



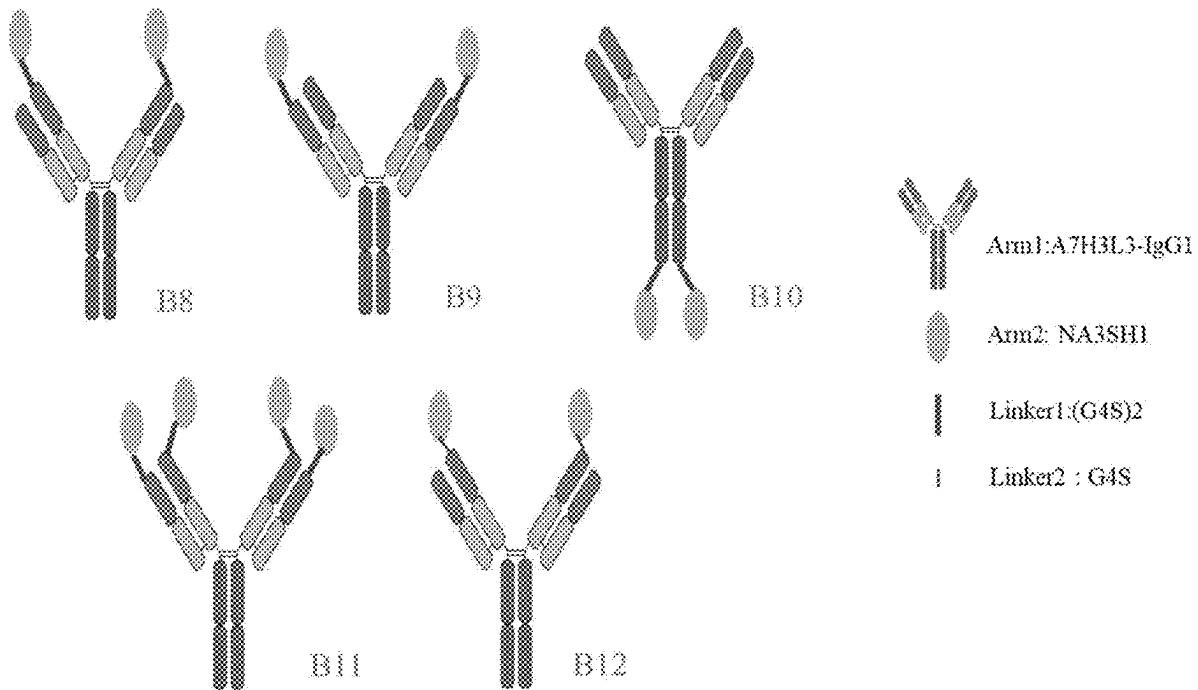
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Huang et al.(10) **Pub. No.: US 2025/0257128 A1**(43) **Pub. Date: Aug. 14, 2025**(54) **ANTI-CD47/ANTI-CLDN18.2 BISPECIFIC
ANTIBODY AND USE THEREOF**(30) **Foreign Application Priority Data**

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(2) Date: **Apr. 18, 2024**(57) **ABSTRACT**The present invention belongs to the field of biomedicine.
Specifically, the present invention relates to an anti-CD47/
anti-CLDN18.2 bispecific antibody and the use thereof.**Specification includes a Sequence Listing.**

