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(54) **ANTI-CD24 ANTIBODY AND USE THEREOF**

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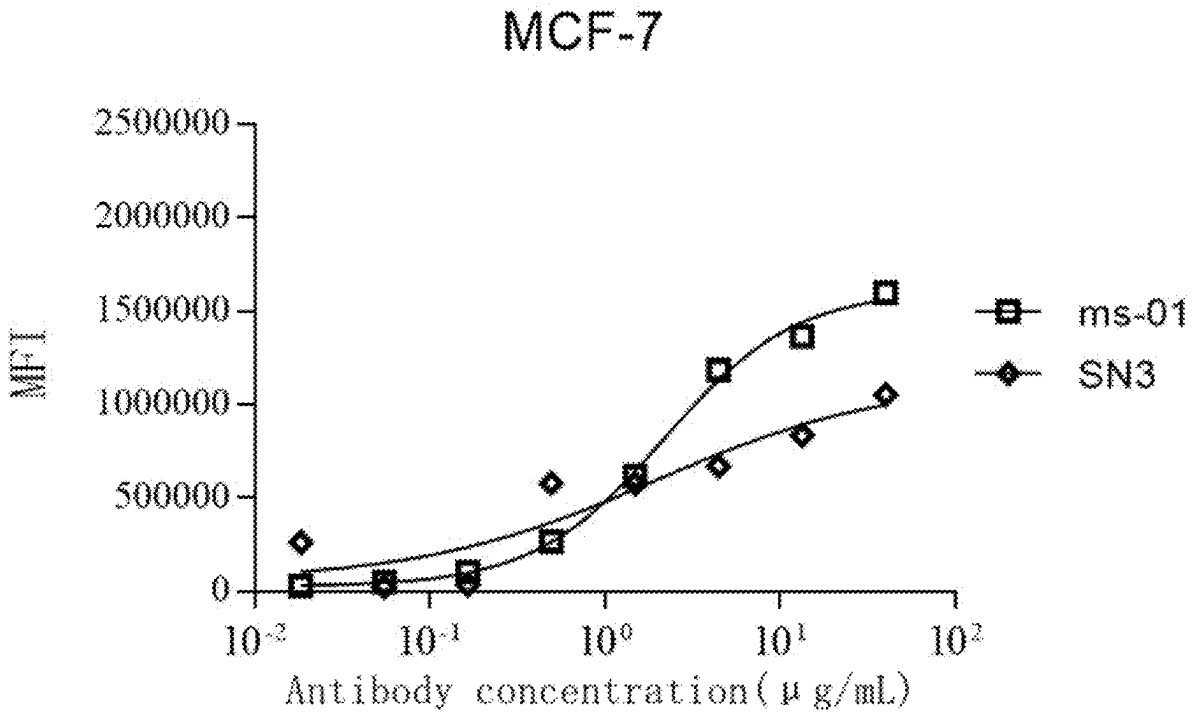
(57) **ABSTRACT**

An anti-CD24 antibody or antigen-binding fragment thereof capable of binding to CD24, and/or capable of exerting an anti-tumor effect through an antibody Fc fragment, and use thereof.

Specification includes a Sequence Listing.



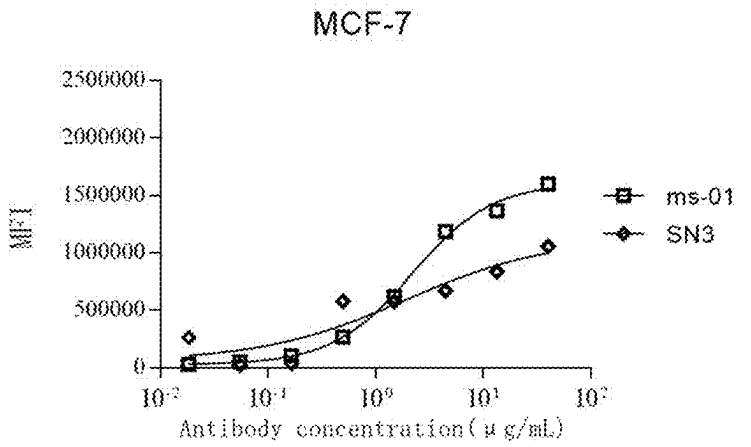


FIG. 1

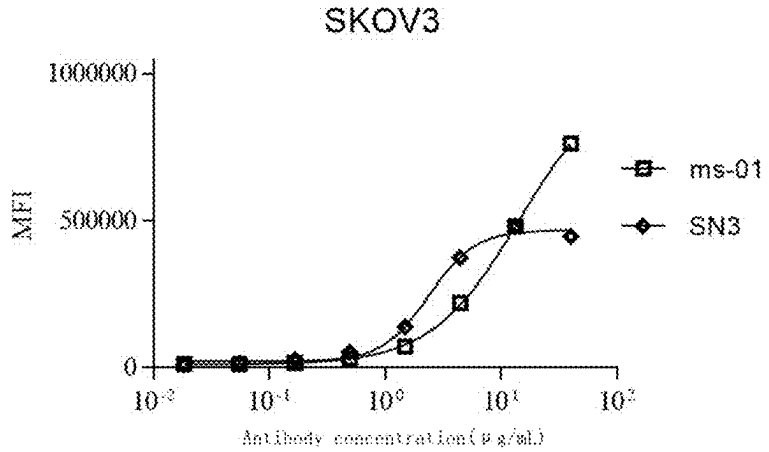
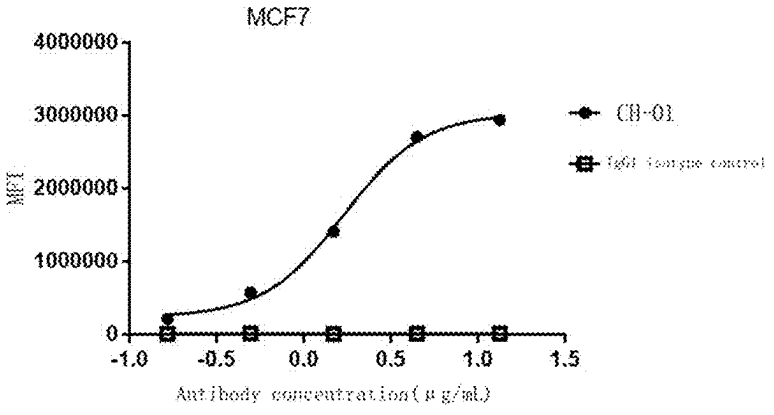


FIG. 2



	CH-01	IgG1
EC50	1.685	~ 0.1417

FIG. 3a

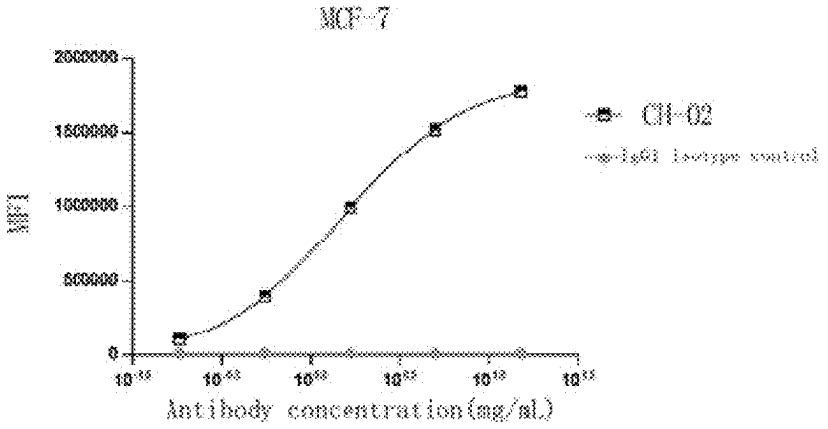
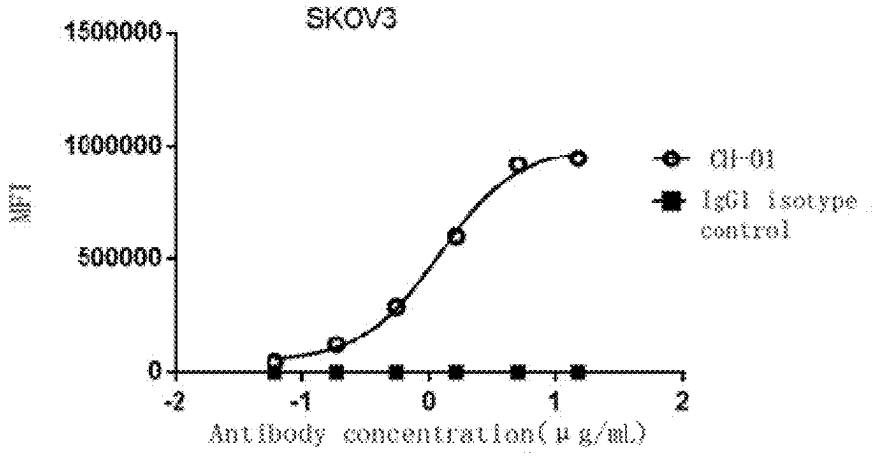


