publishing and Wiley InterScience, New York (1987); Roitt et al., "Immunology (6th Ed.), Mosby/Elsevier, Edinburgh (2001); and Janeway et al., "Immunobiology" (6th Ed.), Garland Science Publishing/Churchill Livingstone, New York (2005), as well as the general background art cited above.

Definitions

As used herein, singular forms “a”, “and,” and “the” include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to “an antibody” includes a plurality of antibodies and reference to “an antibody” in some embodiments includes multiple antibodies, and so forth.

Unless indicated or defined otherwise, the term "comprise", and variations such as "comprises" and "comprising", should be understood to imply the inclusion of a stated elements or step or group of elements or steps but not the exclusion of any other element or step or group of elements or steps.

As used herein, the term “antibody” refers to an immunoglobulin molecule which has the ability to specifically bind to a specific antigen. An antibody often comprises a variable region and a constant region in each of a heavy chain and a light chain. The variable regions of the heavy and light chains of antibodies contain a binding domain that interacts with an antigen. The constant regions of antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (such as effector cells) and components of the complement system such as C1q, the first component in the classical pathway of complement activation. Accordingly, most antibodies have a heavy chain variable region (VH) and a light chain variable region (VL) that together form the portion of the antibody that binds to the antigen.

A “light chain variable region” (VL) or “heavy chain variable region” (VH) consists of a “framework” region interrupted by three “complementarity determining regions” or “CDRs”. The framework regions serve to align the CDRs for specific binding to an epitope of an antigen. The CDRs include the amino acid residues of an antibody that are primarily responsible for antigen binding. From amino-terminus to carboxyl-terminus, both VL and VH domains comprise the following framework (FR) and CDR regions: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. CDRs 1, 2, and 3 of a VL domain are also referred to herein, respectively, as LCDR1, LCDR2, and LCDR3; CDRs 1, 2, and 3 of a VH domain are also referred to herein, respectively, as HCDR1, HCDR2, and HCDR3.

The assignment of amino acids to each VL and VH domain is in accordance with any conventional definition of CDRs. Conventional definitions include, the Kabat definition (Kabat, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, MD, 1987 and 1991), the Chothia definition (Chothia & Lesk, J. Mol. Biol. 196:901-917, 1987; Chothia et al., Nature 342:878-883, 1989); a composite of Chothia Kabat CDR in which CDR-H1 is a composite of Chothia and Kabat CDRs; the AbM definition used by Oxford Molecular’s antibody modelling software; and the CONTACT definition of Martin et al. (world wide web bioinfo.org.uk/abs). Kabat provides a widely used numbering convention (Kabat numbering system) in which corresponding residues between different heavy chains or between different light

chains are assigned the same number. The present disclosure can use CDRs defined according to any of these numbering systems, although preferred embodiments use Kabat defined CDRs.

Based on the amino acid sequence of heavy chain constant regions of the antibody, a immunoglobulin molecule can be divided into five classes (isotypes): IgA, IgD, IgE, IgG, and IgM, and can be further divided into different subtypes, such as IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, etc. The light chain of the antibody can be classified as a lambda (λ) chain or a kappa (κ) chain, based on the amino acid sequence of the light chain.

The term "antibody" as used herein should be understood in its broadest meaning, and includes monoclonal antibodies (including full-length monoclonal antibodies), polyclonal antibodies, antibody fragments, and multi-specific antibodies containing at least two different antigen binding regions (e.g., bispecific antibodies). The antibody may contain additional modifications, such as non-naturally occurring amino acids, mutations in Fc regions, and mutations in glycosylation sites. Antibodies also include post-translation modified antibodies, fusion proteins containing the antigenic determinants of the antibody, and immunoglobulin molecules containing any other modifications to antigen recognition sites, as long as these antibodies exhibit desired biological activity.

The term “bispecific antibody” in the context of the present invention is to be understood as an antibody having two different antigen-binding regions defined by different antibody sequences. This can be understood as different target binding but includes as well binding to different epitopes in one target. The term "bispecific antibody" as used herein should be understood in its broadest meaning, and includes full-length bispecific antibodies and antigen binding fragments thereof. The bispecific antibody may contain additional modifications, such as non-naturally occurring amino acids, mutations in Fc regions, and mutations in glycosylation sites. Bispecific antibodies also include post-translation modified antibodies, fusion proteins containing the antigenic determinants of the antibody, and immunoglobulin molecules containing any other modifications to antigen recognition sites, as long as these antibodies exhibit desired biological activity.

As used herein, the term “antigen binding fragment” of an antibody refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen binding function of an antibody can be performed by fragments of a full-length antibody.

Examples of antigen binding fragments encompassed within the term "antigen binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fab' fragment, which is essentially an Fab with part of the hinge region; (iv) a Fd fragment consisting of the VH and CH1 domains; (v) a Fd' fragment having VH and CH1 domains and one or more cysteine residues at the C-terminus of the CH1 domain; (vi) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (vii) a dAb fragment, which consists of a VH domain; (viii) an isolated complementarity determining region (CDR); and (ix) a nanobody, a heavy chain variable region containing a single variable domain and two constant domains. Furthermore, although the two domains of the Fv fragment, VL and VH are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv)). Such single chain antibodies are also intended to be encompassed within the term "antigen binding fragment" of an antibody. Furthermore, the term also includes a "linear antibody" comprising a pair of tandem Fd segments (VH-CH1-VH-CH1), which forms an antigen binding region together with a complementary light chain polypeptide, and a modified version of any of the foregoing fragments, which retains antigen binding activity.

These antigen binding fragments can be obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

As used herein, the term "binding" or "specifically binding" refers to a non-random binding reaction between two molecules, such as between an antibody and its target antigen. The binding specificity of an antibody can be determined based on affinity and/or avidity. The affinity, represented by the equilibrium constant for the dissociation of an antigen with an antibody (KD), is a measure for the binding strength between an antigenic determinant and an antigen-binding site on the antibody: the lesser the value of the KD, the stronger the binding strength between an antigenic determinant and the antibody. Alternatively, the affinity can also be expressed as the affinity constant (KA), which is 1/KD.

Avidity is the measure of the strength of binding between an antibody and the pertinent antigen. Avidity is related to both the affinity between an antigenic determinant and its

antigen binding site on the antibody and the number of pertinent binding sites present on the antibody. Typically, an antibody will bind to an antigen with a dissociation constant (KD) of 10^{-5} to 10^{-12} M or less, and preferably 10^{-7} to 10^{-12} M or less and more preferably 10^{-8} to 10^{-12} M, and/or with a binding affinity of at least 10^{7} M^{-1}, preferably at least 10^{8} M^{-1}, more preferably at least 10^{9} M^{-1}, such as at least 10^{12} M^{-1}. Any K_D value greater than 10^{-4} M is generally considered to indicate non-specific binding. Specifically binding of an antibody to an antigen or antigenic determinant can be determined in any suitable manner known, including, for example, Scatchard analysis and/or competitive binding assays, such as radioimmunoassays (RIA), enzyme immunoassays (EIA) and sandwich competition assays, and the different variants thereof known in the art.

The term "epitope" refers to a site on an antigen to which an antibody binds. An epitope can be formed from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of one or more proteins. Epitopes formed from contiguous amino acids (also known as linear epitopes) are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding (also known as conformational epitopes) are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. The epitope defines the smallest binding site of an antibody and therefore is the specific target of the antibody or antigen binding fragment thereof.

As used herein, the term "sequence identity" refers to the extent to which two sequences (amino acid) have the same residue at the same positions in an alignment. For example, "an amino acid sequence is X% identical to SEQ ID NO: Y" refers to % identity of the amino acid sequence to SEQ ID NO:Y and is elaborated as X% of residues in the amino acid sequence are identical to the residues of sequence disclosed in SEQ ID NO: Y. Generally, computer programs are employed for such calculations. Exemplary programs that compare and align pairs of sequences, include ALIGN (Myers and Miller, 1988), FASTA (Pearson and Lipman, 1988; Pearson, 1990) and gapped BLAST (Altschul et al., 1997), BLASTP, BLASTN, or GCG (Devereux et al., 1984).

Also, in determining the degree of sequence identity between two amino acid sequences, the skilled person may take into account so-called "conservative" amino acid substitutions, which can generally be described as amino acid substitutions in which an amino acid residue is replaced with another amino acid residue of similar chemical structure and which has little or essentially no

influence on the function, activity or other biological properties of the polypeptide. Such conservative amino acid substitutions are well known in the art.

Such conservative substitutions preferably are substitutions in which one amino acid within the following groups (a) - (e) is substituted by another amino acid residue within the same group: (a) small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro and Gly; (b) polar, negatively charged residues and their (uncharged) amides: Asp, Asn, Glu and Gln; (c) polar, positively charged residues: His, Arg and Lys; (d) large aliphatic, nonpolar residues: Met, Leu, He, Val and Cys; and (e) aromatic residues: Phe, Tyr and Trp.

Particularly preferred conservative substitutions are as follows: Ala into Gly or into Ser; Arg into Lys; Asn into Gln or into His; Asp into Glu; Cys into Ser; Gln into Asn; Glu into Asp; Gly into Ala or into Pro; His into Asn or into Gln; Ile into Leu or into Val; Leu into Ile or into Val; Lys into Arg, into Gln or into Glu; Met into Leu, into Tyr or into Ile; Phe into Met, into Leu or into Tyr; Ser into Thr; Thr into Ser; Trp into Tyr; Tyr into Trp; and/or Phe into Val, into Ile or into Leu.

As used herein, the term "vector" is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked.

As used herein, the term "host cell" refers to a cell into which an expression vector has been introduced.

The term “pharmaceutically acceptable” means that the carrier or excipient is compatible with the other ingredients of the composition and not substantially deleterious to the recipient thereof and/or that such carrier or excipient is approved or approvable for inclusion in a pharmaceutical composition for parenteral administration to humans.

As used herein, the terms "treatment", "treating", “treat”, and the like, refer to administering an agent, or carrying out a procedure, for the purposes of obtaining an effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of effecting a partial or complete cure for a disease and/or symptoms of the disease. "Treatment", as used herein, may include treatment of a disease or disorder (e.g. cancer) in a mammal, particularly in a human, and includes: (a) preventing the disease or a symptom of a disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it (e.g., including diseases that may be associated with or caused by a primary disease); (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease. Treating may refer to any indicia of success in

the treatment or amelioration or prevention of a cancer, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the disease condition more tolerable to the patient; slowing in the rate of degeneration or decline; or making the final point of degeneration less debilitating. The treatment or amelioration of symptoms is based on one or more objective or subjective parameters; including the results of an examination by a physician. Accordingly, the term "treating" includes the administration of the antibodies or compositions or conjugates disclosed herein to prevent or delay, to alleviate, or to arrest or inhibit development of the symptoms or conditions associated with diseases (e.g. cancers). The term "therapeutic effect" refers to the reduction, elimination, or prevention of the disease, symptoms of the disease, or side effects of the disease in the subject.

The term "effective amount" as used herein means the amount that, when administered to a subject for treating a disease, is sufficient to effect treatment for that disease.

The term “subject”, as used herein, refers to any mammalian subject for whom diagnosis, treatment or therapy is desired. "Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and laboratory, zoo, sports, or pet animals, such as dogs, horses, cats, cows, sheep, goats, pigs, mice, rats, rabbits, guinea pigs, monkeys etc.

Bispeicific antibodies

The present disclosure provides a bispecific antibody or an antigen binding fragment thereof, comprising a first antigen binding region binding to EGFR comprising a first light chain variable region (VL1) and a first heavy chain variable region (VH1) and a second antigen binding region binding to LAG3 comprising a second light chain variable region (VL2) and a second heavy chain variable region (VH2), wherein the VL1 comprises LCDRs 1-3 having the amino acid sequences as shown in SEQ ID NOs: 1-3 respectively; the VH1 comprises HCDRs 1-3 having the amino acid sequences as shown in SEQ ID NOs: 5-7 respectively; the VL2 comprises LCDRs 1-3 having the amino acid sequences as shown in SEQ ID NOs: 9-11 respectively; and the VH2 comprises HCDRs 1-3 having the amino acid sequences as shown in SEQ ID NOs: 13-15 respectively.

In some embodiments, CDR sequences are defined according to Kabat numbering system.

When CDR sequences are defined according to Kabat numbering system, the VL1 of the antibody disclosed herein comprises LCDR1, LCDR2 and LCDR3 having the amino acid

sequences as set forth in SEQ ID NO: 1 (QASQDISNYLN), SEQ ID NO: 2 (DASNLET) and SEQ ID NO: 3 (QHFDHLPLA) respectively, the VH1 of the antibody disclosed herein comprises HCDR1, HCDR2 and HCDR3 having the amino acid sequences as set forth in SEQ ID NO: 5 (SGDYYWT), SEQ ID NO: 6 (HIYYSGNTNYNPSLKS) and SEQ ID NO: 7 (DRVTGAFDI) respectively, the VL2 of the antibody disclosed herein comprises LCDR1, LCDR2 and LCDR3 having the amino acid sequences as set forth in SEQ ID NO: 9 (SGSSSNIGSNAVS), SEQ ID NO: 10 (YDDLLPS) and SEQ ID NO: 11 (AAWDDSLNAFV) respectively, and the VH2 of the antibody disclosed herein comprises HCDR1, HCDR2 and HCDR3 having the amino acid sequences as set forth in SEQ ID NO: 13 (SYGIS), SEQ ID NO: 14 (WISAYNGNTNYAQKLQG) and SEQ ID NO: 15 (DSSSWWVDAFDI) respectively.

In some embodiments of the bispecific antibody or the antigen binding fragment thereof disclosed herein, the VL1 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4; the VH1 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 8; the VL2 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 12; and the VH2 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 16.

In some embodiments, the VL1 comprises a functional variant of the amino acid sequence as set forth in SEQ ID NO: 4 formed by insertion, deletion and/or substitution of one or more amino acid(s) therein, provided that the functional variant retains the ability of binding to EGFR. In some embodiments, the VH1 comprises a functional variant of the amino acid sequence as set forth in SEQ ID NO: 8 formed by insertion, deletion and/or substitution of one or more amino acid(s) therein, provided that the functional variant retains the ability of binding to EGFR. In some embodiments, the VL2 comprises a functional variant of the amino acid sequence as set forth in SEQ ID NO: 12 formed by insertion, deletion and/or substitution of one or more amino acid(s) therein, provided that the functional variant retains the ability of binding to LAG3. In some embodiments, the VH2 comprises a functional variant of the amino acid sequence as set forth in SEQ ID NO: 16 formed by insertion, deletion and/or substitution of one or more amino acid(s) therein, provided that the functional variant retains the ability of binding to LAG3.

The functional variant comprises or consists of an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% sequence identity to the amino acid sequence of the parent polypeptide.

In the context of the functional variant, the number of the inserted, deleted and/or substituted amino acid is preferably no more than 40% of the total number of amino acids in the parent amino acid sequence, more preferably no more than 35%, more preferably 1-33%, and more preferably 5- 30%, more preferably 10-25%, more preferably 15-20%. For example, the number of the inserted, deleted and/or substituted amino acid can be 1-20, preferably 1-10, more preferably 1-7, still more preferably 1-5, and most preferably 1-2. In a preferred embodiment, the number of the inserted, deleted and/or substituted amino acid is 1, 2, 3, 4, 5, 6, or 7.

In some embodiments, the insertion, deletion and/or substitution can be performed at framework (FR) regions, e.g., at FR1, FR2, FR3, and/or FR4.

In some embodiments, the substitution of one or more amino acid(s) can be conservative substitution of one or more amino acid(s). Such conservative substitutions preferably are substitutions in which one amino acid within the following groups (a)-(e) is substituted by another amino acid residue within the same group: (a) small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro and Gly; (b) polar, negatively charged residues and their (uncharged) amides: Asp, Asn, Glu and Gln; (c) polar, positively charged residues: His, Arg and Lys; (d) large aliphatic, nonpolar residues: Met, Leu, He, Val and Cys; and (e) aromatic residues: Phe, Tyr and Trp.

Particularly preferred conservative substitutions are as follows: Ala into Gly or into Ser; Arg into Lys; Asn into Gln or into His; Asp into Glu; Cys into Ser; Gln into Asn; Glu into Asp; Gly into Ala or into Pro; His into Asn or into Gln; Ile into Leu or into Val; Leu into Ile or into Val; Lys into Arg, into Gln or into Glu; Met into Leu, into Tyr or into Ile; Phe into Met, into Leu or into Tyr; Ser into Thr; Thr into Ser; Trp into Tyr; Tyr into Trp; and/or Phe into Val, into Ile or into Leu.

In a preferred embodiment, the VL1 comprises an amino acid sequence as set forth in SEQ ID NO: 4; the VH1 comprises an amino acid sequence as set forth in SEQ ID NO: 8; the VL2 comprises an amino acid sequence as set forth in SEQ ID NO: 12; and the VH2 comprises an amino acid sequence as set forth in SEQ ID NO: 16.

The bispecific antibody disclosed herein may comprise an Fc region comprising CH2 and CH3 of an antibody.

The Fc region may be of any isotype, including, but not limited to, IgG1, IgG2, IgG3 and IgG4, and may comprise one or more mutations or modifications. In one embodiment, the Fc region is of IgG1 isotype or derived therefrom, optionally with one or more mutations or modifications. In one embodiment, the Fc region is human IgG1 Fc.

In one embodiment, the Fc region is effector-function-deficient. For example, the Fc region may be of an IgG1 isotype, or a non-IgG1 type, e.g. IgG2, IgG3 or IgG4, which has been mutated such that the ability to mediate effector functions, such as ADCC, has been reduced or even eliminated. Such mutations have e.g. been described in Dall'Acqua WF et al., J Immunol. 177(2):1129-1138 (2006) and Hezareh M, J Virol. ; 75(24):12161-12168 (2001).

In one embodiment, the Fc region comprises a mutation removing the acceptor site for Asn-linked glycosylation or is otherwise manipulated to change the glycosylation properties. For example, in an IgG1 Fc region, an N297Q mutation can be used to remove an Asn-linked glycosylation site. Accordingly, in a specific embodiment, Fc region comprises an IgG1 sequence with an N297Q mutation.

In a further embodiment, the Fc region is glyco-engineered to reduce fucose and thus enhance ADCC, e.g. by addition of compounds to the culture media during antibody production as described in US2009317869 or as described in van Berkel et al. (2010) Biotechnol. Bioeng. 105:350 or by using FUT8 knockout cells, e.g. as described in Yamane-Ohnuki et al. (2004) Biotechnol. Bioeng 87:614. ADCC may alternatively be optimized using the method described by Umaña et al. (1999) Nature Biotech 17:176. In a further embodiment, the Fc region has been engineered to enhance complement activation, e.g. as described in Natsume et al. (2009) Cancer Sci. 100:2411.