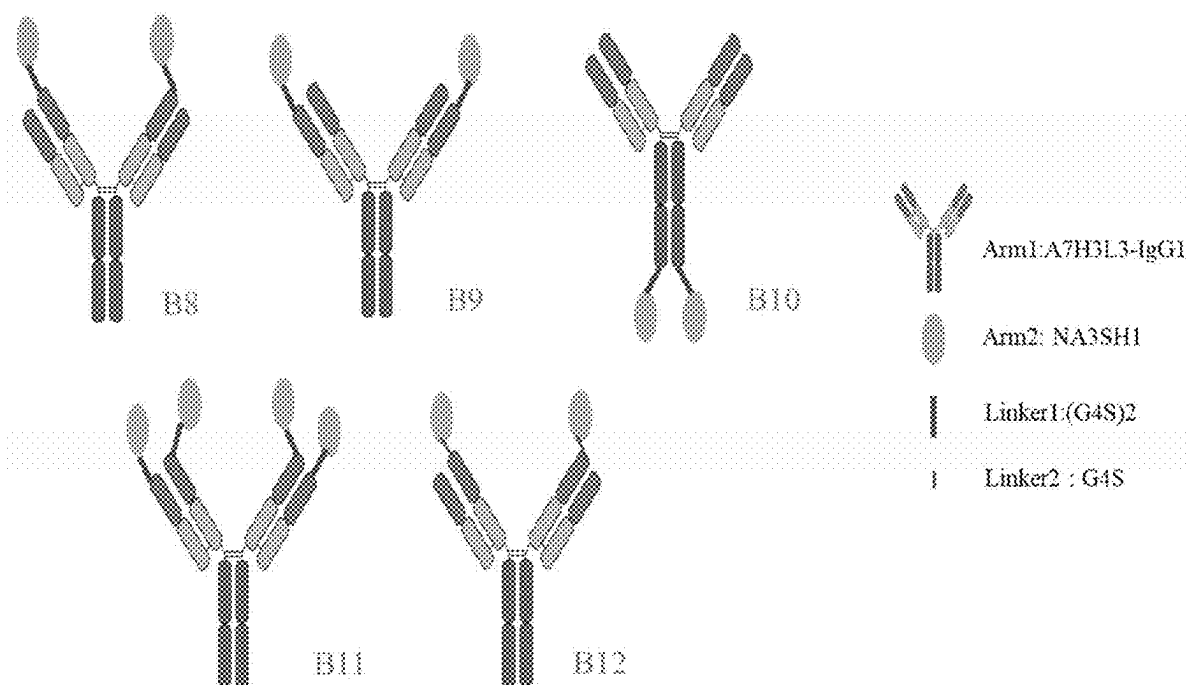


Fig. 1

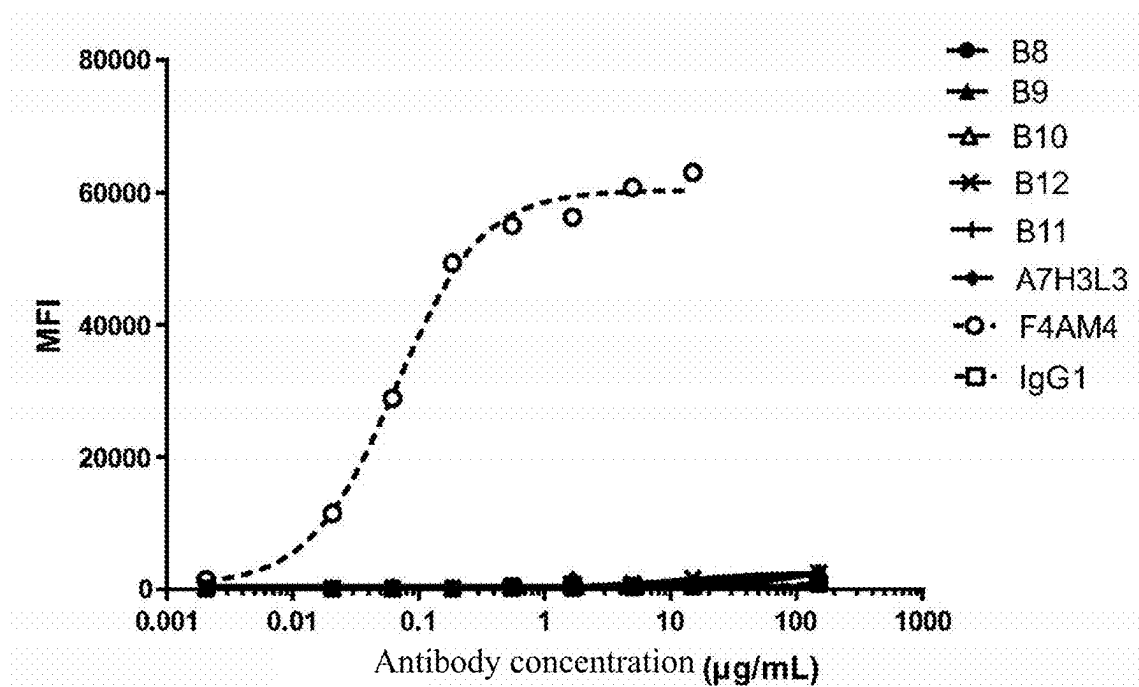


Fig. 2a

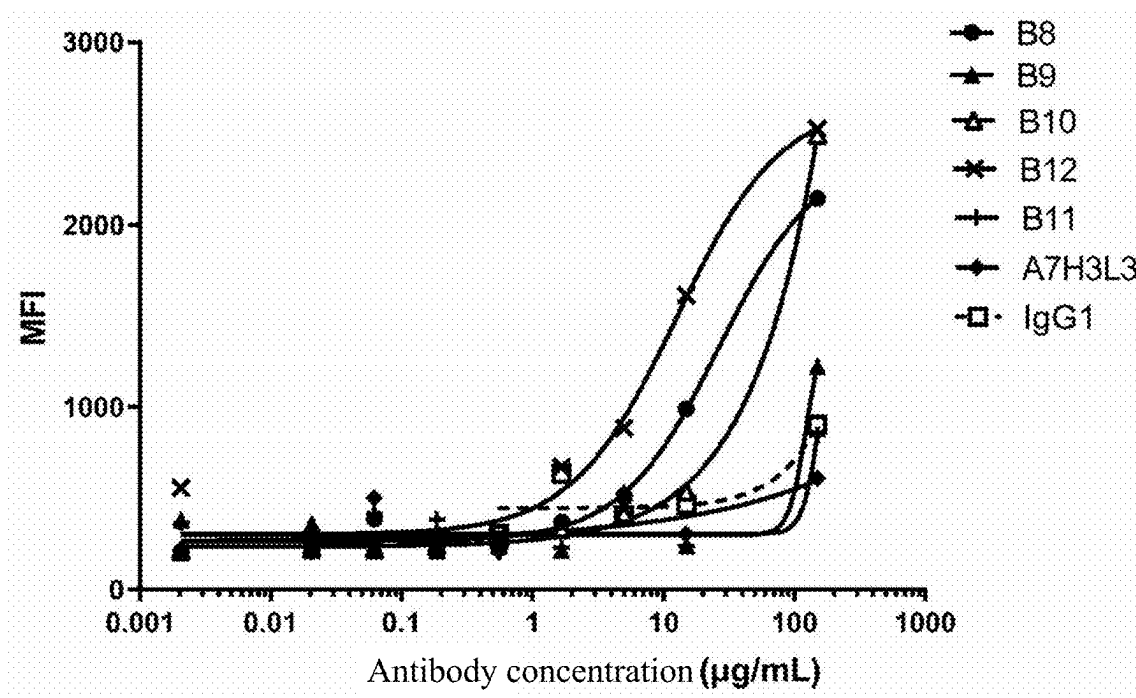


Fig. 2b

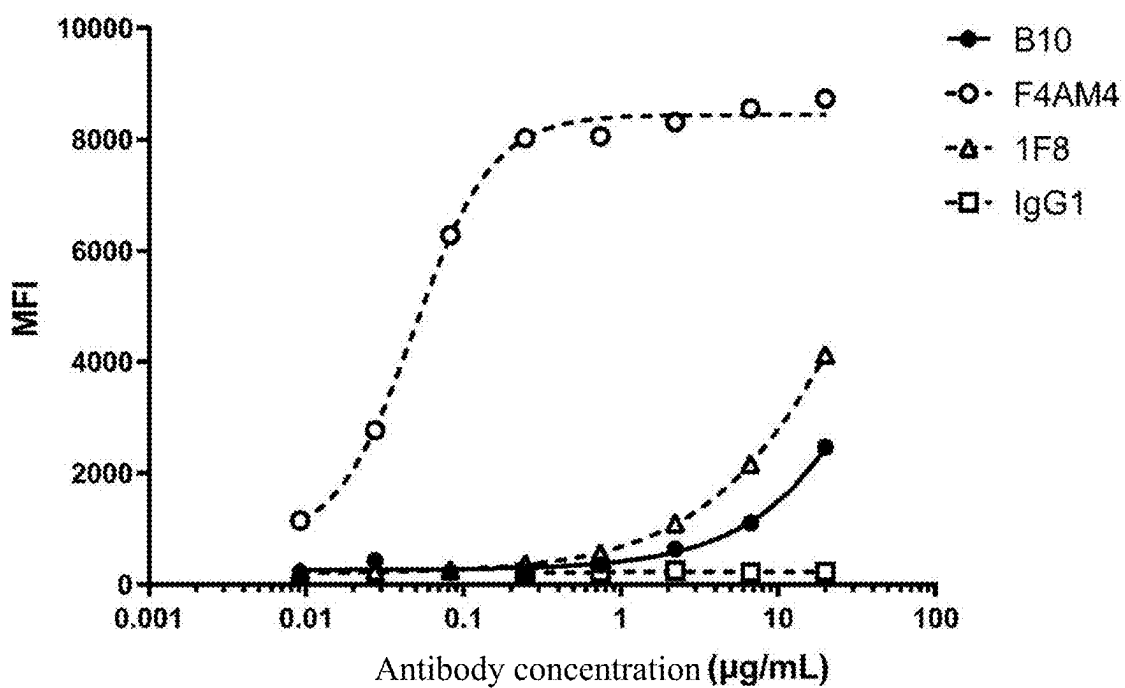


Fig. 3a

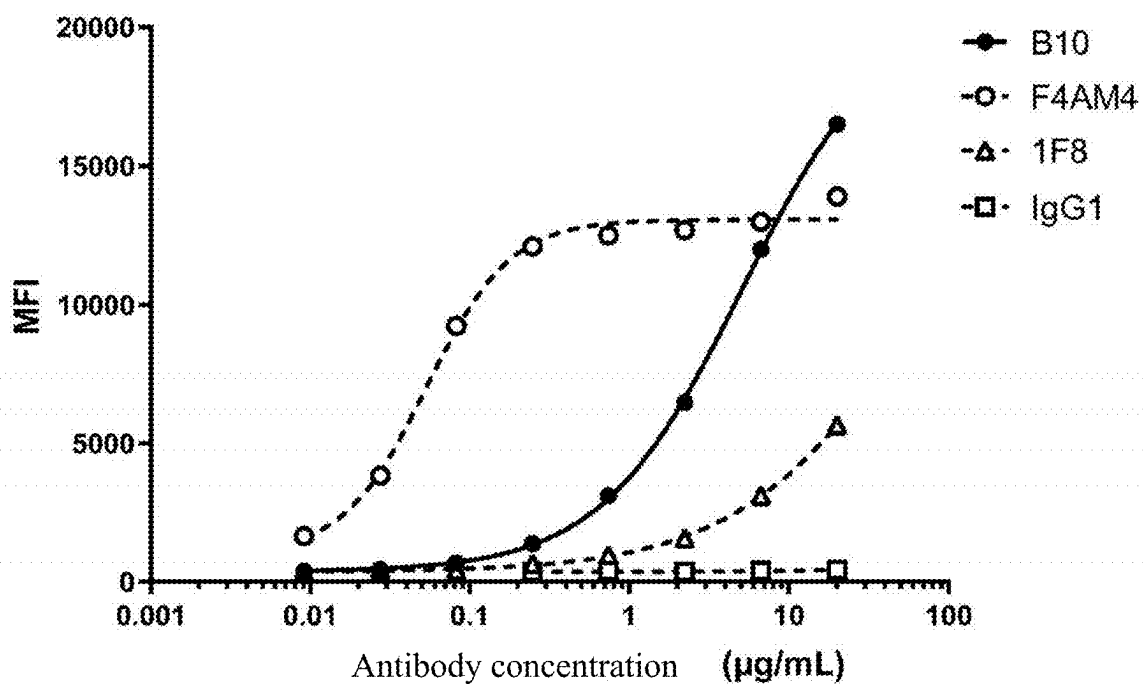


Fig. 3b

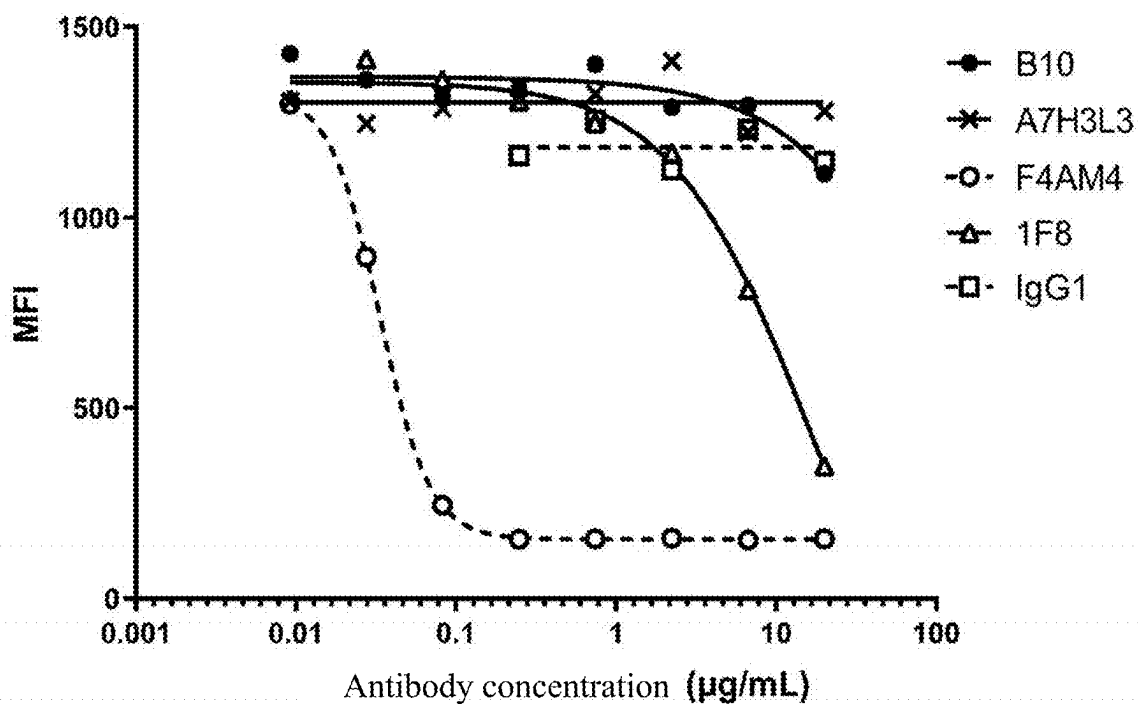


Fig. 4a

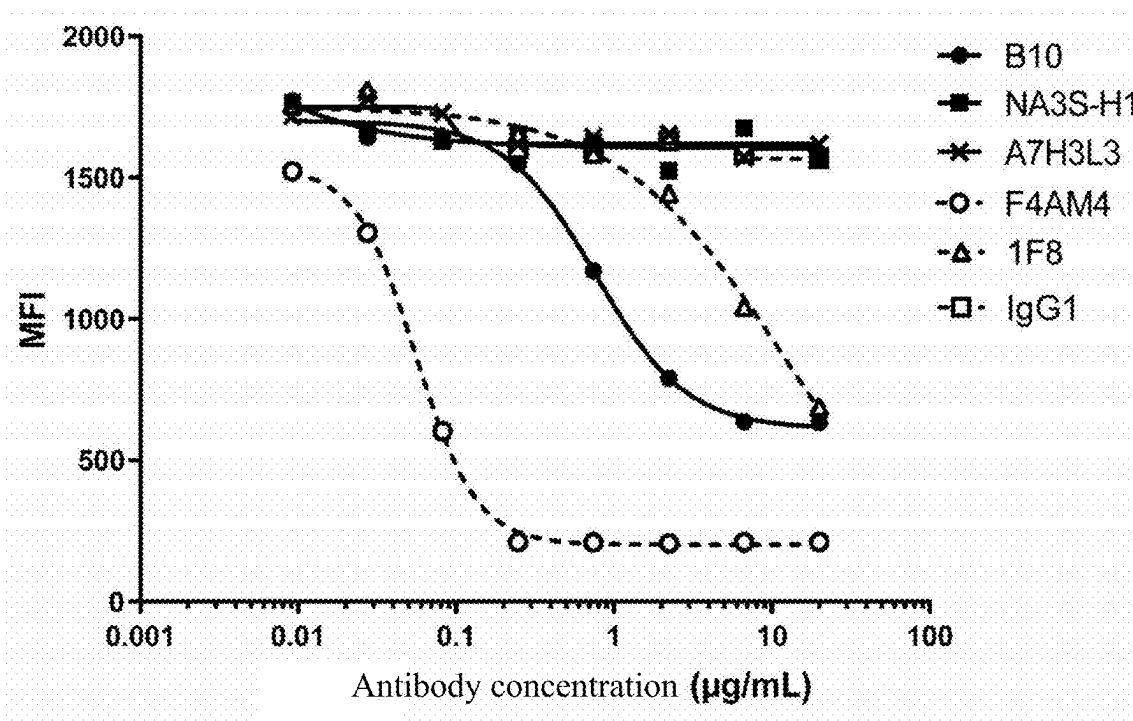


Fig. 4b

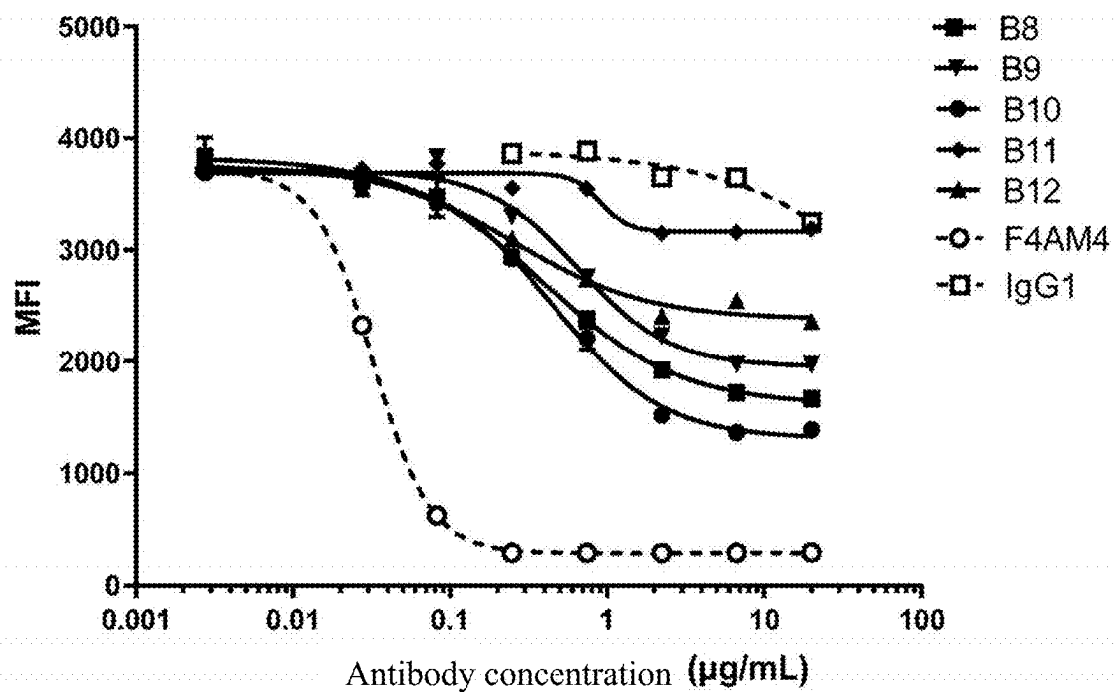


Fig. 4c

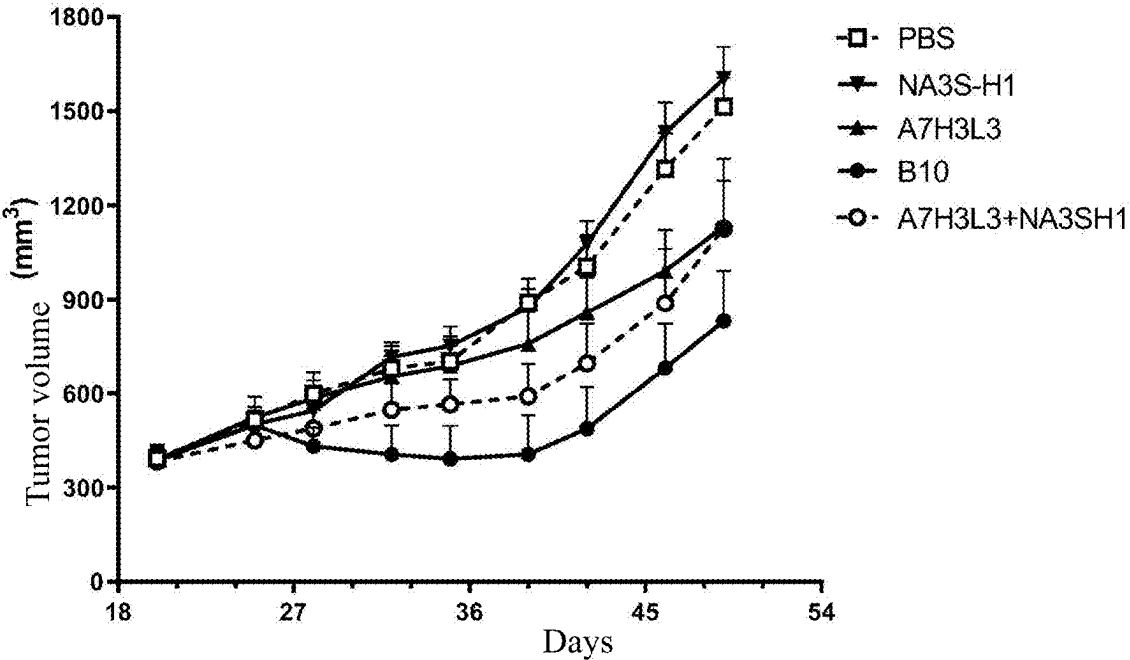


Fig. 5a

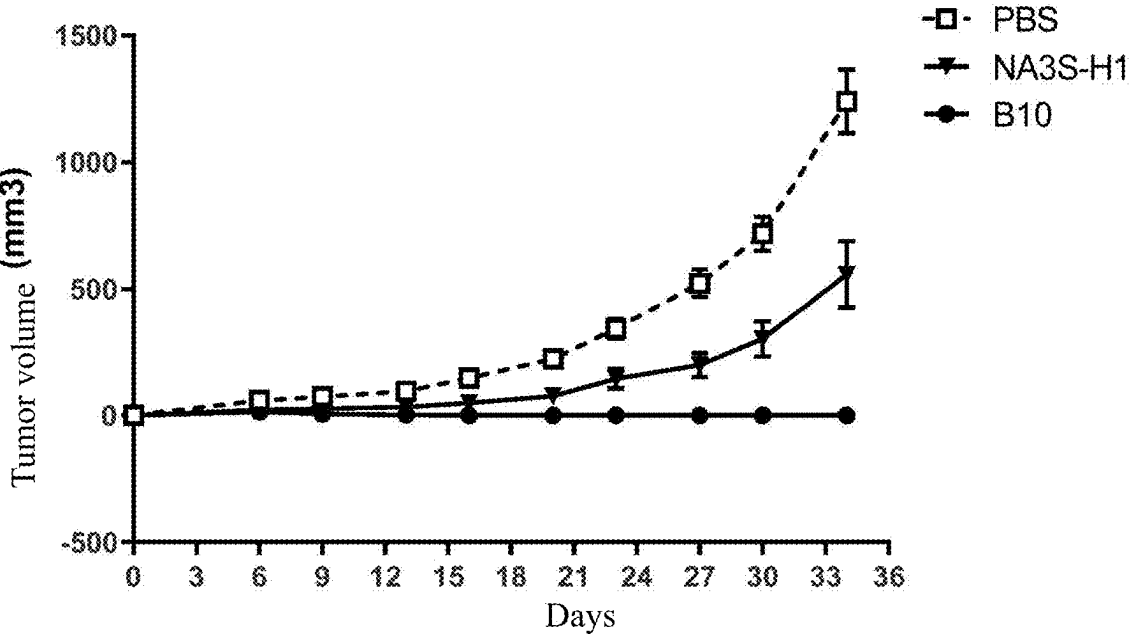


Fig. 5b

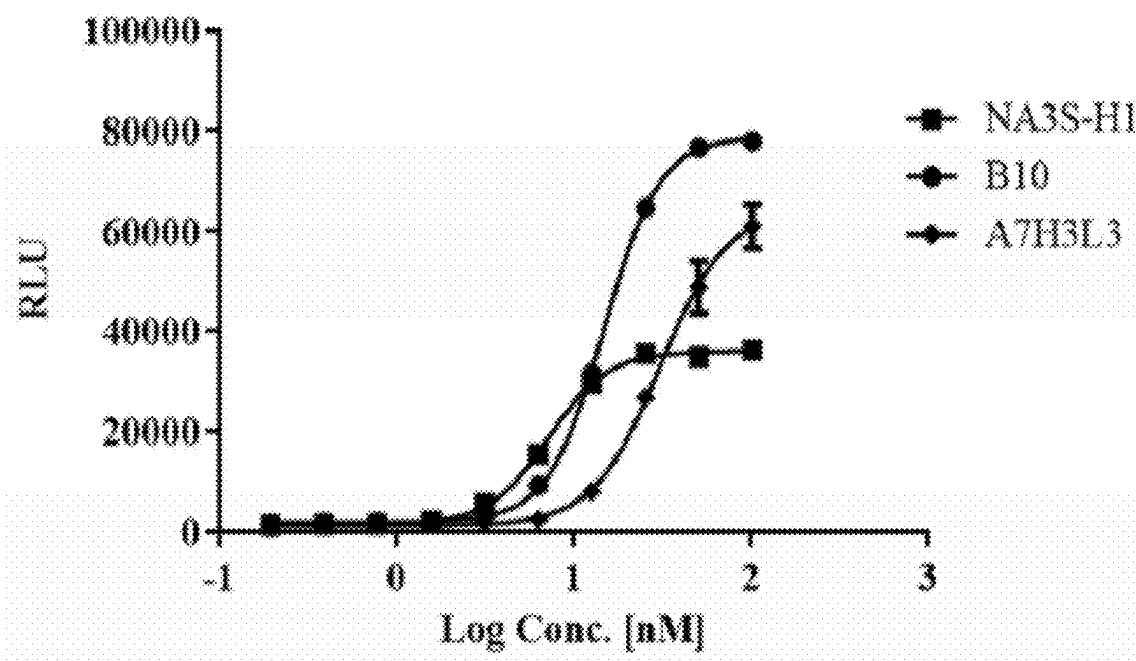


Fig. 6

**ANTI-CD47/ANTI-CLDN18.2 BISPECIFIC
ANTIBODY AND USE THEREOF**

[0001] The present application claims priority to Chinese Patent Application No. 202111214360.8 filed on Oct. 19, 2021, and entitled with “ANTI-CD47/ANTI-CLDN18.2 BISPECIFIC ANTIBODY AND USE THEREOF”, the content of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] The present invention belongs to the field of biomedicine. Specifically, the present invention relates to an anti-CD47/anti-CLDN18.2 bispecific antibody and the use thereof.