FIG. 3b



	CH-01	IgG1
EC50	1.201	~ 49.66

FIG. 4a

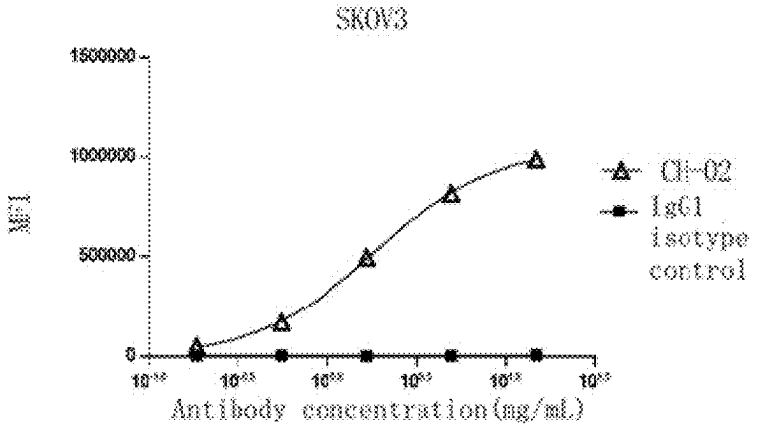


FIG. 4b

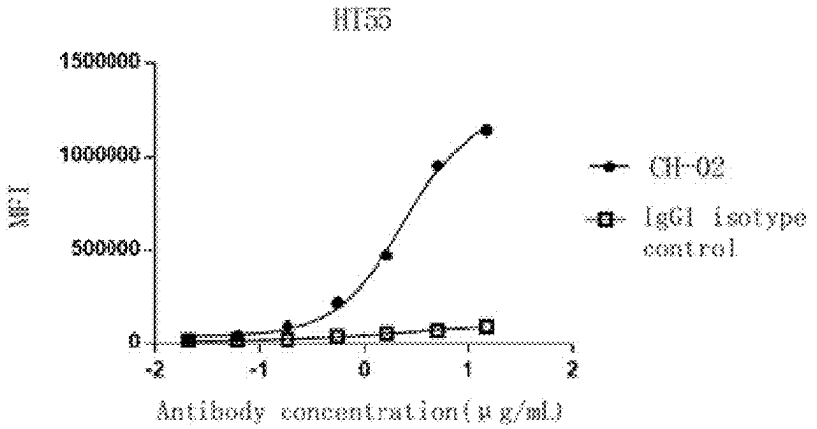


FIG. 5

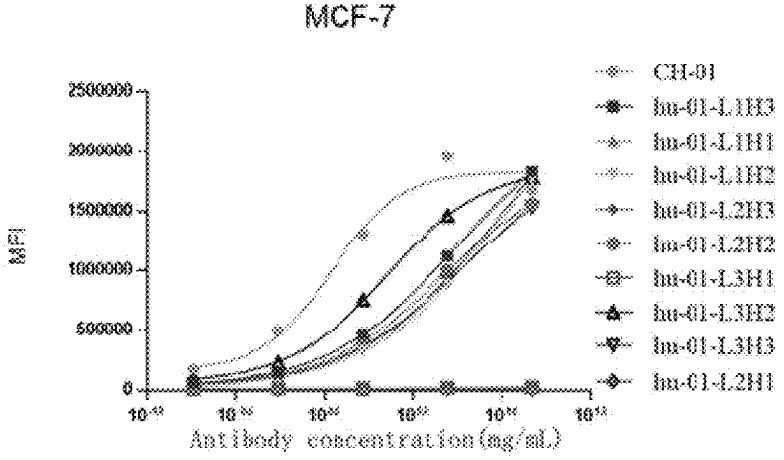


FIG. 6a

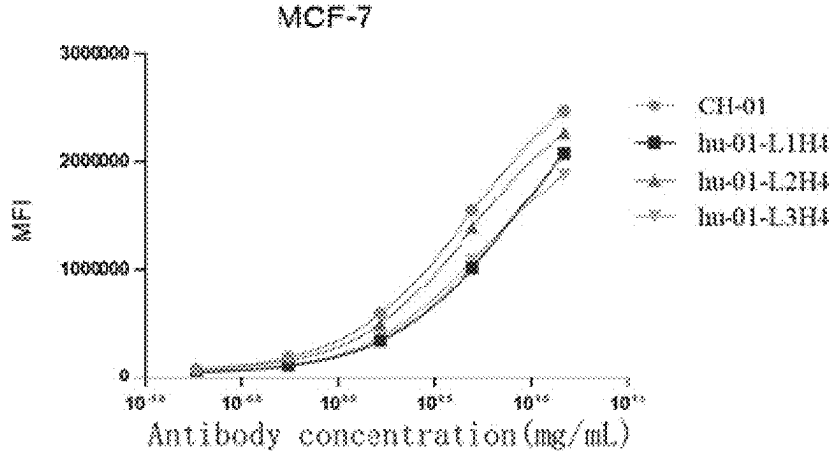


FIG. 6b

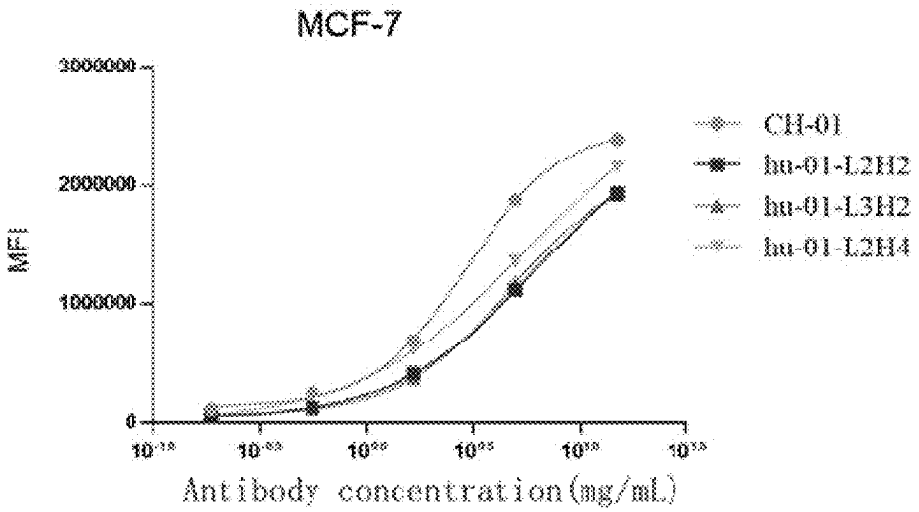


FIG. 6c

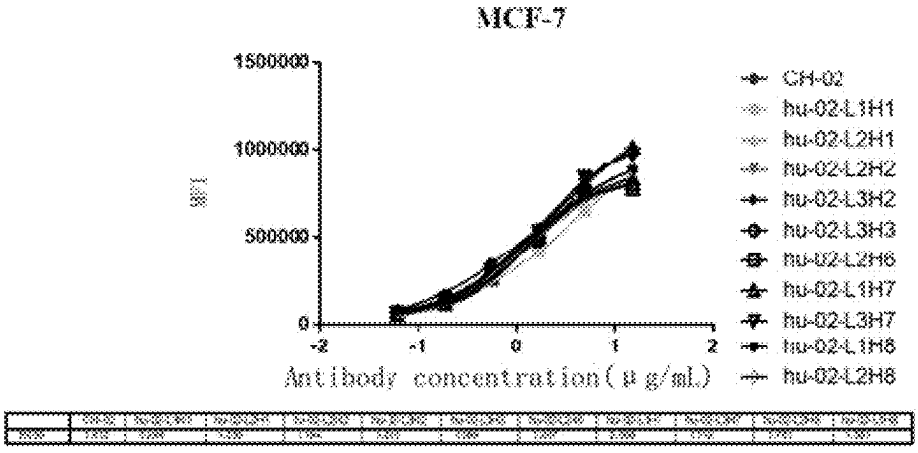


FIG. 6d

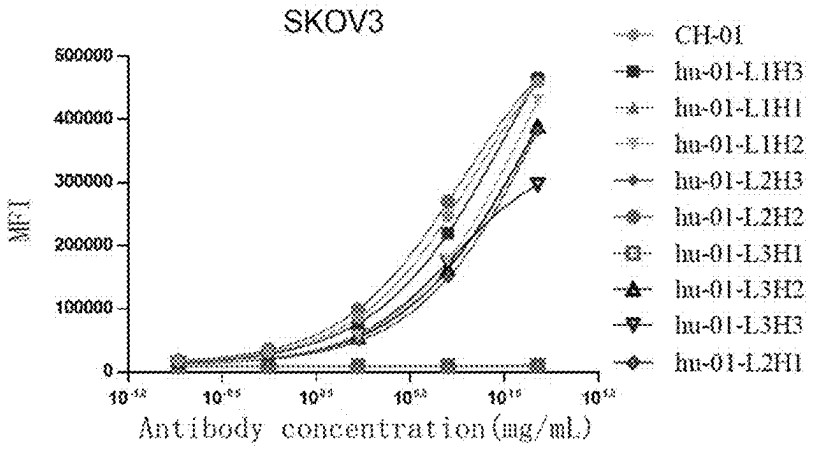


FIG. 7a

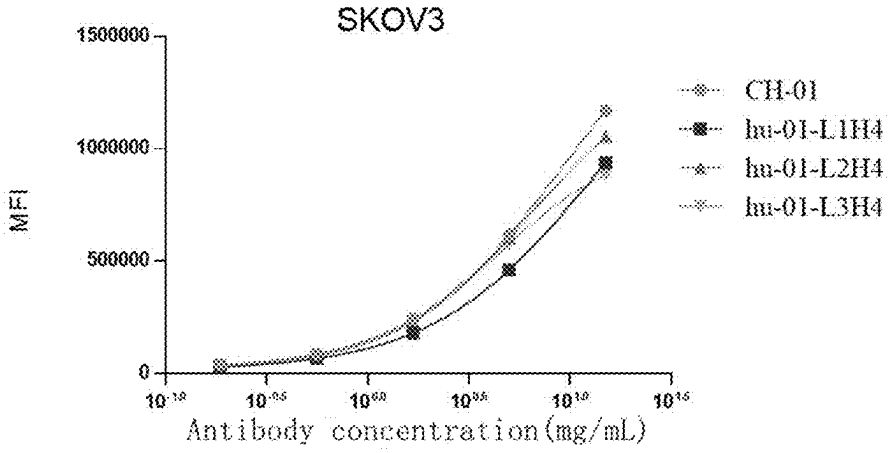

SKOV3
1500000
1000000
500000
0
MFI
CH-01
hu-01-L1H4
hu-01-L2H4
hu-01-L3H4
Antibody concentration(mg/mL)

FIG. 7b

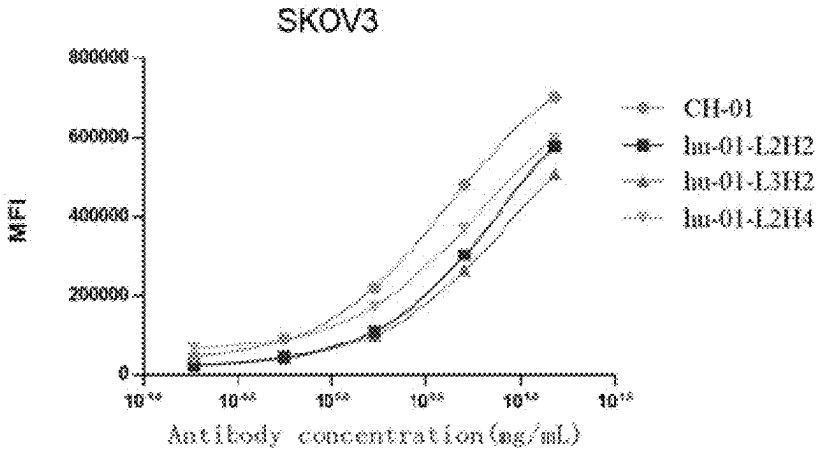
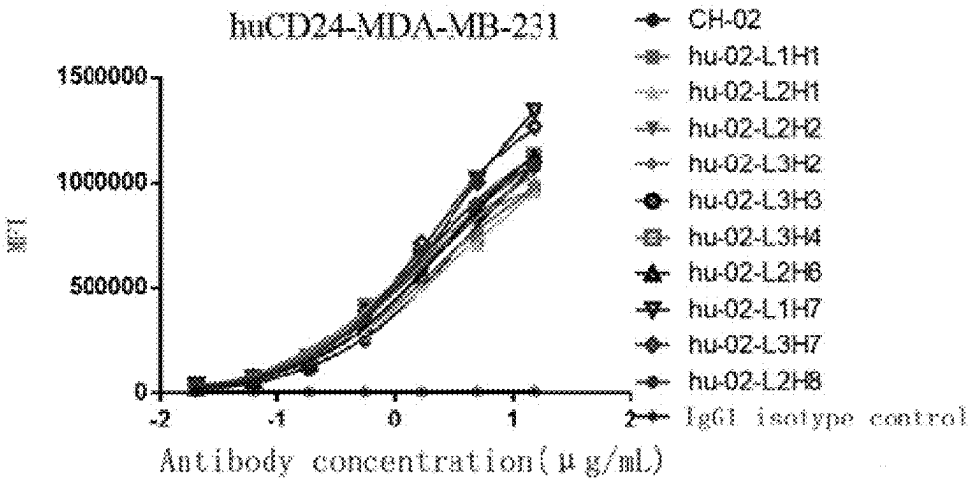

SKOV3
800000
600000
400000
200000
0
MFI
CH-01
hu-01-L2H2
hu-01-L3H2
hu-01-L2H4
Antibody concentration(mg/mL)

FIG. 7c



	CH-02	hu-02-L1H1	hu-02-L2H1	hu-02-L2H2	hu-02-L3H2	hu-02-L3H3	hu-02-L3H4	hu-02-L2H6	hu-02-L1H7	hu-02-L3H7	hu-02-L2H8	IgG1 isotype control
EC50	4.09	2.09	4.21	2.12	1.68	1.99	1.86	2.72	3.01	1.98	1.45	3.46