In some embodiments, the bispecific antibody comprises: a first polypeptide chain comprising from the N terminal to C terminal: VH2-CH1-CH2-CH3-L1-VH1-L2-VL1; and a second polypeptide chain comprising from the N terminal to C terminal: VL2-CL; wherein CH1 represents the first domain of constant region of an immunoglobulin heavy chain; CH2 represents the second domain of constant region of an immunoglobulin heavy chain; CH3 represents the third domain of constant region of an immunoglobulin heavy chain; CL represents constant region of an immunoglobulin light chain; and L1 and L2 each independently represents an optional linker.

In some embodiments, the bispecific antibody comprises two copies of the first polypeptide chain and two copies of the second polypeptide chain.

In some embodiments, each of the CH1, CH2 and CH3 is independently derived from immunoglobulin isotype IgG (e.g. human IgG), preferably derived from IgG subtype selected from the group consisting of IgG1, IgG2 and IgG4 (e.g. human IgG1, IgG2 and IgG4). In a preferred embodiment, each of the CH1, CH2 and CH3 is independently derived from IgG1.

In some embodiments, the CH1-CH2-CH3 comprises the amino acid sequence as shown in any one of SEQ ID NOs: 21-23. In a preferred embodiment, the CH1-CH2-CH3 comprises the amino acid sequence as shown in SEQ ID NO: 21.

In some embodiments, the CL is derived from λ light chain or κ light chain. In a preferred embodiment, the CL is derived from κ light chain.

In some embodiments, the CL comprises the amino acid sequence as shown in any one of SEQ ID NOs: 24-25. In a preferred embodiment, the CL comprises the amino acid sequence as shown in SEQ ID NO: 24.

In some embodiments, the linker comprises an amino acid sequence of (G4S)n, wherein n is an integer selected from 1-5. In some embodiments, the linker may comprise an amino acid sequence of GGGGS (SEQ ID NO: 26). In some embodiments, the linker may comprise an amino acid sequence of GGGGSGGGGS (SEQ ID NO: 27). In some embodiments, the linker may comprise an amino acid sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 28). In some embodiments, the linker may comprise an amino acid sequence of GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 19). In some embodiments, the linker may comprise an amino acid sequence of GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 29). In a preferred embodiment, the linker comprises an amino acid sequence as shown in SEQ ID NO: 19.

In some embodiments, the first polypeptide chain comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 17; and the second polypeptide chain comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 18.

In some embodiments, the first polypeptide chain comprises a functional variant of the amino acid sequence as set forth in SEQ ID NO: 17 formed by insertion, deletion and/or substitution of

one or more amino acid(s) therein, provided that the functional variant retains the ability of binding to EGFR and/or LAG3. In some embodiments, the second polypeptide chain comprises a functional variant of the amino acid sequence as set forth in SEQ ID NO: 18 formed by insertion, deletion and/or substitution of one or more amino acid(s) therein, provided that the functional variant retains the ability of binding to LAG3.

The functional variant comprises or consists of an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% sequence identity to the amino acid sequence of the parent polypeptide.

In some embodiments, the number of the inserted, deleted and/or substituted amino acid is preferably no more than 40% of the total number of amino acids in the parent amino acid sequence, more preferably no more than 35%, more preferably 1-33%, and more preferably 5- 30%, more preferably 10-25%, more preferably 15-20%. For example, the number of the inserted, deleted and/or substituted amino acid can be 1-50, preferably 1-20, more preferably 1-10, still more preferably 1-5. In a preferred embodiment, the number of the inserted, deleted and/or substituted amino acid is 1, 2, 3, 4, 5, 6, or 7.

In some embodiments, the insertion, deletion and/or substitution can be performed at framework (FR) regions, e.g., at FR1, FR2, FR3 and/or FR4; and/or constant regions, e.g., CL, CH1, CH2 and/or CH3.

In some embodiments, the substitution of one or more amino acid(s) can be conservative substitution of one or more amino acid(s). Examples of conservative substitutions are as described above.

In a preferred embodiment, the first polypeptide chain comprises an amino acid sequence as shown in SEQ ID NO: 17; and the second polypeptide chain comprises an amino acid sequence as shown in SEQ ID NO: 18.

Nucleic acids

The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding the bispecific antibody or the antigen binding fragment thereof disclosed herein.

The term "nucleic acid" includes both single-stranded and double-stranded nucleotide polymers. The nucleic acid can be ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. Said modifications include base modifications such as bromouridine and inosine derivatives, ribose modifications such as 2',3'-dideoxyribose, and internucleotide linkage modifications such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phoshoraniladate and phosphoroamidate.

For example, the invention provides nucleic acid molecules encoding any one of the heavy chain variable region sequences disclosed herein. The invention also provides nucleic acid molecules that are at least 90%, at least 95%, at least 98% or at least 99% identical to nucleic acids encoding any one of the heavy chain variable region sequences disclosed herein.

For example, the invention provides nucleic acid molecules encoding any one of the light chain variable region sequences disclosed herein. The invention also provides nucleic acid molecules that are at least 90%, at least 95%, at least 98% or at least 99% identical to nucleic acids encoding any one of the light chain variable region sequences disclosed herein.

For example, the invention provides nucleic acid molecules encoding: (i) any one of the heavy chain variable region sequences disclosed herein and (ii) any one of the light chain variable region sequences disclosed herein. The invention also provides nucleic acid molecules that are at least 90%, at least 95%, at least 98% or at least 99% identical to nucleic acids encoding: (i) any one of the heavy chain variable region sequences disclosed herein and (ii) any one of the light chain variable region sequences disclosed herein.

For example, the invention provides nucleic acid molecules encoding a heavy chain variable region sequence that comprises the CDR sequences of any one of the heavy chain variable region sequences disclosed herein. The invention also provides nucleic acid molecules that encode a heavy chain variable region sequence that comprises CDR sequences that are at least 90%, at least 95%, at least 98% or at least 99% identical to the CDR sequences of any one of the heavy chain variable region sequences disclosed herein.

For example, the invention provides nucleic acid molecules encoding a light chain variable region sequence that comprises the CDR sequences of any one of the light chain variable region sequences disclosed herein. The invention also provides nucleic acid molecules that encode a light chain variable region sequence that comprises CDR sequences that are at least 90%, at least 95%,

at least 98% or at least 99% identical to the CDR sequences of any one of the light chain variable region sequences disclosed herein.

For example, the invention provides nucleic acid molecules encoding: (i) a heavy chain variable region sequence that comprises the CDR sequences of any one of the heavy chain variable region sequences disclosed herein and (ii) a light chain variable region sequence that comprises the CDR sequences of any one of the light chain variable region sequences disclosed herein. The invention also provides nucleic acid molecules that encode: (i) a heavy chain variable region sequence that comprises CDR sequences that are at least 90%, at least 95%, at least 98% or at least 99% identical to the CDR sequences of any one of the heavy chain variable region sequences disclosed herein and (ii) a light chain variable region sequence that comprises CDR sequences that are at least 90%, at least 95%, at least 98% or at least 99% identical to the CDR sequences of any one of the light chain variable region sequences disclosed herein.

In some embodiments, the nucleic acid is ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). In some embodiments, the invention provides a ribonucleic acid (RNA) comprising a nucleotide sequence encoding the bispecific antibody disclosed herein. In some embodiments, the invention provides a deoxyribonucleic acid (DNA) comprising a deoxynucleotide sequence encoding the bispecific antibody disclosed herein.

In some embodiments, the deoxyribonucleic acid (DNA) may be introduced into the cells of a human body in vivo. In some embodiments, the deoxyribonucleic acid (DNA) of the invention is comprised in a vector or a delivering agent. In some embodiments, the deoxyribonucleic acid (DNA) of the invention is integrated into the genome of a cell.

In some embodiments, the ribonucleic acid (RNA) may be introduced into the cells of a human body in vivo. In some embodiments, the ribonucleic acid (RNA) of the invention is comprised in a vector or a delivering agent.

Vectors

The present disclosure provides a vector comprising the nucleic acid disclosed herein.

In some embodiments, the vector is an expression vector capable of expressing a polypeptide comprising a heavy or light chain variable region of the bispecific antibody. For example, the invention provides expression vectors comprising any of the nucleic acid molecules mentioned above.

Any vector may be suitable for the present disclosure. In some embodiments, the vector is a viral vector. In some embodiments, the vector is a retroviral vector, a DNA vector, a murine leukemia virus vector, an SFG vector, a plasmid, a RNA vector, an adenoviral vector, a baculoviral vector, an Epstein Barr viral vector, a papovaviral vector, a vaccinia viral vector, a herpes simplex viral vector, an adenovirus associated vector (AAV), a lentiviral vector, or any combination thereof. Suitable exemplary vectors include e.g., pGAR, pBABE-puro, pBABE-neo largeTcDNA, pBABE-hygro-hTERT, pMKO.1 GFP, MSCV-IRES-GFP, pMSCV PIG (Puro IRES GFP empty plasmid), pMSCV-loxp-dsRed-loxp-eGFP-Puro-WPRE, MSCV IRES Luciferase, pMIG, MDH1-PGK-GFP_2.0, TtRMPVIR, pMSCV-IRES-mCherry FP, pRetroX GFP T2A Cre, pRXTN, pLncEXP, and pLXIN-Luc.

An expression vector may be any suitable recombinant expression vector. Suitable vectors comprise those designed for propagation and expansion or for expression or both, such as plasmids and viruses. For example, a vector may be selected from the pUC series (Fermentas Life Sciences, Glen Burnie, Md.), the pBluescript series (Stratagene, LaJolla, Calif.), the pET series (Novagen, Madison, Wis.), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, Calif.). Bacteriophage vectors, such as λGT10, λGT11, λZapII (Stratagene), λEMBL4, and λNM1149, also may be used. Examples of plant expression vectors useful in the context of the disclosure comprise pBI01, pBI101.2, pBI101.3, pBI121 and pBIN19 (Clontech). Examples of animal expression vectors useful in the context of the disclosure comprise pcDNA, pEUK-Cl, pMAM, and pMAMneo (Clontech).

Recombinant expression vectors may be prepared using standard recombinant DNA techniques described in, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 2001; and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, NY, 1994. Constructs of expression vectors, which are circular or linear, may be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems may be derived, e.g., from ColEl, 2μ plasmid, λ, SV40, bovine papilloma virus, and the like.

For example, the vector may be an adenoviral vector comprising a nucleotide sequence encoding the bispecific antibody disclosed herein. The vector may be administered into the body of a subject, and then enter into a cell of the subject in vivo, thereby the nucleotide sequence

encoding the bispecific antibody disclosed herein is integrated into the genome of the cell, and subsequently the cell expresses the bispecific antibody disclosed herein.

Host Cells

The present disclosure provides a host cell comprising the nucleic acid disclosed herein or the vector disclosed herein.

Any cell may be used as a host cell for the nucleic acids or the vectors of the present disclosure. In some embodiments, the cell can be a prokaryotic cell, fungal cell, yeast cell, or higher eukaryotic cells such as a mammalian cell. Suitable prokaryotic cells include, without limitation, eubacteria, such as Gram-negative or Gram-positive organisms, for example, *Enterobactehaceae* such as *Escherichia*, *e.g.*, *E. coli*; *Enterobacter*; *Erwinia*; *Klebsiella*; *Proteus*; *Salmonella*, e.g., *Salmonella typhimurium*; *Serratia*, e.g., *Serratia marcescans*, and *Shigella*; *Bacilli* such as *B. subtilis* and *B. licheniformis*; *Pseudomonas* such as *P. aeruginosa*; and *Streptomyces*. In some embodiments, the cell is a human cell. In some embodiments, the cell is an immune cell. In some embodiments, host cells include, for example, CHO cells, such as CHOS cells and CHO-K1 cells, or HEK293 cells, such as HEK293A, HEK293T and HEK293FS.

The host cell of the invention is prepared by introducing the vector disclosed herein or the nucleic acid disclosed herein in vitro or ex vivo. The host cell of the invention may be administered into the body of a subject, and the host cell expresses the bispecific antibody disclosed herein in vivo.

The invention provides host cells into which any of the vectors mentioned above have been introduced. The invention further provides a method of preparing the bispecific antibody of the invention, wherein the method comprises a) culturing the host cell of the fourth aspect of the invention under a condition suitable for the production of the bispecific antibody; and b) obtaining the bispecific antibody from the culture.

Pharmaceutical compositions

The present disclosure provides a pharmaceutical composition comprising the bispecific antibody or the antigen binding fragment thereof disclosed herein, and a pharmaceutically acceptable carrier or excipient.

The bispecific antibody or antigen binding fragment thereof or agents of the invention (also referred to herein as "active compounds"), and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the bispecific antibody or antigen binding fragment thereof or agent and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Preferred examples of such carriers or excipients include, but are not limited to, water, saline, ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

In some embodiments, the pharmaceutical composition further comprises a second therapeutic agent. In some embodiments, the second therapeutic agent is selected from an antibody, a chemotherapeutic agent and a small molecule drug. In some embodiments, the second therapeutic agent is selected from a Bruton's tyrosine kinase (BTK) inhibitor, a PI3K inhibitor, a HDAC inhibitor, an ERK inhibitor, a MAPK inhibitor, a PD-1/PD-L1 inhibitor, a CTLA-4 inhibitor, a TIGIT inhibitor, a TIM3 inhibitor, a VEGF inhibitor, and glucocorticoid.

In some embodiments, the therapeutic agent is a chemotherapeutic agent. The chemotherapeutic agents can include, for example, cytotoxic agents, anti-metabolite agents (e.g., folate antagonists, purine analogs, pyrimidine analogs, etc.), topoisomerase inhibitors (e.g., camptothecin derivatives, anthracenedione, anthracyclines, epipodophyllotoxins, quinoline alkaloids, etc.), anti-microtubule agents (e.g., taxanes, vinca alkaloids), protein synthesis inhibitors (e.g., cephalotaxine, camptothecin derivatives, quinoline alkaloids), alkylating agents (e.g., alkyl sulfonates, ethylenimines, nitrogen mustards, nitrosoureas, platinum derivatives, triazenes, etc.), alkaloids, terpenoids, and kinase inhibitors.

The pharmaceutical composition of the invention can be formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (i.e., topical),

transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium

and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The active compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compounds against rapid elimination from the body, such as a controlled release formulation,

including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art.

The invention provides therapeutic compositions comprising the bispecific antibody or antigen binding fragment thereof of the present invention. Therapeutic compositions in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

Conjugates

The present disclosure provides a conjugate comprising the bispecific antibody or the antigen binding fragment thereof disclosed herein, and a chemical moiety conjugated thereto.

In the context of the present disclosure, a "conjugate" is an antibody or antibody fragment (such as an antigen-binding fragment) covalently linked to a chemical moiety. The chemical moiety can be, for example, a drug, toxin, therapeutic agent, detectable label, protein, nucleic acid, lipid, nanoparticle, carbohydrate or recombinant virus. An antibody conjugate is often referred to as an "immunoconjugate". When the conjugate comprises an antibody linked to a drug (e.g., a cytotoxic agent), the conjugate is often referred to as an "antibody-drug conjugate" or "ADC."

The term "conjugated" or "linked" may refer to making two polypeptides into one contiguous polypeptide molecule. In one embodiment, an antibody is joined to a chemical moiety. In another embodiment, an antibody joined to a chemical moiety is further joined to a lipid or other molecule to a protein or peptide to increase its half- life in the body. The linkage can be either by chemical or recombinant means. In one embodiment, the linkage is chemical, wherein a reaction between

the antibody moiety and the chemical moiety has produced a covalent bond formed between the two molecules to form one molecule. A peptide linker (short peptide sequence) can optionally be included between the antibody and the chemical moiety.

A chemical moiety can be linked to the antibody of the invention using any number of means known to those of skill in the art. Both covalent and noncovalent attachment means may be used. The procedure for attaching a chemical moiety to the antibody varies according to the chemical structure of the chemical moiety. Polypeptides typically contain a variety of functional groups; such as carboxylic acid (COOH), free amine (-NH2) or sulfhydryl (-SH) groups, which are available for reaction with a suitable functional group on an antibody to result in the binding of the chemical moiety. Alternatively, the antibody is derivatized to expose or attach additional reactive functional groups. The derivatization may involve attachment of any of a number of known linker molecules. The linker can be any molecule used to join the antibody to the chemical moiety. The linker is capable of forming covalent bonds to both the antibody and to the chemical moiety. Suitable linkers are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. Where the antibody and the chemical moiety are polypeptides, the linkers may be joined to the constituent amino acids through their side groups (such as through a disulfide linkage to cysteine) or to the alpha carbon amino and carboxyl groups of the terminal amino acids.

In some circumstances, it is desirable to free the chemical moiety from the antibody when the immunoconjugate has reached its target site. Therefore, in these circumstances, immunoconjugates will comprise linkages that are cleavable in the vicinity of the target site.

Cleavage of the linker to release the chemical moiety from the antibody may be prompted by enzymatic activity or conditions to which the immunoconjugate is subjected either inside the target cell or in the vicinity of the target site.

In view of the large number of methods that have been reported for attaching a variety of radiodiagnostic compounds, radiotherapeutic compounds, labels (such as enzymes or fluorescent molecules), drugs, toxins, and other agents to antibodies one skilled in the art will be able to determine a suitable method for attaching a given agent to an antibody or other polypeptide.

The antibodies disclosed herein can be derivatized or linked to another molecule (such as another peptide or protein). In general, the antibodies or portion thereof is derivatized such that the binding to the target antigen is not affected adversely by the derivatization or labeling. For

example, the antibody can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (for example, a bi-specific antibody or a diabody), a detection agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a strep tavidin core region or a polyhistidine tag).

One type of derivatized antibody is produced by cross-linking two or more antibodies (of the same type or of different types). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (such as m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (such as disuccinimidyl suberate). Such linkers are commercially available.

In some embodiments of the conjugate disclosed herein, the chemical moiety is selected from the group consisting of a therapeutic agent, a detectable moiety, and an immune stimulatory molecule.

In some embodiments, the therapeutic agent includes but is not limited to immunomodulators, radioactive compounds, enzymes (for example perforin), chemotherapeutic agents (for example cis-platin), or a toxin. In some embodiments, the therapeutic agent can be such as maytansine, geldanamycin, tubulin inhibitors such as tubulin binding agents (e.g., auristatins), or minor groove binding agents such as calicheamicin.

Other suitable therapeutic agents include such as, small molecule cytotoxic agents, i.e. compounds with the ability to kill mammalian cells having a molecular weight of less than 700 Daltons. Such compounds could also contain toxic metals capable of having a cytotoxic effect. Furthermore, it is to be understood that these small molecule cytotoxic agents also include pro-drugs, i.e. compounds that decay or are converted under physiological conditions to release cytotoxic agents. Examples of such agents include cis-platin, maytansine derivatives, rachelmycin, calicheamicin, docetaxel, etoposide, gemcitabine, ifosfamide, irinotecan, melphalan, mitoxantrone, sorfimer sodiumphotofrin II, temozolomide, topotecan, trimetreate glucuronate, auristatin E vincristine and doxorubicin; peptide cytotoxins, i.e. proteins or fragments thereof with the ability to kill mammalian cells, for example, ricin, diphtheria toxin, pseudomonas bacterial exotoxin A, DNase and RNase; radio-nuclides, i.e. unstable isotopes of elements which decay with the concurrent emission of one or more of a or β particles, or γ rays, for example, iodine-131 , rhenium-186, indium-111, yttrium-90, bismuth-210, bismuth-213, actinium-225 and astatine-213; chelating

agents may be used to facilitate the association of these radionuclides to the molecules, or multimers thereof.