BACKGROUND ART

[0003] CD47 is a transmembrane glycoprotein widely expressed on cell surface, belongs to the immunoglobulin superfamily and can interact with signal regulatory protein alpha (SIRP α), thrombospondin-1 (TSP1) and integrins to mediate a series of responses such as cell apoptosis, proliferation and immunization. It has been demonstrated that the phagocytosis of tumor cells can be effectively mediated by blocking the CD47-SIRP α pathway with an anti-CD47 antibody, thereby inhibiting the growth of various hematological malignancies and solid tumors in vivo. However, CD47 is highly expressed not only on tumor cells, but also on normal cells, such as red blood cells. Therapies targeting CD47 may cause undesired side effects. Some anti-CD47 antibodies disclosed in the prior art (see, e.g., US20160304609) bind to red blood cells, which not only causes severe anemia, but also requires an administration dose of up to 30 mg/kg. These characteristics pose a huge challenge to the clinical application of anti-CD47 antibodies.

[0004] CLDN18 belongs to the Claudin family, which was discovered by Shoichiro Tsukita et al. in 1998. It is an important molecule for tight junctions of epithelial cells, determines the epithelial permeability, and also plays a role in blocking the diffusion of proteins and lipids on the surface of cell membranes (Gunzel, D. and A. S. Yu (2013) *Physical Rev.* 932:525-569). The human CLDN18 gene has two different exons 1 and is subject to alternative splicing after transcription to finally generate two protein subtypes CLDN18.1 and CLDN18.2 which merely vary in the N-terminal sequences. CLDN18.2 has currently become a very potential action target for anti-tumor drugs due to the expression specificity in tumor cells and normal tissues. WO 2016165762 A1 discloses an anti-CLDN18.2 antibody IMAB362 (Zolbetuximab), which significantly prolongs survival in a phase II clinical trial of gastric cancer relative to standard chemotherapy (13.2 months vs. 8.4 months) and shows a clear advantage in patients with high CLDN18.2 expression.

[0005] WO 2021003082 A1 discloses an anti-CD47/anti-CLDN18.2 bispecific antibody, which essentially does not bind to human red blood cells. However, there remains a need to develop a new bispecific antibody targeting CLDN18.2 and CD47 to provide more possibilities for cancer treatment.

SUMMARY OF THE INVENTION

[0006] In one aspect, the present invention provides a bispecific antibody comprising a first antigen binding portion that binds to CD47 and a second antigen binding portion that binds to CLDN18.2, wherein the first antigen binding portion comprises a heavy chain variable region (VH) and a light chain variable region (VL), the heavy chain variable region comprises: 1) HCDR1 comprising the amino acid sequence of SEQ ID NO: 4; 2) HCDR2 comprising the amino acid sequence of SEQ ID NO: 5; and 3) HCDR3 comprising the amino acid sequence of SEQ ID NO: 6; and the light chain variable region comprises: 1) LCDR1 comprising the amino acid sequence of SEQ ID NO: 7; 2) LCDR2 comprising the amino acid sequence of SEQ ID NO: 8; and 3) LCDR3 comprising the amino acid sequence of SEQ ID NO: 9.

[0007] In an embodiment, the second antigen binding portion comprises an immunoglobulin single variable domain (VHH) that binds to CLDN18.2. Preferably, the immunoglobulin single variable domain comprises: CDR1 comprising the amino acid sequence of SEQ ID NO: 13; CDR2 comprising the amino acid sequence of SEQ ID NO: 14; and CDR3 comprising the amino acid sequence of SEQ ID NO: 15.

[0008] In yet another aspect, the present invention provides an isolated polynucleotide encoding the bispecific antibody of the present invention.

[0009] The present invention also provides an expression vector comprising the polynucleotide of the present invention.

[0010] In another aspect, the present invention provides a host cell comprising the polynucleotide or expression vector of the present invention.

[0011] The present invention also relates to an antibody conjugate comprising the bispecific antibody of the present invention conjugated with at least one therapeutic agent.

[0012] In yet another aspect, the present invention relates to a pharmaceutical composition comprising the bispecific antibody or the antibody conjugate of the present invention, and a pharmaceutically acceptable carrier.

[0013] The present invention also relates to the use of the bispecific antibody, the antibody conjugate, or the pharmaceutical composition of the present invention in the preparation of a drug for treating cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. 1 shows a schematic structure of the anti-CD47/anti-CLDN18.2 bispecific antibody.

[0015] FIGS. 2a and 2b show the binding activity of the anti-CD47/anti-CLDN18.2 bispecific antibody to CD47 on red blood cells.

[0016] FIGS. 3a and 3b show the binding activity of the anti-CD47/anti-CLDN18.2 bispecific antibody on tumor cells expressing a single target and double targets, wherein FIG. 3a shows the binding activity of the anti-CD47/anti-CLDN18.2 bispecific antibody on NUGC-4 cells, and FIG. 3b shows the binding activity of the anti-CD47/anti-CLDN18.2 bispecific antibody on hCLDN18.2-NUGC-4 cells.

[0017] FIGS. 4a, 4b and 4c show the ability of the anti-CD47/anti-CLDN18.2 bispecific antibody to block the binding of human CD47 to the receptor SIRP α on tumor cells expressing a single target and double targets, wherein

FIG. 4a shows the ability of the anti-CD47/anti-CLDN18.2 bispecific antibody to block the binding of human CD47 to the receptor SIRP α on NUGC-4 cells, and FIGS. 4b and 4c show the ability of the anti-CD47/anti-CLDN18.2 bispecific antibody to block the binding of human CD47 to the receptor SIRP α on hCLDN18.2-NUGC-4 cells.

[0018] FIGS. 5a and 5b show the inhibitory effects of the anti-CD47/anti-CLDN18.2 bispecific antibody on tumor growth in mice.

[0019] FIG. 6 shows the ADCP activity of the anti-CD47/anti-CLDN18.2 bispecific antibody determined by the bioluminescent reporter gene method.

DETAILED DESCRIPTION OF EMBODIMENTS

Definitions

[0020] In the present invention, all scientific and technical terms used herein have the meanings typically understood by a person skilled in the art unless specified otherwise. In addition, the terms and laboratory operation steps related to the protein and nucleic acid chemistry, molecular biology, cell and tissue culture, microbiology and immunology used herein are terms and conventional steps that are widely used in the corresponding art. To better understand the present invention, definitions and explanations of related terms are provided below.

[0021] As used herein, the expressions “including”, “comprising”, “containing” and “having” are open-ended and indicate that the listed elements, steps or components are included, but additional unlisted elements, steps or components are not excluded. The expression “consisting of” does not include any unspecified element, step or component. The expression “consisting essentially of” means that the range is limited to the specified elements, steps or components, and optionally, elements, steps or components that do not significantly affect the basic and novel features of the claimed subject matter. It should be understood that the expressions “consisting essentially of” and “consisting of” are encompassed in the meaning of the expression “including”.

[0022] As used herein, an “antibody” refers to an immunoglobulin or a fragment thereof, which specifically binds to an antigen epitope through at least one antigen binding site. The definition of the antibody herein encompasses an antigen binding fragment. The term “antibody” includes a multispecific antibody (for example, a bispecific antibody), a human antibody, a non-human antibody, a humanized antibody, a chimeric antibody, a single domain antibody, and an antigen binding fragment. The antibody may be synthetic (e.g., produced by chemical or biological coupling), enzymatically treated, or recombinant. The antibody provided herein includes any immunoglobulin type (e.g., IgG, IgM, IgD, IgE, IgA, and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2), or subclass (e.g., IgG2a and IgG2b). The antibody may be “univalent”, “bivalent”, “trivalent” or “tetravalent” antibodies or antibodies with higher valency, which means that the antibody comprises 1, 2, 3, 4 or more antigen binding sites.

[0023] As used herein, a “full-length antibody” typically comprises four polypeptides: two heavy chains (HC) and two light chains (LC). Each light chain comprises a “light chain variable region (VL)” and a “light chain constant region (CL)” from the N-terminus (the amino terminus) to the C-terminus (the carboxyl terminus). Each heavy chain

contains a “heavy chain variable region (VH)” and a “heavy chain constant region (CH)” from the N-terminus to the C-terminus. In general, the heavy chain constant region of a full-length antibody may comprise CH1-hinge-CH2-CH3 from the N-terminus to the C-terminus. In certain immunoglobulin types (e.g., IgM and IgE), the heavy chain constant region may comprise CH1-hinge-CH2-CH3-CH4 from the N-terminus to the C-terminus.

[0024] The light chain variable region and the heavy chain variable region may each comprise three highly variable “complementarity determining regions (CDRs)” and four relatively conserved “framework regions (FRs)”, which are connected from the N-terminus to the C-terminus in the order of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. The CDR of a light chain variable region (CDRL or LCDR) herein may be referred to as LCDR1, LCDR2 and LCDR3, and the CDR of a heavy chain variable region (CDRH or HCDR) may be referred to as HCDR1, HCDR2 and HCDR3.

[0025] In the present invention, the amino acid sequences of CDRs are shown according to the rules of the AbM definition (the sequences in the claims of the present invention are also shown according to the rules of the AbM definition). However, it is well known to those skilled in the art that the CDR of an antibody may be defined by various methods in the art, for example, Chothia based on the three-dimensional structure of antibodies and the topology of CDR loops (see, for example, Chothia, C. et al., *Nature*, 342, 877-883 (1989); and Al-Lazikani, B. et al., *J. Mol. Biol.*, 273, 927-948 (1997)), Kabat based on antibody sequence variability (see, for example, Kabat, E. A. et al. (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242), AbM (Martin, A. C. R. and J. Allen (2007) “Bioinformatics tools for antibody engineering” in S. Dübel (ed.), *Handbook of Therapeutic Antibodies*. Weinheim: Wiley-VCH Verlag, pp. 95-118), Contact (MacCallum, R. M. et al., (1996) *J. Mol. Biol.* 262:732-745), IMGT (Lefranc, M.-P., 2011 (6), IMGT, the International ImMunoGeneTics Information System Cold Spring Harb Protoc.; and Lefranc, M.-P. et al., *Dev. Comp. Immunol.*, 27, 55-77 (2003)), and North CDR definition based on affinity propagation clustering using a large number of crystal structures. It should be understood by a person skilled in the art that unless otherwise specified, the terms “CDR” and “complementarity determining region” of a given antibody or region thereof (e.g., a variable region) should be understood to encompass a complementarity determining region as defined by any of the above-mentioned known schemes described in the present invention. Although the scope of protection as claimed in the claims of the present invention is based on the sequences shown according to the rules of the AbM definition, corresponding amino acid sequences according to the rules of other CDR definitions shall also fall within the scope of protection of the present invention.

[0026] Therefore, when it comes to defining an antibody with specific CDR sequences defined in the present invention, the scope of the antibody also encompasses such an antibody whose variable region sequences comprise the specific CDR sequences but whose claimed CDR boundaries are different from the specific CDR boundaries defined by

the present invention due to the application of a different scheme (for example, rules of different assignment systems or their combinations).

[0027] As used herein, the terms “framework region” and “FR” may be used interchangeably. As used herein, the term “framework region” residue or “FR” residue refers to those amino acid residues in the variable region of the antibody other than the CDR sequences defined above.

[0028] As used herein, a “single domain antibody (sdAb)” or a “nanobody” refers to an antibody comprising a single immunoglobulin variable domain (single variable domain) as a functional antigen binding fragment. Similar to the variable region of a full-length antibody, a single variable domain typically comprises CDR1, CDR2 and CDR3 that form an antigen binding site and a supporting framework region. The single variable domain may be, for example, a variable domain of a heavy chain antibody (VHH), a shark IgNAR variable domain, a human light chain antibody variable domain and a human heavy chain antibody variable domain.

[0029] As used herein, the term “antibody dependent cell-mediated phagocytosis” or “ADCP” refers to a cell-mediated process in which non-specific cytotoxic cells (for example, monocytes, macrophages, neutrophils, and dendritic cells) expressing Fc γ receptors (Fc γ R) recognize antibodies that bind to target cells (such as tumor cells), and then phagocytose target cells (such as tumor cells) as effector cells. In some embodiments, the anti-CD47/anti-CLDN18.2 bispecific antibody of the present invention mediates the ADCP targeting cancer cells expressing CLDN18.2, in particular cancer cells expressing CD47 and CLDN18.2.

[0030] As used herein, the terms “percent (%) sequence identity” and “sequence identity” of amino acid sequences have a well-recognized meaning in the art and refer to the percentage of the identical parts of two polypeptide sequences determined by sequence alignment (for example, by manual inspection or a well-known algorithm). The percentage can be determined using methods known to a person skilled in the art, for example, using an available computer software such as BLAST, BLAST-2, Clustal Omega and FASTA software.

[0031] An amino acid sequence “originated from” or “derived from” a reference amino acid sequence herein is partially or completely identical or homologous to the reference amino acid sequence. For example, an amino acid sequence derived from a human immunoglobulin heavy chain constant region may have at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the wild-type sequence of the human immunoglobulin heavy chain constant region from which the amino acid sequence is originated.