FIG. 8

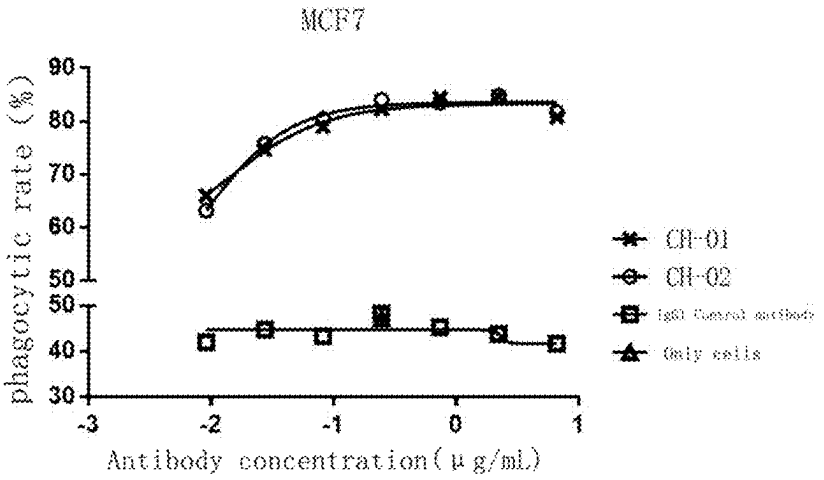


FIG. 9a

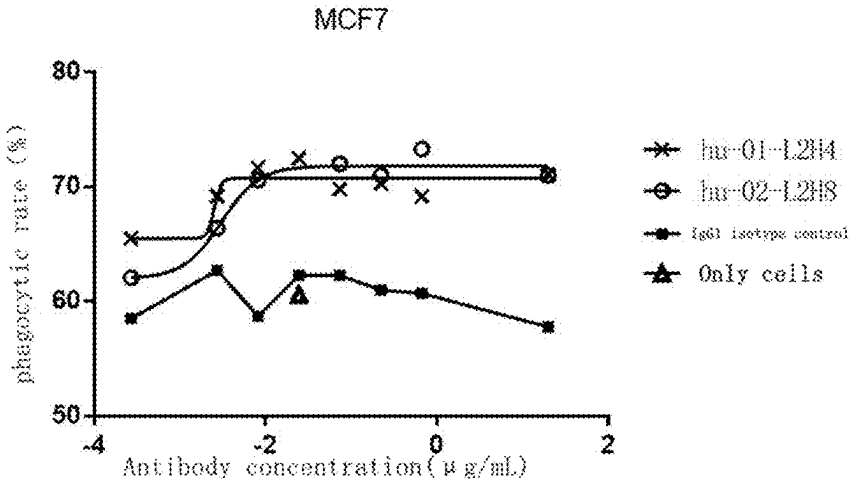


FIG. 9b

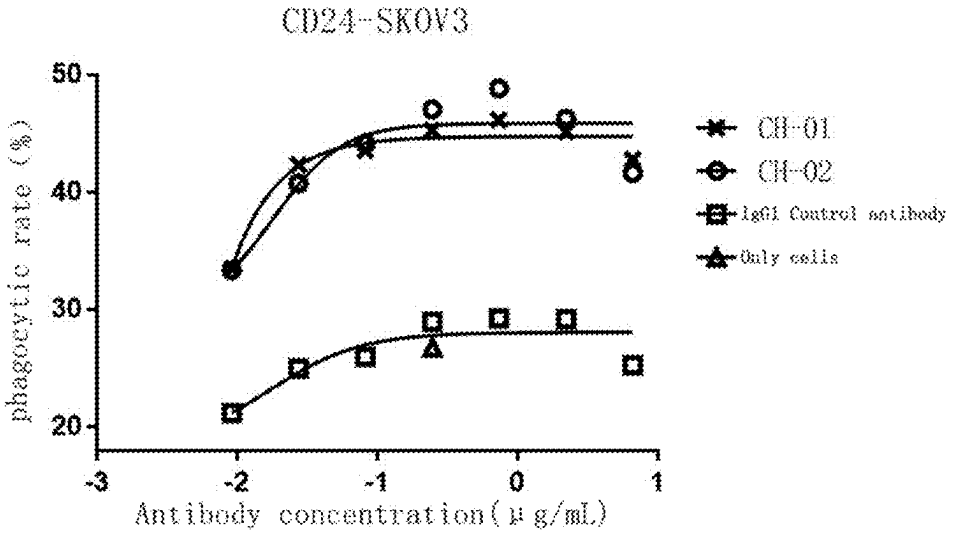


FIG. 10a

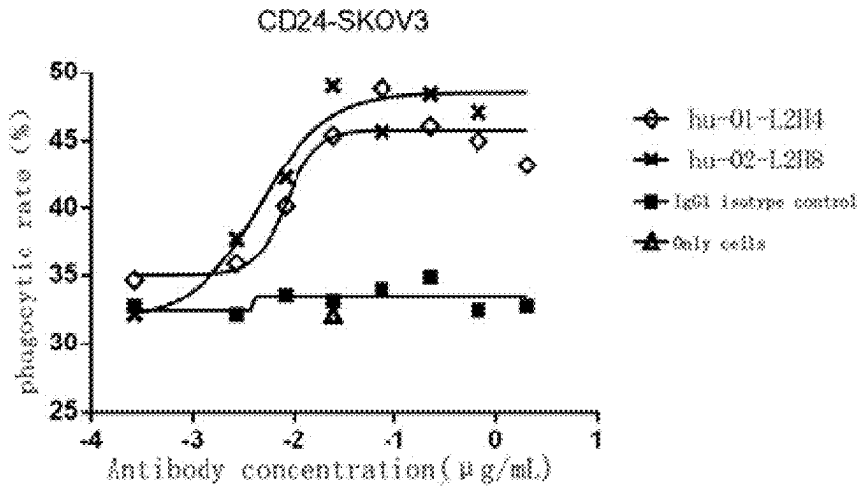


FIG. 10b

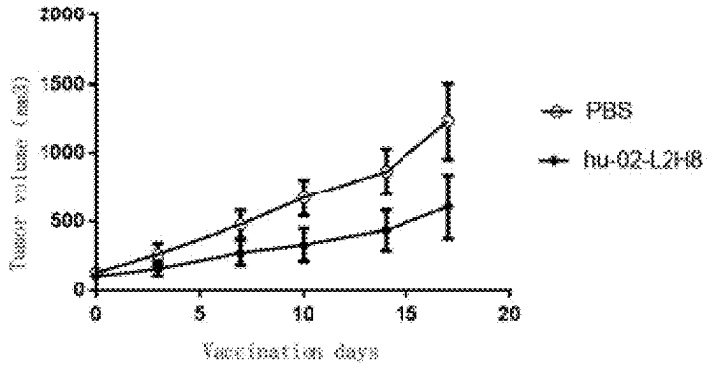


FIG. 11

ANTI-CD24 ANTIBODY AND USE THEREOF

TECHNICAL FIELD

[0001] The present invention belongs to the field of tumor immunotherapy and molecular immunology and specifically relates to an anti-CD24 antibody or antigen-binding fragment thereof.

BACKGROUND

[0002] With the development of a deeper understanding of the immune system and the mechanisms of tumorigenesis, tumor immunotherapy has increasingly become a powerful weapon against tumors.

[0003] Tumor-targeted monoclonal antibodies are one of the most important tools in the field of tumor immunotherapy. Macrophage phagocytosis requires the simultaneous action of two signals: activation of the "eat me" signal on the surface of the target cell, and inactivation of the "don't eat me" signal on the surface of the same cell. The absence of either signal is not sufficient to trigger phagocytosis. There is increasing evidence that CD47 is a class of "don't eat me" signals, and that tumor cells that highly express CD47 release "don't eat me" signals by binding to signal-regulated protein α (SIRPα) on the surface of macrophages, thus preventing tumor cells from being phagocytosed by macrophages.

[0004] CD24, also known as heat-stable antigen, is a highly glycosylated glycosylphosphatidylinositol-anchored surface protein. It has been found that CD24 signaling is often used as a complementary pathway to CD47 signaling in cancers such as blood cancers, which are very susceptible to CD47 signaling blockade and insensitive to CD24 signaling blockade, whereas in other cancers such as ovarian cancers, the opposite is true, which allows the majority of cancers to be attacked by blocking one of these signals. In addition, cancers may be more susceptible to attack if multiple "don't eat me" signals are blocked on the tumor, and of the many primary and secondary "don't eat me" signals, CD24 is the most prominent one. It has been shown that CD24 is used by cancer cells to protect themselves by binding to Siglec-10 on the surface of macrophages and activating the SHP-1/SHP-2-mediated inhibitory signaling pathway, which is a very promising target for the development of cancer immunotherapy. High CD24 expression has been clinically shown to be negatively correlated with the prognosis of breast cancer. The development of anti-CD24 antibodies could target refractory ovarian cancer and triple-negative breast cancer.

[0005] In conclusion, CD24 is an anti-phagocytic signal that is highly expressed in a variety of cancers, and anti-CD24 antibodies have a solid theoretical basis in efficacy and can increase the chemosensitivity of cancer cells, blocking CD24 and macrophage surface Siglec-10 binding, disarming the inhibitory signaling to tumor cells is expected to be a novel immunotherapy.

SUMMARY

[0006] The present invention provides an anti-CD24 antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof has at least one of the following properties:

[0007] (1) capable of binding to CD24; and

[0008] (2) capable of exerting an anti-tumor effect through the antibody Fc fragment.

[0009] In some embodiments, the anti-CD24 antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the heavy chain complementary determining regions HCDR1, HCDR2, and HCDR3, and the light chain variable region comprises the light chain complementary determining regions LCDR1, LCDR2, and LCDR3, wherein

[0010] (a) HCDR1 of the heavy chain variable region is selected from any amino acid sequence of SEQ ID NOs: 2, 17, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 2, 17, or an amino acid sequence having one or more (preferably 2 or 3) conserved amino acid mutations (preferably substitutions, insertions, or deletions) in comparison to any amino acid sequence of SEQ ID NOs: 2, 17;

[0011] (b) HCDR2 of the heavy chain variable region is selected from any amino acid sequence of SEQ ID NOs: 3, 18, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 3, 18, or an amino acid sequence having one or more (preferably 2 or 3) conserved amino acid mutations (preferably substitutions, insertions, or deletions) in comparison to any amino acid sequence of SEQ ID NOs: 3, 18;

[0012] (c) HCDR3 of the heavy chain variable region is selected from any amino acid sequence of SEQ ID NOs: 4, 19, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 4, 19, or an amino acid sequence having one or more (preferably 2 or 3) conserved amino acid mutations (preferably substitutions, insertions, or deletions) in comparison to any amino acid sequence of SEQ ID NOs: 4, 19;

[0013] (d) LCDR1 of the light chain variable region is selected from any amino acid sequence of SEQ ID NOs: 6, 21, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 6, 21, or an amino acid sequence having one or more (preferably 2 or 3) conserved amino acid mutations (preferably substitutions, insertions, or deletions) in comparison to any amino acid sequence of SEQ ID NOs: 6, 21;

[0014] (e) LCDR2 of the light chain variable region is selected from any amino acid sequence of SEQ ID NOs: 7, 22, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 7, 22, or an amino acid sequence having one or more (preferably 2 or 3) conserved amino acid mutations (preferably substitutions, insertions, or deletions) in comparison to any amino acid sequence of SEQ ID NOs: 7, 22; and/or

[0015] (f) LCDR3 of the light chain variable region is selected from any amino acid sequence of SEQ ID NOs: 8, 23, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,

99% or more identity to any amino acid sequence of SEQ ID NOs: 8, 23, or an amino acid sequence having one or more (preferably 2 or 3) conserved amino acid mutations (preferably substitutions, insertions, or deletions) in comparison to any amino acid sequence of SEQ ID NOs: 8, 23;

[0016] In some embodiments, the anti-CD24 antibody or antigen-binding fragment, HCDR1, HCDR2, HCDR3 of the heavy chain variable region consist of the amino acid sequences SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, respectively, and LCDR1, LCDR2, LCDR3 of the light chain variable region consist of SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, respectively.

[0017] In some embodiments, the heavy chain variable region has the amino acid sequence given in SEQ ID NO: 1 and the light chain variable region that has the amino acid sequence given in SEQ ID NO: 5.

[0018] In some embodiments, the heavy chain variable region and light chain variable region are selected from a group of the following (1)-(12) amino acid sequence combinations:

NO.	Heavy chain variable region	Light chain variable region
(1)	SEQ ID NO: 9	SEQ ID NO: 13
(2)	SEQ ID NO: 9	SEQ ID NO: 14
(3)	SEQ ID NO: 9	SEQ ID NO: 15
(4)	SEQ ID NO: 10	SEQ ID NO: 13
(5)	SEQ ID NO: 10	SEQ ID NO: 14
(6)	SEQ ID NO: 10	SEQ ID NO: 15
(7)	SEQ ID NO: 11	SEQ ID NO: 13
(8)	SEQ ID NO: 11	SEQ ID NO: 14
(9)	SEQ ID NO: 11	SEQ ID NO: 15
(10)	SEQ ID NO: 12	SEQ ID NO: 13
(11)	SEQ ID NO: 12	SEQ ID NO: 14
(12)	SEQ ID NO: 12	SEQ ID NO: 15

[0019] In some embodiments, HCDR1, HCDR2, HCDR3 of the heavy chain variable region consist of the amino acid sequences SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively, and LCDR1, LCDR2, LCDR3 of the light chain variable region consist of SEQ ID NO: 21, SEQ ID NO: 22, and SEQ ID NO: 23, respectively.

[0020] In some embodiments, the heavy chain variable region has the amino acid sequence given in SEQ ID NO: 16 and the light chain variable region has the amino acid sequence given in SEQ ID NO: 20.