In some embodiments, the detectable moiety can be selected from the group consisting of biotin, streptavidin, an enzyme or catalytically active fragment thereof, a radionuclide, a nanoparticle, a paramagnetic metal ion, or a fluorescent, phosphorescent, or chemiluminescent molecule. A detectable moiety for diagnostic purposes include for instance, fluorescent labels, radiolabels, enzymes, nucleic acid probes and contrast reagents.

The bispecific antibody can be conjugated with a detectable marker; for example, a detectable marker capable of detection by ELISA, spectrophotometry, flow cytometry, microscopy or diagnostic imaging techniques (such as computed tomography (CT), computed axial tomography (CAT) scans, magnetic resonance imaging (MRI), nuclear magnetic resonance imaging NMRI), magnetic resonance tomography (MTR), ultrasound, fiberoptic examination, and laparoscopic examination). Specific, non-limiting examples of detectable markers include fluorophores, chemiluminescent agents, enzymatic linkages, radioactive isotopes and heavy metals or compounds (for example super paramagnetic iron oxide nanocrystals for detection by MRI). For example, useful detectable markers include fluorescent compounds, including fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-l-napthalenesulfonyl chloride, phycoerythrin, lanthanide phosphors and the like. Bioluminescent markers are also of use, such as luciferase, green fluorescent protein (GFP) and yellow fluorescent protein (YFP).

The bispecific antibody or antigen binding fragment can also be conjugated with enzymes that are useful for detection, such as horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase, glucose oxidase and the like. When a bispecific antibody or antigen binding fragment is conjugated with a detectable enzyme, it can be detected by adding additional reagents that the enzyme uses to produce a reaction product that can be discerned. For example, when the agent horseradish peroxidase is present the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is visually detectable. The bispecific antibody or antigen binding fragment may also be conjugated with biotin, and detected through indirect measurement of avidin or streptavidin binding. It should be noted that the avidin itself can be conjugated with an enzyme or a fluorescent label.

The bispecific antibody may be fused to a self-labelling protein tag (e.g. HaloTag). For example, the protein tag could be cloned at the end of a constant region. HaloTag is a self-labelling

protein tag derived from a bacterial enzyme (a haloalkane dehalogenase), designed to covalently bind to a synthetic ligand. In some instances, the synthetic ligand comprises a chloroalkane linker attached to a fluorophore, such as a near-infrared fluorophore (Los et al. (2008) ACS Chem Biol. 3(6):373-82).

The bispecific antibody may be labeled with a magnetic agent, such as gadolinium. Antibodies can also be labeled with lanthanides (such as europium and dysprosium), and manganese.

Paramagnetic particles such as superparamagnetic iron oxide are also of use as labels. The bispecific antibody may also be labeled with a predetermined polypeptide epitopes recognized by a secondary reporter (such as leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

The bispecific antibody can also be labeled with a radiolabeled amino acid. The radiolabel may be used for both diagnostic and therapeutic purposes. For instance, the radiolabel may be used to detect expression of a target antigen by x-ray, emission spectra, or other diagnostic techniques. Examples of labels for polypeptides include, but are not limited to, the following radioisotopes or radionucleotides: 3H, 14C, 15N, 35S, 90Y, 99Tc, 111In, 125I, 131I.

In some embodiments, the immune stimulatory molecule is an immune effector molecules which stimulate immune response. For example, the immune stimulatory molecule can be cytokines such as IL-2 and IFN-γ, chemokines such as IL-8, platelet factor 4, melanoma growth stimulatory protein, complement activators; viral/bacterial protein domains, or viral/bacterial peptides.

Therapeutic methods

The present disclosure provides a method of treating a cancer in a subject, comprising administering to the subject an effective amount of the bispecific antibody or the antigen binding fragment thereof disclosed herein, the pharmaceutical composition disclosed herein, or the conjugate disclosed herein.

In some embodiments of the method disclosed herein, the cancer is a solid tumor or a hematologic malignancy. In some embodiments, the cancer is an EGFR positive cancer.

Examples of cancers include: leukaemias such as but not limited to, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukaemias such as myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukaemia leukaemias and myelodysplastic syndrome, chronic leukaemias such as but not limited to, chronic myelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, hairy cell leukemia; polycythemia vera; lymphomas such as but not limited to Hodgkin's disease, non-Hodgkin's disease; multiple myelomas such as but not limited to smoldering multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma and extramedullary plasmacytoma; Waldenstrom's macroglobulinemia; monoclonal gammopathy of undetermined significance; benign monoclonal gammopathy; heavy chain disease; bone and connective tissue sarcomas such as but not limited to bone sarcoma, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, metastatic cancers, neurilemmoma, rhabdomyosarcoma, synovial sarcoma; brain tumors such as but not limited to, glioma, glioblastoma, astrocytoma, brain stem glioma, ependymoma, oligodendroglioma, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma, pineocytoma, pineoblastoma, primary brain lymphoma; breast cancer, including, but not limited to, adenocarcinoma, lobular (small cell) carcinoma, intraductal carcinoma, medullary breast cancer, mucinous breast cancer, tubular breast cancer, papillary breast cancer, primary cancers, Paget's disease, and inflammatory breast cancer; adrenal cancer such as but not limited to pheochromocytoma, and adrenocortical carcinoma; thyroid cancer such as but not limited to papillary or follicular thyroid cancer, Medullary thyroid carcinoma, medullary thyroid cancer and anaplastic thyroid cancer; GIST –gastrointestinal stromal tumor; pancreatic cancer such as but not limited to, insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, and carcinoid or islet cell tumor; pituitary cancers such as but limited to Cushing's disease, prolactin-secreting tumor, acromegaly, and diabetes insipius; eye cancers such as but not limited to ocular melanoma such as iris melanoma, choroidal melanoma, and cilliary body melanoma, and retinoblastoma; vaginal cancers such as squamous cell carcinoma, adenocarcinoma, and melanoma; vulvar cancer such as squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, sarcoma, and Paget's disease; cervical cancers such as but not limited to, squamous cell carcinoma, and adenocarcinoma; uterine cancers such as but not limited to endometrial

carcinoma and uterine sarcoma; ovarian cancers such as but not limited to, ovarian epithelial carcinoma, borderline tumor, germ cell tumor, and stromal tumor; esophageal cancers such as but not limited to, squamous cancer, adenocarcinoma, adenoid cyctic carcinoma, mucoepidermoid carcinoma, adenosquamous carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and oat cell (small cell) carcinoma; stomach cancers such as but not limited to, adenocarcinoma, fungating (polypoid), ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma, fibrosarcoma, and carcinosarcoma; colon cancers; rectal cancers; liver cancers such as but not limited to hepatocellular carcinoma and hepatoblastoma, gallbladder cancers such as adenocarcinoma; cholangiocarcinomas such as but not limited to pappillary, nodular, and diffuse; lung cancers such as non-small cell lung cancer (NSCLC), squamous cell carcinoma (epidermoid carcinoma), adenocarcinoma, large-cell carcinoma and small-cell lung cancer (SCLC); testicular cancers such as but not limited to germinal tumor, seminoma, anaplastic, classic (typical), spermatocytic, nonseminoma, embryonal carcinoma, teratoma carcinoma, choriocarcinoma (yolk-sac tumor), prostate cancers such as but not limited to, adenocarcinoma, leiomyosarcoma, and rhabdomyosarcoma; genital cancers such as penile cancer; oral cancers such as but not limited to squamous cell carcinoma; basal cancers; salivary gland cancers such as but not limited to adenocarcinoma, mucoepidermoid carcinoma, and adenoidcystic carcinoma; pharynx cancers such as but not limited to squamous cell cancer, and verrucous; skin cancers such as but not limited to, basal cell carcinoma, squamous cell carcinoma and melanoma, superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, acral lentiginous melanoma; kidney cancers such as but not limited to renal cell cancer, Clear cell renal cell carcinoma, adenocarcinoma, hypernephroma, fibrosarcoma, transitional cell cancer (renal pelvis and/or ureter); Wilms' tumor; bladder cancers such as but not limited to transitional cell carcinoma, squamous cell cancer, adenocarcinoma, carcinosarcoma. In addition, cancers include myxosarcoma, osteogenic sarcoma, endotheliosarcoma, lymphangioendotheliosarcoma, mesothelioma, synovioma, hemangioblastoma, epithelial carcinoma, cystadenocarcinoma, bronchogenic carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma and papillary adenocarcinomas. Preferably, the cancer is selected from breast, melanoma, prostate, ovarian, colorectal, lung or glioma cancer.

In some embodiments, the cancer is selected from the group consisting of colon cancer, kidney cancer, colorectal cancer, lung cancer, gastric cancer, ovarian cancer, breast cancer,

pancreatic cancer, prostate cancer, skin cancer, head and neck cancer, brain cancer, bladder cancer and liver cancer. In preferred embodiments, the cancer is selected from the group consisting of colorectal cancer, head and neck cancer, kidney cancer, and skin cancer.

In some embodiments, dosage administered to a subject may vary with the embodiment, the medicament employed, the method of administration, and the site and subject being treated. However, a dose should be sufficient to provide a therapeutic response. A clinician may determine the effective amount to be administered to a human or other subject in order to treat a medical condition. The precise amount required to be therapeutically effective may depend upon numerous factors, e.g., such as the activity of the antibody, and the route of administration.

A dose of the antibodies, compositions or conjugates described herein may be administered to a mammal at one time or in a series of sub-doses administered over a suitable period of time, e.g., on a daily, semi-weekly, weekly, bi-weekly, semi-monthly, bi-monthly, semi-annual, or annual basis, as needed. A dosage unit comprising an effective amount of antibodies, compositions or conjugates may be administered in a single daily dose, or the total daily dosage may be administered in two, three, four, or more divided doses administered daily, as needed.

A suitable means of administration may be selected by a medical practitioner. Route of administration may be parenteral, for example, administration by injection, transnasal administration, transpulmonary administration, or transcutaneous administration. Administration may be systemic or local by intravenous injection, intramuscular injection, intraperitoneal injection, subcutaneous injection. In some embodiments, the antibodies, compositions or conjugates are selected for parenteral delivery, for inhalation, or for delivery through the digestive tract, such as orally. Dose and method of administration may vary depending on the weight, age, condition, and the like of the subject, and may be suitably selected.

In some embodiments, the method further comprises administering to the subject a second therapeutic agent. In certain embodiments, the antibody, composition or conjugate disclosed herein is administered prior to, substantially simultaneously with, or after the administration of the second therapeutic agent.

In some embodiments, the second therapeutic agent is selected from an antibody, a chemotherapeutic agent and a small molecule drug. In some embodiments, the second therapeutic agent is selected from a Bruton's tyrosine kinase (BTK) inhibitor, a PI3K inhibitor, a HDAC

inhibitor, an ERK inhibitor, a MAPK inhibitor, a PD-1/PD-L1 inhibitor, a CTLA-4 inhibitor, a TIGIT inhibitor, a TIM3 inhibitor, a VEGF inhibitor, and glucocorticoid.

In some embodiments, the second therapeutic agent is a chemotherapeutic agent. The chemotherapeutic agents can include, for example, cytotoxic agents, anti-metabolite agents (e.g., folate antagonists, purine analogs, pyrimidine analogs, etc.), topoisomerase inhibitors (e.g., camptothecin derivatives, anthracenedione, anthracyclines, epipodophyllotoxins, quinoline alkaloids, etc.), anti-microtubule agents (e.g., taxanes, vinca alkaloids), protein synthesis inhibitors (e.g., cephalotaxine, camptothecin derivatives, quinoline alkaloids), alkylating agents (e.g., alkyl sulfonates, ethylenimines, nitrogen mustards, nitrosoureas, platinum derivatives, triazenes, etc.), alkaloids, terpenoids, and kinase inhibitors.

Medical uses

The present disclosure provides use of the bispecific antibody or the antigen binding fragment thereof disclosed herein, the pharmaceutical composition disclosed herein, or the conjugate disclosed herein in the manufacture of a medicament for treating a cancer in a subject.

The present disclosure also provides the bispecific antibody or the antigen binding fragment thereof disclosed herein, the pharmaceutical composition disclosed herein, or the conjugate disclosed herein for use in treating a cancer in a subject.

In some embodiments of the use disclosed herein, the cancer is a solid tumor or a hematologic malignancy. In some embodiments, the cancer is an EGFR positive cancer. In some embodiments, the cancer is selected from the group consisting of colon cancer, kidney cancer, colorectal cancer, lung cancer, gastric cancer, ovarian cancer, breast cancer, pancreatic cancer, prostate cancer, skin cancer, head and neck cancer, brain cancer, bladder cancer and liver cancer. In preferred embodiments, the cancer is selected from the group consisting of colorectal cancer, head and neck cancer, kidney cancer, and skin cancer.

In some embodiments, the bispecific antibody or the antigen binding fragment thereof disclosed herein, the pharmaceutical composition disclosed herein, or the conjugate disclosed herein is in combination with a second therapeutic agent. In some embodiments, the second therapeutic agent is selected from an antibody, a chemotherapeutic agent and a small molecule drug. In some embodiments, the second therapeutic agent is selected from a Bruton's tyrosine kinase (BTK) inhibitor, a PI3K inhibitor, a HDAC inhibitor, an ERK inhibitor, a MAPK inhibitor,

a PD-1/PD-L1 inhibitor, a CTLA-4 inhibitor, a TIGIT inhibitor, a TIM3 inhibitor, a VEGF inhibitor, and glucocorticoid.

Kits

The present disclosure provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions described herein, such as the bispecific antibodies or the antigen binding fragment disclosed herein. Optionally, associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

In a specific embodiment, the kit comprises a first container containing the bispecific antibodies disclosed herein. In a specific embodiment, the kit comprises a first container that is a vial containing the bispecific antibodies as a lyophilized sterile powder under vacuum, and the kit further comprises a second container comprising a pharmaceutically acceptable fluid.

In a specific embodiment, provided herein is an injection device containing the bispecific antibodies. In a specific embodiment, the injection device comprises the bispecific antibody in sterile solution. In a specific embodiment, the injection device is a syringe.

EXAMPLES

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. The present examples, along with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

MC38-hEGFR cancer cells were purchased from Kyinno Biotechnology CO., LTD. Activated T cells were obtained by activating frozen PBMC with the human CD3/CD28 T Cell Activator. Other tumor cell lines including human epidermal cancer cell line A431, human

lymphoma cell line Raji, human colon cancer cell line Caco-2, and kidney cancer cell line Achn were purchased from ATCC.

Human EGF protein and human FGL1 protein were purchased from ACROBiosystems. Human EGFR/HER1/ErbB1 protein and human LAG3 protein were purchased from Sino Biological. Anti-human IgG (γ-chain specific)-R-PE antibody, anti-human IgG (Fc-specific)-peroxidase antibody and monoclonal anti-Flag®M2-peroxidase were purchased from Sigma. PE anti-His Tag antibody was purchased from BioLegend. Anti-EGFR mAb and anti-LAG3 mAb were generated in house.

The whole light chain and heavy chain sequences of Anti-EGFR mAb and anti-LAG3 mAb are shown below.

Anti-EGFR mAb

Heavy chain:

QVQLQESGPGLVKPSETLSLTCTVSGGSVSSGDYYWTWIRQSPGKGLEWIGHIYYS
GNTNYNPSLKSRLTISIDTSKTQFSLKLSSVTAADTAIYYCVRDRVTGAFDIWGQGTMVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 34)

Light chain:

DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYDASNLETG
VPSRFSGSGSGTDFTFTISSLQPEDIATYFCQHFDHLPLAFGGGTKVEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 35)

Anti- LAG3 mAb

Heavy chain:

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAY
NGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSWWVDAFDIW
GQGTMVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKKVESKYGPPCPPC

PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ
VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 36)

Light chain:

SYELTQPPSVSEAPRQRVTISCSGSSSNIGSNAVSWYQQVPRKAPKLLVYYDDLLPS
GVSDRFSGSKSGTSASLAIRGLQSEDEADYYCAAWDDSLNAFVFGTGTKVTVLGQPKA
NPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNN
KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO: 37)

Example 1. Construction and initial characterization of EGFR×LAG3 bispecific antibody

Cloning of EGFR×LAG3 bispecific antibody (BsAb)

The light chain of EGFR×LAG3 BsAb is composed of anti-LAG-3 VL and CL domains. The heavy chain, from N-terminus to C-terminus, comprises anti-LAG-3 VH, CH1 domains, a wild type human IgG1 Fc and an anti-EGFR single chain variable fragment (ScFv). The structure of EGFR×LAG3 BsAb (also termed as CMD005 herein) is as shown in Figure 1. The anti-EGFR ScFv and the light chain of EGFR×LAG3 BsAb were directly synthesized by GENEWIZ. To generate the construct of EGFR×LAG3 BsAb, the following primers which were synthesized by GENEWIZ were used:

vector-HC-FP: 5' TAATTCTAGAGTCGACAATCAACCTCTGG 3' (sense) (SEQ ID NO: 30);
vector-HC-RP: 5' TGATCCACCACCTCCACTACCG 3' (antisense) (SEQ ID NO: 31);
EGFR-HB-pa-VH-FP: 5' GTGGTGGATCACAGGTGCAGCTGCAGGAGAG 3' (sense) (SEQ ID NO: 32);
EGFR-HB-pa-VL-RP: 5' GATTGTCGACTCTAGAATTATTTGATTTCCACCTTGGTTCCG3' (antisense) (SEQ ID NO: 33).

To generate EGFR×LAG3 BsAb, the anti-EGFR ScFv was fused to the C-terminus of the LAG-3 monoclonal antibody via a (G4S)4 linker. The light chain and heavy chain were constructed into a single vector pcDNA3.4 respectively for mammalian cell expression.

Protein expression, purification and initial characterization

EGFR×LAG3 BsAb was expressed in CHO-S cells by using the ExpiFectamine CHO Transfection Kit. The cells were continued to grow for 5-7 days after transfection. The cell culture was harvested by centrifugation at 8000 rpm for 20 min. The culture supernatant containing target proteins was loaded onto Gravity Column with EshmunoA (Merck). The subsequent purification was carried out according to the manufacturer's instructions.

EGFR×LAG3 BsAb was well expressed in transiently transfected CHO-S cells and secreted into the culture supernatant. On a non-reducing SDS-PAGE, CMD005 displays an apparent molecular weight (aMW) of approximately 76.3 kDa. On a reduced SDS-PAGE, the heavy chain and light chain are close to each other with an apparent molecular weight of approximately 23.1 kDa (data not shown).