[0032] Non-critical regions in a polypeptide (for example, CDR regions of an antibody, non-critical amino acids of framework regions, amino acids of constant regions) can be modified, for example, by making substitution, addition and/or deletion of one or more amino acids without altering the function of the polypeptide. A person skilled in the art should understand that amino acids in the non-critical regions of a polypeptide can be substituted with suitable conservative amino acids while retaining its biological activity as a general rule (see, for example, Watson et al.,

Molecular Biology of the Gene, 4th Edition, 1987, The Benjamin/Cummings Pub. co., p. 224). Suitable conservative substitution is well known to a person skilled in the art. In some cases, the amino acid substitution is a non-conservative substitution. A person skilled in the art should understand that the performance of an antibody or an antibody fragment may be altered by making amino acid mutation or modification, for example, the antibody glycosylation type and the ability to form an interchain disulfide bond may be altered, or an active group may be provided for the preparation of an antibody conjugate. Antibodies or antigen binding fragments thereof containing such amino acid mutations or modifications are also encompassed in the scope of the bispecific antibody of the present invention.

[0033] The anti-CD47/anti-CLDN18.2 bispecific antibody or the polynucleotide encoding same according to the present invention may be isolated. As used herein, the expression “isolated” means that a substance (for example, a polynucleotide or a polypeptide) is isolated from the source or environment in which the substance exists, that is, the substance is substantially free of any other ingredients.

[0034] The terms “polynucleotide” and “nucleic acid” herein may be interchangeably used to represent an oligomer or a polymer comprising at least two linked nucleotides or nucleotide derivatives and may typically include deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

[0035] The term “vector” herein is a vehicle used to introduce a foreign polynucleotide into a host cell. When the vector is transformed into an appropriate host cell, the foreign polynucleotide can be amplified or expressed. The vector typically remains free, but may be designed to integrate the gene or a part thereof into the chromosome of the genome. As used herein, the definition of a “vector” encompasses a plasmid, a linearized plasmid, a viral vector, a cosmid, a phage vector, a phagemid, an artificial chromosome (for example, a yeast artificial chromosome and a mammalian artificial chromosome), etc. The viral vector includes but is not limited to a retrovirus vector (including a lentiviral vector), an adenovirus vector, an adeno-associated virus vector, a herpes virus vector, a poxvirus vector, a baculovirus vector, etc.

[0036] As used herein, the term “expression” refers to the production of an RNA and/or a polypeptide.

[0037] As used herein, the term “expression vector” refers to a vector capable of expressing a polynucleotide of interest (including DNA and RNA). For example, in an expression vector, a polynucleotide sequence (including DNA and RNA) encoding a polypeptide of interest may be operably linked to a regulatory sequence (such as a promoter and a ribosome binding site) that can affect the expression of the polynucleotide sequence. The regulatory sequence may comprise promoter and terminator sequences, and may optionally comprise an origin of replication, a selectable marker, an enhancer, a polyadenylation signal, etc. The expression vector may be a plasmid, a phage vector, a recombinant virus or other vector which results in the expression of a polynucleotides of interest upon introduction into an appropriate host cell. Suitable expression vectors are well known to a person skilled in the art. A person skilled in the art can prepare the expression vector as a vector that is replicable in a host cell, remains free in a host cell, or is integrated into the genome of a host cell genome as required.

[0038] As used herein, a “host cell” refers to a cell used to receive, maintain, replicate, or amplify a vector. The host

cell may also be used to express a polypeptide encoded by a polynucleotide or a vector. The host cell may be a eukaryotic cell or a prokaryotic cell. The prokaryotic cell is e.g., *E. coli* or *Bacillus subtilis*, and the eukaryotic cell is e.g., a yeast cell or an *Aspergillus* cell, an insect cell (such as a *Drosophila* S2 cell or an Sf9 cell), and an animal cell (such as a fibroblast, a CHO cell, a COS cell, a HeLa cell, a NSO cell, or a HEK293 cell).

[0039] As used herein, the term “treating” refers to ameliorating diseases/symptoms, for example, reducing or eliminating the diseases/symptoms, or preventing or slowing the occurrence, progression and/or deterioration of the diseases/symptoms. Therefore, the term “treating” includes preventing, treating, and/or curing.

[0040] As used herein, the term “pharmaceutically acceptable carrier” refers to a carrier that is pharmacologically and/or physiologically compatible with the subject and the active ingredient and that is well known in the art (see, for example, Remington’s Pharmaceutical Sciences, edited by Gennaro AR, 19th ed. Pennsylvania: Mack Publishing Company, 1995), including but not limited to: a pH adjusting agent, a surfactant, an adjuvant, an ion strength enhancer, a diluent, a reagent for maintaining the osmotic pressure, an absorption delaying agent, and a preservative. For example, the pH adjusting agent includes but is not limited to a phosphate buffer. The surfactant includes but is not limited to cationic, anionic or nonionic surfactants, such as Tween-80. The ion strength enhancer includes but is not limited to sodium chloride. The preservative includes but is not limited to various antibacterial reagents and antifungal reagents, such as parabens, chlorobutanol, phenol, and sorbic acid. The reagent for maintaining the osmotic pressure includes but is not limited to sugar, NaCl, and an analog thereof. The absorption delaying agent includes but is not limited to monostearate and gelatin. The diluent includes but is not limited to water, aqueous buffers (such as buffered saline), alcohols, polyols (such as glycerol), etc. The preservative includes but is not limited to various antibacterial reagents and antifungal reagents, such as thiomersal, 2-phenoxyethanol, parabens, chlorobutanol, phenol, and sorbic acid. A “stabilizer” has the meaning that is commonly understood by a person skilled in the art and can stabilize the desired activity of the active ingredient in a drug. The stabilizer includes but is not limited to sodium glutamate, gelatin, SPGA (Sucrose-Phosphate-Glutamate-Albumin), sugars (such as sorbitol, mannitol, starch, sucrose, lactose, dextran or glucose), amino acids (such as glutamic acid or glycine), proteins (such as dry whey, albumin or casein), or degradation products thereof (such as lactalbumin hydrolysate), etc.

[0041] As used herein, examples of mammals include but are not limited to human, non-human primate, rat, mouse, cattle, horse, pig, sheep, alpaca, dog, cat, etc. The term “subject” herein refers to a mammal, for example, a human. In some embodiments, the subject is a human. In some embodiments, the subject is a cancer patient, a human or an animal suspected of having cancer or at risk of having cancer.

Anti-CD47/Anti-CLDN18.2 Bispecific Antibody

[0042] The present invention provides an anti-CD47/anti-CLDN18.2 bispecific antibody comprising a first antigen binding portion that binds to CD47 and a second antigen binding portion that binds to CLDN18.2, wherein the first

antigen binding portion comprises a heavy chain variable region (VH) and a light chain variable region (VL), the heavy chain variable region comprises:

[0043] 1) HCDR1 comprising the amino acid sequence of SEQ ID NO: 4 or a variant thereof;

[0044] 2) HCDR2 comprising the amino acid sequence of SEQ ID NO: 5 or a variant thereof; and

[0045] 3) HCDR3 comprising the amino acid sequence of SEQ ID NO: 6 or a variant thereof; and

the light chain variable region comprises:

[0046] 1) LCDR1 comprising the amino acid sequence of SEQ ID NO: 7 or a variant thereof;

[0047] 2) LCDR2 comprising the amino acid sequence of SEQ ID NO: 8 or a variant thereof; and

[0048] 3) LCDR3 comprising the amino acid sequence of SEQ ID NO: 9 or a variant thereof;

[0049] wherein the variant has substitution, addition and/or deletion of one or two amino acids compared with the sequence from which the variant is originated.

[0050] In a specific embodiment, the first antigen binding portion comprises a first antigen binding portion that specifically binds to CD47, and the first antigen binding portion comprises a heavy chain variable region (VH) and a light chain variable region (VL),

the heavy chain variable region comprises:

[0051] 1) HCDR1 comprising the amino acid sequence of SEQ ID NO: 4;

[0052] 2) HCDR2 comprising the amino acid sequence of SEQ ID NO: 5; and

[0053] 3) HCDR3 comprising the amino acid sequence of SEQ ID NO: 6; and

the light chain variable region comprises:

[0054] 1) LCDR1 comprising the amino acid sequence of SEQ ID NO: 7;

[0055] 2) LCDR2 comprising the amino acid sequence of SEQ ID NO: 8; and

[0056] 3) LCDR3 comprising the amino acid sequence of SEQ ID NO: 9.

[0057] In an embodiment, the VH comprises: 1) the amino acid sequence of SEQ ID NO: 10; or 2) an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 10; and/or

[0058] the VL comprises: 1) the amino acid sequence of SEQ ID NO: 11; or 2) an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 11.

[0059] In an embodiment, the VH comprises the amino acid sequence of SEQ ID NO: 10, and the VL comprises the amino acid sequence of SEQ ID NO: 11.

[0060] The first antigen binding portion may comprise any form of an antigen binding fragment, for example, scFv, dsFv, scdsFv, Fab, Fab' or F(ab')₂. According to the present invention, the first antigen binding portion specifically binds to CD47 on the surface of cancer cells, but does not or substantially does not bind to CD47 on red blood cells, so that the bispecific antibody of the present invention does not cause red blood cell agglutination.

[0061] The second antigen binding portion may comprise any form of an antigen binding fragment, including but not limited to scFv, dsFv, scdsFv, Fab, Fab', F(ab')₂, and a single

variable domain. In some embodiments, the second antigen binding portion comprises an immunoglobulin single variable domain that specifically binds to CLD18.2. An immunoglobulin single variable domain specifically binding to CLD18.2 is described in for example, CN 112480248 A and WO 2020238730 A1, the contents of which are incorporated herein by reference in their entireties. In an embodiment, the immunoglobulin single variable domain comprises: CDR1 comprising the amino acid sequence of SEQ ID NO: 13 or a variant thereof; CDR2 comprising the amino acid sequence of SEQ ID NO: 14 or a variant thereof; and CDR3 comprising the amino acid sequence of SEQ ID NO: 15 or a variant thereof, wherein the variant has substitution, addition and/or deletion of one or two amino acids compared with the sequence from which the variant is originated. In a specific embodiment, the immunoglobulin single variable domain comprises: CDR1 comprising the amino acid sequence of SEQ ID NO: 13; CDR2 comprising the amino acid sequence of SEQ ID NO: 14; and CDR3 comprising the amino acid sequence of SEQ ID NO: 15. In an embodiment, the immunoglobulin single variable domain comprises the amino acid sequence of SEQ ID NO: 12. In yet another embodiment, the immunoglobulin single variable domain comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 12.

[0062] In some embodiments, the first antigen binding portion and the second antigen binding portion are connected through a linker. The linker may be a peptide linker or a chemical bond, preferably a peptide linker. An exemplary peptide linker may include but is not limited to polyglycine (G), polyalanine (A), polyserine(S), or a combination thereof, for example, GGAS, GGGS, GGGSG, or $(G-\text{S})_n$, wherein n is an integer of 1-20. Preferably, n is an integer of 1-5. In a specific embodiment, the peptide linker comprises the amino acid sequence of SEQ ID NO: 22 or SEQ ID NO: 23.

[0063] In some embodiments, the anti-CD47/anti-CLDN18.2 bispecific antibody of the present invention further comprises an immunoglobulin constant region. The immunoglobulin constant region may be a heavy chain constant region (CH) and a light chain constant region (CL) of an immunoglobulin of any species. The heavy chain constant region can be derived from a heavy chain constant region of an immunoglobulin of any subtype (e.g., IgA, IgD, IgE, IgG and IgM), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), or subclass (e.g., IgG2a and IgG2b) or a combination thereof. In a preferred embodiment, the heavy chain constant region at least comprises an Fc region, for example, the heavy chain constant region of IgG1 may comprise: all or part of the hinge region-CH2-CH3 or CH1-hinge-CH2-CH3. The light chain constant region may be derived from a 2 (Lambda) light chain constant region or k (Kappa) light chain constant region. In a preferred embodiment, the heavy chain constant region is a human IgG1 heavy chain constant region. In an embodiment, the heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 2. In a preferred embodiment, the light chain constant region is a human kappa light chain constant region. In an embodiment, the light chain constant region comprises the amino acid sequence of SEQ ID NO: 3.

[0064] In an embodiment, the VH and the VL of the first antigen binding portion are respectively fused to the N-terminus of the heavy chain constant region and the light chain constant region, and the single variable domain of the second antigen binding portion is optionally fused to the N-terminus of the VH, the N-terminus of the VL, the C-terminus of the heavy chain constant region or the C-terminus of the light chain constant region through a linker.

[0065] In some embodiments, the anti-CD47/anti-CLDN18.2 bispecific antibody comprises a first polypeptide and a second polypeptide, wherein the first polypeptide comprises the VH and the heavy chain constant region of the first antigen binding portion, the second polypeptide comprises the VL and the light chain constant region of the first antigen binding portion, and the single variable domain of the second antigen binding portion is optionally fused to the N-terminus of the VH or the VL or the C-terminus of the heavy chain constant region or the light chain constant region through a linker. In an embodiment, the single variable domain is optionally fused to the N-terminus of the VH or the C-terminus of the heavy chain constant region through a linker, and the first polypeptide may also be referred to as a fused heavy chain. In an embodiment, the fused heavy chain has the structure of formula (I) or formula (II) as described below. In another embodiment, the single variable domain of the second antigen binding portion is optionally fused to the N-terminus of the VL or the C-terminus of the light chain constant region through a linker, and the second polypeptide may also be referred to as a fused light chain. In an embodiment, the fused light chain has the structure of formula (IV) or formula (VI) as described below.

[0066] In an embodiment, the first polypeptide has the structure of formula (I):

VH-CH-Linker-VHH Formula (I),

the second polypeptide has the structure of formula (II):

VL-CL Formula (II),

wherein

[0067] the VH and the VL are respectively the heavy chain variable region and the light chain variable region of the first antigen binding portion as described above;

[0068] the VHH is the immunoglobulin single variable domain as described above;

[0069] the CH and the CL are respectively the heavy chain constant region and the light chain constant region as described above; and

[0070] the Linker is a linker.

[0071] In an embodiment, the first polypeptide has the structure of formula (I) and comprises the amino acid sequence of SEQ ID NO: 20; and the second polypeptide has the structure of formula (II) and comprises the amino acid sequence of SEQ ID NO: 17.

[0072] In an embodiment, the first polypeptide has the structure of formula (III):

VHH-Linker-VH-CH Formula (III),

and the second polypeptide has the structure of formula (II), wherein

[0073] the VHH is the immunoglobulin single variable domain as described above;

[0074] the VH is the heavy chain variable region of the first antigen binding portion as described above;

[0075] the CH is the heavy chain constant region as described above;

[0076] formula (II) is as defined above; and

[0077] the Linker is a linker.

[0078] In an embodiment, the first polypeptide has the structure of formula (III) and comprises the amino acid sequence of SEQ ID NO: 16; and the second polypeptide has the structure of formula (II) and comprises the amino acid sequence of SEQ ID NO: 17.

[0079] In an embodiment, the first polypeptide has the structure of formula (III) and comprises the amino acid sequence of SEQ ID NO: 21; and the second polypeptide has the structure of formula (II) and comprises the amino acid sequence of SEQ ID NO: 17.