[0021] In some embodiments, the heavy chain variable region and light chain variable region are selected from a group of the following (1)-(24) amino acid sequence combinations:

NO.	Heavy chain variable region	Light chain variable region
(1)	SEQ ID NO: 24	SEQ ID NO: 32
(2)	SEQ ID NO: 24	SEQ ID NO: 33
(3)	SEQ ID NO: 24	SEQ ID NO: 34
(4)	SEQ ID NO: 25	SEQ ID NO: 32
(5)	SEQ ID NO: 25	SEQ ID NO: 33
(6)	SEQ ID NO: 25	SEQ ID NO: 34
(7)	SEQ ID NO: 26	SEQ ID NO: 32
(8)	SEQ ID NO: 26	SEQ ID NO: 33
(9)	SEQ ID NO: 26	SEQ ID NO: 34
(10)	SEQ ID NO: 27	SEQ ID NO: 32
(11)	SEQ ID NO: 27	SEQ ID NO: 33
(12)	SEQ ID NO: 27	SEQ ID NO: 34
(13)	SEQ ID NO: 28	SEQ ID NO: 32

-continued

NO.	Heavy chain variable region	Light chain variable region
(14)	SEQ ID NO: 28	SEQ ID NO: 33
(15)	SEQ ID NO: 28	SEQ ID NO: 34
(16)	SEQ ID NO: 29	SEQ ID NO: 32
(17)	SEQ ID NO: 29	SEQ ID NO: 33
(18)	SEQ ID NO: 29	SEQ ID NO: 34
(19)	SEQ ID NO: 30	SEQ ID NO: 32
(20)	SEQ ID NO: 30	SEQ ID NO: 33
(21)	SEQ ID NO: 30	SEQ ID NO: 34
(22)	SEQ ID NO: 31	SEQ ID NO: 32
(23)	SEQ ID NO: 31	SEQ ID NO: 33
(24)	SEQ ID NO: 31	SEQ ID NO: 34

[0022] In some embodiments, the anti-CD24 antibody or antigen-binding fragment comprises a mouse-derived antibody or antigen-binding fragment thereof, a chimeric antibody or antigen-binding fragment thereof, and/or a humanized antibody or antigen-binding fragment thereof.

[0023] In some embodiments, the anti-CD24 antibody or antigen-binding fragment further comprises an Fc region, and the Fc region is selected from mouse IgG1, IgG2a, IgG2b and/or IgG3, or from rat IgG1, IgG2a, IgG2b and/or IgG2c.

[0024] In some embodiments, the anti-CD24 antibody or antigen-binding fragment further comprises an Fc region, and the Fc region is selected from human IgG1, IgG2, IgG3 and/or IgG4.

[0025] The present invention also provides a nucleic acid molecule encoding any one of the above-described anti-CD24 antibodies or antigen-binding fragments thereof.

[0026] The present invention also provides a recombinant vector comprising the above-described nucleic acid molecules.

[0027] The present invention also provides a recombinant cell comprising the above-described nucleic acid molecules and/or recombinant vectors and capable of expressing the anti-CD24 antibody or antigen-binding fragment thereof.

[0028] The present invention also provides a multifunctional fusion protein comprising any one of the above-described anti-CD24 antibodies or antigen-binding fragments thereof.

[0029] In some embodiments, the multifunctional fusion protein further comprises one or more second antibodies or antigen-binding portions thereof that bind specifically to other antigens.

[0030] In some embodiments, the antigen binding the second antibody or antigen-binding portion thereof is selected from a tumor-associated antigen (TAA) or an immune checkpoint.

[0031] The present invention also provides use of any one of the above-described anti-CD24 antibodies or antigen-binding fragments thereof and the multifunctional fusion proteins in the preparation of a drug for the treatment and/or prevention and/or diagnosis of a disease.

[0032] In some embodiments, the use is achieved by one or more of tumor immunotherapy, cell therapy, and gene therapy.

[0033] The present invention also provides use of any one of the above-described anti-CD24 antibodies or antigen-binding fragments thereof and the multifunctional fusion proteins in the preparation of a drug for the treatment of cancer.

[0034] In some embodiments, the cancer is breast cancer, ovarian cancer, lung cancer, liver cancer, melanoma, malignant glioma, head and neck cancer, colorectal cancer, gastric cancer, bladder cancer, pancreatic cancer, colon cancer, cervical cancer, or a related tumor.

[0035] The present invention also provides a pharmaceutical composition comprising any one of the above-described anti-CD24 antibodies or antigen-binding fragments thereof and an acceptable carrier, diluent or excipient.

[0036] The present invention also provides a pharmaceutical composition comprising any one of the above-described multifunctional fusion proteins and an acceptable carrier, diluent or excipient.

[0037] The anti-CD24 antibodies provided by the present invention have excellent binding activity to MCF7 cells, SKOV3 cells, HT55 cells and huCD24-MDA-MB-231 (breast cancer MDA-MB-231 cells overexpressing CD24). Both the chimeric anti-CD24 antibody and the humanized anti-CD24 antibody provided by the present invention are capable of mediating phagocytosis of MCF7 cells and CD24-SKOV3 cells (SKOV3 cells overexpressing CD24) by macrophages. Moreover, the humanized antibody shows high tumor inhibition in mouse.

[0038] To facilitate understanding of the invention set forth herein, the following explanation of abbreviations and definitions of terms are provided.

[0039] The following abbreviations are used in this article:

[0040] CDR: Complementary determining regions in the variable region of the antibody

[0041] HCDR: Complementary determining regions in the variable region of the antibody heavy chain

[0042] LCDR: Complementary determining regions in the variable region of the antibody light chain

[0043] FR: Antibody construct region, which is the amino acid residues other than CDR residues in the variable region of the antibody

[0044] ELISA: Enzyme-linked immunosorbent assay (ELISA)

[0045] FACS: Fluorescence Activated Cell Sorting

[0046] ADCP: Antibody-dependent cell-mediated phagocytosis

[0047] In this specification, the term "antibody" means a natural immunoglobulin or an immunoglobulin prepared by partial or complete synthesis. The antibody may be isolated from a natural source such as plasma or serum in which the antibody is naturally present, or from the culture supernatant of antibody-producing hybridoma cells, or from the immune serum of an animal, or from reconstruction by phage library screening. Alternatively, they may be partially or completely synthesized by using techniques such as genetic recombination. Preferred antibodies include, for example, antibodies to isoforms of immunoglobulins or subclasses of these isoforms. Human immunoglobulins are known to include nine classes (isotypes) of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, and IgM. Within these isotypes, the antibodies of the present invention may include IgG1, IgG2, IgG3, and/or IgG4.

[0048] The terms "antibody" and "immunoglobulin" are used interchangeably, and some of the antibodies used herein consist of an immunoglobulin molecule comprising two pairs of polypeptide chains, each pair having a light chain (LC) and a heavy chain (HC). Each heavy chain consists of a heavy chain variable region (VH) and a heavy chain constant region (CH). The heavy chain constant region consists of three structural domains (CH1, CH2 and CH3). Each light chain consists of a light chain variable region (VL) and a light chain constant region (CL), or only a light chain constant region (CL). The light chain constant region consists of a structural domain CL. The constant structural domain is not directly involved in antibody-antigen binding, but exhibits a variety of effector functions, such as can mediate the binding of immunoglobulins to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component of the classical complement system (C1q). The VH and VL regions can also be subdivided into regions with high variability (termed complementarity-determining regions (CDRs)), which are scattered with more conserved regions called framing regions (FR). Each VH and VL consists of three CDRs and four FRs arranged from amino-terminal to carboxy-terminal in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of each heavy chain/light chain pair (VH and VL) form the antigen-binding site, respectively.

[0049] The term "antigen-binding fragment" of an antibody refers to a polypeptide fragment of an antibody, such as a polypeptide fragment of a full-length antibody, which maintains the ability to specifically bind the same antigen bound by the full-length antibody and/or to compete with the full-length antibody for specific binding of the antigen, and which is also referred to as an "antigen-binding portion". The antigen-binding fragment of an antibody may be produced by recombinant DNA technology or by enzymatic or chemical breakage of an intact antibody. Non-limiting examples of antigen-binding fragments include Fab, Fab', F(ab')2, Fd, Fv, dAb, and complementary determining region (CDR) fragments, single-chain antibodies (e.g., scFv), chimeric antibodies, diabody, linear antibody, nanoantibodies (e.g., technology from Ablynx), structural domain antibodies (e.g., technology from Domantis), and such polypeptides comprising at least a portion of an antibody sufficient to confer specific antigen-binding ability to the polypeptide.

[0050] The term "polypeptide" refers to a chain of amino acids of any length, irrespective of modification (e.g. phosphorylation or glycosylation). The term polypeptide includes proteins and fragments thereof. Polypeptides may be "exogenous", meaning that they are "heterologous", i.e., foreign to the host cell utilized, such as human polypeptides produced by bacterial cells. Polypeptides are disclosed herein as sequences of amino acid residues. Those sequences are written from left to right in the direction from the amino terminus to the carboxy terminus. According to standard nomenclature, the amino acid residue sequences are named in a three-letter or single-letter code as follows: alanine (Ala, A), arginine (Arg, R), asparagine (Asn, N), aspartate (Asp, D), cysteine (Cys, C), glutamine (Gln, Q), glutamic acid (Glu, E), glycine (Gly, G), histidine (His, H), isoleucine (Ile, I), leucine (Leu, L), lysine (Lys, K), methionine (Met, M), phenylalanine (Phe, F), proline (Pro, P), serine (Ser, S), threonine (Thr, T), tryptophan (Trp, W), tyrosine (Tyr, Y) and valine (Val, V).

[0051] There are a variety of methods/systems in the field to define and characterize CDRs that have been developed and refined over the years, including Kabat, Chothia, IMGT, AbM, and Contact; Kabat is the most commonly used and defines CDRs based on sequence variability; Chothia defines CDRs based on sequence variability based on the position of

the cyclic region of the structure; the IMGT system defines CDRs based on sequence variability and position within the variable domain structure; AbM is defined based on Oxford Molecules' AbM antibody modeling software and is a compromise between Kabat and Chothia; Contact defines CDRs based on the analysis of complex crystal structures, and is similar to Chothia in several respects.

[0052] The amino acid positions are numbered (e.g., amino acid residues in the Fc region) and the target region (e.g., CDR) in the anti-CD24 antibody of the present invention, using the Kabat system.

[0053] With respect to the percentage (%) amino acid sequence "identity" of the reference polypeptide sequence is defined as the percentage of amino acid residues in the candidate sequence that are identical to amino acid residues in the reference polypeptide sequence after comparing the sequences and introducing gaps where necessary to obtain the maximum percentage sequence identity. Comparisons for the purpose of determining percent amino acid sequence identity can be performed in a variety of ways within the skill of the art, such as using publicly available computer software, such as BLAST, BLAST-2, Clustal W, Megalign (DNASTAR) software, or the FASTA program package.

[0054] The term "monoclonal antibody" refers to a homogeneous antibody directed only to a specific antigenic epitope. In contrast to common polyclonal antibody preparations, which typically include different antibodies directed against different antigenic determinants (epitopes), each monoclonal antibody is directed against a single antigenic determinant on the antigen. The modifier "monoclonal" denotes a homogeneous characterization of the antibody and is not to be construed as requiring that the antibody be produced by any particular method. The monoclonal antibodies of the present invention are preferably produced by recombinant DNA methods or obtained by screening methods described elsewhere herein.

[0055] The term "mouse-derived antibody" is used in the present invention to mean a monoclonal antibody prepared according to the knowledge and skill in the art. In some embodiments, the preparation involves injecting a test subject with an antigen and then isolating a hybridoma expressing an antibody having the desired sequence or functional properties. In some embodiments, the desired mouse-derived antibody is obtained by screening a mouse immune library.

[0056] The term "chimeric antibody" refers to an antibody that is formed by fusing a variable region of a mouse-derived antibody with a constant region of a human antibody, which can attenuate the immune response induced by the mouse-derived antibody. In some embodiments, to establish a chimeric antibody, a hybridoma secreting a mouse-derived specific monoclonal antibody is established, then the variable region gene is cloned from the mouse hybridoma cells, and then the constant region gene of the human antibody is cloned as needed, and the mouse variable region gene is inserted into a human vector after linking the mouse variable region gene and the human constant region gene to form a chimeric gene, and then finally, the chimeric antibody molecule is expressed in a eukaryotic industrial system or a prokaryotic industrial system.

[0057] The term "humanized antibody" means an antibody comprising at least one humanized antibody chain (at least one humanized light or heavy chain). The term "humanized antibody chain" ("humanized immunoglobulin chain") refers to an antibody chain (light or heavy chain, respectively) having a variable region, the variable region comprising a substantial variable framework region and complementarity determination of the human antibody. Regions (CDRs) substantially derived from a non-human antibody (e.g., at least one CDR, two CDRs, or three CDRs). In some embodiments, the humanized antibody chain further comprises constant regions (e.g., in the case of a light chain, one constant region or a portion thereof, and in the case of a heavy chain, preferably three constant regions).