The CDR sequences of CMD005 according to the Kabat numbering system are shown in Table 1. The amino acid sequences of light chain variable region (VL) and heavy chain variable region (VH) of CMD005 are shown in Table 2. The whole light chain and heavy chain sequences of CMD005 are shown in Table 3.

Table 1. CDR sequences of CMD005

LCDR1 against EGFR	QASQDISNYLN (SEQ ID NO: 1)
LCDR2 against EGFR	DASNLET (SEQ ID NO: 2)
LCDR3 against EGFR	QHFDHLPLA (SEQ ID NO: 3)
HCDR1 against EGFR	SGDYYWT (SEQ ID NO: 5)
HCDR2 against EGFR	HIYYSGNTNYNPSLKS (SEQ ID NO: 6)
HCDR3 against EGFR	DRVTGAFDI (SEQ ID NO: 7)
LCDR1 against LAG3	SGSSSNIGSNAVS (SEQ ID NO: 9)
LCDR2 against LAG3	YDDLLPS (SEQ ID NO: 10)
LCDR3 against LAG3	AAWDDSLNAFV (SEQ ID NO: 11)
HCDR1 against LAG3	SYGIS (SEQ ID NO: 13)
HCDR2 against LAG3	WISAYNGNTNYAQKLQG (SEQ ID NO: 14)
HCDR3 against LAG3	DSSSWWVDAFDI (SEQ ID NO: 15)

Table 2. VL and VH sequences of CMD005

VL against EGFR	DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKL LIYDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYFCQHFDHLP LAFGGGTKVEIK (SEQ ID NO: 4)
VH against EGFR	QVQLQESGPGLVKPSETLSLTCTVSGGSVSSGDYYWTWIRQSPGKG LEWIGHIYYSGNTNYNPSLKSRLTISIDTSKTQFSLKLSSVTAADTAI YYCVRDRVTGAFDIWGQGTMVTVSS (SEQ ID NO: 8)
VL against LAG3	SYELTQPPSVSEAPRQRVTISCSGSSSNIGSNAVSWYQQVPRKAPKL LVYYDDLLPSGVSDRFSGSKSGTSASLAIRGLQSEDEADYYCAAWD DSLNAFVFGTGTKVTVL (SEQ ID NO: 12)
VH against LAG3	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGL EWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSD DTAVYYCARDSSSWWVDAFDIWGQGTMVTVSS (SEQ ID NO: 16)

Table 3. Heavy chain and light chain sequences of CMD005

Heavy chain	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWIS AYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSW WVDAFDIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGAGGGGSGGGGSGGGGSGGGGSQ VQLQESGPGLVKPSETLSLTCTVSGGSVSSGDYYWTWIRQSPGKGLEWIGHIY YSGNTNYNPSLKSRLTISIDTSKTQFSLKLSSVTAADTAIYYCVRDRVTGAFDI WGQGTMVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTI TCQASQDISNYLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTI SSLQPEDIATYFCQHFDHLPLAFGGGTKVEIK (SEQ ID NO: 17)
Light chain	SYELTQPPSVSEAPRQRVTISCSGSSSNIGSNAVSWYQQVPRKAPKLLVYYDDL LPSGVSDRFSGSKSGTSASLAIRGLQSEDEADYYCAAWDDSLNAFVFGTGTKV TVLGQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVK AGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPT ECS (SEQ ID NO: 18)

Example 2. Binding of EGFR×LAG3 BsAb CMD005 to recombinant EGFR and LAG3

ELISA was performed according to standard protocols to determine the binding affinity of CMD005 to recombinant EGFR and LAG3. Briefly, human antigen EGFR or LAG3 was coated on Corning EIA/RIA high-binding 96-well plates (Corning Inc.) at 100 ng per well overnight at 4°C and blocked with 3% nonfat milk in PBS (pH7.4, MPBS), 3% bovine serum albumin and 1‰ Tween 20 in PBS, respectively. Five-fold serially diluted antibodies were added accordingly and incubated at room temperature for 2 h. The plates were washed with PBS containing 0.05% Tween 20. Bound antibodies were detected by using HRP-conjugated streptavidin (Sino Biological). The assay was developed at room temperature with TMB substrate (Solarbio) and monitored at 450 nm with a microplate reader. The half-maximal binding (EC_{50}) was calculated by fitting the data to the Langmuir adsorption isotherm. Human IgG1 kappa isotype control as negative control. The results were shown in Figures 2A-2B.

The results show that CMD005 can bind to human recombinant EGFR with an EC50 of 0.43 nM, and bind to human recombinant LAG3 with an EC50 of 0.09 nM. In addition, there is no significant variation for the binding activity between the EGFR×LAG3 bispecific antibody CMD005 and anti-EGFR or anti-LAG3 mAb.

Example 3. Binding of EGFR×LAG3 BsAb CMD005 to cell surface-associated EGFR and LAG3

To measure the binding ability of EGFR×LAG3 BsAb CMD005 to cell surface-associated LAG3, flow cytometry was carried out using activated T cells, and human colon cancer cell line Caco-2 was used to measure the binding ability of CMD005 to cell surface-associated EGFR by flow cytometry. About 5×10^5 cells were incubated with five-fold serially diluted antibodies on ice for 1 h. The cells were washed once with PBS containing 0.5% bovine serum albumin (PBSA) and resuspended in 100 μL PBSA. Then 1 μL anti-human IgG (γ-chain specific)-R-Phycoerythrin antibody produced in goat (Sigma) was added and incubated for 30 min. The cells were washed once with PBSA and then used for flow cytometry analysis. Human IgG1 kappa isotype control as negative control. The results were shown in Figures 3A-3B.

The results indicate that EGFR×LAG3 BsAb CMD005 can bind well to Caco-2 and T cells, and the binding affinity was similar to anti-EGFR mAb or anti-LAG3 mAb.

Example 4. Blocking of binding of EGFR to EGF, LAG3 to FGL1, and LAG3 to MHC II mediated by EGFR×LAG3 BsAb CMD005

ELISA assay was performed to determine the ability of EGFR×LAG3 BsAb CMD005 to block the binding of EGFR/EGF and LAG3/FGL1 receptor-ligand pairs.

Human antigen EGFR were coated on Corning EIA/RIA high-binding 96-well plates (Corning Inc.) at 500 ng per well overnight at 4°C and blocked with 3% MPBS (pH7.4). Five-fold serially diluted antibodies were mixed with human antigen EGF (14 μg/mL) in equal volume proportion. The mixture were added to the plates and incubated at room temperature for 2 h. The plates were washed with PBS containing 0.05% Tween 20. Bound antibodies were detected by HRP anti-6X His tag® antibody (Abcam). The assay was developed at room temperature with TMB substrate (Solarbio) and monitored at 450 nm with a microplate reader. The half-maximal binding (EC_{50}) was calculated by fitting the data to the Langmuir adsorption isotherm. Human IgG1 kappa isotype control as negative control. The results were shown in Figure 4A.

Human antigen FGL1 were coated on plates at 100 ng per well overnight at 4°C and blocked with 3% bovine serum albumin and 1‰ Tween 20 in PBS. Five-fold serially diluted antibodies were mixed with human antigen LAG3 (4 μg/mL) in equal volume proportion. The mixture were added the plates and incubated at room temperature for 2 h. The remaining steps was the same as above. Human IgG1 kappa isotype control as negative control. The results were shown in Figure 4B.

The blocking ability of CMD005 on LAG3/MHC II receptor-ligand pair was measured using a competitive cell-based flow cytometry assay. About 5×10^5 Raji cells that expresses MHC II on cell surface were incubated with gradient diluted antibody solutions and biotin-labeled human antigen LAG3 (400 ng) on ice for 1 h. The cells were washed once with 0.5% PBSA and resuspended in 100 μL PBSA. Then 0.1% streptavidin R-PE conjugate (Invitrogen) was added and incubated for 30 min on ice. The cells were washed once with PBSA and then used for flow cytometry analysis. Human IgG1 kappa isotype control as negative control. The results were shown in Figure 5.

The results indicate that EGFR×LAG3 BsAb CMD005 has strong blocking effect on both EGFR/EGF and LAG3/FGL1 receptor-ligand interaction with EC50s of 1.86 nM and 32.85 nM, respectively (Figures 4A-4B), and CMD005 inhibits or competes with MHC II binding to LAG3 with an EC50 of 6.88 nM (Figure 5).

Example 5. EGFR×LAG3 BsAb CMD005 mediated killing against human cancer cell line

In vitro efficacy of EGFR×LAG3 BsAb CMD005 was assessed by CCK8 assay using several cancer cell lines including A431, Caco-2, and Achn. These three cell lines were centrifuged and resuspended in RPMI 1640 complete medium, and then added into a 96-well round-bottom plate at a density of 1000, 4000, and 1500 cells/100 μL, respectively. The cells were incubated in a 37 °C incubator supplying with 5 % CO2 for 24 hours. The second day, 100 μL antibody solutions (10-fold serially diluted from 100 nM) were added into each well, accordingly. 4-10 days after incubation (the cell confluence is 80-90%), the medium was removed from target cells and 100 μL RPMI 1640 complete medium containing 10% CCK-8 was added and incubated for 30 minutes in CO_2 incubator. Cell inhibition activity was measured by using microplate reader according to the manufacturer's instructions. Human IgG1 kappa isotype control as negative control. The results were shown in Figures 6A-6C.

The results show that EGFR×LAG3 BsAb CMD005 displays potent inhibition on tumor cell growth in a dose-dependent manner. There is no significant difference in the inhibition effect of tumor cell growth between EGFR×LAG3 BsAb CMD005 and anti-EGFR mAb.

Example 6. Anti-AXL bispecific antibodies mediated inhibition of tumor growth in mice

Pharmacokinetic measurement in mice

Two BALB/c mice were administered intravenously with 200 μg EGFR×LAG3 BsAb CMD005 on day 0. Plasma samples *were* collected on time points 15hr, 39hr, 63hr, 87hr after treatment and used for measurement of antibody serum concentrations by ELISA with standard curves generated using the original antibody stocks. The results were shown in Figure 7.

The results indicate that the serum concentration of CMD005 was gradually declining but still maintained a *relatively* high level at the end point.

***In vivo* tumor growth inhibition**

The *in vivo* efficacy of EGFR×LAG3 BsAb CMD005 was evaluated by using hLAG3-c57 BL/6 mice. 3×10^6 MC38-hEGFR tumor cells were inoculated subcutaneously into the right side abdomen of hLAG3-c57 BL/6 mice. After palpable tumors were established, randomization was performed on the basis of tumor volume and body weight. The negative control group mice were dosed intravenously with 10 mg/kg human IgG1 kappa isotype control. The experiment groups

contain anti-EGFR mAb group (10 mg/kg), anti-LAG3 mAb group (10 mg/kg), anti-EGFR mAb+anti-LAG3 mAb group (10+10 mg/kg), and CMD005 group (10 mg/kg). These mice were dosed twice times a week. After three weeks of treatment, mice were sacrificed and tumor volumes were measured. Tumor growth inhibition (TGI) rates was calculated using the following formula: TGI (%) = [1 - (T-T_0)/(C-C_0)] × 100 (T and T_0: tumor volumes on a specific experimental day and on the randomization day, respectively; C and C0: corresponding mean tumor volumes for the control group). Values > 100% represent tumor shrinkage. The results were shown in Figures 8A-8B.

The results indicate that EGFR×LAG3 BsAb CMD005 displays potent inhibition on tumor growth, and the inhibitory effect is stronger than that of anti-EGFR mAb, anti-LAG3 mAb, and anti-EGFR mAb+anti-LAG3 mAb (Figure 8A). The CMD005 group (10 mg/kg) has 137.1% inhibition rate of tumor growth, anti-EGFR mAb group (10 mg/kg) has a 27.1% inhibition rate of tumor growth, anti-LAG3 mAb group (10 mg/kg) has a 86.2% inhibition rate of tumor growth, and anti-EGFR mAb+LAG3 mAb group (10+10 mg/kg) has a 60.2% inhibition rate of tumor growth. The body weight of all mice groups only has minor variation (Figure 8B).

In summary, *in vivo* studies have demonstrated that EGFR×LAG3 BsAb CMD005 has a long serum half-life, and it can specifically and potently inhibit the tumor growth. Therefore, this bispecific antibody therapeutics EGFR×LAG3 BsAb CMD005 has great clinical development prospects.

While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments described herein may be employed. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

1. A bispecific antibody or an antigen binding fragment thereof, comprising a first antigen binding region binding to EGFR comprising a first light chain variable region (VL1) and a first heavy chain variable region (VH1) and a second antigen binding region binding to LAG3 comprising a second light chain variable region (VL2) and a second heavy chain variable region (VH2), wherein
the VL1 comprises LCDRs 1-3 having the amino acid sequences as shown in SEQ ID NOs: 1-3 respectively;
the VH1 comprises HCDRs 1-3 having the amino acid sequences as shown in SEQ ID NOs: 5-7 respectively;
the VL2 comprises LCDRs 1-3 having the amino acid sequences as shown in SEQ ID NOs: 9-11 respectively; and
the VH2 comprises HCDRs 1-3 having the amino acid sequences as shown in SEQ ID NOs: 13-15 respectively.

2. The bispecific antibody or the antigen binding fragment thereof according to claim 1, wherein
the VL1 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4;
the VH1 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 8;
the VL2 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 12; and
the VH2 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 16.

3. The bispecific antibody or the antigen binding fragment thereof according to claim 2, wherein
the VL1 comprises an amino acid sequence as set forth in SEQ ID NO: 4;
the VH1 comprises an amino acid sequence as set forth in SEQ ID NO: 8;
the VL2 comprises an amino acid sequence as set forth in SEQ ID NO: 12; and
the VH2 comprises an amino acid sequence as set forth in SEQ ID NO: 16.

4. The bispecific antibody or the antigen binding fragment thereof according to any one of claims 1-3, wherein the bispecific antibody comprises:

a first polypeptide chain comprising from the N terminal to C terminal: VH2-CH1-CH2-CH3-L1-VH1-L2-VL1; and

a second polypeptide chain comprising from the N terminal to C terminal: VL2-CL;

wherein CH1 represents the first domain of constant region of an immunoglobulin heavy chain; CH2 represents the second domain of constant region of an immunoglobulin heavy chain; CH3 represents the third domain of constant region of an immunoglobulin heavy chain; CL represents constant region of an immunoglobulin light chain; and L1 and L2 each independently represents an optional linker.

5. The bispecific antibody or the antigen binding fragment thereof according to claim 4, wherein the bispecific antibody comprises two copies of the first polypeptide chain and two copies of the second polypeptide chain.

6. The bispecific antibody or the antigen binding fragment thereof according to claim 4 or 5, wherein each of the CH1, CH2 and CH3 is independently derived from immunoglobulin isotype IgG (e.g. human IgG), preferably derived from IgG subtype selected from the group consisting of IgG1, IgG2 and IgG4 (e.g. human IgG1, IgG2 and IgG4).

7. The bispecific antibody or the antigen binding fragment thereof according to any one of claims 4-6, wherein the CL is derived from λ light chain or κ light chain.

8. The bispecific antibody or the antigen binding fragment thereof according to any one of claims 4-7, wherein the linker comprises an amino acid sequence of (G4S)n, wherein n is an integer selected from 1-5, preferably the linker comprises an amino acid sequence as shown in SEQ ID NO: 19.

9. The bispecific antibody or the antigen binding fragment thereof according to any one of claims 4-8, wherein

the first polypeptide chain comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 17; and

the second polypeptide chain comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 18.

10. The bispecific antibody or the antigen binding fragment thereof according to claim 9, wherein the first polypeptide chain comprises an amino acid sequence as shown in SEQ ID NO: 17; and the second polypeptide chain comprises an amino acid sequence as shown in SEQ ID NO: 18.

11. A nucleic acid comprising a nucleotide sequence encoding the bispecific antibody or the antigen binding fragment thereof according to any one of claims 1-10.

12. A vector comprising the nucleic acid according to claim 11.

13. A host cell comprising the nucleic acid according to claim 11 or the vector according to claim 12.

14. A pharmaceutical composition comprising the bispecific antibody or the antigen binding fragment thereof according to any one of claims 1-10, and a pharmaceutically acceptable carrier or excipient.

15. The pharmaceutical composition according to claim 14, further comprising a second therapeutic agent.

16. The pharmaceutical composition according to claim 15, wherein the second therapeutic agent is selected from an antibody, a chemotherapeutic agent and a small molecule drug.

17. The pharmaceutical composition according to claim 15 or 16, wherein the second therapeutic agent is selected from a Bruton's tyrosine kinase (BTK) inhibitor, a PI3K inhibitor, a HDAC inhibitor, an ERK inhibitor, a MAPK inhibitor, a PD-1/PD-L1 inhibitor, a CTLA-4 inhibitor, a TIGIT inhibitor, a TIM3 inhibitor, a VEGF inhibitor, and glucocorticoid.

18. A conjugate comprising the bispecific antibody or the antigen binding fragment thereof according to any one of claims 1-10, and a chemical moiety conjugated thereto.

19. The conjugate according to claim 18, wherein the chemical moiety is selected from the group consisting of a therapeutic agent, a detectable moiety, and an immune stimulatory molecule.

20. A method of treating a cancer in a subject, comprising administering to the subject an effective amount of the bispecific antibody or the antigen binding fragment thereof according to any one of

claims 1-10, the pharmaceutical composition according to any one of claims 14-17, or the conjugate according to claim 18 or 19.

21. The method according to claim 20, wherein the cancer is an EGFR positive cancer.

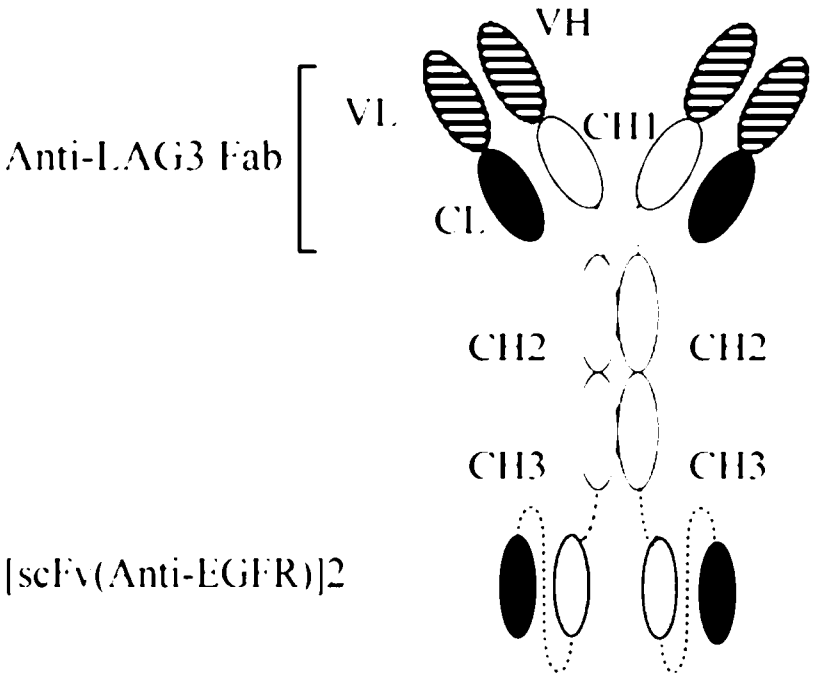
22. The method according to claim 21, wherein the cancer is selected from the group consisting of colon cancer, kidney cancer, colorectal cancer, lung cancer, gastric cancer, ovarian cancer, breast cancer, pancreatic cancer, prostate cancer, skin cancer, head and neck cancer, brain cancer, bladder cancer and liver cancer, preferably colorectal cancer, head and neck cancer, kidney cancer, or skin cancer.