[0080] In yet another embodiment, the first polypeptide has the structure of formula (III), and the second polypeptide has the structure of formula (IV):

VHH-Linker-VL-CL

Formula (IV),

wherein

[0081] formula (III) is as defined above;

[0082] the VHH is the immunoglobulin single variable domain as described above;

[0083] the VL is the light chain variable region of the first antigen binding portion as described above;

[0084] the CL is the light chain constant region as described above; and

[0085] the Linker is a linker.

[0086] In an embodiment, the first polypeptide has the structure of formula (III) and comprises the amino acid sequence of SEQ ID NO: 16; and the second polypeptide has the structure of formula (IV) and comprises the amino acid sequence of SEQ ID NO: 19.

[0087] In another embodiment, the first polypeptide has the structure of formula (V):

VH-CH

Formula (V),

and the second polypeptide has the structure of formula (IV), wherein

[0088] formula (IV) is as defined above;

[0089] the VH is the heavy chain variable region of the first antigen binding portion as described above;

[0090] the CH is the heavy chain constant region as described above; and

[0091] the Linker is a linker.

[0092] In an embodiment, the first polypeptide has the structure of formula (V) and comprises the amino acid sequence of SEQ ID NO: 18; and the second polypeptide has the structure of formula (IV) and comprises the amino acid sequence of SEQ ID NO: 19.

[0093] In another embodiment, the first polypeptide has the structure of formula (V), and the second polypeptide has the structure of formula (VI):

VL-CL-Linker-VHH

Formula (VI),

wherein

formula (V) is as defined above;

[0094] the VHH is the immunoglobulin single variable domain as described above;

[0095] the VL is the light chain variable region of the first antigen binding portion as described above;

[0096] the CL is the light chain constant region as described above; and

[0097] the Linker is a linker.

[0098] In some embodiments, the anti-CD47/anti-CLDN18.2 bispecific antibody of the present invention can:

[0099] 1) block the binding of CD47 on the surface of cancer cells to SIRPα;

[0100] 2) induce macrophages to phagocytose cancer cells expressing CD47 and CLDN18.2; and/or

[0101] 3) bind to cancer cells expressing CD47 and CLDN18.2 but does not or substantially does not bind to red blood cells.

Polynucleotide, Vector and Host Cell

[0102] In another aspect, the present invention provides an isolated polynucleotide, comprising a polynucleotide sequence encoding the anti-CD47/anti-CLDN18.2 bispecific antibody of the present invention.

[0103] The polynucleotide of the present invention can be obtained by using the methods known in the art, for example, by isolating from a phage display library, a yeast display library, an immunized animal, and an immortalized cell (for example, mouse B-cell hybridoma cells, EBV mediated immortalized B cells) or by chemical synthesis. The polynucleotide of the present invention can be codon optimized for a host cell for expression.

[0104] In yet another aspect, the present invention also provides a vector comprising the polynucleotide of the present invention. In some embodiments, the polynucleotide of the present invention is cloned into an expression vector. The expression vector may further comprise an additional polynucleotide sequence, for example, a regulatory sequence and an antibiotic resistance gene. The expression vector may also comprise a polynucleotide sequence encoding an additional polypeptide. The additional polypeptide can be, for example, a polypeptide that facilitates the detection and/or isolation of an antibody or an antigen binding fragment, including but not limited to an affinity tag (e.g., a polyhistidine tag (His₆) or a glutathione S-transferase (GST) tag), a polypeptide comprising a protease cleavage site, and a reporter protein (e.g., a fluorescent protein).

[0105] In an embodiment, the polynucleotide of the present invention is prepared as a recombinant nucleic acid. The recombinant nucleic acid can be prepared using techniques well known in the art, such as chemical synthesis, recombinant DNA technology (for example, polymerase chain reaction (PCR) technology), etc. (see Sambrook, J., E. F. Fritsch, and T. Maniatis. (1989). *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).

[0106] The polynucleotide of the present invention may be present in one or more expression vectors. In some embodiments, the expression vector is a DNA plasmid, for example, a DNA plasmid for expression in a bacterial cell, a yeast cell, or a mammalian cell. In some further embodiments, the expression vector is a viral vector. In other embodiments, the expression vector is a phage vector or a phagemid vector.

[0107] The present invention also provides a host cell comprising at least one polynucleotide or vector as described above. The polynucleotide or expression vector of the present invention can be introduced into a suitable host cell by using various methods known in the art. Such methods include but are not limited to lipofection, electroporation, viral transduction, calcium phosphate transfection, etc.

[0108] In a preferred embodiment, the host cell is used to express the anti-CD47/anti-CLDN18.2 bispecific antibody of the present invention. Examples of the host cell include but are not limited to a prokaryotic cell (such as, bacteria, e.g., *E. coli*) and a eukaryotic cell (such as, yeast, an insect cell, and a mammalian cell).

[0109] A mammalian host cell suitable for antibody expression includes but is not limited to a myeloma cell, a HeLa cell, a HEK cell (for example, a HEK 293 cell), a Chinese hamster ovary (CHO) cell, and other mammalian cells suitable for antibody expression.

[0110] The present invention also provides a method for producing the anti-CD47/anti-CLDN18.2 bispecific antibody of the present invention, which method comprises the following steps:

[0111] (I) cultivating the host cell of the present invention under suitable conditions to express the anti-CD47/anti-CLDN18.2 bispecific antibody, and

[0112] (II) isolating the antibody from the host cell or a culture thereof.

[0113] In some embodiments, a single vector comprising polynucleotide sequences encoding a heavy chain and a light chain is used. In some embodiments, two vectors respectively encoding the antibody light chain and the antibody heavy chain are used. In some embodiments, the host cell also comprises a chaperone plasmid, which may help to improve the solubility, stability, and/or folding of the antibody. Techniques for isolating and purifying an antibody from a host cell or culture media thereof are well known to a person skilled in the art.

Antibody Conjugate

[0114] The present invention also provides an antibody conjugate comprising the anti-CD47/anti-CLDN18.2 bispecific antibody of the present invention conjugated with at least one therapeutic agent. The antibody-drug conjugate (ADC) is a typical antibody conjugate, wherein the therapeutic agent may be, for example, a cytotoxic agent.

[0115] As used herein, “conjugation” refers to the interconnection of two or more parts by covalent or non-covalent interactions. In a preferred embodiment, the conjugation is covalent conjugation.

[0116] The therapeutic agent can be selected from a cytotoxic agent, a therapeutic antibody (e.g., an antibody or an antigen binding fragment thereof specifically binding to an additional antigen), a radioisotope, an oligonucleotide and an analog thereof (e.g., an interfering RNA), a bioactive peptide, a protein toxin (e.g., diphtheria toxin, ricin) and an enzyme (e.g., urease).

[0117] The cytotoxic agent refers to a substance that inhibits or reduces cell activity and function and/or kills the cells. Examples of the cytotoxic agent may include but are not limited to: maytansinoid (e.g., maytansine), auristatins (e.g., MMAF, MMAE, MMAD), duostatin, cryptophycin, vinca alkaloids (e.g., vinblastine, vincristine), colchicines, aplysiatoxins, taxane, taxol, docetaxel, cabazitaxel, enediyne antibiotics, cytochalasins, camptothecins, anthracycline antibiotics (e.g., daunorubicin, dihydroxyanthracin-dione, doxorubicin), cytotoxic antibiotics (e.g., mitomycin, actinomycin, duocarmycin (e.g., CC-1065), auromycin, duomycin, calicheamicin, endomycin, phenomycin), adriamycin, rubidomycin, calicheamicin, cisplatin, ethidium bromide, bleomycin, mitomycin, mithramycin, pladienolide, podophyllotoxin, etoposide, mitoxantrone, 5-fluorouracil,

cytarabine, gemcitabine, mercaptopurine, pentostatin, fludarabine, cladribine, nelarabine, carmustine, lomustine, methotrexate, melphalan, tenoposide, glucocorticoid, etc.

[0118] The radioisotope can be selected from, for example, ²¹²Bi, ²¹³Bi, ¹³¹I, ¹²⁵I, ¹¹¹In, ¹⁷⁷Lu, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁵³Sm, and ⁹⁰Y. A radioisotope-labeled antibody may also be referred to as a radioactive immunoconjugate.

[0119] In a preferred embodiment, the bioactive polypeptide is a polypeptide or protein having a therapeutic activity, binding activity, or enzyme activity. Non-limiting examples of the bioactive polypeptide may include but are not limited to: a protein toxin (e.g., diphtheria toxin, ricin), an enzyme (e.g., urease, horseradish peroxidase), and a cytokine.

[0120] In an embodiment, the therapeutic agent is a molecule having a biological activity against tumors. The molecule having a biological activity against tumors includes but is not limited to a cytotoxic agent, a chemotherapeutic agent, a radioisotope, an immune checkpoint inhibitor, an antibody targeting a tumor-specific antigen and other anti-tumor drugs. In a preferred embodiment, the therapeutic agent is a cytotoxic agent. In yet another preferred embodiment, the therapeutic agent is a radioisotope.

[0121] The therapeutic agent can be conjugated with the anti-CD47/anti-CLDN18.2 bispecific antibody of the present invention through a linker using any technique known in the art. The linker may comprise an active group for covalent conjugation, for example, amine, hydroxylamine, a maleimide group, carboxyl, phenyl, mercaptan, sulfhydryl or hydroxyl. The linker may be cleavable or non-cleavable. A cleavable linker is, for example, an enzymatically cleavable linker (e.g., a peptide comprising a protease cleavage site), a pH-sensitive linker (e.g., a hydrazone linker), or a reducible linker (e.g., a disulfide bond).

[0122] In an embodiment, the linker comprises an active group selected from amine, hydroxylamine, a maleimide group, carboxyl, phenyl, mercaptan, sulfhydryl and hydroxyl. In an embodiment, the linker is a chemical bond. In an embodiment, the linker comprises an amino acid or a peptide consisting of 2-10 amino acids. The amino acid may be natural or non-natural amino acids.

[0123] In some embodiments, the therapeutic agent and the linker are conjugated to form an intermediate prior to conjugation with the anti-CD47/anti-CLDN18.2 bispecific antibody of the present invention. In some embodiments, the intermediate is connected to the anti-CD47/anti-CLDN18.2 bispecific antibody of the present invention by forming a thioether bond with the sulfhydryl group of the bispecific antibody. The structure and preparation method of such intermediates and the method for preparing an antibody conjugate using such intermediates are described in, for example, International Patent Application Publication No. WO 2019114666, the relevant content of which is incorporated herein by reference in its entirety.

Pharmaceutical Composition

[0124] The present invention also provides a pharmaceutical composition comprising the anti-CD47/anti-CLDN18.2 bispecific antibody or the antibody conjugate of the present invention, and a pharmaceutically acceptable carrier.

[0125] The pharmaceutically acceptable carrier includes but is not limited to: a diluent, a binder and an adhesive, a lubricant, a disintegrant, a preservative, a vehicle, a dispersant, a glidant, a sweetener, a coating, an excipient, a preservative, an antioxidant (such as ascorbic acid, cysteine

hydrochloride, sodium bisulfate, sodium pyrosulfite, sodium sulfite, ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, citric acid, ethylene diamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, etc.), a solubilizer, a gelling agent, a softener, a solvent (such as, water, alcohol, acetic acid, and syrup), a buffer (such as, phosphate buffer, histidine buffer, and acetate buffer), a surfactant (for example, a nonionic surfactant, such as polysorbate 80, polysorbate 20, poloxamer or polyethylene glycol), an antibacterial agent, an antifungal agent, an isotonic agent (such as, trehalose, sucrose, mannitol, sorbitol, lactose, and glucose), an absorption retardant, a chelating agent, and an emulsifying agent. For a composition comprising an antibody or an antibody conjugate, a suitable carrier can be selected from a buffer (such as, citrate buffer, acetate buffer, phosphate buffer, histidine buffer, and histidine salt buffer), an isotonic agent (such as, trehalose, sucrose, mannitol, sorbitol, lactose, and glucose), a nonionic surfactant (such as, polysorbate 80, polysorbate 20, and poloxamer), or a combination thereof.

[0126] The pharmaceutical compositions provided herein may be in a variety of dosage forms, including but not limited to solid, semi-solid, liquid, powder or freeze-dried forms. Preferably, the pharmaceutical composition is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (such as by injection or infusion). For a composition comprising an antibody or an antibody conjugate, the preferred dosage form may generally be, for example, an injection and a freeze-dried powder.

[0127] The pharmaceutical composition provided herein may be administered to a subject by any method known in the art, for example, by systemic or local administration. Administration routes include but are not limited to parenteral (e.g., intravenous, intraperitoneal, intradermal, intramuscular, subcutaneous or intracavitary), topical (e.g., intratumoral), epidural or mucosal (e.g., intranasal, oral, vaginal, rectal, sublingual or topical) administration. It should be understood by a person skilled in the art that the exact administration dose will depend on various factors, for example, the pharmacokinetic properties of a pharmaceutical composition, the duration of the treatment, the excretion rate of a specific compound, the purpose of the treatment, administration routes, and the condition of the subject (e.g., the age, health status, body weight, sex, diet, medical history of the patient), and other factors well-known in the medical field. The administration method may be, for example, injection or infusion.

[0128] As a general guide, the administration dose of the anti-CD47/anti-CLDN18.2 bispecific antibody of the present invention may be in the range from about 0.0001 to 100 mg/kg, more typically from 0.01 to 20 mg/kg body weight of the subject. For example, the administration dose may be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight, 10 mg/kg body weight or 20 mg/kg body weight, or within the range of 1-20 mg/kg body weight. An exemplary treatment regimen entails administration once a week, once every two weeks, once every three weeks, once every four weeks, once a month, once every three months, once every 3-6 months, or with a short administration interval at the beginning and then an extended interval later. The mode of administration may be intravenous drip.

Treatment

[0129] In yet another aspect, the present invention relates to the use of the anti-CD47/anti-CLDN18.2 bispecific antibody, the antibody conjugate, or the pharmaceutical composition of the present invention in the preparation of a drug for treating a disease in a subject.

[0130] The present invention also relates to the anti-CD47/anti-CLDN18.2 bispecific antibody, the antibody conjugate, or the pharmaceutical composition of the present invention for use in the treatment of a disease.

[0131] The present invention also provides a method for treating a disease in a subject, which method comprises administering to the subject a therapeutically effective amount of the anti-CD47/anti-CLDN18.2 bispecific antibody, the antibody conjugate, or the pharmaceutical composition of the present invention.

[0132] In an embodiment, the disease as described above is cancer. As used herein, the “cancer” includes but is not limited to blood cancer and solid tumor. The cancer can also be a metastatic cancer. “Metastasis” refers to the spread of cancer cells to other parts of the body from their primary site. For example, the anti-CD47/anti-CLDN18.2 bispecific antibody may be used to treat a cancer that is CLDN18.2 positive. In a preferred embodiment, the anti-CD47/anti-CLDN18.2 bispecific antibody is used to treat a cancer that is CD47 and CLDN18.2 positive. In some embodiments, the cancer is gastric cancer.