[0058] The term "host cell" means a cell that has been or is capable of being transformed with a nucleic acid sequence and thus expressing the selected target gene. The term includes the progeny of the parental cell, whether or not the progeny is morphologically or genetically identical to the original parental cell, as long as the selected target gene is present in the progeny. Commonly used host cells include bacteria, yeast, mammalian cells, etc.

[0059] The term "vector" refers to a nucleic acid molecule capable of proliferating another nucleic acid to which it is attached. The term includes vectors that are self-replicating nucleic acid structures and vectors that are incorporated into the genome of the host cell into which they are introduced. Certain vectors are capable of directing the expression of nucleic acids operably linked to them. Such vectors are referred to herein as "expression vectors".

BRIEF DESCRIPTION OF THE DRAWINGS

[0060] FIG. **1** shows the binding activity of mouse-derived anti-CD24 antibody to MCF7 cells;

[0061] FIG. **2** shows the binding activity of mouse-derived anti-CD24 antibody to SKOV3 cells;

[0062] FIGS. **3***a*-**3***b* show the binding activity of chimeric anti-CD24 antibody to MCF7 cells;

[0063] FIGS. **4***a*-**4***b* show the binding activity of chimeric anti-CD24 antibody to SKOV3 cells;

[0064] FIG. **5** shows the binding activity of chimeric anti-CD24 antibody to HT55 cells;

[0065] FIGS. **6***a*-**6***d* show the binding activity of humanized anti-CD24 antibody to MCF7 cells;

[0066] FIGS. **7***a*-**7***c* show the binding activity of humanized anti-CD24 antibody to SKOV3 cells;

[0067] FIG. **8** shows the binding activity of humanized anti-CD24 antibody to huCD24-MDA-MB-231 cells;

[0068] FIGS. **9***a*-**9***b* show chimeric and humanized anti-CD24 antibody-mediated ADCP effects on MCF7 cells;

[0069] FIGS. **10***a*-**10***b* show chimeric and humanized anti-CD24 antibody-mediated ADCP effects on CD24-SKOV3 cells.

[0070] FIG. **11** shows the in vivo efficacy data of the humanized anti-CD24 antibody.

DETAILED DESCRIPTION

[0071] The present invention is further described below in connection with the accompanying drawings and specific embodiments, the protection of which is not limited to the following embodiments. It should also be understood that the terms used in embodiments of the present invention are intended to describe specific embodiments and are not intended to limit the scope of protection of the present invention. Without departing from the spirit and scope of the inventive concept, variations and advantages that can be envisaged by those skilled in the art are included in the

present invention and the scope of protection of the invention by the appended claims and any equivalents thereof. In the specification and claims of the invention, the singular forms "a", "one" and "this" include the plural forms unless the context expressly states otherwise. The processes, conditions, reagents, experimental methods, etc. for carrying out the invention are, except where specifically mentioned, of general knowledge and common knowledge to those skilled in the art, and the invention is not specifically limited in the present invention.

Example 1 Animal Immunization

[0072] Balb/c mice were immunized with human CD24-his and VLP-huCD24 as immunogens. The immunogen was diluted with PBS, and Fuchs complete adjuvant (Sigma; F5881) was added 1:1 by volume, and vortex emulsified, and the mouse were immunized intraperitoneally after the emulsification was complete. The second immunization was performed half a month after the first immunization, and again every other month after that. Negative serum was taken from mouse 3 days before immunization, and 50 μL of blood was taken from tail clipping 6 days after each immunization. Negative serum and immune serum were diluted proportionally (1:0.1K, 1:1K, 1:10K, 1:100K, 1:1000K, and 1:10,000K), and the breast cancer MDA-MB-231 cells overexpressing CD24 (huCD24-MDA-MB-231) were detected by ELISA for serum potency assay. When the potency result meets the requirement and anti-human CD24 antibody was detected at a dilution of >1:10K, rat spleen and lymph nodes could be harvested.

Example 2 Cell Fusion

[0073] B lymphocytes and lymph node cells for the experiment were obtained from Balb/c mouse immunized four times with recombinant human CD24 protein. The spleens and lymph nodes were placed in a cell sieve, which was then placed in a 50 mL centrifuge tube. DMEM was aspirated and added dropwise to the spleens for grinding to make splenocyte suspension, and centrifuged at 1600 rpm for 10 min, and the supernatant was removed. B cells were resuspended with 2 mL of erythrocyte lysate, and lysed at room temperature for 2 minutes. 30 mL of DMEM was added, mixed and centrifuged at 1600 rpm for 10 min, and counted.

[0074] Myeloma cells SP2/0 (ATCC) were passaged the day before fusion so that the cells were in logarithmic growth phase at the time of the experiment. Splenocytes and SP2/0 were mixed at a ratio of 2:1 and then centrifuged at 1600 rpm for 10 min. The mixed cells were washed twice with fusion solution and centrifuged at 1600 rpm for 10 min. According to the final cell density of 1×10^7, the cells were suspended with fusion solution. Within 5 min, the cell suspensions were transferred to the fusion chamber of the electric fusion apparatus (BTX; ECM 2001) for fusion. After fusion, the cells were transferred from the fusion chamber to complete medium containing HAT and incubated at 37° C. for 60 min. After incubation, the cells were spread in 96-well plates containing feeder cells, and incubated at 37° C. and 5% CO_2.

Example 3 Preliminary Screening of Positive Clones by ELISA Method

[0075] The fusion supernatant was initially screened after 7 days of culture. huCD24-MDA-MB-231 cells were spread into 96 enzyme-labeled plates (1.5×10^4/per well), incubated for 36 h, and hand-washed with PBST twice. It was fixed with 4% paraformaldehyde and closed with 2% BSA. The sealing solution was poured off and washed with the plate washer 3 times. The fusion supernatant was added into the sealed enzyme-labeled plate at 100 μL/well, and incubated at 37° C. for 1 h, and the liquid in the wells were discarded. It was washed with the plate washer 3 times. Sheep anti-mouse secondary antibody-HRP (ABCOM; Ab6789) was diluted with 0.5% BSA at 100 μL/well and incubated at 37° C. It was washed with PBST in the plate washer 6 times, and patted dry on plate paper. Solarbio color development solution (Solarbio; PR1200) was added into the plate wells at 100 μL/well, and wrapped in aluminum foil, and the color was developed at 37° C. without light. The color development reaction was terminated by adding 1 mol/L M HCl, and the data were read at 450 nm on an enzyme marker, and the data were analyzed. The cell lines with OD450>1.0 in the test supernatant result were selected as candidate positive cell lines for initial screening. The culture supernatant of the positive cell lines was aspirated and discarded, and a new HAT complete medium was added.

Example 4 FACS Method for Further Screening of Positive Clones

[0076] huCD24-MDA-MB-231 cells were transferred to a centrifuge tube and centrifuged at 1000 rpm for 5 min. 100 μL of 3×10^5 stably expressing cells were aliquoted into separate tubes and 100 μL of fusion supernatant was added. The cells were incubated at 4° C. for 60 minutes and then washed twice with excess FACS buffer. Cells were resuspended in 100 μL of FACS buffer, and sheep anti-mouse secondary antibody-FITC (ABCOM; ab6785) was added to the sample, incubated for 30 minutes and washed twice with excess FACS buffer. Cells were fixed in fixation buffer and subsequently analyzed by flow cytometry. The antibodies that bind specifically to huCD24-MDA-MB-231 cells were screened by FACS method.

[0077] The hybridoma cells were monoclonalized by two rounds of limited dilution method, and ELISA was used for detection. The monoclones with OD450>1.0 were selected as the definitive candidate cell lines for passage, and clones without monoclonal antibodies were selected with OD450>1.0 for the next subcloning.

Example 5 Small Sample Production of Antibodies to Candidate Cell Lines

[0078] The hybridoma cells were incubated in T75 until the cell coverage was 80-90%. The cell supernatant was discarded from 2 bottles, and 30 mL hybridoma-SFM was added, incubated at 37° C. and 5% CO_2 for 2-3 days, and observed the cell status and medium color. If the medium color turned yellow, 30 mL of new hybridoma-SFM was added and incubated for 6-7 days, and the culture supernatant was collected after low-speed centrifugation for purification.

Example 6 Candidate Antibody Binding Activity to Tumor Cells

[0079] The final positive clones were confirmed by evaluating the binding activity of the candidate antibodies to tumor cells MCF7 (ATCC), SKOV3 (ATCC), HT55 (ATCC), and huCD24-MDA-MB-231 using the FACS

method. The commercial antibody SN3 (ABCOM; ab134375) and IgG1 isotype control were used as control. MCF7, SKOV3, HT55 or huCD24-MDA-MB-231 cells were taken and transferred to a centrifuge tube, and centrifuged at 1000 rpm for 5 min. 100 μL of MCF7, SKOV3, HT55, or huCD24-MDA-MB-231 cells were spread in a 96 U plate, respectively, and washed twice with PBS. 100 μL of the fusion supernatant was added, and diluted for 8 gradients. Cells were incubated at 4° C. for 60 minutes, and then washed twice with excess FACS buffer. Cells were resuspended in 100 μL of FACS buffer and sheep anti-mouse secondary antibody-FITC (ABCOM; ab6785) was added to the samples, incubated for 30 minutes and washed twice with excess FACS buffer. Cells were fixed in fixation buffer and subsequently analyzed by flow cytometry.

[0080] The binding activity of ms-01 and MCF7 cells is shown in FIG. **1**. As can be seen from the results, the binding activity of ms-01 and MCF7 cells is better than the control antibody SN3 or comparable to that of the control antibody SN3.

[0081] The binding activity of ms-01 and SKOV3 cells is shown in FIG. **2**. As can be seen from the results, the binding activity of ms-01 and SKOV3 cells is better than or comparable to that of the control antibody SN3.

Example 7 Construction of the Mouse Immunization Library

[0082] The spleen of immunized mouse was taken, and RNA was extracted from the mouse spleen tissue by chloroform method. After the RNA was identified and the concentration was determined to be of satisfactory quality, the RNA was reverse transcribed according to the method provided by the TaKaRa Reverse Transcription Kit. The immunized mouse antibody gene was amplified by universal concatenated primers, and then the antibody gene was recombined by in vitro ligation, and finally the recombined antibody Fab gene sequence was inserted into PMID-1121B vector, thus the mouse immunized antibody library was obtained. In addition, the library capacity of the antibody library was determined by the dilution spot plate method, and the correct insertion rate of the antibody gene was verified by monoclonal sequencing analysis. The results showed that the library capacity of the antibody library was larger than the designed capacity, and the correct insertion rate of the antibody library was more than 80%, which could reflect the high abundance from CDR3, and the quality of this library construction was qualified and could be used for antibody screening.

Example 8 Mouse Immune Bank Screening

[0083] With Human CD24-his and VLP-huCD24 as screening proteins, Kingfisher method or the immunotube method were used to select in the constructed mouse immune library. Then huCD24-HEK293, huCD24-MDA-MB-231 and huCD24-SKOV-3 were used as screening cells for cell panning of the constructed mouse immune library, and finally positive antibodies were screened by ELISA method. The specific steps were as follows:

1. Kingfisher Method or the Immunotube Method

[0084] Elution: Phage was eluted with Trypsin.

[0085] Immersion library plate: The eluted phage solution was mixed thoroughly with log phase SS320 cells and incubated statically. It was coated on 2YT-C+-T+ plate, and cultured overnight in 37° C. incubator.

[0086] Spot Titer: After elution, the phage solution was diluted with logarithmic SS320 cells in 10-fold gradient and incubated statically. After mixing well, 2 μL was spotted on the plate and incubated in an incubator at 37° C. overnight.

[0087] Statistics: Phage output, input, etc. were calculated, and input phage was prepared by scraping plate, and subsequent screening was carried out. Each round of screening would reduce the concentration of the antigen by 3 times to ensure that Fab with good affinity was screened.

2. Cell Panning Using Live and Fixed Cells Respectively

[0088] (1) Live cell panning: According to the number of rounds of panning and the requirements of cell screening, the cells were pre-distributed into 15 mL centrifuge tubes according to the amount and kept at a low temperature. The closed phage was added into the treated cells. Before that, it is necessary that the closed phage was added into the negative screening cells, mixed, combined, and centrifuged. The supernatant of the phage library after negative screening was added to the corresponding positive screening cells, mixed, combined and centrifuged. The supernatant was discarded and washed with pre-cooled 5% FBS-PBS. Phage library elution was then performed.