23. The method according to any one of claims 20-22, further comprising administering to the subject a second therapeutic agent.

24. The method according to claim 23, wherein the second therapeutic agent is selected from an antibody, a chemotherapeutic agent and a small molecule drug.

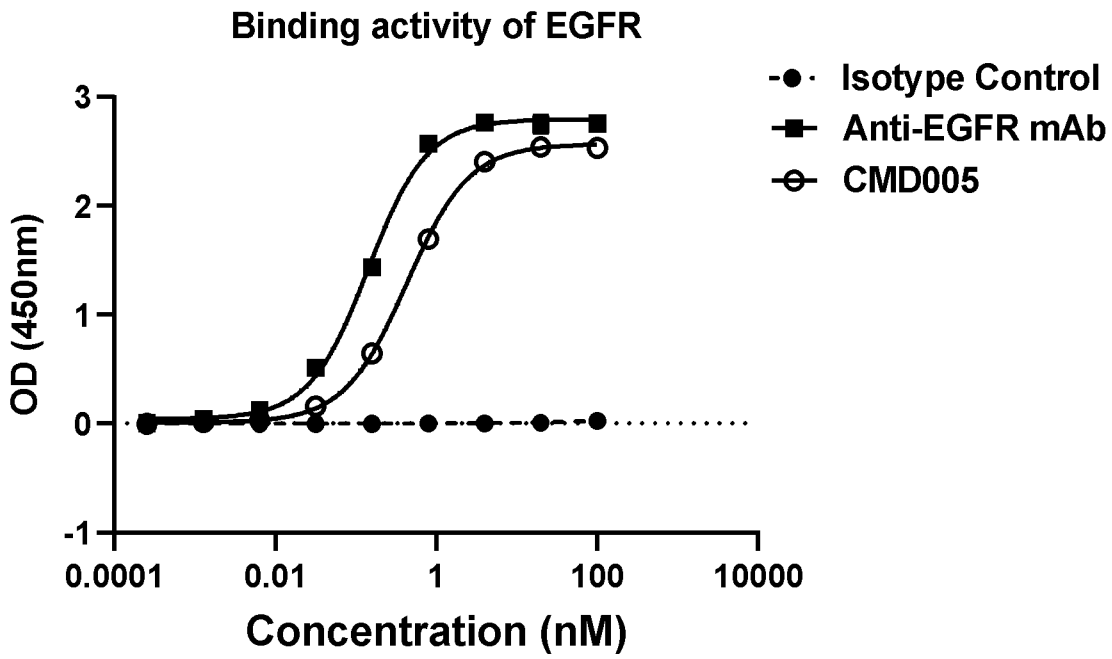
25. The method according to claim 23 or 24, wherein the second therapeutic agent is selected from a Bruton's tyrosine kinase (BTK) inhibitor, a PI3K inhibitor, a HDAC inhibitor, an ERK inhibitor, a MAPK inhibitor, a PD-1/PD-L1 inhibitor, a CTLA-4 inhibitor, a TIGIT inhibitor, a TIM3 inhibitor, a VEGF inhibitor, and glucocorticoid.

Figure 1



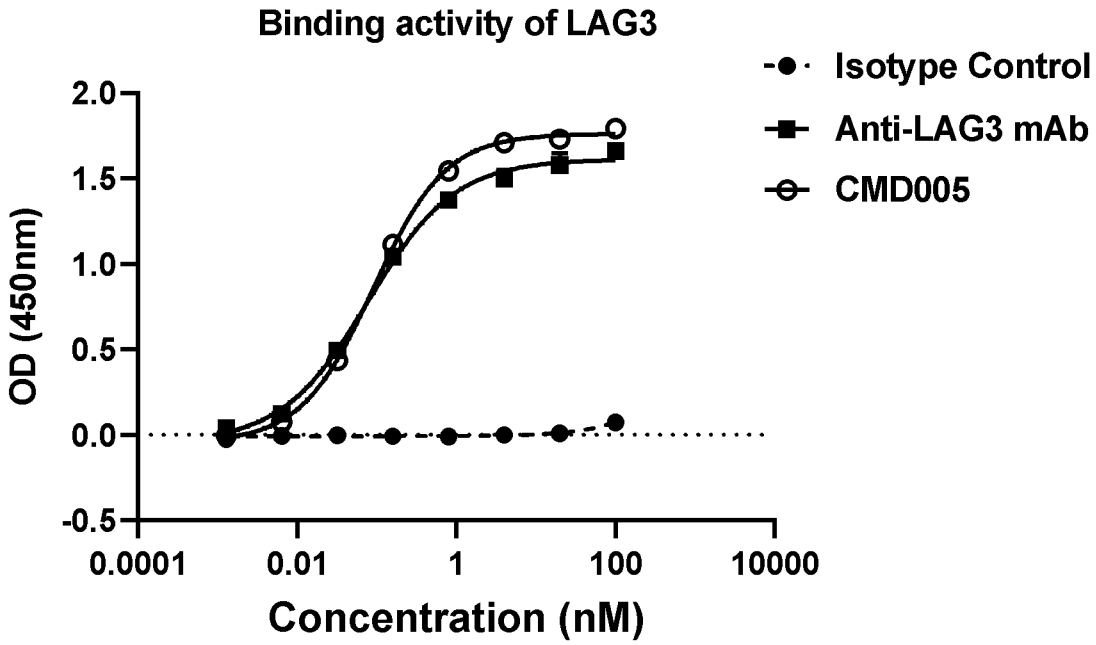
CMD005

Figure 2A



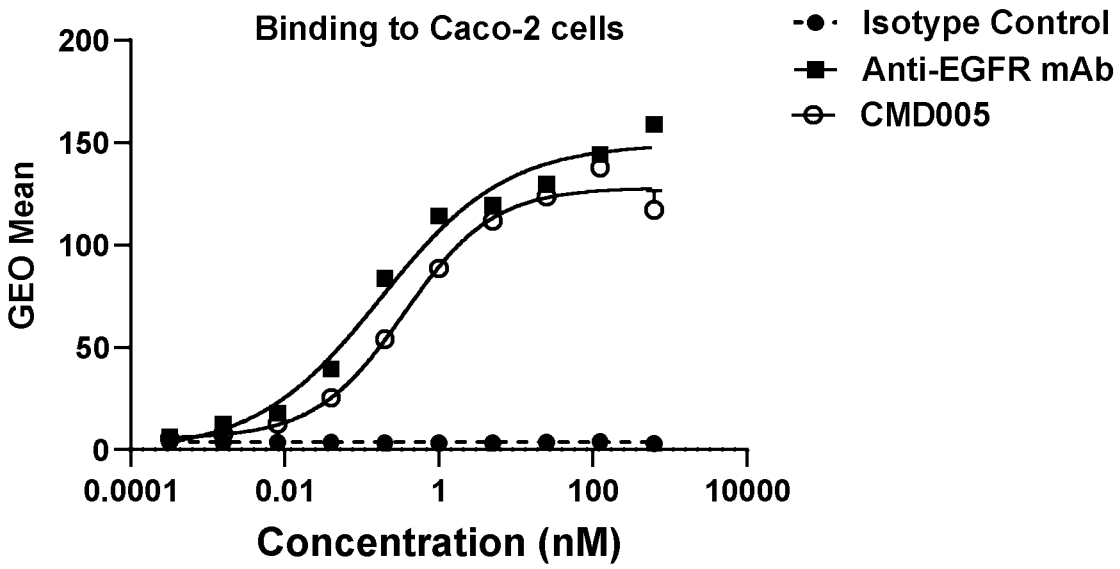
	Anti-EGFR mAb	CMD005
EC50	0.1429	0.4319

Figure 2B



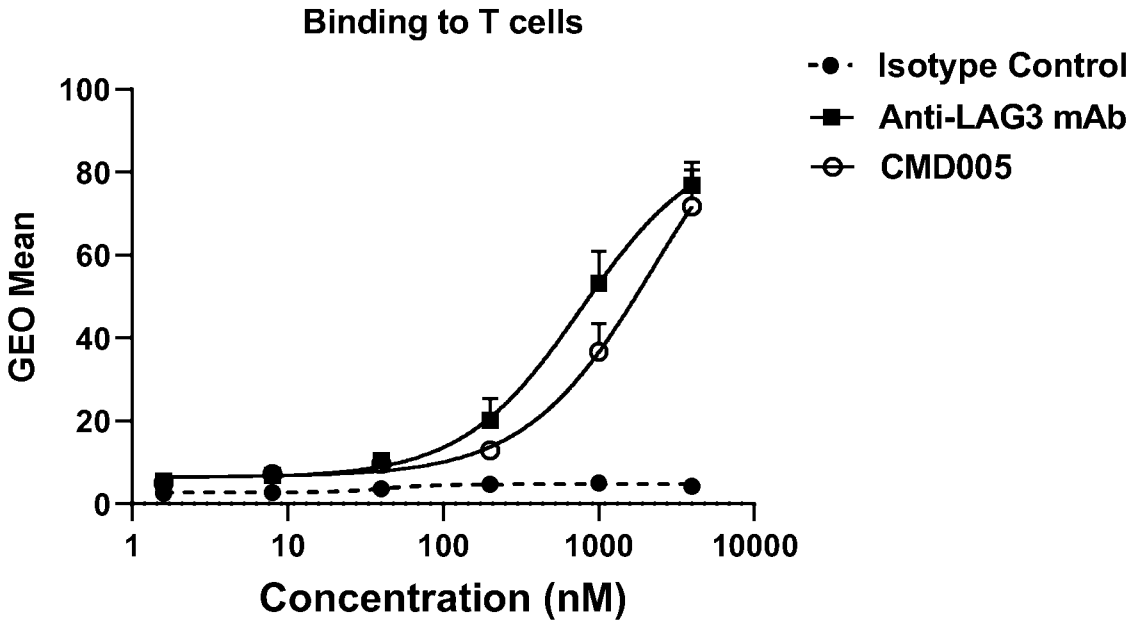
	Anti-LAG3 mAb	CMD005
EC50	0.07835	0.09076

Figure 3A



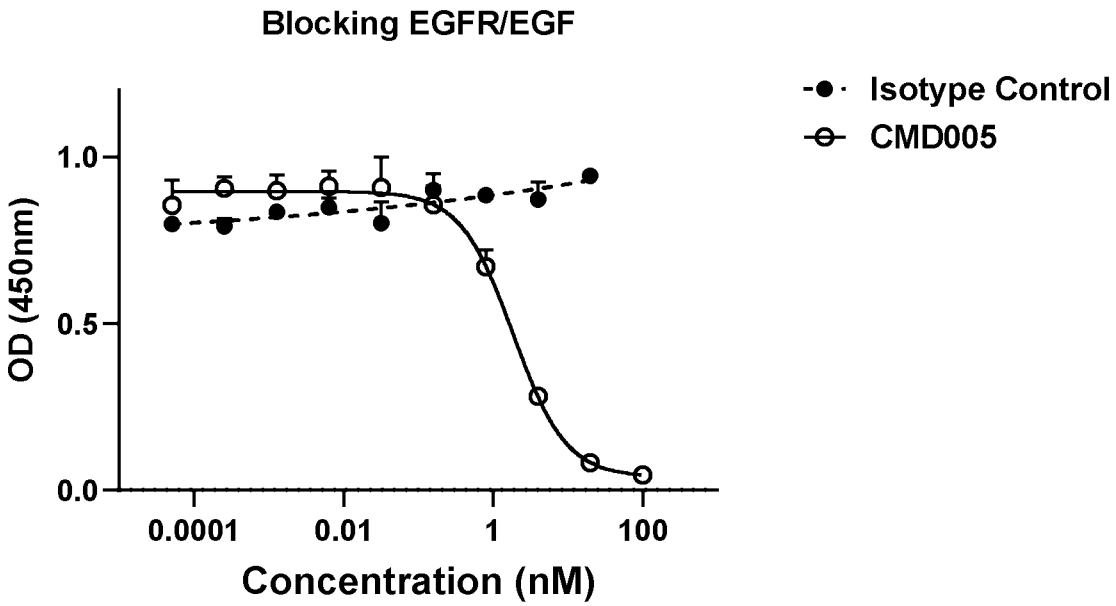
	Anti-EGFR mAb	CMD005
EC50	0.1777	0.3570

Figure 3B



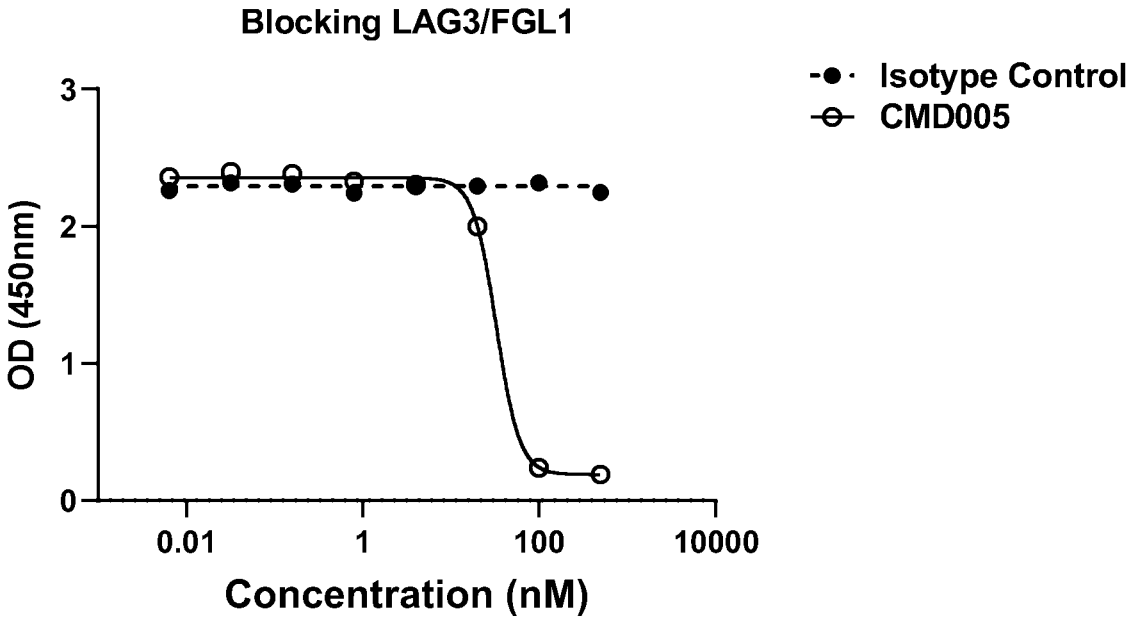
	Anti-LAG3 mAb	CMD005
EC50	774.6	2249

Figure 4A



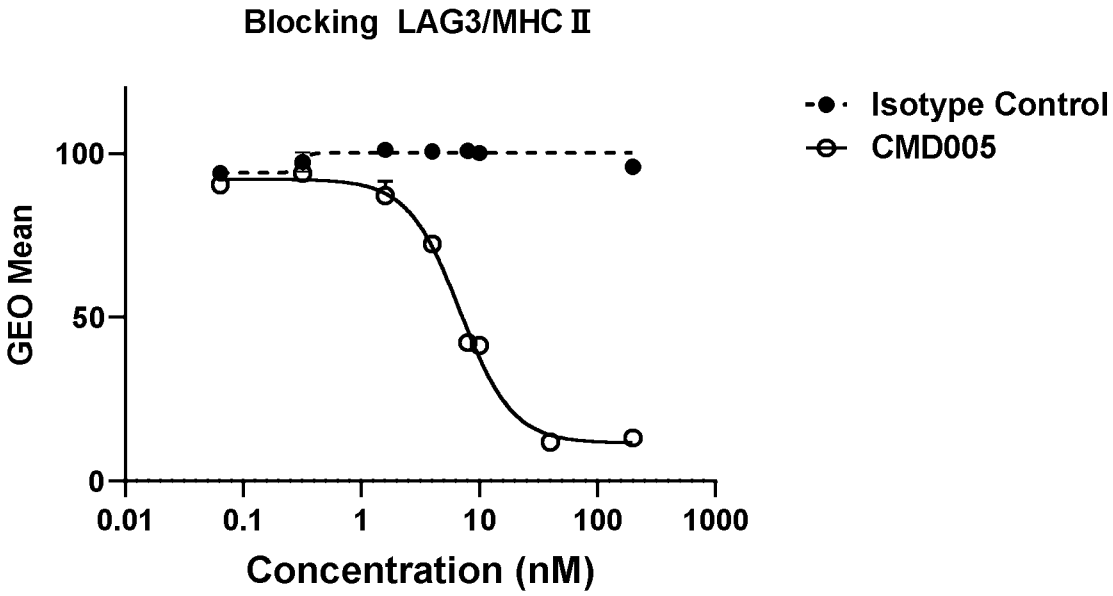
EC50	1.864

Figure 4B



EC50	32.85

Figure 5



EC50	6.878

Figure 6A

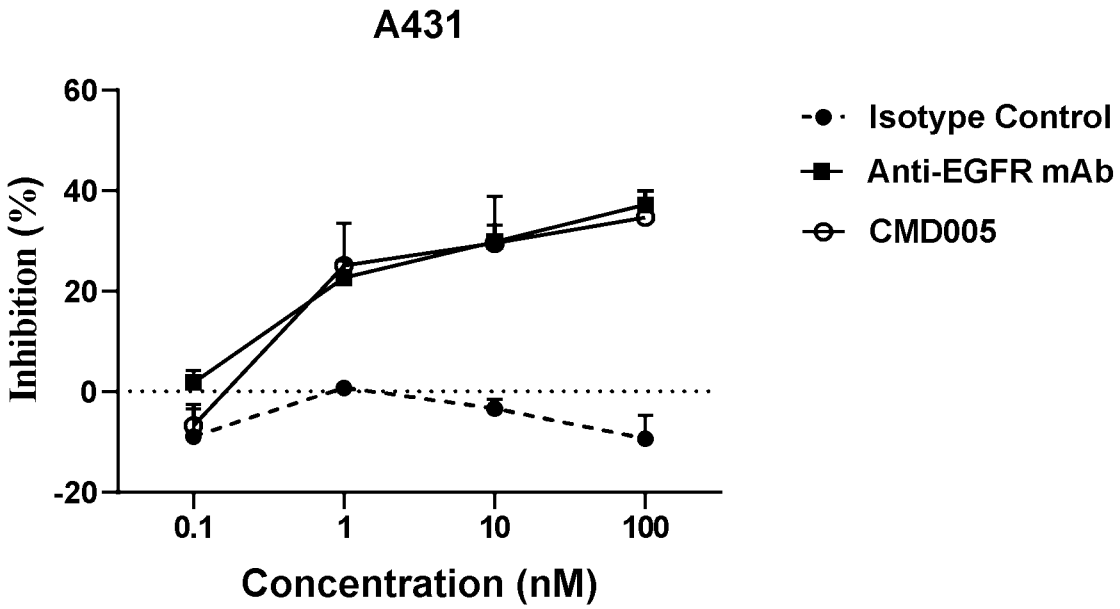

A431
Inhibition (%)
60
40
20
0
-20
0.1
1
10
100
Concentration (nM)
Isotype Control
Anti-EGFR mAb
CMD005