Kit

[0133] The present invention also provides a kit comprising the anti-CD47/anti-CLDN18.2 bispecific antibody, the antibody conjugate, or the pharmaceutical composition of the present invention, and instructions for use. The kit may also comprise a suitable container. In certain embodiments, the kit also comprises an administration device. In general, the kit also includes a label for indicating the intended use and/or method of use of the contents of the kit. The term “label” includes any written or recorded material provided on or with the kit, or which otherwise accompanies the kit.

Beneficial Effects

[0134] The anti-CD47/anti-CLDN18.2 bispecific antibody of the present invention can achieve at least one of the beneficial effects of:

- [0135]** 1) blocking the binding of CD47 on the surface of cancer cells to SIRP α ;
- [0136]** 2) inducing macrophages to phagocytose cancer cells expressing CD47 and CLDN18.2;
- [0137]** 3) mediating ADCP targeting the cancer cells expressing CLDN18.2; and
- [0138]** 4) binding to cancer cells expressing CD47 and CLDN18.2 while not or substantially not binding to red blood cells.

[0139] In addition, compared with monoclonal antibodies targeting CD47 alone, the anti-CD47/anti-CLDN18.2 bispecific antibody of the present invention has a higher binding activity to tumor cells that are CD47 and CLDN18.2 positive, and is more potent in blocking CD47/SIRP α interactions.

EXAMPLES

[0140] The examples below are intended to merely illustrate the present invention and should therefore not be considered to limit the present invention in any way.

Materials and Methods

1. Construction of Antibody Expression Plasmid

[0141] DNA fragments encoding the antibody heavy chain and the antibody light chain were prepared using the recombinant DNA technology and then respectively cloned into the expression vector pcDNA3.4-TOPO (Invitrogen) to obtain the plasmids for expressing the antibody heavy chain and light chain. The plasmids for expressing the antibody heavy chain and light chain were amplified in *E. coli* DH5a and then extracted and purified.

2. Antibody Expression and Purification

[0142] All the antibodies were expressed by using the ExpiCHO transient expression system (Thermo Fisher, A29133) (see WO 2020238730 A1) and affinity purified by using MabSelect SuRe LX (GE, 17547403).

3. Antibody Purity Determination by SDS-PAGE

[0143] Preparation of a reducing solution: 2 μ g of an antibody to be tested or IPI (Ipilimumab; reference) was added to 5 \times SDS loading buffer (containing DTT at a final concentration of 5 mM). The resulting mixture was heated in a dry bath at 100° C. for 10 min, cooled to room temperature, and then centrifuged at 12000 rpm for 5 min, and the supernatant was taken. The supernatant was added to Bis-tris 4-15% gradient gel (Genscript) for protein gel electrophoresis, followed by Coomassie brilliant blue staining for color development of protein bands. After destaining, the protein bands were scanned by EPSON V550 color scanner. The purity was calculated for the protein bands by the peak area normalization method through ImageJ.

4. Antibody Purity Determination by SEC-HPLC

[0144] Agilent HPLC 1100 chromatographic column (XBridge BEH SEC 3.5 μ m, 7.8 mm I.D. \times 30 cm, Waters); the flow rate was set as 0.8 mL/min; injection volume: 20 μ L; and detector (VWD) wavelength: 280 nm and 214 nm. Mobile phase: 150 mmol/L phosphate buffer, pH 7.4. All samples were diluted to 0.5 mg/mL with the mobile phase, and then a blank solution and a sample solution were injected in sequence. The percentages of high polymer aggregate, antibody monomer and low molecular aggregate in the sample were calculated according to the area normalization method.

5. Antibody Stability Determination by Differential Scanning Fluorimetry

[0145] Differential scanning fluorimetry (DSF) can provide the information about the protein structure stability according to the fluorescence change process in a protein profile, detect the change of the protein configuration, and obtain a melting temperature (T_m) of the protein. 0.2 mg/mL antibody sample solution to be tested was prepared, and PBS and IPI (Ipilimumab; 0.2 mg/mL) were used as reference. Test sample was added in triplicate at 19 μ L/well to a

96-well plate (Nunc), and then 1 μ L of 100 \times SYPRO orange dye was added to each well. A pipette was used for mixing by pipetting until ready for testing. ABI 7500 FAST RT-PCR instrument was used for the thermal stability test of the sample, melting curve was selected as the test type, and a continuous mode was adopted with the scan temperature ranging 25-95° C. at the heating rate of 1%. Equilibration was allowed at 25° C. for 5 min and data was collected during the heating process. "ROX" was selected as the reporter group and "None" as the quenching group. The reaction volume was 20 μ L. The temperature corresponding to the first peak of the first derivative of the melting curve was determined as the antibody melting temperature T_m .

Example 1 Design of Anti-CD47/Anti-CLDN18.2 Bispecific Antibody

[0146] This example describes an exemplary anti-CD47/anti-CLDN18.2 bispecific antibody, wherein the arm that binds to CLDN18.2 adopts the anti-CLDN18.2 single domain antibody NA3S-H1 that specifically recognizes human CLDN18.2 but does not recognize CLDN18.1 (the amino acid sequences of CDR1, CDR2 and CDR3 are respectively set forth in SEQ ID NOs: 13, 14 and 15; and the amino acid sequence of VHH is set forth in SEQ ID NO: 12), and the arm that binds to CD47 adopts the antigen binding domain of the anti-CD47 humanized antibody A7H3L3 (the amino acid sequences of HCDR1, HCDR2 and HCDR3 are respectively set forth in SEQ ID NOs: 4, 5 and 6; the amino acid sequences of LCDR1, LCDR2 and LCDR3 are respectively set forth in SEQ ID NOs: 7, 8 and 9; and the amino acid sequence of the heavy chain variable region is set forth in SEQ ID NO: 10, and the amino acid sequence of the light chain variable region is set forth in SEQ ID NO: 11). The heavy chain variable region of the antibody A7H3L3 was fused to the human IgG1 heavy chain constant region (SEQ ID NO: 2) to form the heavy chain of the antibody A7H3L3, and the light chain variable region was fused to the human kappa light chain constant region (SEQ ID NO: 3) to form the light chain of the antibody A7H3L3. The anti-CLDN18.2 single domain antibody NA3S-H1 has been published in WO 2020238730 A1 and exhibited excellent efficacies in the in vitro cell killing (ADCC and CDC) assay and tumor inhibition trial on human CLDN18.2-HEK29T-SCID tumor transplantation model. The humanized antibody A7H3L3 binds poorly to CD47 on red blood cells and is an ideal candidate antibody for the bispecific antibody. Meanwhile, due to the binding to CLDN18.2, the design of the bispecific antibody enables the binding arm of the anti-CD47 antibody A7H3L3 to better bind to the tumor cells expressing double targets and to better block the SIRP α suppressive signal.

[0147] The bispecific antibody was designed according to the valence number, position, and linker length of the anti-CLDN18.2 single domain antibody (VHH) NA3S-H1. Five bispecific antibodies (B8-B12 respectively) were designed. The structures of the exemplary bispecific antibodies are shown in Table 1 and FIG. 1, and the corresponding amino acid sequences are provided in Table 2, wherein the sequence of Linker1 is GGGGSGGGGS (SEQ ID NO: 22), and the sequence of Linker2 is GGGGS (SEQ ID NO: 23).

TABLE 1

Structure of anti-CD47/anti-CLDN18.2 bispecific antibody		
Antibody name	Heavy chain structure	Light chain structure
B8	VHH ^{CLDN18.2} -Linker1-VH ^{CD47} -CH1-Hinge-CH2-CH3	VL ^{CD47} -CL
B9	VH ^{CD47} -CH1-Hinge-CH2-CH3	VHH ^{CLDN18.2} -Linker1-VL ^{CD47} -CL
B10	VH ^{CD47} -CH1-Hinge-CH2-CH3-Linker1-VHH ^{CLDN18.2}	VL ^{CD47} -CL
B11	VHH ^{CLDN18.2} -Linker1-VH ^{CD47} -CH1-Hinge-CH2-CH3	VHH ^{CLDN18.2} -Linker1-VL ^{CD47} -CL
B12	VHH ^{CLDN18.2} -Linker2-VH ^{CD47} -CH1-Hinge-CH2-CH3	VL ^{CD47} -CL

TABLE 2

Amino acid sequences of anti-CD47/anti-CLDN18.2 bispecific antibody		
Antibody name	Heavy chain amino acid sequence	Light chain amino acid sequence
B8	SEQ ID NO: 16	SEQ ID NO: 17
B9	SEQ ID NO: 18	SEQ ID NO: 19
B10	SEQ ID NO: 20	SEQ ID NO: 17
B11	SEQ ID NO: 16	SEQ ID NO: 19
B12	SEQ ID NO: 21	SEQ ID NO: 17

were diluted to 1×10⁷/mL with PBS, pipetted at 50 μL/well and added to a 96-well round bottom cell culture plate. Then an equal volume of the serially diluted antibody to be tested was added, fully mixed, and incubated at 4° C. for 1 h. After the culture was rinsed with FACS buffer for three times, 0.5 μg of PE-labeled goat anti-human IgG Fc antibody (Abcam, ab98596) was added, followed by incubation at 4° C. for 1 h. Subsequently, the cells were rinsed with FACS buffer for three times and then resuspended by adding 200 μL of FACS buffer, and finally the amount of the antibody binding to the red blood cells (expressed as mean fluorescence intensity (MFI)) was measured by a flow cytometer (Beckman, Cyto-FLEX AOO-1-1102).

[0150] The binding activity of the antibody to red blood cells is shown in FIGS. 2a and 2b. As shown in FIGS. 2a and 2b, even at a very high concentration (150 μg/mL), almost all the anti-CD47/anti-CLDN18.2 bispecific antibodies did not bind to the red blood cells or had a very low binding activity relative to the strong binding of F4AM4-IgG1 on red blood cells; and at a high concentration (15 μg/mL), there was no significant difference in the binding of red blood cells between B9, B10 and B11 and the negative control IgG1. The specific values are shown in Table 3. It can be seen therefrom that the bispecific antibody of the present invention has a low binding activity to CD47 on red blood cells, and would not substantially cause red blood cell agglutination.

TABLE 3

Mean fluorescence intensity (MFI) for binding of bispecific antibody on red blood cells								
	B8	B9	B10	B11	B12	A7H3L3	IgG1	F4AM4-IgG1
15 μg/mL MFI	988	239	534	238	1615	463	303	63000
150 μg/mL MFI	2145	1220	2493	847	2526	608	901	>63000

Example 2 Binding Activity of Anti-CD47/Anti-CLDN18.2 Bispecific Antibody to CD47 on Red Blood Cells

[0148] Flow cytometry was used to determine whether the bispecific antibody was bound to red blood cells. Another anti-CD47 antibody F4AM4-IgG1 (abbreviated as F4AM4 in the accompanying drawings; the amino acid sequence of the heavy chain is SEQ ID NO: 24, and the amino acid sequence of the light chain is SEQ ID NO: 25) developed by the applicant was used as a positive control antibody. F4AM4-IgG1 exhibited strong binding to CD47 of red blood cells in a series of functional validation experiments (conducted prior to the experiment in this example), and hence was selected as a positive control in this example.

[0149] The specific method was as follows: red blood cells were isolated from 1 mL of anticoagulant human blood, and the supernatant was aspirated following centrifugation. After the cells were rinsed twice with PBS, 1 mL of PBS was added to resuspend the red blood cells. The red blood cells

Example 3 Binding Activity of Anti-CD47/Anti-CLDN18.2 Bispecific Antibody on Tumor Cells Expressing Single Target and Double Targets

[0151] The binding activities of the anti-CD47/anti-CLDN18.2 bispecific antibody B10 to NUGC-4 cells (expressing endogenous CD47, purchased from BeNa Culture Collection (BNCC), No. BNCC341962) and hCLDN18.2-NUGC-4 cells (gastric cancer cell line NUGC-4 overexpressing exogenous human CLDN18.2 (amino acid sequence SEQ ID NO: 1) and also expressing endogenous CD47, which cell line was constructed by transfection with lentiviruses) were determined by flow cytometry. As a comparison, the binding activities of the antibody 1F8 (the 1F8 antibody in WO 2018075857 A1) and F4AM4-IgG1 to the two cell lines were also determined. Human IgG1 was used as an isotype negative control.

[0152] The specific method was as follows: 1×10⁵ NUGC-4 cells or hCLDN18.2-NUGC-4 cells were taken and centrifuged at a low speed (300 g), and the supernatant was removed. The cells at the bottom of the centrifuge tube were rinsed once with a formulated FACS buffer (1×PBS buffer containing 2% by volume of FBS). Subsequently, the

serially diluted antibody to be tested was added to the rinsed cells, which were then incubated at 4° C. for 1 h. After the culture was rinsed with the above-mentioned FACS buffer for three times, 0.5 µg of PE-labeled goat anti-human IgG Fc antibody (Abcam, ab98596) was added, followed by incubation at 4° C. for 1 h. Subsequently, the cells were rinsed with FACS buffer for three times and then resuspended with 200 µL of FACS buffer, and finally the amount of the antibody binding to the red blood cells (expressed as mean fluorescence intensity (MFI)) was measured by a flow cytometer (Beckman, CytoFLEX AOO-1-1102).

[0153] The binding activities of the antibody to the NUGC-4 cells and hCLDN18.2-NUGC-4 cells are respectively shown in FIGS. 3a and 3b. As shown in FIG. 3a, the binding activity of the bispecific antibody B10 on the tumor cell NUGC-4 was poor and was slightly weaker than that of the antibody 1F8. As shown in FIG. 3b, the bispecific antibody B10 had a significantly better binding activity on the hCLDN18.2-NUGC-4 cells expressing both CD47 and CLDN18.2 than the antibody 1F8. On this basis, although the binding of the bispecific antibody B10 on cells expressing the single target of CD47 is weaker than that of 1F8, its binding on cells expressing the double targets of CD47 and CLDN18.2 is superior to the binding of 1F8. The results demonstrate that the bispecific antibody B10 can bind to CLDN18.2 and CD47 in the tumor cells at the same time, and has an increased tumor cell-binding ability.

Example 4 Ability of Anti-CD47/Anti-CLDN18.2 Bispecific Antibody to Block SIRPα/CD47 Binding on Tumor Cells Expressing Single Target and Double Targets

[0154] Flow cytometry was used to determine the ability of the anti-CD47/anti-CLDN18.2 bispecific antibody to block the binding of CD47 on the NUGC-4 cells and hCLDN18.2-NUGC-4 cells to SIRPα. As a comparison, the ability of the antibodies 1F8, F4AM4-IgG1 and A7H3L3 to block the binding of CD47 on the NUGC-4 tumor cells and hCLDN18.2-NUGC-4 tumor cells to SIRPα was also determined.