[0089] (2) Fixed cell panning: the closed phage was absorbed with a disposable pipette and slowly added to the treated cell bottle on the side wall. Before that, negative screening was required. The closed phage was first added into the negative screening cells and placed on the track-type shaker for mixing and combining for 1 h. After combination, the cell culture bottle was gently stood up, and the phage library after negative screening was sucked out and added to the corresponding positive screening cell bottle using a disposable pipette. It was mixed and combined on the track-type shaker at room temperature for 2 h. The supernatant in the culture bottle was discarded, and 5% FBS-PBS was added. Then the cell plate was placed in the upright position, and carefully suck out with a disposable pipette to complete the washing. Phage library elution was then performed.

3. Preliminary Screening of Monoclones by ELISA

[0090] The second and third rounds of clones were selected and cultured overnight in 96-well deep-well plates. The supernatant was centrifuged for ELISA screening. 30 μL of antigen-coated synthetic CD24 extracellular region peptide, huCD24-his and VLP-hCD24 at 2 μg/mL were added to each well at 4° C. overnight. The plate was washed 3 times with PBST. 5% PBSM was added and blocked at room temperature for 1 h. The plate was washed 3 times with PBST. Monoclones were added at 30 μL/well at room temperature for 1 h, and the plate was washed 3 times with PBST. 30 μL of secondary antibody Anti-M13-HRP (1:8000) was added at room temperature for 1 h, and the plate was washed 9 times with PBST. 30 μL of TMB was added to develop the color at room temperature for 5-10 min, after which the reaction was terminated by adding 30 μL of 2 M HCl. The data were read by the enzyme marker at OD450.

4. Quantification of Phage Lysate by ELISA

[0091] Anti-mouse-Fab was diluted with PBS to 1 μg/mL, added at 30 μL per well and left to stand at 4° C. overnight.

Plates were washed 3 times with PBST. It was blocked with 5% PBSM at room temperature for 1 h, and the plate was washed 3 times with PBST. 30 μL of gradient diluted phage lysate was added at room temperature for 1 h, and the plate was washed 3 times with PBST. 30 μL of diluted secondary antibody Anti-mouse-Fab-HRP (1:5000) was added at room temperature for 1 h, and the plate was washed 9 times with PBST. 30 μL of TMB was added to develop the color at room temperature for 5-20 min, after which the reaction was terminated by adding 30 μL of 2 mol/L HCl. The data were read by the enzyme marker at OD450.

Example 9 Sequencing of Monoclonal Antibodies

[0092] The murine anti-CD24 antibody ms-01 with good binding activity to tumor cells MCF7 and SKOV3 was obtained by hybridoma screening, and Fab fragment of the murine anti-CD24 antibody ms-02 was obtained by mouse immune library screening. The amino acid sequences of murine anti-CD24 antibody ms-01 and ms-02 were finally obtained by sequencing ms-01 and ms-02, respectively:

[0093] (1) The amino acid sequences of ms-01 are as follows:

[0094] The amino acid sequence of the heavy chain variable region of ms-01 is SEQ ID NO: 1;

[0095] The amino acid sequences of HCDR1, HCDR2 and HCDR3 of the heavy chain variable region of ms-01 are SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, respectively;

[0096] The amino acid sequence of the light chain variable region of ms-01 is SEQ ID NO: 5;

[0097] The amino acid sequences of LCDR1, LCDR2 and LCDR3 of the light chain variable region of ms-01 are SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, respectively.

[0098] (2) The amino acid sequences of ms-02 are as follows:

[0099] The amino acid sequence of the heavy chain variable region of ms-02 is SEQ ID NO: 16;

[0100] The amino acid sequences of HCDR1, HCDR2 and HCDR3 of the heavy chain variable region of ms-02 are SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, respectively;

[0101] The amino acid sequence of the light chain variable region of ms-02 is SEQ ID NO: 20;

[0102] The amino acid sequences of LCDR1, LCDR2 and LCDR3 of the light chain variable region of ms-02 are SEQ ID NO: 21, SEQ ID NO: 22 and SEQ ID NO: 23, respectively.

Example 10 Construction and Expression of Chimeric Antibodies

[0103] The antibody fragment obtained by sequencing in Example 9 was subjected to gene synthesis and constructed into a human IgG framework, and then the antibody fragment was inserted into a PCDNA3.1 vector using molecular cloning technology, constructed into a mammalian cell expression plasmid, and introduced into a host cell line CHO cell by liposome transfection. The fermentation supernatant was obtained by cell fed-batch. The supernatant of the fermentation fluid was taken for purification by affinity chromatography, ion exchange chromatography and a series of other steps, and the constructed chimeric antibody was finally purified.

[0104] (1) The amino acid sequences of CH-01 are as follows:

[0105] The amino acid sequence of the heavy chain variable region of CH-01 is SEQ ID NO: 1;

[0106] The amino acid sequences of HCDR1, HCDR2 and HCDR3 of the heavy chain variable region of CH-01 are SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, respectively;

[0107] The amino acid sequence of the heavy chain constant region of CH-01 is SEQ ID NO: 35;

[0108] The amino acid sequence of the light chain variable region of CH-01 is SEQ ID NO: 5;

[0109] The amino acid sequences of LCDR1, LCDR2 and LCDR3 of the light chain variable region of CH-01 are SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, respectively;

[0110] The amino acid sequence of the light chain constant region of CH-01 is SEQ ID NO: 36.

[0111] (2) The amino acid sequences of CH-02 are as follows:

[0112] The amino acid sequence of the heavy chain variable region of CH-02 is SEQ ID NO: 16;

[0113] The amino acid sequences of HCDR1, HCDR2 and HCDR3 of the heavy chain variable region of CH-02 are SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, respectively;

[0114] The amino acid sequence of the heavy chain constant region of CH-02 is SEQ ID NO: 35;

[0115] The amino acid sequence of the light chain variable region of CH-02 is SEQ ID NO: 20;

[0116] The amino acid sequences of LCDR1, LCDR2 and LCDR3 of the light chain variable region of CH-02 are SEQ ID NO: 21, SEQ ID NO: 22 and SEQ ID NO: 23, respectively;

[0117] The amino acid sequence of the light chain constant region of CH-02 is SEQ ID NO: 36.

Example 11 Binding Activity of Chimeric Antibodies to Tumor Cells

[0118] The binding activity of the chimeric antibodies CH-01 and CH-02 and tumor cells was detected according to the method of Example 6.

[0119] The binding activity of the chimeric antibodies and MCF7 cells is shown in FIGS. **3***a*-**3***b*. As can be seen from the results, the binding activity of CH-01 and CH-02 and MCF7 cells was significantly better than that of the IgG1 isotype control.

[0120] The binding activity of the chimeric antibodies and SKOV3 cells is shown in FIGS. **4***a*-**4***b*. As can be seen from the results, the binding activity of CH-01 and CH-02 and SKOV3 cells was significantly better than that of the IgG1 isotype control.

[0121] The binding activity of the chimeric antibodies and HT55 cells is shown in FIG. **5**. As can be seen from the results, the binding activity of CH-02 and HT55 cells was significantly better than that of the IgG1 isotype control.

Example 12 Antibody Humanization

[0122] The IgBLAS tool was used to align the murine sequence obtained in Example 9 with the human Germline sequence. The results showed that the heavy chain variable region Framework 1-3 contained 18 murine sites (V genes), and the light chain variable region Framework 1-3 contained

23 murine sites (V genes). The heavy chain design template selected the IGHV7 category, and the light chain design template selected the IGHV1 category. Humanized sequences were designed and the sequences are mutated into humanized sequences. 4 heavy chains and 3 light chains of the humanized antibody were obtained from the ms-01, and 8 heavy chains and 3 light chains of the humanized antibody were obtained from the ms-02. The designed humanized sequences were compared with the human Germline sequences and the percentage of humanization degree of the antibodies was calculated to be above 80%.

[0123] (1) Four humanized antibody heavy chains H1, H2, H3 and H4 with all three identical HCDRs were obtained from ms-01, and the amino acid sequences of HCDR1, HCDR2 and HCDR3 in the variable region of the heavy chains were SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, respectively; and three humanized antibody light chains L1, L2 and L3 were obtained which were identical to the three LCDRs, and the amino acid sequences of HCDR1, HCDR2 and HCDR3 in the variable regions of their light chains were SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, respectively.

[0124] The amino acid sequences of the variable regions of the heavy chains H1, H2, H3 and H4 of the four humanized antibodies are shown in Table 1:

TABLE 1

Heavy chain numbers	Variable region amino acid sequences
H1	SEQ ID NO: 9
H2	SEQ ID NO: 10
H3	SEQ ID NO: 11
H4	SEQ ID NO: 12

[0125] The constant regions of the heavy chains H1, H2, H3 and H4 of the four humanized antibodies are identical, and the amino acid sequence of their constant regions is SEQ ID NO: 35.

[0126] The amino acid sequences of the variable regions of the light chains L1, L2 and L3 of the three humanized antibodies are shown in Table 2:

TABLE 2

Light chain numbers	Variable region amino acid sequences
L1	SEQ ID NO: 13
L2	SEQ ID NO: 14
L3	SEQ ID NO: 15

[0127] The constant regions of the three humanized antibody light chains L1, L2 and L3 are identical, and the amino acid sequence of their constant regions is SEQ ID NO: 36.

[0128] The above four heavy chains and three light chains were grouped into 12 humanized antibodies, namely: hu-01-L1H1, hu-01-L2H1, hu-01-L3H1, hu-01-L1H2, hu-01-L2H2, hu-01-L3H2, hu-01-L1H3, hu-01-L2H3, hu-01-L3H3, hu-01-L1H4, hu-01-L2H4, and hu-01-L3H4, respectively.

[0129] (2) Eight humanized antibodies H1, H2, H3, H4, H5, H6, H7 and H8 with all three identical HCDRs were obtained from ms-02, and the amino acid sequences of HCDR1, HCDR2 and HCDR3 in the variable region of the heavy chain were SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, respectively; and three humanized antibody light chains L1, L2, and L3 with the same LCDR were obtained. The amino acid sequences of HCDR1, HCDR2 and HCDR3 in the variable regions of the light chains of the three humanized antibodies were SEQ ID NO: 21, SEQ ID NO: 22 and SEQ ID NO: 23, respectively.

[0130] The amino acid sequences of the variable regions of the heavy chains H1, H2, H3, H4, H5, H6, H7 and H8 of the eight humanized antibodies are shown in Table 3:

TABLE 3

Heavy chain numbers	Variable region amino acid sequences
H1	SEQ ID NO: 24
H2	SEQ ID NO: 25
H3	SEQ ID NO: 26
H4	SEQ ID NO: 27
H5	SEQ ID NO: 28
H6	SEQ ID NO: 29
H7	SEQ ID NO: 30
H8	SEQ ID NO: 31

[0131] The constant regions of the heavy chains H1, H2, H3, H4, H5, H6, H7 and H8 of the eight humanized antibodies are identical, and the amino acid sequences of their constant regions is SEQ ID NO: 35.

[0132] The amino acid sequences of the variable regions of the light chains L1, L2 and L3 of the three humanized antibodies are shown in Table 4:

TABLE 4

Light chain numbers	Variable region amino acid sequences
L1	SEQ ID NO: 32
L2	SEQ ID NO: 33
L3	SEQ ID NO: 34

[0133] The constant regions of the three humanized antibody light chains L1, L2 and L3 are identical, and the amino acid sequence of their constant regions is SEQ ID NO: 36.

[0134] The above eight heavy chains and three light chains were grouped into 24 humanized antibodies, namely: hu-02-L1H1, hu-02-L2H1, hu-02-L3H1, hu-02-L1H2, hu-02-L2H2, hu-02-L3H2, hu-02-L1H3, hu-02-L2H3, hu-02-L3H3, hu-02-L3H3, hu-02-L1H4, hu-02-L2H4, hu-02-L3H4, hu-02-L1H5, hu-02-L2H5, hu-02-L3H5, hu-02-L1H6, hu-02-L2H6, hu-02-L3H6, hu-02-L1H7, hu-02-L2H7, hu-02-L3H7, hu-02-L1H8, hu-02-L2H8, and hu-02-L3H8.

Example 13 Affinity Assay for Humanized Antibodies

[0135] ForteBio Biofilm Interference (BLI) was used to detect the affinity of humanized antibodies. The sample to be tested (solidified) was diluted to 20 μg/mL with 0.02% PBST (buffer). The analyte human CD24 protein (ACRO) was diluted to 100 nM with 0.02% PBST (buffer) as the initial concentration, and then diluted by double gradient for 7 gradients starting from this concentration, and a negative control (buffer) was performed.