Figure 6B

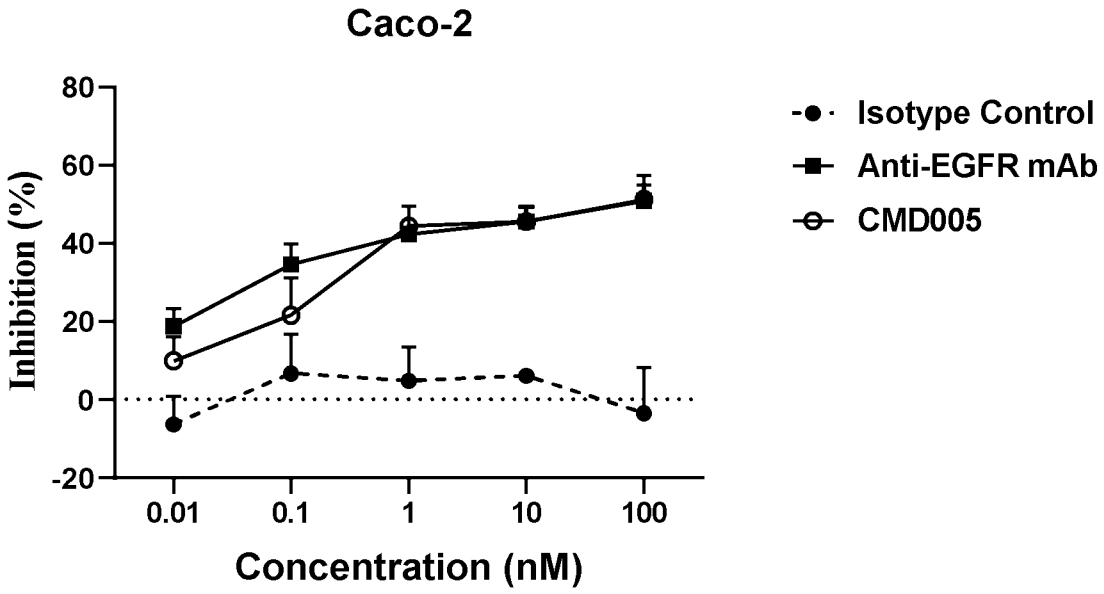

Caco-2
Inhibition (%)
80
60
40
20
0
-20
0.01
0.1
1
10
100
Concentration (nM)
Isotype Control
Anti-EGFR mAb
CMD005

Figure 6C

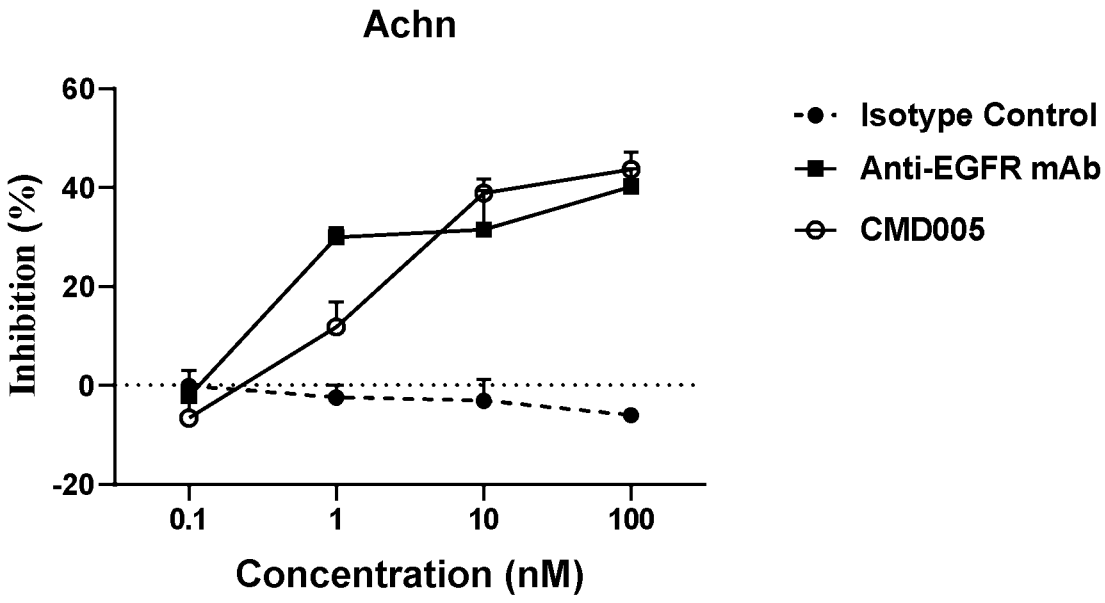

Achn
Inhibition (%)
60
40
20
0
-20
0.1
1
10
100
Concentration (nM)
Isotype Control
Anti-EGFR mAb
CMD005

Figure 7

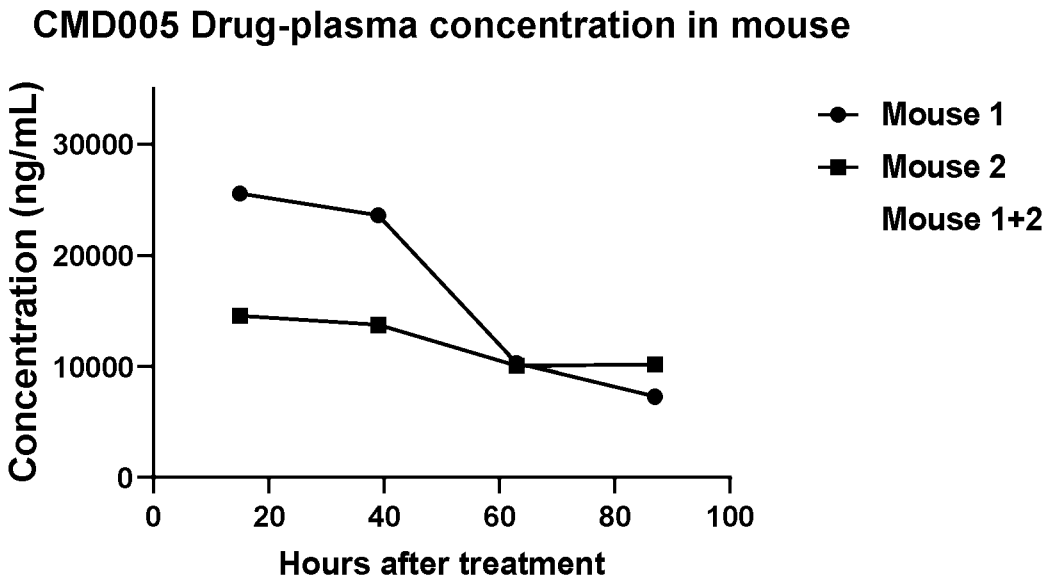

CMD005 Drug-plasma concentration in mouse
Concentration (ng/mL)
30000
20000
10000
0
0
20
40
60
80
100
Hours after treatment
Mouse 1
Mouse 2
Mouse 1+2

Figure 8A

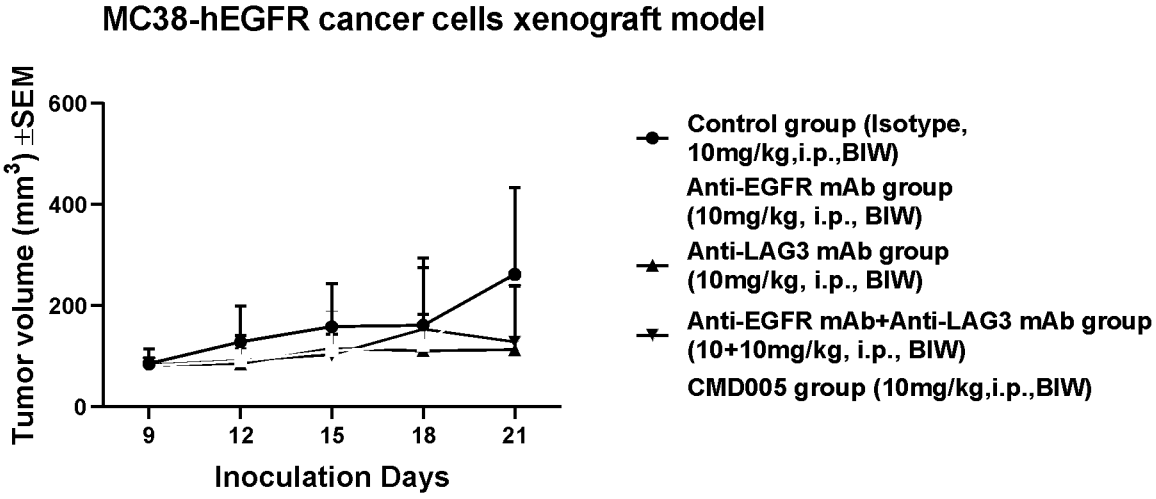
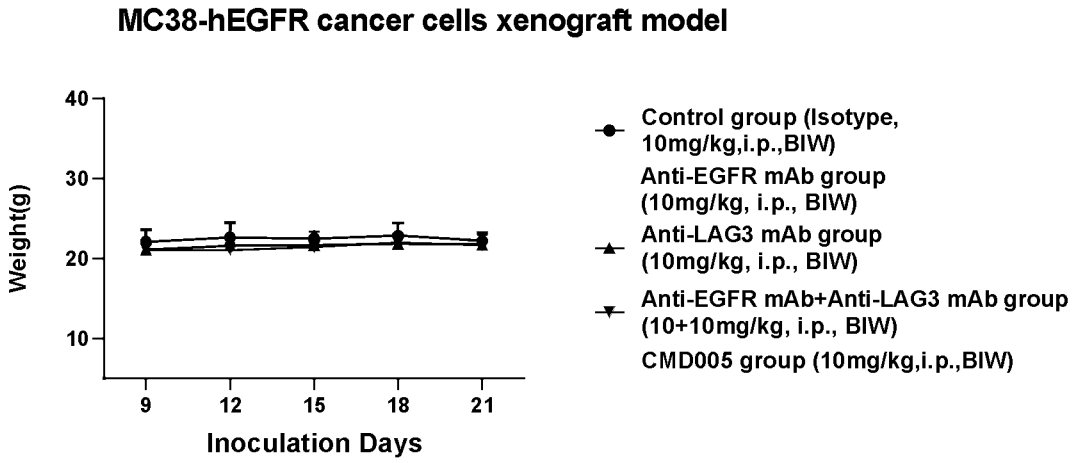

MC38-hEGFR cancer cells xenograft model
Tumor volume (mm3) ±SEM
600
400
200
0
9
12
15
18
21
Inoculation Days
Control group (Isotype, 10mg/kg,i.p.,BIW)
Anti-EGFR mAb group (10mg/kg, i.p., BIW)
Anti-LAG3 mAb group (10mg/kg, i.p., BIW)
Anti-EGFR mAb+Anti-LAG3 mAb group (10+10mg/kg, i.p., BIW)
CMD005 group (10mg/kg,i.p.,BIW)

Figure 8B


MC38-hEGFR cancer cells xenograft model
Weight(g)
40
30
20
10
9
12
15
18
21
Inoculation Days
Control group (Isotype, 10mg/kg,i.p.,BIW)
Anti-EGFR mAb group (10mg/kg, i.p., BIW)
Anti-LAG3 mAb group (10mg/kg, i.p., BIW)
Anti-EGFR mAb+Anti-LAG3 mAb group (10+10mg/kg, i.p., BIW)
CMD005 group (10mg/kg,i.p.,BIW)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2023/076189

A. CLASSIFICATION OF SUBJECT MATTER

C07K16/28(2006.01)i; C07K16/24(2006.01)i; C07K16/46(2006.01)i; C12N15/63(2006.01)i; C12N5/10(2006.01)i; A61K39/395(2006.01)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: C07K C12N 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, CNKI, CNTXT, DWPI, SIPOABS, EPTXT, USTXT, WOTXT, JPTXT, ISI web of Knowledge, PubMed, Genbank, EMBL, Retrieving System for Biological Sequence of Chinese Patent and searched items: Epidermal growth factor receptor, EGFR, Lymphocyte activation gene-3, LAG3, CD223, antibody, bispecific, tetravalent, SEQ ID Nos: 1-18

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CN 113105553 A (BAIL-N) BAILI-BIO CHENGDU PHARM CO LTD (SICH-N) SICHUAN BAILI PHARM CO LTD (SICH-N) SICHUAN BIOKIN PHARM CO LTD (SYST-N) SYSTIMMUNE INC13 July 2021 (2021-07-13) see claims, table 1 and the sequence listing	1-25
Y	CN 110621337 A (CENT-N) CENTRYMED PHARM INC (SMET-N) SMET PHARM INC (ZHEJ-N) ZHEJIANG SHIMAI PHARM CO LTD27 December 2019 (2019-12-27) see claims and the sequence listing	1-25
A	US 2019233518 A1 (MERCK SHARP & DOHME;ZYMEWORKS INC;) 01 August 2019 (2019-08-01) see claims	1-25
A	US 2017369587 A1 (SYSTIMMUNE INC) 28 December 2017 (2017-12-28) see claims	1-25
Y	WO 2017015560 A2 (SORRENTO THERAPEUTICS INC) 26 January 2017 (2017-01-26) see page 48, line 25 to page 49, line 3	1-25

☑ 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
23 August 2023	**02 September 2023**

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**	**WANG,XiangYu** Telephone No. **(+86) 010-62089318**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2023/076189

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2019149716 A1 (HOFFMANN LA ROCHE;HOFFMANN LA ROCHE;) 08 August 2019 (2019-08-08) see claims	1-25

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2023/076189

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/CN2023/076189

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.: **20-25**
because they relate to subject matter not required to be searched by this Authority, namely:

Methods for treating diseases (Rule 39.1(iv) PCT).

The international search report is based on the subject-matter that could reasonably be expected to be claimed, i.e., the preparation of a medicament for treating cancer.

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/CN2023/076189

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	113105553	A	13 July 2021	None			
CN	110621337	A	27 December 2019	EP	3621642	A1	18 March 2020
				EP	3621642	A4	06 January 2021
				US	2021115135	A1	22 April 2021
				US	11339218	B2	24 May 2022
				WO	2018208868	A1	15 November 2018
US	2019233518	A1	01 August 2019	None			
US	2017369587	A1	28 December 2017	None			
WO	2017015560	A2	26 January 2017	TW	201713692	A	16 April 2017
				TWI	733687	B	21 July 2021
				EP	3325009	A2	30 May 2018
				EP	3325009	A4	05 December 2018
				US	2017022273	A1	26 January 2017
				US	9902772	B2	27 February 2018
				AR	105444	A1	04 October 2017
				CA	2993177	A1	26 January 2017
				WO	2017015560	A3	02 March 2017
WO	2019149716	A1	08 August 2019	None			

项目名称: C23W3660.01CN

状态: 已生成

创建日期: 2023-03-30

Last modified: 2023-03-30

基本信息

当前申请

申请号: before

知识产权局: CN
申请号:
申请日:
申请人档案名: C23W3660.01CN

发明名称

语言	发明人姓名
zh	EGFR和LAG3双靶向的双特异性抗体及其用途

申请人和发明人:

孝作祥
语言: zh
拉丁名称: XIAO Zuoxiang
居住地址:
通信地址:

申请人姓名或名称: 浙江时迈药业有限公司
语言: zh
拉丁名称: Zhejiang Shimai Pharmaceutical Co., Ltd.
居住地址:
通信地址:

序列

序列 1: "C23W3660.01CN-SL-20230308_seq_1"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
11	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符

特征键	特征位置	限定符
REGION	1..11	note = CMD005 anti-EGFR VL CDR1
source	1..11	mol_type = protein organism = synthetic construct

残基

QASQDISNYL N 11

序列 2: "C23W3660.01CN-SL-20230308_seq_2"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
7	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..7	note = CMD005 anti-EGFR VL CDR2
source	1..7	mol_type = protein organism = synthetic construct

残基

DASNLET 7

序列 3: "C23W3660.01CN-SL-20230308_seq_3"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
9	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..9	note = CMD005 anti-EGFR VL CDR3
source	1..9	mol_type = protein organism = synthetic construct

残基

QHFDHLPLA 9

序列 4: "C23W3660.01CN-SL-20230308_seq_4"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
107	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..107	note = CMD005 anti-EGFR VL
source	1..107	mol_type = protein organism = synthetic construct

残基

```
DIQMTQSPSS LSASVGDRVT ITCQASQDIS NYLNWYQQKP GKAPKLLIYD ASNLETGVPS    60
RFSGSGSGTD FTFTISSLQP EDIATYFCQH FDHLPLAFGG GTKVEIK                 107
```

序列 5: "C23W3660.01CN-SL-20230308_seq_5"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
7	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..7	note = CMD005 anti-EGFR VH CDR1
source	1..7	mol_type = protein organism = synthetic construct

残基

```
SGDYYWT                                                             7
```

序列 6: "C23W3660.01CN-SL-20230308_seq_6"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
16	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..16	note = CMD005 anti-EGFR VH CDR2
source	1..16	mol_type = protein organism = synthetic construct

残基

```
HIYYSGNTNY NPSLKS                                                  16
```

序列 7: "C23W3660.01CN-SL-20230308_seq_7"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
9	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..9	note = CMD005 anti-EGFR VH CDR3
source	1..9	mol_type = protein organism = synthetic construct

残基

```
DRVTGAFDI                                                    9
```

序列 8: "C23W3660.01CN-SL-20230308_seq_8"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
119	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..119	note = CMD005 anti-EGFR VH
source	1..119	mol_type = protein organism = synthetic construct

残基

```
QVQLQESGPG LVKPSETLSL TCTVSGGSVS SGDYYWTWIR QSPGKGLEWI GHIYYSGNTN    60
YNPSLKSRLT ISIDTSKTQF SLKLSSVTAA DTAIYYCVRD RVTGAFDIWG QGTMVTVSS     119
```

序列 9: "C23W3660.01CN-SL-20230308_seq_9"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
13	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..13	note = CMD005 anti-LAG3 VL CDR1
source	1..13	mol_type = protein organism = synthetic construct

残基

```
SGSSSNIGSN AVS                                               13
```

序列 10: "C23W3660.01CN-SL-20230308_seq_10"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
7	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..7	note = CMD005 anti-LAG3 VL CDR2
source	1..7	mol_type = protein organism = synthetic construct