[0155] The specific method was as follows: 1×10^5 NUGC-4 cells or hCLDN18.2-NUGC-4 cells were taken and centrifuged at a low speed (300 g), and the supernatant was removed. The cells at the bottom of the centrifuge tube were rinsed once with a formulated FACS buffer (1×PBS buffer containing 2% FBS). Subsequently, the serially diluted antibody to be tested was added to the rinsed cells, which were then incubated for 1 h. After the cells were rinsed twice with FACS buffer, 100 µL of 1 µg/mL SIRPα-mFc (ACRO, SIA-H52A8) was added, followed by incubation at 4° C. for 1 h. After the cells were rinsed with FACS buffer for three times, 100 µL of 1:200 diluted PE-labeled goat anti-mouse Fc (Abcam, ab98742) was added as the secondary antibody, followed by incubation at 4° C. for 1 h. After centrifugation, the supernatant was removed, the cells were resuspended by adding 200 µL of FACS buffer, and finally the amount of SIRPα-mFc binding to the cells (expressed as mean fluorescence intensity (MFI)) was measured by a flow cytometer (Beckman, CytoFLEX AOO-1-1102).

[0156] The results obtained from the NUGC-4 cells are as shown in FIG. 4a: the antibody F4AM4-IgG1 can effectively block the binding of CD47 and SIRPα with an IC_{50} of 0.033 µg/mL (0.226 nM); the antibody 1F8 had a weak blocking

effect with an IC_{50} of 15.36 µg/mL (105.6 nM); however, the bispecific antibody B10 had barely any blocking ability.

[0157] The results obtained from the hCLDN18.2-NUGC-4 cells are as shown in FIG. 4b: the antibody F4AM4-IgG1 also had a strong blocking ability on this tumor cell with an IC_{50} of 0.056 µg/mL (0.383 nM); compared with the blocking ability on NUGC-4 cells, the blocking ability of the bispecific antibody B10 on the hCLDN18.2-NUGC-4 cells was significantly improved and was superior to that of the antibody 1F8; and B10 and 1F8 blocked the binding of CD47 on hCLDN18.2-NUGC-4 to SIRPα with the IC_{50} of 0.765 µg/mL (4.476 nM) and 11.98 µg/mL (82.34 nM). Besides, due to the fact that the anti-CLDN18.2 single domain antibody NA3S-H1 was only bound to CLDN18.2, the antibody had no blocking effect. On this basis, although the blocking activity of the bispecific antibody B10 on cells expressing the single target of CD47 is weaker than that of the antibody 1F8, its blocking activity on cells expressing the double targets of CD47 and CLDN18.2 is significantly superior to that of the antibody 1F8 and hence the bispecific antibody has a stronger ability to block the binding of CD47 to the receptor SIRPα.

[0158] The blocking ability of other bispecific antibodies on the hCLDN18.2-NUGC-4 cells was also determined. The results are as shown in FIG. 4c. The bispecific antibody B10 among B8-B12 has the highest blocking activity.

Example 5 In Vivo Tumor Inhibition Experiment of Anti-CD47/Anti-CLDN18.2 Bispecific Antibody

5.1 In Vivo Tumor Inhibition Experiment 1

[0159] Female nude mice (6-7 weeks old, body weight: 16-18 g) were housed in an independent ventilated box with constant temperature and humidity. The rearing chamber was at the temperature of 21-24° C. and the humidity of 30-53%. The nude mice were injected with 3×10^6 hCLDN18.2-NUGC-4 cells subcutaneously in the left armpit (day 0). When the subcutaneous tumor of the mice reached the volume of around 300-400 mm³ (day 20), the mouse samples with too large or too small tumor volumes were culled. Randomized grouping was carried out according to the tumor volume (8 mice per group): PBS treatment group, NA3S-H1 monoclonal antibody administration group, A7H3L3 monoclonal antibody administration group, NA3S-H1+A7H3L3 co-administration group and bispecific antibody B10 administration group, respectively. With NA3S-H1 monoclonal antibody at 5 mg/kg as the standard, all the other drugs were dosed at equimolar dosages, namely, A7H3L3 monoclonal antibody: 9.4 mg/kg, the combination NA3S-H1+A7H3L3: 5 mg/kg+9.4 mg/kg, and the bispecific antibody B10: 10.6 mg/kg. Twice weekly dosing was given by alternate intraperitoneal injection (i.p.) and intravenous injection (i.v.). The length (mm) and width (mm) of the tumor were observed and recorded at any time, and the tumor volume (V) thereof was calculated, using the equations: $V = (\text{length} \times \text{width}^2) / 2$, tumor inhibition rate TGI (%) = $(1 - \text{average tumor volume in the administration group} / \text{average tumor volume in the PBS treatment group}) \times 100\%$.

[0160] The tumor inhibition results of the antibody are as shown in FIG. 5a and Table 4, from which it can be seen that: at this equimolar dosage, the NA3S-H1 monoclonal antibody administration group exhibited barely any tumor inhibition effect, while the other groups exhibited a certain tumor inhibition effect. The bispecific antibody B10, as the

best among them, achieved a tumor inhibition rate close to 54.54% before day 39 with the tumor size close to the initial tumor volume on day 20, which was also superior to the results of the combined administration (A7H3L3+NA3S-H1 (9.4+5 mpk)). This suggests that the binding of the bispecific antibody B10 to CLDN18.2 can enhance its blocking effect on the binding of CD47 and SIRPα.

TABLE 4

Tumor inhibition rate of bispecific antibody in mice				
Days	NA3S-H1	A7H3L3	B10	A7H3L3 + NA3S-H1
25	2.96%	-1.31%	3.41%	12.73%
28	8.76%	2.17%	27.69%	18.40%
32	-5.33%	3.73%	40.26%	19.30%
35	-6.94%	2.10%	44.31%	19.49%
39	2.01%	15.00%	54.54%	33.71%
42	-7.26%	14.65%	51.45%	30.84%
46	-8.69%	24.65%	48.33%	32.59%
49	-5.94%	25.18%	45.12%	25.62%

5.2 In Vivo Tumor Inhibition Experiment 2

[0161] Randomized grouping (8 mice per group): PBS treatment group, monoclonal antibody NA3S-H1 administration group, and bispecific antibody B10 administration group, respectively. The first administration was given on the day of inoculation (day 0), followed by a twice weekly dosing regimen. With NA3S-H1 monoclonal antibody at 2.5 mg/kg as the standard, the bispecific antibody B10 was dosed at an equimolar dosage, namely 5.3 mg/kg. The rest was consistent with Example 5.1.

[0162] The results are as shown in FIG. 5b. At this equimolar dosage, the NA3S-H1 monoclonal antibody administration group exhibited a certain tumor inhibition effect with a tumor inhibition rate of 54.99% (day 34); and in the bispecific antibody B10 group, complete tumor inhibition was achieved in all the mice and the tumor inhibition rate was nearly 100% (day 34). The results demonstrate that the tumor inhibition effect of the bispecific antibody B10 is significantly superior to that of the anti-CLDN18.2 monoclonal antibody NA3S-H1.

Example 6 Determination of Physicochemical Properties of Anti-CD47/Anti-CLDN18.2 Bispecific Antibody

6.1 Purity Determination by SDS-PAGE

[0163] The purity of the bispecific antibody B10 was determined using reducing SDS-PAGE. The apparent relative molecular weights of the main bands of the heavy and light chains of the bispecific antibody B10 were about 65 kD and 25 kD respectively, which were in line with the expected size, and the purity was about 90%.

6.2 Purity Determination by SEC-HPLC

[0164] The monomer purity of the bispecific antibody B10 was determined using SEC-HPLC. The monomer purity of the bispecific antibody B10 determined by SEC-HPLC was greater than 94%.

6.3 Thermal Stability Test of Bispecific Antibody B10 by DSF

[0165] The T_m value of the bispecific antibody B10 was determined using the DSF method to evaluate its thermal stability. The results show that the bispecific antibody B10 had two melting peaks, the T_m value of the first peak was 69.85±0.06° C., and the T_m value of the second peak was 80.60±0.16° C., indicating that the bispecific antibody B10 has a good thermal stability.

Example 7 ADCP Activity of Anti-CD47/Anti-CLDN18.2 Bispecific Antibody

[0166] Antibody dependent cell-mediated phagocytosis (ADCP) is an important mechanism by which therapeutic antibodies fight against viral infection or tumor cells. In this experiment, the ADCP activity of the bispecific antibody of the present invention was evaluated by the bioluminescent reporter gene method. In this method, genetically engineered Jurkat cells (BPS Bioscience Inc., 71273), which stably express FcγRIIa receptors and are driven by NFAT response elements to express luciferase (Int Immunopharmacol. 2021 November; 100:108-112), were used as effector cells. After recognizing the target cell, the antibody activates the NFAT response element in the cell by binding to FcγRIIa on the surface of the effector cell, which in turn drives the expression of luciferase. The activity of luciferase can be quantified by the bioluminescence method.

[0167] The samples to be tested (B10, A7H3L3, and NA3S-H1) were diluted to an initial reaction concentration of 200 nM, and 10 dilution gradients were made in 2-fold gradient. The diluted samples were added to a 96-well white bottom plate at 50 μL/well. Three replicate wells were set for each concentration gradient. PBS solution (150 μL/well) was supplemented to the edge wells of the 96-well white bottom plate. Then hCLDN18.2-NUGC-4 cells (target cells, 5×10⁵ cells/mL) were added to the 96-well white bottom plate at 50 μL/well and incubated at room temperature for 30 min. Then effector cells as described above (1.5×10⁶ cells/mL) were added at 50 μL/well. The 96-well white bottom plate was incubated in a 37° C., 5% CO₂ incubator for 6 h. Detection: the 96-well white bottom plate was taken out and was let to stand for 30 min at room temperature. The detection reagent luciferase substrate (Bio-Glo™ Luciferase Assay System Promega G7940) was added at 50 μL/well and allowed to react away from light at room temperature for 5-10 min, and the luminescence intensity (expressed as relative light unit (RLU)) was measured with a microplate reader. The log value of the antibody concentration was plotted against the RLU value at the corresponding concentration, the data was analyzed using Graphpad Prism software, and the EC₅₀ value was calculated.

[0168] Experimental results: it can be seen from FIG. 6 and Table 5 that when the target cells were hCLDN18.2-NUGC-4 cells, the bispecific antibody B10 of the present invention exhibited a higher ADCP activity compared with the anti-CD47 monoclonal antibody A7H3L3 and the anti-CLDN18.2 monoclonal antibody NA3S-H1.

TABLE 5

ADCP activity of bispecific antibody			
	NA3S-H1	B10	A7H3L3
EC ₅₀	7.169	11.69	25.64
R ²	0.9948	0.9895	0.9962

[0169] Although the specific embodiments of the present invention have been described in detail, it will be understood by a person skilled in the art that: according to all the teachings disclosed herein, various modifications and changes can be made to the details, and these changes are all within the protection scope of the present invention. The protection scope of the present invention is given by the appended claims and any equivalents thereof.

SEQUENCE LISTING

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<211> LENGTH: 261

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

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Gly Ile Ala Gly Ile Ile Ala Ala Thr Cys Met Asp Gln Trp Ser Thr
                20           25           30

Gln Asp Leu Tyr Asn Asn Pro Val Thr Ala Val Phe Asn Tyr Gln Gly
35           40           45

Leu Trp Arg Ser Cys Val Arg Glu Ser Ser Gly Phe Thr Glu Cys Arg
50           55           60

Gly Tyr Phe Thr Leu Leu Gly Leu Pro Ala Met Leu Gln Ala Val Arg
65           70           75           80

Ala Leu Met Ile Val Gly Ile Val Leu Gly Ala Ile Gly Leu Leu Val
85           90           95

Ser Ile Phe Ala Leu Lys Cys Ile Arg Ile Gly Ser Met Glu Asp Ser
100          105          110

Ala Lys Ala Asn Met Thr Leu Thr Ser Gly Ile Met Phe Ile Val Ser
115          120          125

Gly Leu Cys Ala Ile Ala Gly Val Ser Val Phe Ala Asn Met Leu Val
130          135          140

Thr Asn Phe Trp Met Ser Thr Ala Asn Met Tyr Thr Gly Met Gly Gly
145          150          155          160

Met Val Gln Thr Val Gln Thr Arg Tyr Thr Phe Gly Ala Ala Leu Phe
165          170          175

Val Gly Trp Val Ala Gly Gly Leu Thr Leu Ile Gly Gly Val Met Met
180          185          190

Cys Ile Ala Cys Arg Gly Leu Ala Pro Glu Glu Thr Asn Tyr Lys Ala
195          200          205

Val Ser Tyr His Ala Ser Gly His Ser Val Ala Tyr Lys Pro Gly Gly
210          215          220

Phe Lys Ala Ser Thr Gly Phe Gly Ser Asn Thr Lys Asn Lys Lys Ile
225          230          235          240

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<210> SEQ ID NO 2

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Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
115 120 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
130 135 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145 150 155 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225 230 235 240

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
245 250 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
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Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
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Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
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<223> OTHER INFORMATION: Human Kappa light chain constant region

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20 25 30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
35 40 45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
50 55 60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
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Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
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Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
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<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: A7H3L3-HCDR1

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<212> TYPE: PRT

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<220> FEATURE:

<223> OTHER INFORMATION: A7H3L3-HCDR2

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<210> SEQ ID NO 7

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Tyr Thr Ser Arg Ile His Ser
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<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: A7H3L3-VH

<400> SEQUENCE: 10

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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Ile
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Tyr Ile Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Lys Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp Gln Lys Phe
50 55 60

Gln Gly Arg Ala Thr Ile Thr Ala Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80

Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
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Val Thr Val Ser Ser
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<210> SEQ ID NO 11
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<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: A7H3L3-VL

<400> SEQUENCE: 11

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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn His
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Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
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Tyr Tyr Thr Ser Arg Ile His Ser Gly Val Pro Ser Ser Phe Arg Gly
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Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
   65                               70                               75                               80

Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Tyr Thr Leu Pro Phe
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Val Met Gly Trp Tyr Arg Gln Ala Pro Gly Lys Gln Arg Glu Leu Val
      35      40      45

Ala Gly Ile Ser Thr Gly Gly Thr Thr Asn Tyr Gly Asp Ser Val Lys
      50      55      60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
      65      70      75      80

Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn
           85      90      95

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<223> OTHER INFORMATION: NA3S-H1 CDR1

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<212> TYPE: PRT
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<223> OTHER INFORMATION: NA3S-H1 CDR2

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<400> SEQUENCE: 16

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Val Met Gly Trp Tyr Arg Gln Ala Pro Gly Lys Gln Arg Glu Leu Val
35 40 45
Ala Gly Ile Ser Thr Gly Gly Thr Thr Asn Tyr Gly Asp Ser Val Lys
50 55 60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80
Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn
85 90 95
Val Leu Val Val Ser Gly Ile Gly Ser Thr Leu Glu Val Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125
Gly Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro
130 135 140
Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys
145 150 155 160
Asp Ile Tyr Ile Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu
165 170 175
Trp Ile Gly Lys Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp Gln
180 185 190
Lys Phe Gln Gly Arg Ala Thr Ile Thr Ala Asp Thr Ser Thr Asn Thr
195 200 205
Ala Tyr Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr
210 215 220
Tyr Cys Ala Arg Gly Tyr Gly Ser Gly Phe Ala Tyr Trp Gly Gln Gly
225 230 235 240
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
245 250 255
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
260 265 270
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
275 280 285