[0136] The molecular interaction analysis instrument (ForteBio; Octet RED96e) was operated, and 200 μL of

curing buffer (0.02% PBST) was added to a pre-wetted plate, and the sensor was pre-wetted for 5-10 min. Diluted samples and other reagents for assay were added into wells in the sample plate corresponding to the pre-wetted sensor.

[0137] Through the analysis of the experimental data, the affinity assay data for the humanized antibodies were obtained as shown in Table 5 and Table 6.

TABLE 5

Antibody Name	K_D (mol/L)
CH-01	8.08×10^{-09}
hu-01-L1H1	No bonding
hu-01-L1H2	5.58×10^{-09}
hu-01-L1H3	1.00×10^{-08}
hu-01-L1H4	4.82×10^{-08}
hu-01-L2H1	No bonding
hu-01-L2H2	1.05×10^{-08}
hu-01-L2H3	9.03×10^{-09}
hu-01-L2H4	3.91×10^{-08}
hu-01-L3H1	No bonding
hu-01-L3H2	8.29×10^{-09}
hu-01-L3H3	1.24×10^{-08}
hu-01-L3H4	4.12×10^{-08}

TABLE 6

Antibody Name	K_D (mol/L)
CH-02	1.19×10^{-08}
hu-02-L1H1	6.27×10^{-08}
hu-02-L2H1	3.27×10^{-08}
hu-02-L3H1	4.47×10^{-08}
hu-02-L1H2	Untested
hu-02-L2H2	3.58×10^{-08}
hu-02-L3H2	2.27×10^{-08}
hu-02-L1H7	3.00×10^{-08}
hu-02-L2H3	3.25×10^{-08}
hu-02-L3H3	4.35×10^{-08}
hu-02-L1H4	Untested
hu-02-L2H4	2.79×10^{-08}
hu-02-L3H4	3.26×10^{-08}
hu-02-L1H5	Untested
hu-02-L2H5	2.68×10^{-08}
hu-02-L3H5	2.45×10^{-08}
hu-02-L1H6	Untested
hu-02-L2H6	2.58×10^{-08}
hu-02-L3H6	2.95×10^{-08}
hu-02-L1H7	2.05×10^{-08}
hu-02-L2H7	1.23×10^{-08}
hu-02-L3H7	1.35×10^{-08}
hu-02-L1H8	Untested
hu-02-L2H8	8.23×10^{-09}
hu-02-L3H8	1.00×10^{-08}

Example 14 Binding Activity of Humanized Antibodies to Tumor Cells

[0138] The binding activity of the humanized antibodies to the tumor cells was detected according to the method of Example 8.

[0139] The binding activity of the humanized antibodies and MCF7 cells is shown in FIGS. **6***a*-**6***d*. As can be seen from the results, except for hu-01-L1H1 and hu-01-L3H1, which have poor binding activity and MCF7 cells, all other humanized antibodies have good binding activity and MCF7 cells, which are comparable to the chimeric antibodies.

[0140] The binding activity of the humanized antibodies and SKOV3 cells is shown in FIGS. **7***a*-**7***c*. As can be seen from the results, except for hu-01-L1H1 and hu-01-L3H1, which have poor binding activity and SKOV3 cells, all other humanized antibodies have good binding activity and MCF7 cells, which are comparable to the chimeric antibodies.

[0141] The binding activity of the humanized antibodies and huCD24-MDA-MB-231 cells is shown in FIG. **8**. As can be seen from the results, the binding activity of the humanized anti-CD24 antibodies and huCD24-MDA-MB-231 cells are significantly better than those of the chimeric antibody CH-02 and the control antibody.

Example 15 ADCP Effects Mediated by Chimeric and Humanized Antibodies

[0142] Antibodies were diluted with FACS buffer into diluents with different concentration gradients. Antibody diluents were added into the 96-well round-bottom plate using a 100 μL pipette displacement gun at 50 μL per well.

[0143] After the cells were evenly dispersed and counted, the cells were washed with PBS. The cell density of tumor cells (MCF7 cells or SKOV3 cells) was adjusted to 1×10^6 cells/mL with pre-warmed PBS, and CFSE was added to make its concentration to 1 μM. The cells were incubated at 37° C. for 20 min, and centrifuged to remove the supernatant. The cells were resuspended by adding 5 mL of 1640+10% FBS complete medium. The cells were centrifuged after the incubation was completed, and the cells were resuspended with 1640+10% FBS complete medium and counted The cell density was adjusted to 2×10^6 cells/mL for use. The diluted cells were poured into a sterile sampling tank, and the cells were added to the above cell plates using a 300 μL 12-channel discharge gun at 50 μL per well, and the number of tumor cells per well was 1×10^5/well.

[0144] The monocytes were induced into macrophages, and cells were digested by accutase. Macrophages were made into cell suspension, and centrifuged, and supernatant was discarded. RPMI1640 complete medium was added for resuspension counting, and the cell density was adjusted to 1×10^6 cells/mL. Antibody diluent was added into the 96-well round-bottomed plate with a 100 μL pipette volley gun, each well was 100 μL, and gently blew a few times. The two cells and antibody diluent were mixed, and co-incubated at 37° C. for 3 h.

[0145] Adding the secondary antibody: the incubated 96-well round-bottomed plate was put into the centrifuge and centrifuged. The supernatant was discarded. The secondary antibody CD14 Monoclonal Antibody (61D3) APC was diluted at 1:100 with FACS buffer, and it was added to each well at 100 μL/well, and incubated at 4° C. for 20 min. The plate was washed twice with FACS buffer. The samples were detected and analyzed. The results are shown in FIGS. **9***a*-**9***b* and FIGS. **10***a*-**10***b*.

[0146] As can be seen from the results, CH-01, CH-02, hu-01-L2H4 and hu-02-L2H8 all mediate the phagocytosis of MCF7 cells and CD24-SKOV3 cells by macrophages.

Example 16 in Vivo Pharmacodynamic Experiments with Humanized Antibodies

[0147] A subcutaneous tumor model of ovarian cancer SKOV3 cells in BALB/C nude mice was established to evaluate in vivo anti-tumor efficacy of the humanized antibody hu-02-L2H8. SKOV3 cells were resuscitated, cell culture was performed, and cell suspension was prepared after digestion. When the cells were cultured to the logarithmic growth phase, the cells were collected, and the tumor

cell suspension was injected into the subcutaneous of BALB/C nude mice, each of which was inoculated with 100 μL of cell suspension containing 5×10^6 cells. Tumor growth was observed and tumor growth curves were plotted.

[0148] When the subcutaneous tumor grew to about 500 mm^3, the subcutaneous tumor was stripped, divided into 1 mm^3 tumor tissue blocks in the medium, and transplanted to the subcutaneous back of BALB/C nude mice. When the average volume of subcutaneous tumors reached 75-125 mm^3, the animals were randomly grouped according to tumor volume and given anti-CD24 antibody treatment.

[0149] The grouped tumor-bearing mice were given intraperitoneal injections of the antibodies hu-02-L2H8 and PBS, respectively, and 10.0 mg/kg was administered twice a week for a total of five times.

[0150] The tumor-suppressive efficacy of the compounds was evaluated by TGI (%). The formula for calculating TGI (%) was: TGI (%)=[1−(average tumor volume at the end of administration of a treatment group−average tumor volume at the beginning of administration of this treatment group)/(average tumor volume at the end of treatment of solvent control group−average tumor volume at the beginning of treatment of solvent control group)]×100%.

[0151] The tumor growth curves of the mouse ovarian cancer SKOV3 cell subcutaneous tumor model of hormonal mice given the antibody are described in FIG. **8**, where the number of days after the start of treatment was indicated by the horizontal coordinate and the tumor volume was indicated by the vertical coordinate. The tumor suppression rate TGI (%) was greater than 50%.

[0152] As shown in FIG. **11**, the humanized antibody hu-02-L2H8 had a high tumor inhibitory effect, which was significantly better than that of the PBS group.

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

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

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

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
65                  70                  75                  80

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

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            100                 105                 110
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Gly

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1 5 10 15

Glu Glu Ile Thr Leu Thr Cys Ser Ala Ser Ser Ser Val Ser Ser Met
20 25 30

His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Leu Leu Ile Tyr
35 40 45

Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Phe Tyr Ser Leu Thr Ile Ser Ser Val Glu Ser Glu
65 70 75 80

Asp Ala Ala Asp Tyr Tyr Cys His His Trp Thr Ser Tyr Met Tyr Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

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1 5 10 15

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

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

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

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

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Val His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105 110

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1 5 10 15

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

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

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

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

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Thr Ser Gly Val His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105 110

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1 5 10 15

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

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

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

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

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Thr Ser Gly Val His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105 110

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1 5 10 15

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

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

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

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

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Thr Ser Gly Val His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105 110

<210> SEQ ID NO 13
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1 5 10 15

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            20                  25                  30

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

Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys His His Trp Thr Ser Tyr Met Tyr Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
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Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Ser Met
            20                  25                  30

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

Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Glu Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys His His Trp Thr Ser Tyr Met Tyr Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105


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Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Ser Met
            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Glu Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys His His Trp Thr Ser Tyr Met Tyr Thr
                85                  90                  95
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Ser Leu Ser Leu Thr Cys Thr Val Thr Gly Tyr Ser Ile Thr Ser Gly
            20                  25                  30

Tyr Thr Trp His Trp Ile Arg Gln Phe Pro Gly Asn Lys Leu Glu Trp
        35                  40                  45

Met Gly His Ile Gln Tyr Thr Gly Asn Thr Lys Tyr Asn Pro Ser Leu
    50                  55                  60

Glu Ser Arg Ile Ser Ile Thr Arg Glu Thr Ser Lys Asn Gln Phe Phe
65                  70                  75                  80

Leu Gln Leu Asn Ser Val Thr Thr Glu Asp Thr Gly Thr Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Ser Thr Asn Ser Asn Asp Tyr Trp Gly Gln Gly Thr Ser
            100                 105                 110

Val Thr Val Ser Ser
        115


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1               5                   10                  15


<210> SEQ ID NO 19
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1 5 10 15

Glu Lys Val Thr Val Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

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

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Thr Ser Val Lys Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Phe Ile Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
100 105 110

Lys

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Ala

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<400> SEQUENCE: 22

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<210> SEQ ID NO 23
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1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Gly
20 25 30

Tyr Thr Trp His Trp Ile Arg Gln His Pro Gly Lys Gly Leu Glu Trp
35 40 45

Ile Gly His Ile Gln Tyr Thr Gly Asn Thr Lys Tyr Asn Pro Ser Leu
50 55 60

Glu Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Ser Thr Asn Ser Asn Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

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1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Gly
20 25 30

Tyr Thr Trp His Trp Ile Arg Gln His Pro Gly Lys Gly Leu Glu Trp
35 40 45

Ile Gly His Ile Gln Tyr Thr Gly Asn Thr Lys Tyr Asn Pro Ser Leu
50 55 60

Glu Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Thr Tyr Tyr Cys
85 90 95

Ala Arg Gly Ser Thr Asn Ser Asn Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

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1               5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Gly
            20                  25                  30

Tyr Thr Trp His Trp Ile Arg Gln His Pro Gly Asn Gly Leu Glu Trp
        35                  40                  45

Ile Gly His Ile Gln Tyr Thr Gly Asn Thr Lys Tyr Asn Pro Ser Leu
    50                  55                  60

Glu Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
65                  70                  75                  80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Thr Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Ser Thr Asn Ser Asn Asp Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110

Val Thr Val Ser Ser
        115


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<223> OTHER INFORMATION: VH

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Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Gly
            20                  25                  30

Tyr Thr Trp His Trp Ile Arg Gln His Pro Gly Asn Lys Leu Glu Trp
        35                  40                  45

Ile Gly His Ile Gln Tyr Thr Gly Asn Thr Lys Tyr Asn Pro Ser Leu
    50                  55                  60

Glu Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
65                  70                  75                  80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Thr Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Ser Thr Asn Ser Asn Asp Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110

Val Thr Val Ser Ser
        115


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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 28

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1               5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Gly
            20                  25                  30

Tyr Thr Trp His Trp Ile Arg Gln Phe Pro Gly Asn Lys Leu Glu Trp
        35                  40                  45
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Ile Gly His Ile Gln Tyr Thr Gly Asn Thr Lys Tyr Asn Pro Ser Leu
50 55 60

Glu Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Thr Tyr Tyr Cys
85 90 95

Ala Arg Gly Ser Thr Asn Ser Asn Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> SEQ ID NO 29
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VH