残基

```
YDDLLPS                                                         7
```

序列 11: "C23W3660.01CN-SL-20230308_seq_11"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
11	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..11	note = CMD005 anti-LAG3 VL CDR3
source	1..11	mol_type = protein organism = synthetic construct

残基

```
AAWDDSLNAF V                                                    11
```

序列 12: "C23W3660.01CN-SL-20230308_seq_12"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
110	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..110	note = CMD005 anti-LAG3 VL
source	1..110	mol_type = protein organism = synthetic construct

残基

```
SYELTQPPSV SEAPRQRVTI SCSGSSSNIG SNAVSWYQQV PRKAPKLLVY YDDLLPSGVS    60
DRFSGSKSGT SASLAIRGLQ SEDEADYYCA AWDDSLNAFV FGTGTKVTVL              110
```

序列 13: "C23W3660.01CN-SL-20230308_seq_13"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
5	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..5	note = CMD005 anti-LAG3 VH CDR1
source	1..5	mol_type = protein organism = synthetic construct

残基

```
SYGIS                                                                    5
```

序列 14: "C23W3660.01CN-SL-20230308_seq_14"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
17	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..17	note = CMD005 anti-LAG3 VH CDR2
source	1..17	mol_type = protein organism = synthetic construct

残基

```
WISAYNGNTN YAQKLQG                                                      17
```

序列 15: "C23W3660.01CN-SL-20230308_seq_15"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
12	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..12	note = CMD005 anti-LAG3 VH CDR3
source	1..12	mol_type = protein organism = synthetic construct

残基

```
DSSSWWVDAF DI                                                           12
```

序列 16: "C23W3660.01CN-SL-20230308_seq_16"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
121	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..121	note = CMD005 anti-LAG3 VH
source	1..121	mol_type = protein organism = synthetic construct

残基

```
EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYGISWVRQA PGQGLEWMGW ISAYNGNTNY    60
AQKLQGRVTM TTDTSTSTAY MELRSLRSDD TAVYYCARDS SSWWVDAFDI WGQGTMVTVS    120
S                                                                    121
```

序列 17: "C23W3660.01CN-SL-20230308_seq_17"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
717	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..717	note = CMD005 heavy chain
source	1..717	mol_type = protein organism = synthetic construct

残基

```
EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYGISWVRQA PGQGLEWMGW ISAYNGNTNY    60
AQKLQGRVTM TTDTSTSTAY MELRSLRSDD TAVYYCARDS SSWWVDAFDI WGQGTMVTVS    120
SASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS    180
SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKKVE PKSCDKTHTC PPCPAPELLG    240
GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY    300
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD    360
ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR    420
WQQGNVFSCS VMHEALHNHY TQKSLSLSPG AGGGGSGGGG SGGGGSGGGG SQVQLQESGP    480
GLVKPSETLS LTCTVSGGSV SSGDYYWTWI RQSPGKGLEW IGHIYYSGNT NYNPSLKSRL    540
TISIDTSKTQ FSLKLSSVTA ADTAIYYCVR DRVTGAFDIW GQGTMVTVSS GGGGSGGGGS    600
GGGGSGGGGS DIQMTQSPSS LSASVGDRVT ITCQASQDIS NYLNWYQQKP GKAPKLLIYD    660
ASNLETGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQH FDHLPLAFGG GTKVEIK       717
```

序列 18: "C23W3660.01CN-SL-20230308_seq_18"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
216	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..216	note = CMD005 light chain
source	1..216	mol_type = protein

特征键	特征位置	限定符
		organism = synthetic construct

残基

```
SYELTQPPSV SEAPRQRVTI SCSGSSSNIG SNAVSWYQQV PRKAPKLLVY YDDLLPSGVS    60
DRFSGSKSGT SASLAIRGLQ SEDEADYYCA AWDDSLNAFV FGTGTKVTVL GQPKANPTVT    120
LFPPSSEELQ ANKATLVCLI SDFYPGAVTV AWKADGSPVK AGVETTKPSK QSNNKYAASS    180
YLSLTPEQWK SHRSYSCQVT HEGSTVEKTV APTECS                              216
```

序列 19: "C23W3660.01CN-SL-20230308_seq_19"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
20	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..20	note = Linker
source	1..20	mol_type = protein organism = synthetic construct

残基

```
GGGGSGGGGS GGGGSGGGGS                                                20
```

序列 20: "C23W3660.01CN-SL-20230308_seq_20"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
6	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..6	note = Conserved six amino acid motif of LAG3
source	1..6	mol_type = protein organism = synthetic construct

残基

```
KIEELE                                                               6
```

序列 21: "C23W3660.01CN-SL-20230308_seq_21"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
330	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..330	note = Amino acid sequence of CH1-CH2-CH3
source	1..330	mol_type = protein organism = synthetic construct

残基

```
ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS    60
GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG    120
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN    180
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE    240
LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW    300
QQGNVFSCSV MHEALHNHYT QKSLSLSPGA                                     330
```

序列 22: "C23W3660.01CN-SL-20230308_seq_22"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
330	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..330	note = Amino acid sequence of CH1-CH2-CH3
source	1..330	mol_type = protein organism = synthetic construct

残基

```
ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS    60
GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG    120
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN    180
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE    240
LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW    300
QQGNVFSCSV MHEALHNHYT QKSLSLSPGK                                     330
```

序列 23: "C23W3660.01CN-SL-20230308_seq_23"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
327	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..327	note = Amino acid sequence of CH1-CH2-CH3
source	1..327	mol_type = protein organism = synthetic construct

残基

```
ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS    60
GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKKVES KYGPPCPPCP APEFLGGPSV    120
FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY    180
RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK    240
NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG    300
NVFSCSVMHE ALHNHYTQKS LSLSLGK                                        327
```

序列 24: "C23W3660.01CN-SL-20230308_seq_24"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
106	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..106	note = Amino acid sequence of CL
source	1..106	mol_type = protein organism = synthetic construct

残基

```
GQPKANPTVT LFPPSSEELQ ANKATLVCLI SDFYPGAVTV AWKADGSPVK AGVETTKPSK    60
QSNNKYAASS YLSLTPEQWK SHRSYSCQVT HEGSTVEKTV APTECS                  106
```

序列 25: "C23W3660.01CN-SL-20230308_seq_25"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
107	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..107	note = Amino acid sequence of CL
source	1..107	mol_type = protein organism = synthetic construct

残基

```
RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD    60
SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC                 107
```

序列 26: "C23W3660.01CN-SL-20230308_seq_26"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
5	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..5	note = linker
source	1..5	mol_type = protein organism = synthetic construct

残基

```
GGGGS                                                               5
```

序列 27: "C23W3660.01CN-SL-20230308_seq_27"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
10	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..10	note = linker
source	1..10	mol_type = protein organism = synthetic construct

残基

```
GGGGSGGGGS                                                    10
```

序列 28: "C23W3660.01CN-SL-20230308_seq_28"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
15	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..15	note = linker
source	1..15	mol_type = protein organism = synthetic construct

残基

```
GGGGSGGGGS GGGGS                                              15
```

序列 29: "C23W3660.01CN-SL-20230308_seq_29"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
25	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..25	note = linker
source	1..25	mol_type = protein organism = synthetic construct

残基

GGGGSGGGGS GGGGSGGGGS GGGGS 25

序列 30: "C23W3660.01CN-SL-20230308_seq_30"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
29	DNA	synthetic construct	否	否

特征

特征键	特征位置	限定符
misc_feature	1..29	note = Primer
source	1..29	mol_type = other DNA organism = synthetic construct

残基

taattctaga gtcgacaatc aacctctgg 29

序列 31: "C23W3660.01CN-SL-20230308_seq_31"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
22	DNA	synthetic construct	否	否

特征

特征键	特征位置	限定符
misc_feature	1..22	note = primer
source	1..22	mol_type = other DNA organism = synthetic construct

残基

tgatccacca cctccactac cg 22

序列 32: "C23W3660.01CN-SL-20230308_seq_32"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
31	DNA	synthetic construct	否	否

特征

特征键	特征位置	限定符
misc_feature	1..31	note = primer
source	1..31	mol_type = other DNA organism = synthetic construct

残基

```
gtggtggatc acaggtgcag ctgcaggaga g                                    31
```

序列 33: "C23W3660.01CN-SL-20230308_seq_33"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
42	DNA	synthetic construct	否	否

特征

特征键	特征位置	限定符
misc_feature	1..42	note = primer
source	1..42	mol_type = other DNA organism = synthetic construct

残基

```
gattgtcgac tctagaatta tttgatttcc accttggttc cg                        42
```

序列 34: "C23W3660.01CN-SL-20230308_seq_34"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
449	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..449	note = Anti-EGFR mAb Heavy chain
source	1..449	mol_type = protein organism = synthetic construct

残基

```
QVQLQESGPG LVKPSETLSL TCTVSGGSVS SGDYYWTWIR QSPGKGLEWI GHIYYSGNTN     60
YNPSLKSRLT ISIDTSKTQF SLKLSSVTAA DTAIYYCVRD RVTGAFDIWG QGTMVTVSSA    120
STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW NSGALTSGVH TFPAVLQSSG    180
LYSLSSVVTV PSSSLGTQTY ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP    240
SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS    300
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL    360
TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ    420
QGNVFSCSVM HEALHNHYTQ KSLSLSPGK                                      449
```

序列 35: "C23W3660.01CN-SL-20230308_seq_35"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
214	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..214	note = Anti-EGFR mAb Light chain

特征键	特征位置	限定符
source	1..214	mol_type = protein organism = synthetic construct

残基

```
DIQMTQSPSS LSASVGDRVT ITCQASQDIS NYLNWYQQKP GKAPKLLIYD ASNLETGVPS    60
RFSGSGSGTD FTFTISSLQP EDIATYFCQH FDHLPLAFGG GTKVEIKRTV AAPSVFIFPP   120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT   180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC                               214
```

序列 36: "C23W3660.01CN-SL-20230308_seq_36"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
448	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..448	note = Anti- LAG3 mAb Heavy chain
source	1..448	mol_type = protein organism = synthetic construct

残基

```
EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYGISWVRQA PGQGLEWMGW ISAYNGNTNY    60
AQKLQGRVTM TTDTSTSTAY MELRSLRSDD TAVYYCARDS SSWWVDAFDI WGQGTMVTVS   120
SASTKGPSVF PLAPCSRSTS ESTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS   180
SGLYSLSSVV TVPSSSLGTK TYTCNVDHKP SNTKVDKKVE SKYGPPCPPC PAPEFLGGPS   240
VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV DGVEVHNAKT KPREEQFNST   300
YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP SSIEKTISKA KGQPREPQVY TLPPSQEEMT   360
KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE   420
GNVFSCSVMH EALHNHYTQK SLSLSLGK                                      448
```

序列 37: "C23W3660.01CN-SL-20230308_seq_37"

长度	分子类型	生物体	含有 DNA 和 RNA 片段	跳过的序列
216	AA	synthetic construct	否	否

特征

特征键	特征位置	限定符
REGION	1..216	note = Anti- LAG3 mAb Light chain
source	1..216	mol_type = protein organism = synthetic construct

残基

```
SYELTQPPSV SEAPRQRVTI SCSGSSSNIG SNAVSWYQQV PRKAPKLLVY YDDLLPSGVS    60
DRFSGSKSGT SASLAIRGLQ SEDEADYYCA AWDDSLNAFV FGTGTKVTVL GQPKANPTVT   120
LFPPSSEELQ ANKATLVCLI SDFYPGAVTV AWKADGSPVK AGVETTKPSK QSNNKYAASS   180
YLSLTPEQWK SHRSYSCQVT HEGSTVEKTV APTECS                             216
```

序列表

1	序列表信息	
1-1	文件名	**C23W3660.01CN-SL-20230308.xml**
1-2	DTD版本	**V1_3**
1-3	软件名称	**WIPO Sequence**
1-4	软件版本	**2.2.0**
1-5	生产日期	**2023-03-08**
2	基本信息	
2-1	当前申请: 知识产权局	**CN**
2-2	当前申请: 申请号	
2-3	当前申请: 申请日	
2-4	当前申请: 申请人档案名	**C23W3660.01CN**
2-5	最早优先权申请: 知识产权局	
2-6	最早优先权申请: 申请号	
2-7	最早优先权申请: 申请日	
2-8zh	申请人姓名或名称	浙江时迈药业有限公司
2-8en	申请人姓名或名称: 拉丁名称	**Zhejiang Shimai Pharmaceutical Co., Ltd.**
2-9zh	发明人姓名	孝作祥
2-9en	发明人姓名: 拉丁名称	**XIAO Zuoxiang**
2-10zh	发明名称	**EGFR**和**LAG3**双靶向的双特异性抗体及其用途
2-11	序列总量	**37**
3-1	序列	
3-1-1	序列号 [ID]	**1**
3-1-2	分子类型	**AA**
3-1-3	长度	**11**
3-1-4-1	特征 位置/限定符	
		REGION 1..11 **note=CMD005 anti-EGFR VL CDR1**
3-1-4-2	特征 位置/限定符	
		source 1..11 **mol_type=protein** **organism=synthetic construct**
3-1-5	残基	**QASQDISNYL N 11**
3-2	序列	
3-2-1	序列号 [ID]	**2**
3-2-2	分子类型	**AA**
3-2-3	长度	**7**
3-2-4-1	特征 位置/限定符	
		REGION 1..7 **note=CMD005 anti-EGFR VL CDR2**
3-2-4-2	特征 位置/限定符	

序列表

		source 1..7 **mol_type=protein** **organism=synthetic construct**
3-2-5	残基	**DASNLET 7**
3-3	序列	
3-3-1	序列号 [ID]	**3**
3-3-2	分子类型	**AA**
3-3-3	长度	**9**
3-3-4-1	特征 位置/限定符	
		REGION 1..9 **note=CMD005 anti-EGFR VL CDR3**
3-3-4-2	特征 位置/限定符	
		source 1..9 **mol_type=protein** **organism=synthetic construct**
3-3-5	残基	**QHFDHLPLA 9**
3-4	序列	
3-4-1	序列号 [ID]	**4**
3-4-2	分子类型	**AA**
3-4-3	长度	**107**
3-4-4-1	特征 位置/限定符	
		REGION 1..107 **note=CMD005 anti-EGFR VL**
3-4-4-2	特征 位置/限定符	
		source 1..107 **mol_type=protein** **organism=synthetic construct**
3-4-5	残基	**DIQMTQSPSS LSASVGDRVT ITCQASQDIS 30** **NYLNWYQQKP GKAPKLLIYD ASNLETGVPS 60** **RFSGSGSGTD FTFTISSLQP EDIATYFCQH 90** **FDHLPLAFGG GTKVEIK 107**
3-5	序列	
3-5-1	序列号 [ID]	**5**
3-5-2	分子类型	**AA**
3-5-3	长度	**7**
3-5-4-1	特征 位置/限定符	
		REGION 1..7 **note=CMD005 anti-EGFR VH CDR1**
3-5-4-2	特征 位置/限定符	
		source 1..7 **mol_type=protein**

序列表

		organism=synthetic construct
3-5-5	残基	**SGDYYWT 7**
3-6	序列	
3-6-1	序列号 [ID]	**6**
3-6-2	分子类型	**AA**
3-6-3	长度	**16**
3-6-4-1	特征 位置/限定符	
		REGION 1..16
		note=CMD005 anti-EGFR VH CDR2
3-6-4-2	特征 位置/限定符	
		source 1..16
		mol_type=protein
		organism=synthetic construct
3-6-5	残基	**HIYYSGNTNY NPSLKS 16**
3-7	序列	
3-7-1	序列号 [ID]	**7**
3-7-2	分子类型	**AA**
3-7-3	长度	**9**
3-7-4-1	特征 位置/限定符	
		REGION 1..9
		note=CMD005 anti-EGFR VH CDR3
3-7-4-2	特征 位置/限定符	
		source 1..9
		mol_type=protein
		organism=synthetic construct
3-7-5	残基	**DRVTGAFDI 9**
3-8	序列	
3-8-1	序列号 [ID]	**8**
3-8-2	分子类型	**AA**
3-8-3	长度	**119**
3-8-4-1	特征 位置/限定符	
		REGION 1..119
		note=CMD005 anti-EGFR VH
3-8-4-2	特征 位置/限定符	
		source 1..119
		mol_type=protein
		organism=synthetic construct
3-8-5	残基	**QVQLQESGPG LVKPSETLSL TCTVSGGSVS 30** **SGDYYWTWIR QSPGKGLEWI GHIYYSGNTN 60** **YNPSLKSRLT ISIDTSKTQF SLKLSSVTAA 90** **DTAIYYCVRD RVTGAFDIWG QGTMVTVSS 119**
3-9	序列	

序列表

3-9-1	序列号 [ID]	**9**
3-9-2	分子类型	**AA**
3-9-3	长度	**13**
3-9-4-1	特征 位置/限定符	
		REGION 1..13
		note=CMD005 anti-LAG3 VL CDR1
3-9-4-2	特征 位置/限定符	
		source 1..13
		mol_type=protein
		organism=synthetic construct
3-9-5	残基	**SGSSSNIGSN AVS 13**
3-10	序列	
3-10-1	序列号 [ID]	**10**
3-10-2	分子类型	**AA**
3-10-3	长度	**7**
3-10-4-1	特征 位置/限定符	
		REGION 1..7
		note=CMD005 anti-LAG3 VL CDR2
3-10-4-2	特征 位置/限定符	
		source 1..7
		mol_type=protein
		organism=synthetic construct
3-10-5	残基	**YDDLLPS 7**
3-11	序列	
3-11-1	序列号 [ID]	**11**
3-11-2	分子类型	**AA**
3-11-3	长度	**11**
3-11-4-1	特征 位置/限定符	
		REGION 1..11
		note=CMD005 anti-LAG3 VL CDR3
3-11-4-2	特征 位置/限定符	
		source 1..11
		mol_type=protein
		organism=synthetic construct
3-11-5	残基	**AAWDDSLNAF V 11**
3-12	序列	
3-12-1	序列号 [ID]	**12**
3-12-2	分子类型	**AA**
3-12-3	长度	**110**
3-12-4-1	特征 位置/限定符	
		REGION 1..110