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Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
 290                295                300

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
305                310                315                320

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
      325                330                335

Ser Asn Thr Lys Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp Lys
      340                345                350

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
      355                360                365

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
      370                375                380

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
385                390                395                400

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
      405                410                415

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
      420                425                430

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
      435                440                445

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
      450                455                460

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
465                470                475                480

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
      485                490                495

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
      500                505                510

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
      515                520                525

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
      530                535                540

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
545                550                555                560

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
      565                570                575

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Lys

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<210> SEQ ID NO 17
<211> LENGTH: 214
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: B8/B10/B12 light chain

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<400> SEQUENCE: 17

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1                5                10                15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn His
      20                25                30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
      35                40                45

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Tyr	Tyr	Thr	Ser	Arg	Ile	His	Ser	Gly	Val	Pro	Ser	Ser	Phe	Arg	Gly
50						55					60				
Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro
65					70					75					80
Glu	Asp	Ile	Ala	Thr	Tyr	Phe	Cys	Gln	Gln	Gly	Tyr	Thr	Leu	Pro	Phe
				85						90				95	
Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala
			100					105					110		
Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly
		115					120					125			
Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala
	130					135					140				
Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln
145					150					155					160
Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser
			165						170					175	
Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr
		180						185					190		
Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser
		195					200					205			
Phe	Asn	Arg	Gly	Glu	Cys										
		210													

<210> SEQ ID NO 18
 <211> LENGTH: 447
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: B9 heavy chain

<400> SEQUENCE: 18

Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ala
1				5					10					15	
Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Phe	Asn	Ile	Lys	Asp	Ile
		20					25						30		
Tyr	Ile	Tyr	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
	35					40						45			
Gly	Lys	Ile	Asp	Pro	Ala	Asn	Gly	Asn	Thr	Lys	Tyr	Asp	Gln	Lys	Phe
	50					55					60				
Gln	Gly	Arg	Ala	Thr	Ile	Thr	Ala	Asp	Thr	Ser	Thr	Asn	Thr	Ala	Tyr
65				70						75					80
Leu	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
			85						90					95	
Ala	Arg	Gly	Tyr	Gly	Ser	Gly	Phe	Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Leu
		100						105					110		
Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu
		115						120				125			
Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys
	130					135					140				
Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser
145					150					155					160
Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser
			165					170						175	

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Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser		
			180					185					190				
Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn		
		195					200					205					
Thr	Lys	Val	Asp	Lys	Lys	Ala	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His		
	210					215					220						
Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val		
	225				230						235				240		
Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr		
			245						250					255			
Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu		
			260					265					270				
Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys		
		275					280					285					
Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser		
	290					295					300						
Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys		
	305				310					315					320		
Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile		
			325					330						335			
Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro		
		340						345					350				
Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu		
		355					360					365					
Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn		
	370					375					380						
Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser		
	385				390					395					400		
Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg		
			405						410					415			
Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu		
			420					425					430				
His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys			
		435					440					445					

<210> SEQ ID NO 19

<211> LENGTH: 344

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: B9/B11 light chain

<400> SEQUENCE: 19

Gln	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly		
1				5						10				15			
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Ser	Ile	Phe	Asn	Ile	Pro		
		20						25					30				
Val	Met	Gly	Trp	Tyr	Arg	Gln	Ala	Pro	Gly	Lys	Gln	Arg	Glu	Leu	Val		
		35					40					45					
Ala	Gly	Ile	Ser	Thr	Gly	Gly	Thr	Thr	Asn	Tyr	Gly	Asp	Ser	Val	Lys		
	50					55					60						
Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Thr	Val	Tyr	Leu		
	65				70					75					80		

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Gln	Met	Asn	Ser	Leu	Lys	Pro	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Asn
			85						90					95	
Val	Leu	Val	Val	Ser	Gly	Ile	Gly	Ser	Thr	Leu	Glu	Val	Trp	Gly	Gln
			100					105					110		
Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly
		115						120					125		
Gly	Ser	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser
	130					135					140				
Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	Asp	Ile	Ser
145					150					155					160
Asn	His	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu
			165						170					175	
Leu	Ile	Tyr	Tyr	Thr	Ser	Arg	Ile	His	Ser	Gly	Val	Pro	Ser	Ser	Phe
		180						185					190		
Arg	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Thr	Leu	Thr	Ile	Ser	Ser	Leu
		195					200					205			
Gln	Pro	Glu	Asp	Ile	Ala	Thr	Tyr	Phe	Cys	Gln	Gln	Gly	Tyr	Thr	Leu
	210					215					220				
Pro	Phe	Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Thr	Val
225					230					235					240
Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys
				245					250					255	
Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg
			260				265						270		
Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn
		275					280					285			
Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser
	290					295					300				
Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys
305					310					315					320
Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr
			325						330					335	
Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys								
			340												

<210> SEQ ID NO 20

<211> LENGTH: 577

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: B10 heavy chain

<400> SEQUENCE: 20

Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ala
1			5						10					15	
Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Phe	Asn	Ile	Lys	Asp	Ile
		20						25					30		
Tyr	Ile	Tyr	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35				40						45			
Gly	Lys	Ile	Asp	Pro	Ala	Asn	Gly	Asn	Thr	Lys	Tyr	Asp	Gln	Lys	Phe
	50					55					60				
Gln	Gly	Arg	Ala	Thr	Ile	Thr	Ala	Asp	Thr	Ser	Thr	Asn	Thr	Ala	Tyr
65					70					75					80

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Leu	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	85	90	95
Ala	Arg	Gly	Tyr	Gly	Ser	Gly	Phe	Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	100	105	110
Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	115	120	125
Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	130	135	140
Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	145	150	155
Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	165	170	175
Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	180	185	190
Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	195	200	205
Thr	Lys	Val	Asp	Lys	Lys	Ala	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	210	215	220
Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	225	230	235
Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	245	250	255
Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	260	265	270
Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	275	280	285
Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	290	295	300
Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	305	310	315
Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	325	330	335
Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	340	345	350
Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	355	360	365
Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	370	375	380
Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	385	390	395
Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	405	410	415
Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	420	425	430
His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	Gly	435	440	445
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gln	Val	Gln	Leu	Val	Glu	Ser	450	455	460
Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	465	470	475
Ala	Ser	Gly	Ser	Ile	Phe	Asn	Ile	Pro	Val	Met	Gly	Trp	Tyr	Arg	Gln	480		

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	485		490		495
Ala Pro Gly	Lys Gln Arg Glu Leu Val Ala Gly Ile Ser Thr Gly Gly				
	500		505		510
Thr Thr Asn Tyr Gly Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg					
	515		520		525
Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro					
	530		535		540
Glu Asp Thr Ala Val Tyr Tyr Cys Asn Val Leu Val Val Ser Gly Ile					
545		550		555	560
Gly Ser Thr Leu Glu Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser					
	565		570		575

Ser

<210> SEQ ID NO 21
 <211> LENGTH: 572
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: B12 heavy chain

<400> SEQUENCE: 21

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly					
1	5		10		15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Ile Phe Asn Ile Pro					
	20		25		30
Val Met Gly Trp Tyr Arg Gln Ala Pro Gly Lys Gln Arg Glu Leu Val					
	35		40		45
Ala Gly Ile Ser Thr Gly Gly Thr Thr Asn Tyr Gly Asp Ser Val Lys					
	50		55		60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu					
65		70		75	80
Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn					
	85		90		95
Val Leu Val Val Ser Gly Ile Gly Ser Thr Leu Glu Val Trp Gly Gln					
	100		105		110
Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gln Val Gln					
	115		120		125
Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys					
	130		135		140
Val Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Ile Tyr Ile Tyr					
145		150		155	160
Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly Lys Ile					
	165		170		175
Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp Gln Lys Phe Gln Gly Arg					
	180		185		190
Ala Thr Ile Thr Ala Asp Thr Ser Thr Asn Thr Ala Tyr Leu Glu Leu					
	195		200		205
Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly					
	210		215		220
Tyr Gly Ser Gly Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val					
225		230		235	240
Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser					
	245		250		255

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Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys
 260 265 270
 Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu
 275 280 285
 Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu
 290 295 300
 Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr
 305 310 315 320
 Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val
 325 330 335
 Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro
 340 345 350
 Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
 355 360 365
 Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
 370 375 380
 Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 385 390 395 400
 Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 405 410 415
 Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
 420 425 430
 Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 435 440 445
 Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
 450 455 460
 Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
 465 470 475 480
 Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
 485 490 495
 Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 500 505 510
 Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
 515 520 525
 Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
 530 535 540
 Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 545 550 555 560
 Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 565 570

<210> SEQ ID NO 22
 <211> LENGTH: 10
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Linker1

<400> SEQUENCE: 22

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 1 5 10

<210> SEQ ID NO 23

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<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker2

<400> SEQUENCE: 23

Gly Gly Gly Gly Ser
1             5

<210> SEQ ID NO 24
<211> LENGTH: 452
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: F4AM4-IgG1 heavy chain

<400> SEQUENCE: 24

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1             5             10             15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Ser
                20             25             30

Val Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
        35             40             45

Gly Tyr Ile Asn Pro Tyr Thr Asp Gly Thr Lys Tyr Ala Gln Lys Phe
        50             55             60

Gln Gly Arg Ala Thr Leu Thr Ser Asp Lys Ser Thr Ser Thr Ala Tyr
65             70             75             80

Met Glu Phe Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85             90             95

Gly Arg Pro Tyr Tyr Gly Thr Arg Tyr Gly Ser Trp Phe Ala Tyr Trp
        100             105             110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
        115             120             125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
        130             135             140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145             150             155             160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
        165             170             175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
        180             185             190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
        195             200             205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Ala Glu Pro Lys Ser
        210             215             220

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225             230             235             240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
        245             250             255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        260             265             270

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His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 275 280 285
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 290 295 300
 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 305 310 315 320
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 325 330 335
 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 340 345 350
 Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
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 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 370 375 380
 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 385 390 395 400
 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 405 410 415
 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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 <223> OTHER INFORMATION: F4AM4-IgG1 light chain
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 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
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 35 40 45
 Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Asn Leu Gln Pro
 65 70 75 80
 Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Gly Lys Asn Tyr Pro Phe
 85 90 95
 Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
 100 105 110
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125

-continued

Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala
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Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln
145					150					155					160
Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser
				165					170					175	
Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr
				180				185					190		
Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser
	195						200				205				
Phe	Asn	Arg	Gly	Glu	Cys										
210															

1. A bispecific antibody comprising a first antigen binding portion that binds to CD47 and a second antigen binding portion that binds to CLDN18.2, wherein the first antigen binding portion comprises a heavy chain variable region (VH) and a light chain variable region (VL),

the heavy chain variable region comprises:

- 1) HCDR1 comprising the amino acid sequence of SEQ ID NO: 4;
- 2) HCDR2 comprising the amino acid sequence of SEQ ID NO: 5; and
- 3) HCDR3 comprising the amino acid sequence of SEQ ID NO: 6; and

the light chain variable region comprises:

- 1) LCDR1 comprising the amino acid sequence of SEQ ID NO: 7;
- 2) LCDR2 comprising the amino acid sequence of SEQ ID NO: 8; and
- 3) LCDR3 comprising the amino acid sequence of SEQ ID NO: 9.

2. The bispecific antibody of claim 1, wherein

the heavy chain variable region comprises: 1) the amino acid sequence of SEQ ID NO: 10; or 2) an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 10; and/or

the light chain variable region comprises: 1) the amino acid sequence of SEQ ID NO: 11; or 2) an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 11.

3. The bispecific antibody of claim 1, wherein the second antigen binding portion comprises an immunoglobulin single variable domain (VHH) that binds to CLDN18;

wherein the immunoglobulin single variable domain comprises:

- CDR1 comprising the amino acid sequence of SEQ ID NO: 13;
- CDR2 comprising the amino acid sequence of SEQ ID NO: 14; and
- CDR3 comprising the amino acid sequence of SEQ ID NO: 15.

4. The bispecific antibody of claim 3, wherein the first antigen binding portion and the second antigen binding portion are connected through a linker.

5. The bispecific antibody of claim 3, which further comprises an immunoglobulin heavy chain constant region (CH) and an immunoglobulin light chain constant region (CL).

6. The bispecific antibody of claim 1, which comprises a first polypeptide and a second polypeptide, wherein

the first polypeptide has the following structure from the N-terminus to the C-terminus:

VH-CH-Linker-VHH,

the second polypeptide has the following structure from the N-terminus to the C-terminus:

VL-CL,

wherein

the VH and the VL are respectively the heavy chain variable region and the light chain variable region as defined in claim 1;

the VHH is an immunoglobulin single variable domain that binds to CLDN18.2, wherein the immunoglobulin single variable domain comprises:

CDR1 comprising the amino acid sequence of SEQ ID NO: 13;

CDR2 comprising the amino acid sequence of SEQ ID NO: 14; and

CDR3 comprising the amino acid sequence of SEQ ID NO: 15;

the CH and the CL are respectively a heavy chain constant region and a light chain constant region; and the Linker is a linker.

7. An isolated polynucleotide encoding the bispecific antibody of claim 1.

8. An expression vector comprising the polynucleotide of claim 7.

9. A host cell comprising the expression vector of claim 8.

10. An antibody conjugate comprising the bispecific antibody of claim 1 conjugated with at least one therapeutic agent.

11. A pharmaceutical composition comprising the bispecific antibody of claim 1, and a pharmaceutically acceptable carrier.

12. A method treating cancer in a subject in need thereof, comprising administering to the subject an effective amount of the bispecific antibody of claim 1; preferably, the cancer is gastric cancer.

13. The bispecific antibody of claim 1, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 10, and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 11.

14. The bispecific antibody of claim 3, wherein the immunoglobulin single variable domain comprises: 1) the amino acid sequence of SEQ ID NO: 12; or 2) an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 12.

15. The bispecific antibody of claim 3, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 10, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 11, and/or the immunoglobulin single variable domain (VHH) comprises the amino acid sequence of SEQ ID NO: 12.

16. The bispecific antibody of claim 15, which further comprises an immunoglobulin heavy chain constant region

(CH) and an immunoglobulin light chain constant region (CL), wherein the heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 2; and/or the light chain constant region comprises the amino acid sequence of SEQ ID NO: 3.

17. The bispecific antibody of claim 5, wherein the heavy chain constant region is a human IgG1 heavy chain constant region, and/or the light chain constant region is a human kappa light chain constant region.

18. The bispecific antibody of claim 5, wherein the heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 2; and/or the light chain constant region comprises the amino acid sequence of SEQ ID NO: 3.

19. The bispecific antibody of claim 6, wherein the VH comprises the amino acid sequence of SEQ ID NO: 10, the VL comprises the amino acid sequence of SEQ ID NO: 11, and/or the VHH comprises the amino acid sequence of SEQ ID NO: 12.

20. The bispecific antibody of claim 6, wherein the first polypeptide comprises the amino acid sequence of SEQ ID NO: 20, and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 17.

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