<400> SEQUENCE: 29

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

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Gly
20 25 30

Tyr Thr Trp His Trp Ile Arg Gln Phe Pro Gly Asn Lys Leu Glu Trp
35 40 45

Ile Gly His Ile Gln Tyr Thr Gly Asn Thr Lys Tyr Asn Pro Ser Leu
50 55 60

Glu Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Thr Tyr Tyr Cys
85 90 95

Ala Arg Gly Ser Thr Asn Ser Asn Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> SEQ ID NO 30
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VH

<400> SEQUENCE: 30

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

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Gly
20 25 30

Tyr Thr Trp His Trp Ile Arg Gln Phe Pro Gly Asn Lys Leu Glu Trp
35 40 45

Ile Gly His Ile Gln Tyr Thr Gly Asn Thr Lys Tyr Asn Pro Ser Leu
50 55 60

Glu Ser Arg Ile Thr Ile Ser Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Thr Tyr Tyr Cys

-continued

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                85                  90                  95

Ala Arg Gly Ser Thr Asn Ser Asn Asp Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110

Val Thr Val Ser Ser
        115


<210> SEQ ID NO 31
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VH

<400> SEQUENCE: 31

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

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Gly
            20                  25                  30

Tyr Thr Trp His Trp Ile Arg Gln Phe Pro Gly Asn Lys Leu Glu Trp
        35                  40                  45

Met Gly His Ile Gln Tyr Thr Gly Asn Thr Lys Tyr Asn Pro Ser Leu
    50                  55                  60

Glu Ser Arg Ile Thr Ile Ser Arg Asp Thr Ser Lys Asn Gln Phe Ser
65                  70                  75                  80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Thr Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Ser Thr Asn Ser Asn Asp Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110

Val Thr Val Ser Ser
        115


<210> SEQ ID NO 32
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VL

<400> SEQUENCE: 32

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

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
            20                  25                  30

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

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
                85                  90                  95

Tyr Phe Ile Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu
            100                 105                 110

Lys
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-continued

<210> SEQ ID NO 33
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VL

<400> SEQUENCE: 33

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

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

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

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Phe Ile Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu
100 105 110

Lys

<210> SEQ ID NO 34
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VL

<400> SEQUENCE: 34

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

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

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

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Phe Ile Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu
100 105 110

Lys

<210> SEQ ID NO 35
<211> LENGTH: 330
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Heavy chain constant region

<400> SEQUENCE: 35

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys

-continued

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1               5                   10                  15

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 Val 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
    290                 295                 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330


<210> SEQ ID NO 36
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Light chain constant region

<400> SEQUENCE: 36

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1               5                   10                  15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
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-continued

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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
65                  70                  75                  80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
                85                  90                  95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100                 105
```

1. (canceled)

2. An anti-CD24 antibody or antigen-binding fragment thereof, comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the heavy chain complementary determining regions HCDR1, HCDR2, and HCDR3, and the light chain variable region comprises the light chain complementary determining regions LCDR1, LCDR2, and LCDR3, wherein

(a) HCDR1 of the heavy chain variable region is selected from any amino acid sequence of SEQ ID NOs: 2, 17, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 2, 17, or an amino acid sequence having one or more conserved amino acid mutations in comparison to any amino acid sequence of SEQ ID NOs: 2, 17;

(b) HCDR2 of the heavy chain variable region is selected from any amino acid sequence of SEQ ID NOs: 3, 18, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 3, 18, or an amino acid sequence having one or more conserved amino acid mutations in comparison to any amino acid sequence of SEQ ID NOs: 3, 18;

(c) HCDR3 of the heavy chain variable region is selected from any amino acid sequence of SEQ ID NOs: 4, 19, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 4, 19, or an amino acid sequence having one or more conserved amino acid mutations in comparison to any amino acid sequence of SEQ ID NOs: 4, 19;

(d) LCDR1 of the light chain variable region is selected from any amino acid sequence of SEQ ID NOs: 6, 21, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 6, 21, or an amino acid sequence having one or more conserved amino acid mutations in comparison to any amino acid sequence of SEQ ID NOs: 6, 21;

(e) LCDR2 of the light chain variable region is selected from any amino acid sequence of SEQ ID NOs: 7, 22, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 7, 22, or an amino acid sequence having one or more conserved amino acid mutations in comparison to any amino acid sequence of SEQ ID NOs: 7, 22; and/or

(f) LCDR3 of the light chain variable region is selected from any amino acid sequence of SEQ ID NOs: 8, 23, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 8, 23, or an amino acid sequence having one or more conserved amino acid mutations in comparison to any amino acid sequence of SEQ ID NOs: 8, 23.

3. The anti-CD24 antibody or antigen-binding fragment thereof according to claim 2, wherein HCDR1, HCDR2, and HCDR3 of the heavy chain variable region consist of the amino acid sequences SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4, respectively, and LCDR1, LCDR2, and LCDR3 of the light chain variable region consist of SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8, respectively.

4. The anti-CD24 antibody or antigen-binding fragment thereof according to claim 2, wherein the heavy chain variable region has the amino acid sequence given in SEQ ID NO: 1 and the light chain variable region that has the amino acid sequence given in SEQ ID NO: 5.

5. The anti-CD24 antibody or antigen-binding fragment thereof according to claim 2, wherein the heavy chain variable region and light chain variable region are selected from a group consisting of the following (1)-(12) amino acid sequence combinations:

NO.	Heavy chain variable region	Light chain variable region
(1)	SEQ ID NO: 9	SEQ ID NO: 13
(2)	SEQ ID NO: 9	SEQ ID NO: 14
(3)	SEQ ID NO: 9	SEQ ID NO: 15
(4)	SEQ ID NO: 10	SEQ ID NO: 13
(5)	SEQ ID NO: 10	SEQ ID NO: 14
(6)	SEQ ID NO: 10	SEQ ID NO: 15
(7)	SEQ ID NO: 11	SEQ ID NO: 13
(8)	SEQ ID NO: 11	SEQ ID NO: 14
(9)	SEQ ID NO: 11	SEQ ID NO: 15
(10)	SEQ ID NO: 12	SEQ ID NO: 13
(11)	SEQ ID NO: 12	SEQ ID NO: 14
(12)	SEQ ID NO: 12	SEQ ID NO: 15.

6. The anti-CD24 antibody or antigen-binding fragment thereof according to claim 2, wherein HCDR1, HCDR2, and HCDR3 of the heavy chain variable region consist of the amino acid sequences SEQ ID NO: 17, SEQ ID NO: 18, and

SEQ ID NO: 19, respectively, and LCDR1, LCDR2, and LCDR3 of the light chain variable region consist of SEQ ID NO: 21, SEQ ID NO: 22, and SEQ ID NO: 23, respectively.

7. The anti-CD24 antibody or antigen-binding fragment thereof according to claim **5**, wherein the heavy chain variable region has the amino acid sequence given in SEQ ID NO: 16 and the light chain variable region has the amino acid sequence given in SEQ ID NO: 20.

8. The anti-CD24 antibody or antigen-binding fragment thereof according to claim **56**, wherein the heavy chain variable region and light chain variable region are selected from a group consisting of the following (1)-(24) amino acid sequence combinations:

NO.	Heavy chain variable region	Light chain variable region
(1)	SEQ ID NO: 24	SEQ ID NO: 32
(2)	SEQ ID NO: 24	SEQ ID NO: 33
(3)	SEQ ID NO: 24	SEQ ID NO: 34
(4)	SEQ ID NO: 25	SEQ ID NO: 32
(5)	SEQ ID NO: 25	SEQ ID NO: 33
(6)	SEQ ID NO: 25	SEQ ID NO: 34
(7)	SEQ ID NO: 26	SEQ ID NO: 32
(8)	SEQ ID NO: 26	SEQ ID NO: 33
(9)	SEQ ID NO: 26	SEQ ID NO: 34
(10)	SEQ ID NO: 27	SEQ ID NO: 32
(11)	SEQ ID NO: 27	SEQ ID NO: 33
(12)	SEQ ID NO: 27	SEQ ID NO: 34
(13)	SEQ ID NO: 28	SEQ ID NO: 32
(14)	SEQ ID NO: 28	SEQ ID NO: 33
(15)	SEQ ID NO: 28	SEQ ID NO: 34
(16)	SEQ ID NO: 29	SEQ ID NO: 32
(17)	SEQ ID NO: 29	SEQ ID NO: 33
(18)	SEQ ID NO: 29	SEQ ID NO: 34
(19)	SEQ ID NO: 30	SEQ ID NO: 32
(20)	SEQ ID NO: 30	SEQ ID NO: 33
(21)	SEQ ID NO: 30	SEQ ID NO: 34
(22)	SEQ ID NO: 31	SEQ ID NO: 32
(23)	SEQ ID NO: 31	SEQ ID NO: 33
(24)	SEQ ID NO: 31	SEQ ID NO: 34.

9. The anti-CD24 antibody or antigen-binding fragment thereof according to claim **7**, wherein the antibody or antigen-binding fragment thereof comprises a mouse-derived antibody or antigen-binding fragment thereof, a chimeric antibody or antigen-binding fragment thereof, and/or a humanized antibody or antigen-binding fragment thereof.

10. The anti-CD24 antibody or antigen-binding fragment thereof according to claim **5**, further comprising an Fc region, and the Fc region is selected from mouse IgG1, IgG2a, IgG2b and/or IgG3, or from rat IgG1, IgG2a, IgG2b and/or IgG2c.

11. The anti-CD24 antibody or antigen-binding fragment thereof according to claim **7**, further comprising an Fc region, and the Fc region is selected from human IgG1, IgG2, IgG3 and/or IgG4.

12. (canceled)

13. (canceled)

14. (canceled)

15. A multifunctional fusion protein comprising the anti-CD24 antibody or antigen-binding fragment thereof of claim **7**.

16. The multifunctional fusion protein according to claim **15**, which further comprises one or more second antibodies or antigen-binding portions thereof that bind specifically to other antigens.

17. The multifunctional fusion protein according to claim **16**, wherein the antigen binding the second antibody or antigen-binding portion thereof is selected from a tumor-associated antigen (TAA) or an immune checkpoint.

18. (canceled)

19. (canceled)

20. Use of the anti-CD24 antibody or antigen-binding fragment thereof claim **2** comprising:

a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the heavy chain complementary determining regions HCDR1, HCDR2, and HCDR3, and the light chain variable region comprises the light chain complementary determining regions LCDR1, LCDR2, and LCDR3, wherein

(g) HCDR1 of the heavy chain variable region is selected from any amino acid sequence of SEQ ID NOs: 2, 17, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 2, 17, or an amino acid sequence having one or more conserved amino acid mutations in comparison to any amino acid sequence of SEQ ID NOs: 2, 17;

(h) HCDR2 of the heavy chain variable region is selected from any amino acid sequence of SEQ ID NOs: 3, 18, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 3, 18, or an amino acid sequence having one or more conserved amino acid mutations in comparison to any amino acid sequence of SEQ ID NOs: 3, 18;

(i) HCDR3 of the heavy chain variable region is selected from any amino acid sequence of SEQ ID NOs: 4, 19, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 4, 19, or an amino acid sequence having one or more conserved amino acid mutations in comparison to any amino acid sequence of SEQ ID NOs: 4, 19;

(j) LCDR1 of the light chain variable region is selected from any amino acid sequence of SEQ ID NOs: 6, 21, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 6, 21, or an amino acid sequence having one or more conserved amino acid mutations in comparison to any amino acid sequence of SEQ ID NOs: 6, 21;

(k) LCDR2 of the light chain variable region is selected from any amino acid sequence of SEQ ID NOs: 7, 22, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 7, 22, or an amino acid sequence having one or more conserved amino acid mutations in comparison to any amino acid sequence of SEQ ID NOs: 7, 22; and/or

(l) LCDR3 of the light chain variable region is selected from any amino acid sequence of SEQ ID NOs: 8, 23, or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to any amino acid sequence of SEQ ID NOs: 8, 23, or an amino acid sequence having one or more conserved amino acid mutations in comparison to any amino acid sequence of SEQ ID NOs: 8, 23, and

and the multifunctional fusion protein claim **15** in the preparation of a drug for the treatment of cancer.

21. The use according to claim **20**, wherein the cancer is breast cancer, ovarian cancer, lung cancer, liver cancer, melanoma, malignant glioma, head and neck cancer, colorectal cancer, gastric cancer, bladder cancer, pancreatic cancer, colon cancer, cervical cancer, or a related tumor.

22. (canceled)

23. (canceled)

* * * * *