序列表

		note=CMD005 anti-LAG3 VL
3-12-4-2	特征 位置/限定符	
		source 1..110
		mol_type=protein
		organism=synthetic construct
3-12-5	残基	**SYELTQPPSV SEAPRQRVTI SCSGSSSNIG 30** **SNAVSWYQQV PRKAPKLLVY YDDLLPSGVS 60** **DRFSGSKSGT SASLAIRGLQ SEDEADYYCA 90** **AWDDSLNAFV FGTGTKVTVL 110**
3-13	序列	
3-13-1	序列号 [ID]	**13**
3-13-2	分子类型	**AA**
3-13-3	长度	**5**
3-13-4-1	特征 位置/限定符	
		REGION 1..5
		note=CMD005 anti-LAG3 VH CDR1
3-13-4-2	特征 位置/限定符	
		source 1..5
		mol_type=protein
		organism=synthetic construct
3-13-5	残基	**SYGIS 5**
3-14	序列	
3-14-1	序列号 [ID]	**14**
3-14-2	分子类型	**AA**
3-14-3	长度	**17**
3-14-4-1	特征 位置/限定符	
		REGION 1..17
		note=CMD005 anti-LAG3 VH CDR2
3-14-4-2	特征 位置/限定符	
		source 1..17
		mol_type=protein
		organism=synthetic construct
3-14-5	残基	**WISAYNGNTN YAQKLQG 17**
3-15	序列	
3-15-1	序列号 [ID]	**15**
3-15-2	分子类型	**AA**
3-15-3	长度	**12**
3-15-4-1	特征 位置/限定符	
		REGION 1..12
		note=CMD005 anti-LAG3 VH CDR3
3-15-4-2	特征 位置/限定符	

序列表

		source 1..12
		mol_type=protein
		organism=synthetic construct
3-15-5	残基	**DSSSWWVDAF DI 12**
3-16	序列	
3-16-1	序列号 [ID]	**16**
3-16-2	分子类型	**AA**
3-16-3	长度	**121**
3-16-4-1	特征 位置/限定符	
		REGION 1..121
		note=CMD005 anti-LAG3 VH
3-16-4-2	特征 位置/限定符	
		source 1..121
		mol_type=protein
		organism=synthetic construct
3-16-5	残基	**EVQLVQSGAE VKKPGASVKV SCKASGYTFT 30** **SYGISWVRQA PGQGLEWMGW ISAYNGNTNY 60** **AQKLQGRVTM TTDTSTSTAY MELRSLRSDD 90** **TAVYYCARDS SSWWVDAFDI WGQGTMVTVS 12** **0** **S 121**
3-17	序列	
3-17-1	序列号 [ID]	**17**
3-17-2	分子类型	**AA**
3-17-3	长度	**717**
3-17-4-1	特征 位置/限定符	
		REGION 1..717
		note=CMD005 heavy chain
3-17-4-2	特征 位置/限定符	
		source 1..717
		mol_type=protein
		organism=synthetic construct
3-17-5	残基	**EVQLVQSGAE VKKPGASVKV SCKASGYTFT 30** **SYGISWVRQA PGQGLEWMGW ISAYNGNTNY 60** **AQKLQGRVTM TTDTSTSTAY MELRSLRSDD 90** **TAVYYCARDS SSWWVDAFDI WGQGTMVTVS 12** **0** **SASTKGPSVF PLAPSSKSTS GGTAALGCLV 150** **KDYFPEPVTV SWNSGALTSG VHTFPAVLQS 180** **SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP 210** **SNTKVDKKVE PKSCDKTHTC PPCPAPELLG 240** **GPSVFLFPPK PKDTLMISRT PEVTCVVVDV 270** **SHEDPEVKFN WYVDGVEVHN AKTKPREEQY 30**

序列表

		0 **NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK 330** **ALPAPIEKTI SKAKGQPREP QVYTLPPSRD 360** **ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ 390** **PENNYKTTPP VLDSDGSFFL YSKLTVDKSR 420** **WQQGNVFSCS VMHEALHNHY TQKSLSLSPG 450** **AGGGGSGGGG SGGGGSGGGG SQVQLQESGP 480** **GLVKPSETLS LTCTVSGGSV SSGDYYWTWI 510** **RQSPGKGLEW IGHIYYSGNT NYNPSLKSRL 540** **TISIDTSKTQ FSLKLSSVTA ADTAIYYCVR 570** **DRVTGAFDIW GQGTMVTVSS GGGGSGGGGS 600** **GGGGSGGGGS DIQMTQSPSS LSASVGDRVT 630** **ITCQASQDIS NYLNWYQQKP GKAPKLLIYD 660** **ASNLETGVPS RFSGSGSGTD FTFTISSLQP 690** **EDIATYFCQH FDHLPLAFGG GTKVEIK 717**
3-18	序列	
3-18-1	序列号 [ID]	**18**
3-18-2	分子类型	**AA**
3-18-3	长度	**216**
3-18-4-1	特征 位置/限定符	
		REGION 1..216 **note=CMD005 light chain**
3-18-4-2	特征 位置/限定符	
		source 1..216 **mol_type=protein** **organism=synthetic construct**
3-18-5	残基	**SYELTQPPSV SEAPRQRVTI SCSGSSSNIG 30** **SNAVSWYQQV PRKAPKLLVY YDDLLPSGVS 60** **DRFSGSKSGT SASLAIRGLQ SEDEADYYCA 90** **AWDDSLNAFV FGTGTKVTVL GQPKANPTVT 120** **LFPPSSEELQ ANKATLVCLI SDFYPGAVTV 150** **AWKADGSPVK AGVETTKPSK QSNNKYAASS 180** **YLSLTPEQWK SHRSYSCQVT HEGSTVEKTV 210** **APTECS 216**
3-19	序列	
3-19-1	序列号 [ID]	**19**
3-19-2	分子类型	**AA**
3-19-3	长度	**20**
3-19-4-1	特征 位置/限定符	
		REGION 1..20 **note=Linker**

序列表

3-19-4-2	特征 位置/限定符	
		source 1..20
		mol_type=protein
		organism=synthetic construct
3-19-5	残基	**GGGGSGGGGS GGGGSGGGGS 20**
3-20	序列	
3-20-1	序列号 [ID]	**20**
3-20-2	分子类型	**AA**
3-20-3	长度	**6**
3-20-4-1	特征 位置/限定符	
		REGION 1..6
		note=Conserved six amino acid motif of LAG3
3-20-4-2	特征 位置/限定符	
		source 1..6
		mol_type=protein
		organism=synthetic construct
3-20-5	残基	**KIEELE 6**
3-21	序列	
3-21-1	序列号 [ID]	**21**
3-21-2	分子类型	**AA**
3-21-3	长度	**330**
3-21-4-1	特征 位置/限定符	
		REGION 1..330
		note=Amino acid sequence of CH1-CH2-CH3
3-21-4-2	特征 位置/限定符	
		source 1..330
		mol_type=protein
		organism=synthetic construct
3-21-5	残基	**ASTKGPSVFP LAPSSKSTSG GTAALGCLVK 30** **DYFPEPVTVS WNSGALTSGV HTFPAVLQSS 60** **GLYSLSSVVT VPSSSLGTQT YICNVNHKPS 90** **NTKVDKKVEP KSCDKTHTCP PCPAPELLGG 120** **PSVFLFPPKP KDTLMISRTP EVTCVVVDVS 150** **HEDPEVKFNW YVDGVEVHNA KTKPREEQYN 18** **0** **STYRVVSVLT VLHQDWLNGK EYKCKVSNKA 21** **0** **LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE 240** **LTKNQVSLTC LVKGFYPSDI AVEWESNGQP 270** **ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW 300** **QQGNVFSCSV MHEALHNHYT QKSLSLSPGA 330**
3-22	序列	
3-22-1	序列号 [ID]	**22**

序列表

3-22-2	分子类型	**AA**
3-22-3	长度	**330**
3-22-4-1	特征 位置/限定符	
		REGION 1..330
		note=Amino acid sequence of CH1-CH2-CH3
3-22-4-2	特征 位置/限定符	
		source 1..330
		mol_type=protein
		organism=synthetic construct
3-22-5	残基	**ASTKGPSVFP LAPSSKSTSG GTAALGCLVK 30** **DYFPEPVTVS WNSGALTSGV HTFPAVLQSS 60** **GLYSLSSVVT VPSSSLGTQT YICNVNHKPS 90** **NTKVDKKVEP KSCDKTHTCP PCPAPELLGG 120** **PSVFLFPPKP KDTLMISRTP EVTCVVVDVS 150** **HEDPEVKFNW YVDGVEVHNA KTKPREEQYN 18** **0** **STYRVVSVLT VLHQDWLNGK EYKCKVSNKA 21** **0** **LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE 240** **LTKNQVSLTC LVKGFYPSDI AVEWESNGQP 270** **ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW 300** **QQGNVFSCSV MHEALHNHYT QKSLSLSPGK 330**
3-23	序列	
3-23-1	序列号 [ID]	**23**
3-23-2	分子类型	**AA**
3-23-3	长度	**327**
3-23-4-1	特征 位置/限定符	
		REGION 1..327
		note=Amino acid sequence of CH1-CH2-CH3
3-23-4-2	特征 位置/限定符	
		source 1..327
		mol_type=protein
		organism=synthetic construct
3-23-5	残基	**ASTKGPSVFP LAPCSRSTSE STAALGCLVK 30** **DYFPEPVTVS WNSGALTSGV HTFPAVLQSS 60** **GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS 90** **NTKVDKKVES KYGPPCPPCP APEFLGGPSV 120** **FLFPPKPKDT LMISRTPEVT CVVVDVSQED 150** **PEVQFNWYVD GVEVHNAKTK PREEQFNSTY 180** **RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS 21** **0** **SIEKTISKAK GQPREPQVYT LPPSQEEMTK 240** **NQVSLTCLVK GFYPSDIAVE WESNGQPENN 270** **YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG 300**

序列表

		NVFSCSVMHE ALHNHYTQKS LSLSLGK 327
3-24	序列	
3-24-1	序列号 [ID]	**24**
3-24-2	分子类型	**AA**
3-24-3	长度	**106**
3-24-4-1	特征 位置/限定符	**REGION 1..106** **note=Amino acid sequence of CL**
3-24-4-2	特征 位置/限定符	**source 1..106** **mol_type=protein** **organism=synthetic construct**
3-24-5	残基	**GQPKANPTVT LFPPSSEELQ ANKATLVCLI 30** **SDFYPGAVTV AWKADGSPVK AGVETTKPSK 60** **QSNNKYAASS YLSLTPEQWK SHRSYSCQVT 90** **HEGSTVEKTV APTECS 106**
3-25	序列	
3-25-1	序列号 [ID]	**25**
3-25-2	分子类型	**AA**
3-25-3	长度	**107**
3-25-4-1	特征 位置/限定符	**REGION 1..107** **note=Amino acid sequence of CL**
3-25-4-2	特征 位置/限定符	**source 1..107** **mol_type=protein** **organism=synthetic construct**
3-25-5	残基	**RTVAAPSVFI FPPSDEQLKS GTASVVCLLN 30** **NFYPREAKVQ WKVDNALQSG NSQESVTEQD 60** **SKDSTYSLSS TLTLSKADYE KHKVYACEVT 90** **HQGLSSPVTK SFNRGEC 107**
3-26	序列	
3-26-1	序列号 [ID]	**26**
3-26-2	分子类型	**AA**
3-26-3	长度	**5**
3-26-4-1	特征 位置/限定符	**REGION 1..5** **note=linker**
3-26-4-2	特征 位置/限定符	**source 1..5** **mol_type=protein** **organism=synthetic construct**

序列表

3-26-5	残基	**GGGGS 5**
3-27	序列	
3-27-1	序列号 [ID]	**27**
3-27-2	分子类型	**AA**
3-27-3	长度	**10**
3-27-4-1	特征 位置/限定符	
		REGION 1..10
		note=linker
3-27-4-2	特征 位置/限定符	
		source 1..10
		mol_type=protein
		organism=synthetic construct
3-27-5	残基	**GGGGSGGGGS 10**
3-28	序列	
3-28-1	序列号 [ID]	**28**
3-28-2	分子类型	**AA**
3-28-3	长度	**15**
3-28-4-1	特征 位置/限定符	
		REGION 1..15
		note=linker
3-28-4-2	特征 位置/限定符	
		source 1..15
		mol_type=protein
		organism=synthetic construct
3-28-5	残基	**GGGGSGGGGS GGGGS 15**
3-29	序列	
3-29-1	序列号 [ID]	**29**
3-29-2	分子类型	**AA**
3-29-3	长度	**25**
3-29-4-1	特征 位置/限定符	
		REGION 1..25
		note=linker
3-29-4-2	特征 位置/限定符	
		source 1..25
		mol_type=protein
		organism=synthetic construct
3-29-5	残基	**GGGGSGGGGS GGGGSGGGGS GGGGS 25**
3-30	序列	
3-30-1	序列号 [ID]	**30**
3-30-2	分子类型	**DNA**
3-30-3	长度	**29**

序列表

3-30-4-1	特征 位置/限定符	
		misc_feature 1..29
		note=Primer
3-30-4-2	特征 位置/限定符	
		source 1..29
		mol_type=other DNA
		organism=synthetic construct
3-30-5	残基	**taattctaga gtcgacaatc aacctctgg 29**
3-31	序列	
3-31-1	序列号 [ID]	**31**
3-31-2	分子类型	**DNA**
3-31-3	长度	**22**
3-31-4-1	特征 位置/限定符	
		misc_feature 1..22
		note=primer
3-31-4-2	特征 位置/限定符	
		source 1..22
		mol_type=other DNA
		organism=synthetic construct
3-31-5	残基	**tgatccacca cctccactac cg 22**
3-32	序列	
3-32-1	序列号 [ID]	**32**
3-32-2	分子类型	**DNA**
3-32-3	长度	**31**
3-32-4-1	特征 位置/限定符	
		misc_feature 1..31
		note=primer
3-32-4-2	特征 位置/限定符	
		source 1..31
		mol_type=other DNA
		organism=synthetic construct
3-32-5	残基	**gtggtggatc acaggtgcag ctgcaggaga 30**
		g 31
3-33	序列	
3-33-1	序列号 [ID]	**33**
3-33-2	分子类型	**DNA**
3-33-3	长度	**42**
3-33-4-1	特征 位置/限定符	
		misc_feature 1..42
		note=primer
3-33-4-2	特征 位置/限定符	

序列表

		source 1..42 **mol_type=other DNA** **organism=synthetic construct**
3-33-5	残基	**gattgtcgac tctagaatta tttgatttcc 30** **accttggttc cg 42**
3-34	序列	
3-34-1	序列号 [ID]	**34**
3-34-2	分子类型	**AA**
3-34-3	长度	**449**
3-34-4-1	特征 位置/限定符	**REGION 1..449** **note=Anti-EGFR mAb Heavy chain**
3-34-4-2	特征 位置/限定符	**source 1..449** **mol_type=protein** **organism=synthetic construct**
3-34-5	残基	**QVQLQESGPG LVKPSETLSL TCTVSGGSVS 30** **SGDYYWTWIR QSPGKGLEWI GHIYYSGNTN 60** **YNPSLKSRLT ISIDTSKTQF SLKLSSVTAA 90** **DTAIYYCVRD RVTGAFDIWG QGTMVTVSSA 120** **STKGPSVFPL APSSKSTSGG TAALGCLVKD 150** **YFPEPVTVSW NSGALTSGVH TFPAVLQSSG 180** **LYSLSSVVTV PSSSLGTQTY ICNVNHKPSN 210** **TKVDKKVEPK SCDKTHTCPP CPAPELLGGP 240** **SVFLFPPKPK DTLMISRTPE VTCVVVDVSH 270** **EDPEVKFNWY VDGVEVHNAK TKPREEQYNS 30** **0** **TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL 33** **0** **PAPIEKTISK AKGQPREPQV YTLPPSRDEL 360** **TKNQVSLTCL VKGFYPSDIA VEWESNGQPE 390** **NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ 420** **QGNVFSCSVM HEALHNHYTQ KSLSLSPGK 449**
3-35	序列	
3-35-1	序列号 [ID]	**35**
3-35-2	分子类型	**AA**
3-35-3	长度	**214**
3-35-4-1	特征 位置/限定符	**REGION 1..214** **note=Anti-EGFR mAb Light chain**
3-35-4-2	特征 位置/限定符	**source 1..214** **mol_type=protein**

序列表

		organism=synthetic construct
3-35-5	残基	**DIQMTQSPSS LSASVGDRVT ITCQASQDIS 30** **NYLNWYQQKP GKAPKLLIYD ASNLETGVPS 60** **RFSGSGSGTD FTFTISSLQP EDIATYFCQH 90** **FDHLPLAFGG GTKVEIKRTV AAPSVFIFPP 120** **SDEQLKSGTA SVVCLLNNFY PREAKVQWKV 150** **DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 180** **LSKADYEKHK VYACEVTHQG LSSPVTKSFN 210** **RGEC 214**
3-36	序列	
3-36-1	序列号 [ID]	**36**
3-36-2	分子类型	**AA**
3-36-3	长度	**448**
3-36-4-1	特征 位置/限定符	
		REGION 1..448
		note=Anti- LAG3 mAb Heavy chain
3-36-4-2	特征 位置/限定符	
		source 1..448
		mol_type=protein
		organism=synthetic construct
3-36-5	残基	**EVQLVQSGAE VKKPGASVKV SCKASGYTFT 30** **SYGISWVRQA PGQGLEWMGW ISAYNGNTNY 60** **AQKLQGRVTM TTDTSTSTAY MELRSLRSDD 90** **TAVYYCARDS SSWWVDAFDI WGQGTMVTVS 12** **0** **SASTKGPSVF PLAPCSRSTS ESTAALGCLV 150** **KDYFPEPVTV SWNSGALTSG VHTFPAVLQS 180** **SGLYSLSSVV TVPSSSLGTK TYTCNVDHKP 210** **SNTKVDKKVE SKYGPPCPPC PAPEFLGGPS 240** **VFLFPPKPKD TLMISRTPEV TCVVVDVSQE 270** **DPEVQFNWYV DGVEVHNAKT KPREEQFNST 300** **YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP 33** **0** **SSIEKTISKA KGQPREPQVY TLPPSQEEMT 360** **KNQVSLTCLV KGFYPSDIAV EWESNGQPEN 390** **NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE 420** **GNVFSCSVMH EALHNHYTQK SLSLSLGK 448**
3-37	序列	
3-37-1	序列号 [ID]	**37**
3-37-2	分子类型	**AA**
3-37-3	长度	**216**
3-37-4-1	特征 位置/限定符	
		REGION 1..216
		note=Anti- LAG3 mAb Light chain

序列表

3-37-4-2	特征 位置/限定符	
		source 1..216
		mol_type=protein
		organism=synthetic construct
3-37-5	残基	**SYELTQPPSV SEAPRQRVTI SCSGSSSNIG 30** **SNAVSWYQQV PRKAPKLLVY YDDLLPSGVS 60** **DRFSGSKSGT SASLAIRGLQ SEDEADYYCA 90** **AWDDSLNAFV FGTGTKVTVL GQPKANPTVT 120** **LFPPSSEELQ ANKATLVCLI SDFYPGAVTV 150** **AWKADGSPVK AGVETTKPSK QSNNKYAASS 180** **YLSLTPEQWK SHRSYSCQVT HEGSTVEKTV 210** **APTECS 216**

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(54)发明名称

EGFR和LAG3双靶向的双特异性抗体及其用途

(57)摘要

本申请公开了针对EGFR和LAG3的双特异性抗体、包含该抗体的核酸、包含该核酸的载体以及包含该核酸或载体的宿主细胞。本申请还公开了包含该抗体的药物组合物和抗体-药物缀合物,以及使用该抗体的治疗方法。

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